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P01

Reproductive Genetics

P01.002.B PPARG1 gene methylation in granulosa cells relation to hyperinsulinemia and hyperandrogenism in women with polycystic ovary syndrome

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Background/Objective: one of the most prevalence endocrine disorders in women is Polycystic Ovary Syndrome (PCOS). This syndrome is heterogeneous, and recent evidence suggested that epigenetic events could responsible for the occurrence of this syndrome. one of the most significant epigenetic modifications is DNA methylation, which could decrease gene expression. There is a close connection between energy metabolism and female fertility. PPARG gene plays an important role in lipid and glucose metabolism, and it shows significant role in the evolution of the reproductive system growth and development in embryonic stage. This study aimed to investigate the frequency PPARG1 gene methylation in granulosa cells in PCOS patients with hyperinsulinemia and hyperandrogenism.

Methods: The follicular fluid of 30 normal individuals, 25 women with polycystic ovary syndrome only, 20 PCO women with hyperandrogenism (HA), and 20 PCO women with insulin resistance (IR) were collected. After isolation of granulosa cells from follicular fluid, genomic DNA extracted and after bisulfite DNA treatment, CpG-rich region in PPARG1 gene promoter was selected and methylation-specific polymerase chain reaction (MS-PCR) was performed on bisulfite DNA.

Results: The results analysis revealed a significant difference between the methylation of the PPARG1 gene in four patient groups ($P < 0.005$), indicating that the PPARG1 gene promoter is hypermethylated more significantly in PCO-IR and PCO-HA patients.

Conclusion: The frequency of PPARG1 gene promoter methylation in PCO-IR and PCO-HA individuals were higher than in other groups. So higher methylation would decrease PPARG1 gene expression in ovary, which could cause PCO-IR and PCO-HA in women in reproductive age.

Conflict of Interest: None declared

P01.004.D Severe premature ovarian insufficiency: don't forget about men

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Background/objectives: Primary ovarian insufficiency (POI) is defined by FSH levels > 20UI/l associated with amenorrhea in women before 40 years old. As infertility is on the forefront of clinical manifestations, familial descriptions of POI in several generations is rare. Here, we describe the case of two families with *TP63* variants transmitted by unaffected males.

Methods: Exome analysis was first performed on proband in the family. Segregation study by Sanger sequencing was then proposed to the relatives. A functional validation on H12999 cells was previously performed by BN-PAGE.

Results: Exome analysis identified heterozygous missense variants in family 1 and 2: NM_003722.4:c.290G>C p.(Arg97Pro) and NM_003722.4:c.1939C>T p.(Arg647Cys) respectively, both absent from population databases and predicted to be deleterious by in-silico tools. Functional studies have revealed an impact on the in vitro dimerization of TP63 (published data). To strengthen the involvement of the variants in the ovarian phenotype, extensive familial studies were performed. In family 1, all four tested paternal aunts carry the variant and report infertility, atrophic ovaries and very early secondary amenorrhea. Infertility was also reported in older generation of the family. The proband's fertile brother also carries the variant. In family 2, the proband inherited the variant from her father. Familial segregation is in progress (proband's fertile sister and paternal uncles)

Conclusion: The description of these families allows the description of an unusual mode of transmission by unaffected men to affected women and confirms *TP63* to be responsible for dominant form of POI.

Grant References: https://doi.org/10.1002/humu.24432

Conflict of Interest: None declared

P01.005.A Role of mtDNA deletions in the background of insulin resistance, PCOS and infertility

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Background: Recent literature suggests that mitochondrial dysfunction plays an important role in the pathomechanisms of insulin resistance, polycystic ovary syndrome (PCOS) and infertility associated with these disorders. In our study, we investigated the presence of the most common mtDNA abnormalities in the background of insulin resistance (IR), infertility and PCOS.

Methods: 91 female patients with infertility and IR or PCOS (mean age 35.6 years) were analyzed. Patients whose clinical picture and family history raised the possibility of mitochondrial dysfunction were included. DNA isolation was performed from blood and urine squamous cells. Mitochondrial DNA deletions were analyzed by long-range PCR.

Results: Of the 91 patients studied, 47 patients were found to have multiple deletions of mtDNA and 10 patients had single deletions, resulting in a total of 57 confirmed mtDNA deletions.

Conclusion: Based on our results so far, 62.6% of our patients with insulin resistance or polycystic ovary syndrome and infertility were found to have multiple or single mtDNA deletions, suggesting a role of mitochondrial dysfunction in the background of their clinical symptoms. Our studies may help to understand the molecular pathomechanism of insulin resistance and polycystic ovary syndrome. Systematic monitoring of mitochondrial pathways may allow us later to identify biomarkers and therapeutic targets for standardized treatment of IR and PCOS patients.

Grant References: This study was supported by the NKFIH_132812, UNKP-22-5 and STIA-OTKA-2021 grants.

Conflict of Interest: None declared

P01.006.B Clinical implications of changes in endometrial microbiome, revealed by Real time PCR analysis

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Background: In recent years the relevance of a healthy endometrial microbiome has shown significant importance in patients with infertility. A severe dysbiosis in the microbiome has been revealed to have a possible connection to implantation failures and miscarriages.

Materials and methods: We investigated 33 women with infertility for the content of endometrial microbiome. The test included quantification of total bacterial mass and Lactobacillus, as well as facultative and obligate anaerobic microorganisms, yeast-like fungi, mycoplasmas and pathogenic microorganisms. It was performed by real-time polymerase chain reaction.

Results: Totally, in 18.2% of samples a normal microbiome was established, in 33.3% there was reduced amount and in 15% lack of *Lactobacillus*, in 21.2% - presence of *Enterobacteriaceae*, in 18.2% - *Gardnerella vaginalis*+*Prevotella bivia*+*Porphyromonas spp.* (obligate anaerobic). According to pregnancy achievement, a normal microbiome was present in 0 of abortions cases, 42.8% of pregnant cases ($p = 0.008$) and 20% of cases with no pregnancy (NP). Reduced amount of *Lactobacillus* was detected in 57% of cases with miscarriages, 28.5% of cases with clinical pregnancy and 10% of NP. Lack of *Lactobacillus* had in 14.2% of abortions, 28.5% of pregnant and 10% of NP cases. *Enterobacteriaceae* were found in 28.5% of abortions, 14.3% of pregnant and 20% of NP. *Obligate anaerobic* were found in 28.5% of abortions ($p = 0.11$), 0 of pregnant women and 20% of NP cases.

Conclusion: The normal endometrial microbiome is significantly associated with clinical pregnancy rate. The presence of obligate anaerobic microorganisms showed trend for statistical association with the occurrence of miscarriages.

Conflict of Interest: None declared

P01.008.D Leveraging a combination of traditional to advanced genetic testing methods to explore genetic landscape of Recurrent Pregnancy Loss

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Background/Objectives: Recurrent Pregnancy Loss (RPL) is one of the most common pregnancy-related complications, which can be stressful and emotionally draining for a couple. Genetic alterations which are responsible for RPL, can be present in either of 3 genomes: mother, father and fetus. Our goal was to evaluate the clinical utility of a combination of traditional and advanced genetic testing to understand the underlying etiology of RPL.

Methods: We recruited 61 RPL couples and 31 products of conceptions (POCs). Karyotyping and chromosomal microarray was done to identify CNVs. NGS (Couple and TRIO exomes) was done to establish single-gene causes of RPL.

Results: In 6 individuals out of 61 couples, abnormality in karyotype was detected. Among 116 normal karyotypes, there were 11 heteromorphisms (9.5%). Out of 31 POCs, 10 were excluded because of MCC (around 30%) and one had major aneuploidy. CMA in POCs identified pathogenic CNVs in 25% of cases (5/20), VUS in 20% of cases (4/20). Couple exome sequencing was done in 20 couples, 14 were found to be carriers for autosomal recessive conditions. Putative causative variants were identified in 37.5% of TRIO cases (3/8). A few important genes (SRP54, ERBB4, NEB, ALMS, ALAD, MTHFR, F5, APOE) which are involved in vital pathways, early embryonic development and fetal demise were identified in fetus.

Conclusion: It enhances our understanding of prenatal phenotypes of many Mendelian disorders. This study shows the utilization of a combination of techniques in improving our understanding of cause of early embryonic lethality in humans.

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Conflict of Interest: Priyanka Srivastava Full time, Principal Investigator, Consumables and contingency, Chitra Bhardwaj Part-time, Senior Research Fellow, Seema Chopra Full time, Collaborator, Minakshi Rohilla Full time, consultant, Chakshu Choudhry Part-time, Anupriya Kaur Full time, Collaborator, Inusha Panigrahi Full time, collaborator, Kausik Mandal Full time, collaborator

P01.009.A High level and whole chromosome embryo mosaicism increase in older women

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Background/Objectives: Embryo mosaicism is a common event in embryos derived from preimplantation genetic testing for aneuploidy (PGT-A). Although the rate of embryo aneuploidy increases with maternal age, embryonic mosaicism appears to be slightly more prevalent in younger women. The aim of this study was to determine if maternal age influences the prevalence of distinct embryo mosaic classification.

Methods: A retrospective study including 7881 embryos from 2084 couples (January 2017-December 2022). The trophoectoderm biopsies were analysed by NGS (Veriseq, Illumina). The different type of mosaicism was defined as low (25-40%) or high (40-50%) and isolated or complex mosaicism when a single or multiples chromosomes were involved.

Results: The average female age was 35.56 + 6.37 (18-47y). Overall, the embryo mosaicism decrease with female age (OR = 0.975; 95%CI:1.007-1.042). However, the prevalence of high and whole mosaicism showed an increase with maternal age (OR = 1.024; 95%CI:1.007-1.041 and OR = 1.035; 95%CI:1.014-1.056). In contrast, mosaicism that involves a partial arm of chromosome decrease with maternal age (OR = 0.975; 95%CI:0.967-0.992). The prevalence of the isolated or complex results showed no differences with maternal age. Moreover, no correlation was found between maternal age and the specific chromosomes involved in the mosaicism.

Conclusions: Our results showed an increase in the high level and whole chromosome mosaicism in older women. Despite previous research reporting better pregnancy outcomes from low and segmental mosaic embryos, our research suggests that, although older women are not at a higher risk for mosaic embryos compared to younger women, the different types of mosaic prevalence still indicate a poorer reproductive outcome.

Conflict of Interest: Belen Lledó Instituto Bernabeu, Jose A. Ortiz Instituto Bernabeu, Ruth Morales Instituto Bernabeu, Eva Garcia-Hernadez Instituto Bernabeu, Jorge Ten Instituto Bernabeu, Andrea Bernabeu: None declared, Rafael Bernabeu: None declared

P01.010.B Primary ciliary dyskinesia with female infertility as a result of a DRC1 variant

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Objectives: We here present a female case with primary ciliary dyskinesia (PCD) and infertility. In this report we also present the evaluation of the patient family, including her twin sister, also with PCD and infertility.

Methods: Confirmation of the PCD clinical diagnosis was performed through assessment of cilia motility by high-speed video microscopy (HSVM), axoneme ultrastructure by transmission electron microscopy (TEM), and genetic characterization by whole exome sequence (WES). Gene expression studies used qPCR for mRNA expression and immunofluorescence to determine cell protein localization.

Results: We identified a homozygous nonsense variant in the *DRC1* gene (NM 145038.5:c.352C>T (p.Gln118Ter)) in the female patient with PCD and infertility, that fit the model of autosomal recessive genetic transmission. This variant eventually resulted in a dyskinetic ciliary beat with a lower frequency and a partial lack of both dynein arms as revealed by TEM analysis. Moreover, this variant implied a decrease in the expression of DRC1 mRNA and protein. Additionally, expression analysis suggested that DRC1 may interact with other DRC elements.

Conclusions: Our findings suggest that the *DRC1* null variant leads to PCD associated to infertility, likely caused by defects in the axoneme of Fallopian tube cilia. Overall, our outcomes contribute to a better understanding of the genetic factors involved in the pathophysiology of PCD and infertility and highlight the interaction of different genes in the patient

phenotype, which should be investigated further because it may explain the high heterogeneity observed in PCD patients.

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Conflict of Interest: None declared

P01.011.C Aneuploidy detection in pooled polar bodies using rapid nanopore sequencing

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Background/Objectives: In many countries, like Austria, the genetic testing of embryos is regulated by law. The analysis of polar bodies (PBs) is, in many cases, the only option for pre-implantation genetic testing, but the detection of copy number variations (CNVs) using array comparative genomic hybridization (aCGH) requires some adaptations as most commercial software products assume a diploid set of chromosomes. Currently, no protocols or studies address the sensitivity and detectability of aneuploidy in PBs using Oxford Nanopore Technology (ONT).

Methods: We performed a proof of concept pilot study with pooled first and second PBs of 102 oocytes from women undergoing IVF treatment. The samples were whole-genome-amplified and analyzed by both aCGH and ONT. An automated bioinformatics pipeline was developed in house to process nanopore sequencing data, compute ploidy states and detect CNVs.

Results: Aneuploidy analysis of ONT vs aCGH showed concordance in more than 94% (96/102, 36 euploid, 60 aneuploid) of the analyzed samples. In addition, the copy number states of all tested chromosomes were equivalent in 93% (2176/2346), with an excess of aneuploid chromosomes using ONT. Furthermore, partial chromosomal gains and losses down to about 15 Mb could also be detected.

Conclusion: Analysis of the ploidy state per chromosome of PBs with rapid nanopore sequencing as well as the detection of shorter duplications and deletions depending on the sequencing depth has advantages over standard commercial methods, which cannot handle non-diploid samples without recalculation.

Conflict of Interest: Silvia Madritsch full, Vivienne Arnold full, Martha Haider full, Julia Bosenge part-time, Mateja Smogavec full, Beatrix Weil full, Manuela Zechmeister part-time, Jürgen Neesen full, co-founder and co-owner of the HLN-Genetik GmbH, Franco Laccone full, co-founder and co-owner of the HLN-Genetik GmbH

P01.012.D Successful birth following preimplantation genetic testing (PGT) for the rare mitochondrial DNA mutation m.10197G>A

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Introduction: PGT for mitochondrial DNA disorder is identifying embryos with mutant load (ML) below the threshold level but the existence of a genetic bottleneck during oogenesis and variation of threshold level between mutations makes it difficult to select a disease-free embryo. Successful birth after PGT has not been reported for the m.10197G>A mutation, a rare variant responsible for Leigh encephalopathy.

Material and Methods: A 32-year-old female carrier of the m.10197G>A mutation and her husband requested PGT to fulfill their desire for a healthy child. Their son died 7 months after birth due to Leigh encephalopathy. ML of the son and the mother were >99% and 11%, respectively. Controlled ovarian stimulation and oocyte retrieval was performed. Fertilized ooctytes that developed to the blastocyst stage embryo were biopsied. Pyrosequencing assay was used to determine the ML of the embryo.

Results: From four PGT cycles, 15 oocytes were retrieved and 9 embryos were biopsied. Two of 9 had >95% ML and were predicted to be affected. Five embryos with ML of <5% were determined to be unaffected and eligible for transfer. Four attempts of embryo transfer resulted in a viable pregnancy. Amniocentesis confirmed the PGT diagnosis and a healthy girl was born at 39 weeks of gestation.

Conclusion: This is the first successful PGT for the m.10197G>A mutation and only report suggesting the possibility of a skewed segregation pattern in the inheritance of this rare variant. We conclude that PGT can be an useful reproductive option for carriers of rare mitochondrial DNA mutations.

Conflict of Interest: None declared

P01.013.A Study of spermal microbiome by real-time PCR in men with spermatogenic failure

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Background/Objectives: Evidence is constantly accumulating for the clinical significance of the spermal microbiome in relation to semen quality and male fertility. The aim of our study was to determine the microbiocenosis and the type and frequency of microorganisms in sperm of men with different degree of spermatogenic failure.

Methods: We analyzed the spermal microbiome in 86 patients with spermatogenic failure, using real-time PCR. The presence of fourteen different pathogens was inspected, including *Mycobacteria spp*., *Ureaplasma spp*., *Candida*, *Enterobacteriaceae spp*., *Trichomonas vaginalis* and *Corynebacterium*.

Results: Normocenosis was found in 16.7% of azoospermia, 29.8% of oligoasthenozoospermia and 38.1% of asthenozoospermia cases. Transitory microflora was detected in 16.7%, 14.9% and 14.3%, respectively. Dysbiosis was established in 66.7% of azoospermia, 55.3% of oligoasthenozoospermia and 47.6% of asthenozoospermia cases, without statistical significance. *Corynebacterium* was detected in 83.7% of all patients with spermatogenic failure, *Streptococcus spp*. in 45.6%, *Staphylococcus* in 27.2%, *Candida* in 19.6%, *Gardnerella vaginalis* in 18.5%, without significant difference between groups. We detected significantly higher frequency of *Enterobacteriaceae spp*. in azoospermia patients (66.7%), compared to patients in the two other groups (36.8%) ($p = 0.02$).

Discussion: Spermal microbiome is strongly associated with oxidative stress, spermal DNA fragmentations and sperm morphology.

Conclusion: Our study revealed high levels of spermal dysbiosis in patients with spermatogenic failure and found an association between the presence of *Enterobacteriaceae spp*. and azoospermia. These are considered to be common pathogens of the urogenital tract and their role in sperm damage is worth further investigation.

Conflict of Interest: None declared

P01.014.B Diagnostic potential of circulating Exosomal miRNA signatures in Idiopathic Recurrent Pregnancy Loss

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Background: Recurrent pregnancy loss (RPL) is one of the common and adverse pregnancy complication, however its exact etiology is poorly understood. Recently, exosomes derived from placenta into the maternal circulation have shown to play an important role in maternal-fetal communication. We tested the hypothesis that RPL may affect the miRNA cargo within circulating exosomes in maternal blood.

Methods: Exosomes were extracted form peripheral blood samples (plasma) of gestational age-matched 10 idiopathic RPL and 5 healthy pregnant women, followed by miRNA isolation. miRNA sequencing was done using Illumina NovaSeq 6000 to identify differentially expressed miRNAs (DEmiRNAs). Gene target prediction and KEGG pathway enrichment analysis was done to search for key signaling pathways.

Results: The extracted exosomes were positive for CD9, CD63, CD81 surface markers, showed round/oval-like structures on TEM and diameters in the range of 90-165nm (NTA). miRNA sequencing revealed 2181 DEmiRNAs between patients and controls, out of which 55 miRNAs were significantly expressed (known = 26, novel = 29). Among them, 48 miRNAs were overexpressed while 7 were downregulated. Most significantly overexpressed miRNAs belong to C19MC (miR-181, miR-498, miR-518, miR-519) and C14MC (miR-376, miR-1185), known to express exclusively within trophoblast and are exported into maternal circulation. Pathway enrichment analysis showed that related target genes are enriched in embryo implantation and early embryonic development.

Conclusion: Exosomes isolated from idiopathic RPL women exhibit a unique miRNA profile, suggesting their role in maternal-fetal communication and could be potential biomarkers to provide insight into pathophysiology of RPL and play a pivotal role in disease diagnostics.

Grant Reference: ICMR:5/10/FR/12/2021-RBMCH

Conflict of Interest: Chitra Bhardwaj Full time PhD Scholar, Principal Investigator, From the Research Contigency funds, Priyanka Srivastava Assistant Professor, Collaborator, Contigency support, Minakshi Rohilla Professor, Collaborator, Anupriya Kaur Associate Professor, Collaborator, yes, Kausik Mandal Professor, Collaborator, Yes

P01.015.C Shingles infection and recurrent miscarriage – using genetics to dissect shared genetic susceptibility and causal relationships

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The loss of two or more pregnancies before 24 weeks of gestation is defined as recurrent pregnancy loss (RPL) and at any time as recurrent miscarriage (RM). We investigated the causality between infection from the *herpes zoster* virus (shingles) and subsequent risk of RPL and RM.

We performed two-sample Mendelian randomization (MR) analysis. As instruments for shingles, we used summary statistics from 23andMe genome-wide association study (GWAS; PMID:28928442). As the outcome data, we used two RPL/RM datasets: (1)LUCAR (Lviv Ukrainian Cohort for Advancing Reproductive Health) study from the Western Ukraine, including 350 women with confirmed idiopathic RPL and 458 control women with at least one healthy child; (2)UK Biobank (UKBB) dataset, consisting of 15,338 women with RM and 136,274 control women with at least one (self-reported) healthy-born child. The LUCAR/UKBB genome-wide array datasets were QCed, imputed to TopMED/HRC reference panels density. *PRSice-2* was used for clumping of shingles GWAS summary statistics ($P\text{-value} \le 5 \times 10^{-5}$). MR was done using *TwoSampleMR0*.**5.2** package.

The MR using only two established lead shingles SNPs as instruments (rs2523591, rs7047299) did not show causality (*P-value*>0.5) between shingles and RPL/RM. We then expanded the sets of independent instruments to 73/183 for LUCAR/UKBB RPL/RM GWAS ($P\text{-value} \le 5 \times 10^{-5}$), respectively. After performing MR analyses in each study, we meta-analysed them and detected a nominally significant (OR[95%CI] = 0.998[0.996-1.0000], *P-value* = 0.055) protective causal effect of shingles on risk of RPL/RM. Our findings suggest that exposure to *herpes zoster* infections should be investigated further for its protective effect on susceptibility to idiopathic pregnancy loss.

Conflict of Interest: None declared

P01.016.D Relevance of the synaptonemal complex in male infertility: Variants in central element genes and meiotic arrest

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Background/Objectives: Disturbed meiotic processes, like homologous chromosome synapsis mediated by the synaptonemal complex, cause meiotic arrest and spermatogenic failure. Variants disrupting the central element (CE) components SYCE1 and SIX6OS1 (*C14orf39*) – but not SYCE2, SYCE3 and TEX12 – have been described as genetic cause of male infertility.

Methods: Exome sequencing of >2,000 men, of whom the majority has pathomechanistically unexplained infertility after standard diagnostics including karyotyping and AZF analysis, was queried for rare (MAF <0.01 gnomAD) bi-allelic loss-of-function or missense variants (CADD >20) in *C14orf39*, *SYCE1*, *SYCE2*, *SYCE3*, *TEX12*. Findings were validated by Sanger sequencing and classified according to clinical guidelines (ClinGen/ACMG).

Results: We identified variants in two of the five CE genes likely causing the individual's spermatogenic failure: one patient carried bi-allelic variants in *SYCE1* (Wyrwoll, Andrology 2022) and the following four patients in *C14orf39*. Two unrelated azoospermic patients (one with meiotic arrest) were homozygous for c.1059-2A>G likely affecting splicing (pathogenic). One man with meiotic arrest carried compound-heterozygous pathogenic frameshift variants (c.[325_326del];[1154_1157del]). Another man with severe oligozoospermia carried a pathogenic frameshift

(c.453_454del) and a predicted splice region variant (c.511+3A>G, uncertain significance) in compound-heterozygous state.

Conclusion: Loss-of-function variants in *C14orf39* and *SYCE1* broaden the genetic landscape of male infertility due to impaired chromosome synapsis essential for recombination and segregation during meiosis. Sufficient evidence (ClinGen: strong) justifies their inclusion in diagnostic panels for male infertility, whereas the other genes remain interesting candidates and merit further investigation.

Grant References: This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells'.

Conflict of Interest: None declared

P01.017.A Preimplantation genetic testing in carriers of chromosomal translocations – analysis of unbalanced and sporadic chromosomal aberrations

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Background: Balanced chromosomal rearrangements (BCR), with an estimated incidence of 0.2% in the general population, occur about 10 times more frequently in patients with reproductive problems - infertility, miscarriage, stillbirth and liveborn with a chromosomal abnormality. Preimplantation genetic testing for structural rearrangements (PGT-SR) is a useful approach for reducing miscarriage and increase successful pregnancy rate in such couples. The aim of our study was to determine the frequency and spectrum of chromosomal aberrations in embryos after PGT-SR in carriers of BCR, looking for the incidence of sporadic aberrations as well.

Materials and methods: Eighteen couples underwent IVF cycles, followed by PGT-A due to BCR. Embryonic cells were analyzed by Next Generation Sequencing after Whole Genome Amplification using PG-Seq™ Rapid kit (Perkin Elmer) protocol.

Results: Overall, 77 embryos were subjected to trophectoderm biopsy - 4.3 embryos/cycle on average. We found 18 euploid embryos in total (23.4%). In 22.2% of the couples no euploid embryo was detected. In 57% of the cases aberrations involved the chromosomes from the parent's translocation. In 14% the karyotype included abnormalities in derivative chromosomes along with some other chromosomes. Only sporadic aberrations were found in 27% of the cases.

Discussion: Our results confirmed an increase in segregation defects involving chromosomes other than those involved in a particular translocation.

Conclusion: We detected high sporadic aneuploidy rate both in Robertsonian and reciprocal translocation. This is an important issue in genetic counseling of couples with BCR

Conflict of Interest: None declared

P01.018.B Aneuploid human embryos are associated with increased maternal age, poor embryo morphology, high mitochondrial content and advanced development day of the biopsy: an analysis of 15'000 embryonic genomes

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Using annotated database of low-coverage, whole-genome sequences of trophectoderm biopsies from 15'012 human blastocyst-stage embryos from Medical Genomics LLC (Moscow, Russia), we analyzed factors, associated with embryo aneuploidy. The multiple logistic regression model, where aneuploid embryos were coded as 1 and euploid embryos as 0, demonstrated significant (all p-values < 8.0e-13) association with four factors. The strongest and the well known factor was the increased maternal age (the scaled coefficient is 0.459); the second factor was poor embryo morphology, numerically coded from 2 till 11 (the numerical embryo quality scoring index) where low numbers correspond to the poor morphology (-0.357); the third factor was the mtDNA content (0.292) and the fourth one was the advanced development day, when the biopsy was performed (0.180). Altogether, three factors beside the maternal age contribute to the risk of aneuploidy much more than the maternal age solely. We demonstrate that the analysis of large datasets obtained from in-vitro fertilization (IVF) cliniques can lead to numerous discoveries, advancing both basic and translational research (https://doi.org/10.1101/2022.10.14.512116). We invite IVF cliniques and laboratories around the whole globe to join our research consortim INITIATOR (IN vItro ferTIlizATion fOr Research) in order to advance the knowledge in human embryogenesis.

Conflict of Interest: Maxim Ri full, collaborator, Natalia Ree: None declared, Maria Tofilo: None declared, Anastasia Kirillova: None declared, Irina Zvereva: None declared, Ilya Mazunin: None declared, Konstantin Popadin full, principal investigator

P01.019.C Family-based methods to identify genes implicated in fetal viability

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Background: Early pregnancy losses often occur before the mother is even aware of her pregnancy, making it difficult to investigate genetic variants associated with early fetal viability. One hypothesis is that assisted reproductive technology (ART) might increase survival rates in the earlier stages of pregnancy. Consequently, the distribution of alleles in children conceived by ART might differ from naturally conceived (non-ART) children.

Methods: We used data from the Norwegian Mother, Father, and Child Cohort Study (MoBa), hereunder genotype data on 18,357 family triads and dyads, one of the largest samples available. 1,334 children were conceived using ART. We estimated fetal, maternal, and parent-of-origin effects associated with ART, applying family-based log-linear models implemented in the R package HAPLIN. Additionally, we also stratified for different ART methods, such as in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and fresh and frozen embryo transfers.

Results: Our analyses reveal significant associations between single nuclear polymorphisms (SNPs) and ART. When stratifying for IVF, ICSI, fresh, and frozen transfers, we identified additional

associations. The SNPs identified are in genes such as *ATP6V1E1*, *ARVCF*, and *SYN3*.

Conclusion: Our results indicate a discordance in the distribution of alleles in ART vs. non-ART children. Further replications are necessary to confirm these findings.

References:

Magnus et al. Cohort profile update: the Norwegian Mother and Child Cohort study (MoBa). Int J Epidemiol. 2016.

Gjessing & Lie. Case-parent Triads: Estimating Single-and Double-dose Effects of Fetal and Maternal Disease Gene Haplotypes. Annals of Human Genetics. 2006.

Grants:

Norwegian Research Council (grant 262700)

Conflict of Interest: None declared

P01.020.D Genetics of congenital hypogonadotropic hypogonadism in the Portuguese population

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Background/Objectives: Congenital hypogonadotropic hypogonadism (CHH) is a rare reproductive endocrine disorder characterised by failure of pubertal development and infertility, due to deficiency of gonadotropin-releasing hormone (GnRH). CHH is genetically heterogeneous and so far, 160 genes have been implicated with the disease.

Methods: The study sequenced 79 Portuguese CHH patients by whole exome sequencing (WES). Pathogenic variants were searched in a list of 160 genes.

Results: We found that 29.1% (23/79) of patients carried at least one pathogenic or likely pathogenic variant. These occurred in 12 genes, namely *GNRHR* (5/79), *ANOS1*, *CHD7* and *FGFR1* (3/79 each), *BBS10* and *PROK2* (2/79 each), and *DCC*, *GNRH1*, *IGFALS*, *POLR3B*, *PROKR2* and *RBM28* (one patient each). Oligogenic inheritance was observed in one patient. When considering variants of uncertain significance (VUS) together with pathogenic and likely pathogenic variants, the proportion of patients with potentially harmful variants increased to 93.7% (74/79) and the proportion of oligogenicity increased to 69.6% (55/79).

Conclusion: Genetic analysis identified a pathogenic variant in 29.1% of the patients. The most involved genes were *GNRHR* followed by *ANOS1*, *CHD7* and *FGFR1*, which confirm the weight of classic CHH-genes in the aetiology of the disease. The high number of VUS and oligogenicity suggest the involvement of other genes in the pathogenesis of this complex disorder.

Grant References: Portuguese Foundation for Science and Technology (PTDC/SAU-GMG/098419/2008, UIDB/00709/2020, and 2020.04924.BD), and Sidra Medicine (SDR400038).

Conflict of Interest: None declared

P01.021.A Results of KIR-HLAC genotyping among Ukrainian married couples with early reproductive losses and in the material spontaneously aborted embryos

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The interactions between the Killer Immunoglobulin-like Receptor and Human Leukocyte Antigen-C molecules is of particular interest in the study of the mechanisms of maternal-fetal allorecognition. As both maternal *KIR* and fetal *HLA-C* are highly polymorphic, there will be different maternal/fetal genetic combinations in each pregnancy. *KIR-HLAC* genotyping allows determining whether there is a good compatibility between KIR uterine receptors and the "foreign" HLA-C presented by the embryo. The **aim** of the study was *KIR-HLAC* genotyping in married couples with early reproductive losses (RL) and/or unsuccessful in vitro fertilization and in the material of spontaneously aborted embryos.

Methods: *KIR-HLAC* genotyping was performed by SSP-PCR. The experimental group consisted of 342 couples with ≥2 consecutive miscarriages and/or unsuccessful IVF and 151 spontaneously eliminated embryos.

Results: The spectrum of *KIR* genes was analyzed and the frequency of *KIR* genotypes in women with RL was established. The *AB* genotype is the most common (71.64%), the *AA* genotype was established in 95 women out of 342 surveyed (27.78%), and the *BB* genotype was established in 0.58% women. *HLAC* genotyping of couples with RSA showed the *C2/C2* genotype of the *HLAC* gene in 20.76% of women, 23.10% of men and 16.56% of embryos. According to the results of *KIR-HLAC* analysis of genotyping of couples with RSA, a significant risk of reproductive losses of immunological origin was found in 52.34% of cases.

Conclusions. *KIR-HLAC* genotyping is a genetic test that allows to evaluate the risk of an embryo being rejected by the mother's immune system.

Conflict of Interest: None declared

P01.023.C Preimplantation Genetic Testing (PGT) for BRCA1/2 carriers: Lessons from a large cohort in a centralized unit

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PGT for prevention of cancer in BRCA1/2 carriers became recently optional yet debatable.We present the experience of a centralized unit performing PGT for carriers of BRCA1/2 pathogenic variants.

Between 2006–2022, overall 197 couples performed PGT for preventing fetus with BRCA1/2 pathogenic variants: 119 (60%) carriers of BRCA1 mutation and 72 (40%) carriers of BRCA2 mutation. Six couples had combinations of BRCA1/2 pathogenic variants in one or both spouses. Of the BRCA1 carriers, 60% were females and 40% males. Of the BRCA2 carriers, 70% were females and 30% males. All patients presented a family history of breast/ovarian/ pancreatic cancer. Overall, 12 of the woman had breast cancer prior to PGT counseling. The majority (63%) did not have children. Forty (20%) couples had fertility problems. For BRCA1, 216 PGT cycles were performed. For BRCA2, 174 PGT cycles were performed. Overall, 13 PGT+aneuploidy cycles were performed for both BRCA1/2. Of BRCA1 carriers, 100% requested to freeze males embryos carriers and 24% requested to transfer a male carrier. Of BRCA2, 40% requested to freeze males embryos carriers but no male carrier embryo of BRCA2 was transferred. Couples that had both a BRCA1/2 preferred to freeze males carrying the BRCA1 but not the BRCA2.

Differences between BRCA1 and BRCA2 carriers were reflected in the male embryos carriers requested to be frozen and/or returned. While this application for PGT becomes acceptable, professionals involved in the process should be aware and address the specific issues and concerns of this group regarding medical, emotional and moral aspects.

Conflict of Interest: None declared

P01.024.D Time to change: CNV and SNV analysis using exome sequencing data as a first-tier genetic test for male infertility

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Background/Objectives: For years, patients with severe oligo/azoospermia are offered karyotyping, AZF deletion screening and/or *CFTR* testing. For other sperm abnormalities, like oligoasthenoteratozoospermia or globozoospermia, no genetic diagnostics are recommended. The objective of this study was to determine whether whole exome sequencing (WES) with combined Copy Number Variant (CNV) and Single Nucleotide Variant (SNV) analysis is a reliable first-tier method to replace current methods, with the option to expand the testing strategy with (novel) male infertility genes.

Methods: WES was performed on DNA of patients with AZF deletions (n = 16), (non-)mosaic sex chromosomal aneuploidies or structural anomalies (n = 35), *CFTR* variants (n = 22), or variants in known infertility genes (n = 4), and >100 controls. The data were analyzed using our standard diagnostic pipeline, including CNV callers ConiFER and ExomeDepth, with a bioinformatic filter for >130 male infertility genes.

Results: All previously reported variants in the validation cohort were correctly identified. CNV analysis of genes in regions encompassing commonly used STS-loci in each AZF region reliably detected clinically relevant Y-chromosomal microdeletions.

Conclusion: Our study shows that our WES-based strategy performs equally well or even superior compared to currently used methods. It is a reliable method to detect the most common causes of male infertility; karyotyping will still remain necessary for detecting e.g. balanced translocations or low-grade mosaicism. Aside from replacing several tests by a single strategy, WES also allows for easily expanding the analysis by adding novel genes to the bioinformatic filter, offering a comprehensive diagnostic option to a wide range of males with fertility issues.

Conflict of Interest: None declared

P01.025.A Clinical utility of periconception carrier screening by genome sequencing

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Background/Objectives: Current ACMG guidelines recommend periconception expanded carrier-screening (PCS) of recessive/X-linked genes with a carrier-frequency of at least 1:200 in all couples and independent of carrier-frequencies in consanguineous couples. Communication of residual risk was not supported due to lack of data. We therefore studied clinical utility of PCS concerning its potential to reduce the risk of affected offspring with neurodevelopmental disorders (NDDs). In a retrospective, blinded exome-sequencing study we showed that PCS could have reduced this risk by virtually half in consanguineous couples, and by ~5% in non-consanguineous couples when analysing all genes, and by ~2% when following the ACMG guidelines (Boonsawat et al. NPJ Genomic Medicine 2022). Since this study revealed a false negative rate of ~5%, we now prospectively assessed PCS by genome-sequencing, due to its superior detecting of copy-number (CNV), structural, intronic variants, and variants in genes within complex regions.

Methods: We analyzed known recessive/X-linked (>3,600) genes using genome-sequencing in 94 parents-to-be-couples.

Results: We identified 25/94~27% of couples being at-risk for affected offspring. Consanguinity was enriched among the 20 autosomal at-risk couples and most of the genes found in at-risk constellations were linked to NDD. In comparison to exome-based PCS, genome-based PCS revealed a significant higher autosomal as well as X-linked at-risk rate. For ~24% of these at-risk couples, at-risk constellations were contributed by CNV, variants in difficult or far intronic regions.

Conclusion: Our findings indicate the superior sensitivity of genome-based PCS, which might therefore be considered for routine PCS screening.

Grant References: UZH CRPP *praeclare*.

Conflict of Interest: None declared

P01.026.B Genetic testing for monogenic forms of male infertility contributes to the clinical diagnosis of men with idiopathic severe male infertility

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Background/Objectives: Male infertility is a disease of the male reproductive system, affecting about 5 % of adult males. Because of its complexity, the cause is in many cases never identified. In recent years, many genes have been proposed to be associated with this condition, however, testing of monogenic forms has not yet been routinely implemented in the diagnosis of severe forms of idiopathic male infertility. Aiming to examine whether genetic testing for monogenic forms of male infertility could contribute to the clinical diagnosis of men with idiopathic severe male infertility we investigated men with unexplained infertility with whole exome sequencing analysing panel of genes selected based on ClinGen curation protocol.

Methods: We performed WES on 194 infertile men. DNA was prepared based on the Twist CORE exome protocol and sequenced on Illumina NovaSeq 6000 platform. All interesting variant results were classified using ACMG/AMG Practice Guidelines for Variant Classification in Rare Diseased.

Results: We identified potential monogenic disease-causing variants in 4 infertile men. We identified pathogenic/likely pathogenic variants in STAG3 (c.2776C>T, p.Arg926*; c.2817delG,

p.Leu940fs), MSH4 (c.1392delG, p.Ile465fs; c.2261C>T, p.Ser754-Leu), TEX15 (c.6848_6849delGA, p.Arg2283fs; c.6271dupA, p.Arg2091fs) and TEX14 (c.1021C>T, p.Arg341*) genes.

Conclusion: In our study, we identified potential monogenic causes in 2 % of infertile men. We confirmed the utility of monogenic testing and propose the clinical use of monogenic testing for men with severe forms of idiopathic male infertility.

Grant References: This study was supported by grant P3-0326 from the Slovenian Research Agency.

Conflict of Interest: None declared

P01.027.C A robust, accurate and cost-effective detection of alpha-thalassemia in an expanded multigene carrier testing

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Background/Objectives: Thalassemia is one of the most common monogenic diseases with a carrier frequency of 1-5% worldwide. Molecular analysis for globin gene mutation is important for the definitive diagnosis. NGS allows variant calling at multiple genes simultaneously, however alpha-thalassemia screening with NGS is challenging, due to high homology in HBA gene cluster region. Our aim is to include alpha-thalassemia in our carrier screening test for the simultaneous detection of multiple inherited conditions using our proprietary NGS based technology.

Methods: The assay consists of a targeted in-solution hybridization NGS protocol that covers all coding regions of 230 genes related to inherited conditions. An in-house algorithm was designed and trained on 200 individuals of known carrier status. Subsequently, a blind validation study was performed on 202 individuals of unknown variant status and individuals who carry an alpha-thalassemia deletion previously identified by an independent lab to set the sensitivity and specificity of alpha thalassemia detection.

Result: Variants of alpha-thalassemia were detected at sensitivity of 100% (CI: 92-100%) and specificity of 100% (CI: 88-96%). All deletions/duplications were confirmed by an orthogonal method. Sensitivity and specificity of SNVs and INDELs for remaining conditions remained at 100% (CI: 98-100%) and 100% (CI: 99.9-100%) respectively.

Conclusion: We have validated a targeted in-solution hybridization protocol for the detection of CNVs within HBA cluster, a high homology region, along with the simultaneous variant detection of multiple inherited conditions. This is a robust, accurate and cost-effective end-to-end CE-IVD solution that eliminates the need of additional testing for alpha-thalassemia.

Conflict of Interest: Skevi Kyriakou full time employment at Medicover Genetics, Michaella Georgiadou full time employment at Medicover Genetics, Achilleas Achilleos full time employment at Medicover Genetics, Christos Lemesios full time employment at Medicover Genetics, Christodoulos Savva full time employment at Medicover Genetics, Chrystalla Havadjia full time employment at Medicover Genetics, Kyriakos Tsangaras full time employment at Medicover Genetics, Gaetan Billioud full time employment at Medicover Genetics, Chrysovalando Sotiriou full time employment at Medicover Genetics, Louisa Constantinou full time employment at Medicover Genetics, Haris Kkoufou full time employment at Medicover Genetics, Lygia Ioannou full time employment at Medicover Genetics, Michalis Spyrou full time employment at Medicover Genetics, Stelia Pissaridou full time employment at Medicover Genetics, Antonia Matsentidou full time employment at Medicover Genetics, Christos Prokopi full time employment at Medicover Genetics, Melina Vaki full time employment at Medicover Genetics, Styliana Georgiou full time employment at Medicover Genetics, Elena Kypri full time employment at Medicover Genetics, Marios Ioannides full time employment at Medicover Genetics, George Koumbaris full time employment at Medicover Genetics, Philippos Patsalis full time employment at Medicover Genetics

P01.028.D Estimating carrier and at-risk couple rates across a 1000-Greek Exome Sequencing Data cohort for the ACMG-ACOG proposed 176 Expanded Carrier Screening panel

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Background/Objectives: High-throughput genomic technologies facilitate Expanded Carrier Screening (ECS) and informed reproductive choices. However, selecting the genes/conditions for optimal clinical utility is challenging and possibly population-specific. We estimated the frequency of pathogenic variants, in the 176, autosomal recessive (AR) and X-linked, genes proposed by ACMG and ACOG, among in-house Exome Sequencing (ES) data.

Materials/Methods: ES data from 1000 unrelated individuals were assessed for pathogenic SNVs and CNVs. Variant filtering used 5% MAF, ClinVar submission and ACMG criteria classification. Evaluation of at-risk couple rate, hypothesized that both partners carried variants in the same gene.

Results: Amongst the 1000 participants, 35,4% were carriers for at least one disorder and 21,2% for 2, or more, whereby 396 unique pathogenic or likely pathogenic heterozygous variants in 136/176 genes were identified. Genes with the highest carrier rates include *CYP21A2, HBB, HBA1/2, GJB2, CFTR, PAH, ATP7B, PMM2, PKHD1 and CAPN3*. Based on this cohort, a minimum 2% of couples would be at-risk for at least one AR condition, extrapolating to 1828 couples for 85000 annual births in Greece.

Discussion: This study provides data confirming that the ACMG/ACOG pan-ethnic ECS list of 176 genes is suitable for carrier screening in Greece, and aids counseling prospective parents for residual risk. The top-10 genes were as expected, although it should be noted that many common conditions (hemoglobinopathies, SMA, Fragile-X) may escape NGS-based detection requiring alternative methods. The ACMG-ACOG ECS panel is appropriate for the Greek population, but should be supported by appropriate interpretation, genetic-counselling and reproductive options.

Conflict of Interest: None declared

P01.029.A Unraveling the role of endocrine disruptors in the etiopathogenesis of endometriosis: interplay between hormones, immune mediators and epigenome in menstrual effluent

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Background/Objectives: Endocrine Disruptor Chemicals (EDCs) mimic or block endogenous hormones and have been related with hormone-related conditions including endometriosis. Endometriosis is one of the most prevalent gynecological diseases characterized by the extrauterine growth of endometrial tissue, causing chronic pelvic pain and infertility. Growing evidence relates ECD exposure with endometriosis, but the underlying mechanisms are still unexplored. In this regard, aberrant DNA methylation has been suggested as a mediator through which an endocrine/immune-system disruption might occur. Our aim is to determine whether changes in DNA methylation might be promoted by EDCs, and whether these changes, in turn, may alter hormonal and immune responses that trigger endometriosis development and progression.

Methods: We will measure EDC levels, hormone status, immune-activation and DNA methylation patterns in Menstrual Effluent (ME), a highly relevant matrix, and apply an integrated statistical approach to unravel the role of each player in the disease.

Results: We have created a multidisciplinary group with expertise in genomics, environmental epidemiology, reproductive physiology, statistics and gynecology. Results from the optimization phase indicate that ME collected overnight between day 1 and 2 of menses is the most suitable and yields sufficient serum and plasma volumes for subsequent analyses. We have obtained high quality DNA in sufficient concentration from plasma samples and will shortly optimize RNA isolation.

Conclusion: The results of this project could be applied to preventive strategies and to the design of novel targeted treatments that could improve the quality of life of affected women.

Grant references: SAN2020111043, 12-4-ID22, COLAB22/01, IT1739-22, PID2019-106382RB-I00, 2021-000007-01EXTF-00209.

Conflict of Interest: None declared

P01.030.B Whole-genome sequencing provides novel insights into the role of de novo mutations in male infertility

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Background/objectives: De novo mutations (DNMs) have been shown to play a prominent role in sporadic disorders with reduced fitness and genetic heterogeneity, such as male infertility (PMID: 35013161). In this study, we show additional evidence to support the role of de novo mutations (DNMs) in severe forms of male infertility.

Methods: In this study, we perform whole-genome sequencing in a unique cohort of 242 males presenting with azoospermia or severe oligozoospermia and their fertile parents.

Results: On average of 1.22 rare DNMs were found in the coding region and 96.8 rare DNM in the non-coding region of the genomes of these infertile men. We identified 57 recurrently mutated genes of which 5 harbour more than one protein-altering DNM (*AKAP11, FIZ1, HTT, EMC7, RP1L1*). Following a systematic analysis assessing the mutational impact and protein function, 38 out of the 295 coding DNMs were classified as possibly causative, with a further 73 predicted pathogenic DNMs requiring additional information to potentially connecting them to male infertility. Several genes were found to be involved in mRNA splicing as previously reported but also to DNA repair. Furthermore, we found 267 rare DNM within promoter and enhancer region of genes, which have the potential to disrupt gene function and spermatogenesis.

Conclusion: Our findings provide additional evidence for the role of de novo mutations in severe male infertility and point to many new candidate genes for male infertility. In addition, we provide the first insight into the role of de novo non-coding mutation in male infertility.

Conflict of Interest: None declared

P01.031.C The importance of using qCarrier Plus® Extended Compatibility test to reduce residual risks in the field of reproductive medicine

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Background/Objectives: In the context of assisted reproduction technologies, genetic matching refers to the assignment of a donor to a receptor couple guided by the minimization of disease risk for the offspring. The heterogeneity in gene composition of the different available carrier screening tests, complicates the matching of patients and donors tested with different assays. We aimed to determine the benefits of using an extended carrier screening that captures 2.898 OMIM associated genes with recessive and X-linked inheritance, in genetic matching processes.

Methods: Observational retrospective study in more than 400 matching requests between individuals tested with qCarrier Plus® Extended Compatibility test and others from third-party laboratories during 2022. Our test uses massive sequencing technology and other molecular tests to screen for mutations in 303 genes causing autosomal recessive and X-linked diseases. The capture of 2.595 additional genes allows the analysis of genes upon need and makes matching possible with tests from different companies.

Results: We show that in 45% (175/389) of cases, the receptor was a carrier of at least one mutation in a gene not analyzed in the qCarrier Plus® test. In these cases, analysis of additional genes in the donor was necessary to reduce reproductive risk. Only in 3% (5/175) of the cases a coincidence of pathogenic variants in the same gene was found.

Conclusions: Using an extensive carrier screening test allows matching between gametes tested with different carrier tests and considerably reduces reproductive risks.

Grant References: not applicable.

Conflict of Interest: Andrea Domingo Gallego Full, Anna Borgia Full, Lydia Madrid Cortés full, Raquel Garcia full, Lidia Carreño full, Neus Fornés full, Nina Bosch Pagès: None declared, Maria Segura-Puimedon full, LLuís Armengol full

P01.032.D Analysis of the placental transcriptome in pregnancy losses

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Background: Pregnancy loss (PL) has a strong clinical and social impact. It occurs in about 15% of clinically recognized pregnancies and has heterogeneous etiologies. However, approximately half of recurrent PL remains unexplained. It extremely relevant uncover molecular mechanisms behind PL to develop new clinical strategies.

Methods: We performed transcriptomic analysis of 11 placentas from 2nd trimester [6 controls vs 5 idiopathic PL (iPL)] and 13 placentas from 3rd trimester [5 controls vs 3 placentas from normal term pregnancies (TP) vs 5 iPL) by bulk RNA Sequencing.

Results: We observed similar transcriptomes among placentas from 2nd trimester. However, differential expression analyses of 3rd trimester samples detected 14 downregulated and 19 upregulated genes, comparing controls with iPL. Gene ontology analysis showed that iPL had decreased transcript levels of pregnancy specific glycoproteins and chorionic somatomammotropin hormone genes involved in the reproductive process and growth placental functions. Additionally, this analysis showed a positive regulation of signalling pathways via JAK-STAT. Comparing TP with iPL, we identified 672 DEGs, of which 375 downregulated and 297 upregulated in iPL. Downregulated genes are linked to reproductive process and upregulated genes are involved in regulation of cellular and apoptotic processes.

Conclusion: Identification of transcriptomic profiles and biological pathways involved in failed pregnancy is crucial for the clinical benefit of patients. Complementary studies on the function of these genes in PL pathophysiology remain necessary.

Grant References: This work is funded by National funds through FCT–Fundação para a Ciência e Tecnologia, I.P., within the scope of the project "EXPL/MED-GEN/1261/2021" FCT-[SFRH/BD/147440/2019]

Conflict of Interest: None declared

P01.033.A Analysis of the human oocyte transcriptome throughout meiotic maturation

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Background: Important transcriptional and epigenetic reprogramming events occur during oocyte maturation. We aim to characterize the transcriptome of human oocytes in different maturation stages – in prophase I (GV-Germinal Vesicle), in metaphase I (MI) and in metaphase II (MII).

Methods: Human oocytes were collected from surplus material of oocyte donations, after written informed consent from the donors. We performed RNA-Seq in 6 GV, 6 MI and 7 MII.

Results: Transcripts from a total of 10568 genes in GV, 9707 in MI and 8796 in MII, were detected, with 8366 overlapping in all stages. Comparison between GV and MI stages revealed 27 DEGs, 4 downregulated and 23 upregulated; between MI and MII, no DEGs were detected; and between GV and MII a higher number of DEGs were observed – 601, with 398 showing decreased expression and 203 increased expression. Genes that are more expressed in GV are associated with GO terms such as DNA synthesis and cell cycle mitotic phases whereas genes more expressed in MII are linked with RNA metabolism and nucleocytoplasmic transport. Regarding epigenetic regulators, *DNMT1* and *TET3* were highly expressed, with *DNMT1* being increased in MII oocytes.

Conclusion: We here contribute to the molecular characterization of human oocyte maturation as only few studies focused on this theme, which is important for oocyte in vitro maturation and other assisted reproduction techniques.

Grant References: This work is funded by National funds through FCT–Fundação para a Ciência e Tecnologia, I.P., within the scope of the project "EXPL/MED-GEN/1261/2021" FCT-[SFRH/BD/141855/2018]

Conflict of Interest: None declared

P01.034.C Monogenic infertility within Israeli population isolates

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Monogenic infertility within Israeli population isolates

Objectives: After ruling out chromosomal rearrangements, various monogenic etiologies are recently recognized as in infertility. Unfortunately, the clinical investigation for infertile couples does not routinely incorporate next generation sequencing (NGS) platforms as a part of the workup. We present our in-house cohort of infertile couples investigated using exome sequencing.

Methods: Between 2018-2022 we recruited patients diagnosed with infertility for genetic evaluation by NGS. The etiologies for infertility were classified into four categories: primary ovarian insufficiency (POI, 22 patients), oocyte maturation arrest (OMA, four patients), disorders of sex development (DSD, one patient) and male factor (three patients).

Results: Our cohort included 30 infertile individuals originating from 20 families (3 males, 27 females, ages 18-40). We characterized two molecular definitive diagnoses of POI within two families (*MCM9*, *NR5A1*), one with OMA *(PATL2*), one diagnosis

of DSD (*NR5A1*) and individuals with a phenotype of male factor for biallelic pathogenic variant in *CFAP44* and in *DNHD1*. Some of the results have fundamental consequences on patients, such as a recommendation to abandon IVF treatment for sisters with biallelic *PATL2* variants, cancer susceptibility in patients with biallelic *MCM9* variants and fertility preservation possibility for young sister of POI patients which is also homozygous for NR5A1 variant but still has ovarian function. There are some variants in candidate genes awaiting further studies.

Conclusions: We encourage clinicians facing unexplained infertility to search for the monogenic missing link by implementing NGS into the infertility workup

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P01.035.B Should genes for non syndromic hearing loss be included in population wide reproductive genetic carrier screening: views of those with lived experience of deafness

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Background: Some couples wish to avoid having a child born deaf, even though there are effective interventions and supports. There is no consensus on whether deafness should be included in reproductive genetic carrier screening (RGCS). This is problematic as governments consider implementation of population RGCS programs.

Aim: This study explored views of those with lived experience of deafness on the acceptability of including genes associated with non-syndromic hearing loss (NSHL) in RGCS in Australia.

Methods: Qualitative interviews were held with 27 participants: 14 who identified as deaf and 13 parents of a deaf child. Interview transcripts were analysed thematically.

Results: This study reveals the complexity of attitudes within these groups. The goal of supporting reproductive autonomy is in tension with concerns about potential harms, especially negative messages about deafness and an existential threat to Deaf culture. Deaf participants who supported carrier screening emphasised the need for accurate and current information on deafness. This stakeholder group acknowledged the complexity of defining the severity of deafness, especially when compared to other conditions included in RGCS.

Conclusion: The findings provide some support for inclusion of genes associated with NSHL but identified strong concerns that need to be addressed in the development of a RGCS program.

Conflict of Interest: None declared

P02

Prental Genetics

P02.001.A Isolation of circulating trophoblasts for a comprehensive fetal genome profiling for the detection of pathogenic submicroscopic CNVs

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Background/Objectives: The analysis of cfDNA for non-invasive prenatal testing (NIPT) is limited by the poor resolution for the detection of submicroscopic variants. Here we present a semi-automated approach for the isolation of circulating extravillous trophoblasts (cEVT) from maternal blood for copy number variant (CNV) detection.

Methods: Single cEVTs were isolated from maternal blood, fetal-origin and CNVs were assessed by proprietary algorithms from the same low-pass NGS raw data.

Results: Single cEVTs analysis provided full concordance for CNVs detection with clinical CMA. In a cohort of 61 cases with fetal chromosomal microarray analysis (CMA) there were 2 cases with pathogenic CNVs. One was a 4q26 duplication of 1.5Mb that could not be confirmed since the 2 isolated cEVTs were suitable only for aneuploidy classification. The second pCNV was a 16p13.11 deletion of 800kb detected in 2/2 isolated cEVTs. In the population with normal CMA results there were 6 (likely) benign CNVs, ranging from 900Kb to 3Mb in size, all identified by single cell analysis.

Conclusions: The analysis of cEVTs revealed imbalances as small as ~1Mb in size, which includes most of the clinically relevant micro-imbalances. Compared with cfDNA testing, the non-invasive analysis of pure unfragmented gDNA is highly advantageous, as it avoids confounding factors related to maternal genetic make-up and allows a high resolution noninvasive comprehensive fetal genomic profile to reduce the residual risk of fetal pathogenic CNVs.

Conflict of Interest: Anna Doffini Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Claudio Forcato Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Chiara Mangano Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Debora Lattuada: None declared, Roberta Aversa Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Chiara Maranta Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Emilia D. Giovannone Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Genny Buson Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Chiara Bolognesi Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Rebecca Maiocchi Consultant for A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Martina Dori Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Liyana Jamal Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Raidah B. Ahmad Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., George S. H. Yeo: None declared, Tai Wai Yeo: None declared,

Silvia Saragozza: None declared, rosamaria silipigni: None declared, Marta Serafini: None declared, Andrea Biondi: None declared, Sofia Perego: None declared, Patrizia Vergani: None declared, Enrico Ferrazzi Consultant for A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Paola Ricciardi-Castagnoli Consultant for A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Thomas J. Musci Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Francesca Romana Grati Employee of A. Menarini Biomarkers Singapore Pte Ltd, a Menarini Company., Between 2020 and 2022, Francesca Romana Grati was an advisory board member and consultant for A. Menarini Biomarkers Singapore Pte Ltd., while she was a full-time employee of TOMA laboratory.

P02.002.B Relative genotype dosage: enabling non-invasive prenatal diagnosis of Mendelian disorders in consanguineous couples

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Background: Non-invasive prenatal diagnosis (NIPD) relies on the presence in maternal blood of circulating cell-free fetal DNA released by apoptotic trophoblast cells. Widely used for aneuploidy screening, it can also be applied to Mendelian diseases to detect known parental mutations. Assessing the presence of a maternally-transmitted mutation in the fetus is achieved via relative haplotype dosage (RHDO), a method relying on "type-4" SNPs (heterozygous in the mother, homozygous in the father) to determine the maternal haplotype transmitted to the fetus. Consequently, there is an inherent risk of failure by lack of informative SNPs around the gene of interest, an event particularly likely in consanguineous couples, who often share common haplotypes over large genomic regions.

Methods: To bypass the above predicament, we propose a novel approach, "relative genotype dosage" (RGDO), which directly assesses fetal genotype by using type-5 SNPs (heterozygous in both parents, thus frequent in consanguineous couples).

Results: We present our results with various clinical situations involving consanguineous and non-consanguineous couples, typically both carriers of a recessive disease. We then show that the sensitivity of RGDO is similar to that of RHDO and that it performs well over a wide range of fetal fractions and DNA amounts.

Conclusion: We demonstrate that our RGDO method enables prenatal diagnosis in situations where standard RHDO analysis fails, thereby opening molecular NIPD to most consanguineous couples. In addition, it can also be applied to non-consanguineous couples, to complement RHDO in case there aren't enough informative SNPs to achieve diagnosis with only one method.

Conflict of Interest: None declared

P02.003.C Advancing our understanding of confined placental mosaicism: deep genome sequencing reveal both spatial & chronological genetic heterogeneity

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Background/Objectives: Confined placental mosaicism is a well-known biological phenomenon that has the potential to affect the results of prenatal genetic testing. Confined placental mosaicism is characterized by a discrepancy between the genetic composition of the placental and fetal cells, with an estimated prevalence of 1-2% of ongoing pregnancies for chromosomal aberrations. Our aim is to assess possible genetic heterogeneity of the placenta at different time points during the pregnancy in relation to de novo sequence variants.

Methods: We compared whole genome sequencing results from 1st trimester chorionic villus or 2nd trimester amniotic fluid samples, four placental biopsies, and a fetal tissue sample obtained after termination or delivery of the pregnancy in each of the six in the study included cases. In five of these cases, deep exome sequencing was performed on circulating DNA from maternal plasma.

Results: Overall, the analysis showed that the majority of de novo variants in the fetus were present in all placental samples. Different de novo variant signatures among placental biopsies indicated subclonal evolution and chronological changes. The analysis could also be used to reveal maternal contamination in the placental biopsies.

Conclusion: Our study provides new insights into the heterogeneity of the placenta in relation to de novo sequence changes in both coding and non-coding regions, highlighting the extent of chronological changes in the placenta. The study adds important knowledge to the understanding and awareness of sequence-level mosaicism when sequencing-based analyses are used in the prenatal diagnostics.

Grant References: Region Syddanmarks Forskningspulje(20/14085); Fonden til Lægevidenskabens Fremme(19-L-0370).

Conflict of Interest: None declared

P02.005.A The yield of exome sequencing in low-risk pregnancies with normal CMA – a systematic review

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Introduction: The yield of prenatal chromosomal microarray analysis (CMA) has been widely explored, both in pregnancies with sonographic anomalies, as well as in fetuses with normal ultrasound. On the contrary, the usefulness of prenatal exome sequencing has been mainly explored in pregnancies with structural anomalies, while evidence for the value of exome in pregnancies with normal sonography and CMA is scarce. Thus, the objective of this study was to perform a systematic literature review, examining the yield of exome sequencing in pregnancies with normal ultrasound and normal CMA.

Methods: Search was conducted in MEDLINE and Google Scholar databases, using the terms ((("normal pregnancies") OR ("normal fetuses")) OR ("low risk pregnancies")) AND (exome), with no language or time restrictions. Relevant articles were accessed in full text, including manual search of the references. The yield of exome sequencing was summarized using percentages with 95% confidence intervals (CI).

Results: Of the six potential papers, only two met the inclusion criteria, encompassing a total of 642 exome tests in pregnancies with normal ultrasound and normal CMA. Of these, five abnormal

results were noted – 0.78% (95%CI 0.28-1.9). The variants included three de novo heterozygous variants (in *KCNK4*, *SPRED1* and *FGFR3* genes), and two compound heterozygous genotypes for *ATP7B* and *NR2E3* variants.

Conclusions: Exome sequencing detects clinically significant findings in one of every 128 low-risk pregnancies with normal CMA. This data is important for geneticists, obstetricians, and pregnant women, to enhance informed decision-making on the issue of prenatal testing.

Conflict of Interest: None declared

P02.006.B Examining the association between fetal HLA-C and maternal KIR haplotypes and birthweight in the UK biobank

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Background: Human birthweight is under stabilizing selection, seeking balance between extremes of high and low, thereby reducing fetal and maternal perinatal mortality risk. Certain combinations of maternal killer immunoglobulin-like receptor (*KIR*) and paternally-derived fetal human leuokocyte antigen-C (*HLA-C*) alleles have been previously associated with increased risk of high and low birthweight in studies with limited sample size ($n = 1316$). Using recently-developed methods to impute *HLA* and *KIR* haplotypes using single nucleotide polymorphism genotype data, we tested associations of fetal *HLA* and maternal *KIR* genotypes with offspring birthweight in mother-offspring pairs from the UK Biobank (UKB).

Methods: We imputed *KIR* haplotypes using the KIR*IMP imputation software in a sample of 3165 UKB mother-offspring pairs, excluding multiple births and birthweight outliers (<2.5 and >4.5 kg). Using mixed linear regression models to account for mothers with multiple children, we tested associations between maternal *KIR* A vs B haplotypes (AA, AB/BA, BB genotypes) in the presence of fetal *HLA* C1/C2 alleles, and offspring birthweight.

Results: Imputation accuracy of 99% was achieved for the A vs B haplotypes. There was no evidence for a change in birthweight for each additional maternal *KIR* B allele in the presence of fetal *HLA-C2* (25g lower birthweight per allele [95%CI: -71, 20], $p = 0.28$).

Conclusions: We did not replicate the previously reported association between offspring birthweight and maternal *KIR* haplotypes in the presence of fetal *HLA-C2*. Four additional birth cohorts (adding ~10,000 mother-offspring pairs) will be similarly analysed to further elucidate findings.

Grant References: QUEX PhD studentship (2020-2023)

Conflict of Interest: None declared

P02.007.C Non-Invasive Prenatal Testing using Oxford Nanopore and the Phivea® platform for the screening of a comprehensive panel of genetic disorders using artificial intelligence

Carmen Garrido Navas[1;2;3], *Aleksandar Nikov*[4], *David Galevski*[4], *Gjorgji Madjarov*[2;4;5], *Rafal Kalka*[2], *Anne Kristine Schack*[2;6], *Karmele Alapont*[2;6], *Lukasz Krych*[6], *Marija Chaushevska*[2;4;5], *Dimitrios Kyriakidis*[2], *Chris Kyriakidis*[2], *Zoran Velkoski*[2]

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Background: The aim of prenatal screening is to offer information about the health status of the fetus, anticipating damaging (or even lethal) conditions. When abnormal results are found, invasive tests such as amniocentesis or chorionic villus sampling are needed to confirm the diagnosis, which might have a miscarriage risk. Non-invasive prenatal tests (NIPTs) based on cell-free fetal DNA (cffDNA) enable detection of genetic disorders reducing the need for invasive tests. However, NIPTs mainly focus on chromosomal abnormalities (trisomies 21, 13 and 18 or sexual chromosomal aberrations) and analysis is still extremely manual, showing high false discovery rates that increase for low-frequency diseases.

Methods: Having demonstrated that the Phivea® platform can detect chromosomal abnormalities such as Klinefelter syndrome (XXY) with high sensitivity/specificity (>99%) and good diagnostic accuracy, gMendel is developing a NIPT based on long-read sequencing and artificial intelligence. This NIPT does not only focus on chromosomal abnormalities but it also analyses the most common genetic disorders included in European screening programs.

Results: The project, funded by the European Commission, aims a) to develop this gMendel®Test NIPT, b) to verify its clinical utility in two cohorts of pregnant women (both low and high risk) and c) to further validate the results in an independent cohort.

Conclusions: NIPT based on long-read sequencing and artificial intelligence enhances the number of diseases screened for reducing price and false discovery rates.

Conflict of Interest: Carmen Garrido Navas Co-PI of a Eureka Eurostars project for this purpose, Co-owner of IPR, Consultant at gMendel, Aleksandar Nikov: None declared, David Galevski: None declared, Gjorgji Madjarov Consultant at gMendel, Rafal Kalka: None declared, Anne Kristine Schack Employed by gMendel as industrial PhD student, Karmele Alapont Employed by gMendel as industrial PhD student, Lukasz Krych Consultant at gMendel, Marija Chaushevska: None declared, Dimitrios Kyriakidis: None declared, Chris Kyriakidis Employed at gMendel, Co-PI at the Eureka Eurostars research project, Owner of the Phivea platform and gMendel technology, Zoran Velkoski Employed at gMendel, Owner of the Phivea platform and gMendel technology

P02.008.B IVDR performance studies demonstrate a sensitive and accurate aneuploidy test

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Background/Objectives: Capillary electrophoresis has proven a stable and simple method in the context of prenatal diagnostics. Devyser Compact employs this technique for aid in diagnosis of the most common aneuploidies, such as Down, Edwards, Patau, Klinefelter, Turner, Triple X and XYY syndrome. The test serves as a diagnostic standard in many countries. In the present study, we set out to lift the test to the next level, an IVDR (In Vitro Diagnostics Regulation 2017/746) -certified product, fully validated and ready for use under the stepped-up requirements of the IVDR.

Methods: The performance parameters of the legacy product were reassessed according to international standards and guidelines. Analytical and clinical studies were set up together with clinical collaborators in comparison to karyotyping as the gold standard. Both amniotic fluid and chorionic villus samples were assessed. The scientific validity of the product was evaluated with hindsight to more than a decade on the market.

Results: Thorough testing of precision and trueness demonstrated 100% accuracy of the assay and analytical sensitivity testing revealed an LoD of 0.3 ng/µL. Important clinical parameters such as the diagnostic sensitivity and specificity as well as positive and negative predictive value consistently yielded 100%. The accuracy, clinical performance and rapid turnaround time prove the validity of the product.

Conclusion: The performance of rapid aneuploidy detection test Devyser Compact holds highest standards according to enhanced regulatory requirements. The test proofs stability and reliability in a routine diagnostic background and provides diagnostic laboratories with a fully validated method certified under IVDR.

Conflict of Interest: Bernadette Schreiner Full-time at Devyser, stocks of Devyser, Emma Lindström full-time at Devyser, stocks, Toheeb Adigun full-time at Devyser, Ulrika Flock full-time at Devyser, Jennie Strindlund full-time at Devyser, Olle Myrberg full-time at Devyser, stocks

P02.009.A Prenatal gender customized head circumference nomograms result in reclassification of microcephaly and macrocephaly

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Objective: Local and worldwide prenatal charts for estimated fetal weight and postnatal charts for head circumference (HC) are gender specific. However, prenatal HC nomograms are not gender customized. We aimed to create gender-customized curves to assess between-gender HC differences and to study the clinical significance of using such gender-customized curves.

Methods: A single-center retrospective study was conducted between 2012-2020.

Prenatal HC measurements were obtained from routine estimated fetal weight ultrasound scans. Postnatal HC measurement at birth and gender were retrieved from computerized neonatal files. HC curves were created, and the normal range was defined for the male and female subpopulations. The clinical outcome of cases classified as microcephaly and macrocephaly according to non- gender customized curves, that were reclassified as normal according to gender specific curves was assessed.

Results: The cohort included 6,000 males and 5,404 females. The curve for male HC was significantly higher than the female curve for all gestational weeks ($p < 0.0001$). Applying gender customized curves resulted in fewer cases of male fetuses defined as two above +2SD the normal range and female fetuses defined as below -2SD of the normal range. Cases reclassified as normal HC following the application of gender customized curves were not related to increased adverse post-natal outcomes.

Conclusions: Prenatal gender customized curves for head circumference can reduce overdiagnosis of microcephaly in females and macrocephaly in males without compromising the clinical yield of prenatal measurements. We suggest that gender-specific curves be used to avoid unnecessary workup and parental anxiety.

Conflict of Interest: None declared

P02.010.B Presence of two low-likelihood ratio soft markers does not increase the risk for clinically significant copy number variants

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Objective: To estimate the risk for clinically significant copy number variants (CNVs) in pregnancies with two low-likelihood ratio (LR) soft markers.

Methods: This retrospective cohort study included all prenatal microarray tests performed in authors' country during 2013-2021, due to demonstration of two soft markers associated with LR of 1-1.5 (namely: echogenic intracardiac foci, choroid plexus cyst, single umbilical artery, and mild pyelectasis). The rates of clinically significant (pathogenic and likely pathogenic) microarray findings was compared to a previously published cohort of 7235 pregnancies with normal ultrasound, in which 87 (1.2%) abnormal CNVs were noted.

Results: Of the 150 pregnancies with two low-LR soft markers, two (1.3%) clinically significant CNVs were found. The rate of abnormal microarray findings did not differ from baseline risk in pregnancies with normal ultrasound – relative risk of 1.11 (95% confidence interval 0.28-4.40).

Discussion: Our results undermine the current national policy of genetic counseling and Ministry of Health-funded invasive testing in pregnancies with combination of two low-LR soft markers. These findings are important for additional countries with similar management, and may facilitate the genetic counseling and informed decision making in such cases.

Conflict of Interest: None declared

P02.011.C Prenatal phenotype of SHOX gene deletion- clinical description of 14 unpublished cases

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Abstract

Objective: SHOX gene resides in the pseudoautosomal region of both sex chromosomes and can be paternally or maternally inherited. SHOX haploinsufficiency has been commonly found in isolated short stature and Leri–Weill dyschondrosteosis. Few

publications have described the genetic analysis and clinical characteristics of fetuses with SHOX haploinsufficiency.

Methods: All fetuses with prenatal diagnosis of SHOX gene and enhancer deletion in band Xp22.33, detected in chromosomal microarray analysis (CMA), were included. Data regarding CNV size, parental origin, sonographic characteristics, and pregnancy outcome were described.

Results: During the years 2013–2023, 14 fetuses from 13 families were detected. 13/14 detected in amniocentesis (17-32 weeks of gestational) and 1/14 in chorionic villus sampling. Deletions length ranged from 65 kb to 8 Mb. 8/14 were males and 6/14 females. Only 6/14 (42.8%) were detected by ultrasound scan due to short, long bones (4/6 females, 2/8 males), most in the 3rd trimester. 6/14 (42.8%) were inherited from their previously undiagnosed parents (3 paternally and 3 maternally), two were de-novo. 6/14 of the fetuses were liveborn and in 4/14 the pregnancy was terminated due to various reasons. 4/14 cases were lost to follow-up.

Conclusion: The fetal phenotype of SHOX haploinsufficiency is highly variable. Skeletal prenatal phenotype was detected in the minority of cases and only in the third trimester of pregnancy. Parental CMA is vital as most cases are inherited. Considering the different size of the deletions, the etiological mechanism is probably not nonhomologous recombination due to low copy repeats.

Conflict of Interest: None declared

P02.012.D Declassification of an RB1 splice site variant detected as a potential secondary finding in prenatal exome sequencing

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Objective: A healthy couple was referred for genetic counselling during their first pregnancy following an early anatomy scan revealing an isolated finding of unilateral polycystic dysplastic kidney. Amniocentesis with chromosomal microarray (CMA) was normal. Trio exome sequencing (ES) did not detect any pathogenic/likely pathogenic (P/LP) variants that could explain the fetal finding. However, the fetus was found to carry a novel heterozygote, likely pathogenic *RB1* variant (c.501-2A>G, NM_000321.2), inherited from a phenotypically healthy father, with no evidence of mosaicism. Our aim was to elucidate the risk related to this splice variant.

Methods: RNA was extracted from paternal mononuclear cells and cDNA was synthesized and sequenced (Invitae). Segregation analysis was carried out on other healthy family members.

Results: RNA analysis demonstrated that this variant results in exon 5 skipping (loss of 13 amino acids), while preserving the reading-frame. This likely results in a shortened, but functional protein. Segregation analysis revealed the splice variant in both the healthy paternal grandfather & uncle of the fetus.

Conclusions: The *RB1* gene is associated with autosomal dominant retinoblastoma. In the vast majority of families with heritable retinoblastoma, the penetrance is very high. Less than 10% of families show a "low-penetrance" phenotype. Our study highlights the importance of pursuing further testing including segregation and RNA analysis in situations where a secondary finding of a P/LP variant is detected, especially in a prenatal setting. In our case, this resulted in a potential down-grading of the *RB1* variant, allowing a more reassuring genetic counseling.

Conflict of Interest: None declared

P02.013.A Clinical outcomes [PD1] of chromosome anomalies other than trisomies 21, 18, and 13 and sex chromosome aneuploidies using genome-wide cell-free DNA based non-invasive prenatal testing: a general population cohort of over 50,000 singleton pregnancies

Luigia De Falco[1], *giovanni savarese*[1], *pasquale savarese*[1], *monica ianniello*[1], *nadia petrillo*[1], *eloisa evangelista*[1], *raffaella ruggiero*[1], *teresa suero*[1], *mariasole bruno*[1], *rossella castiello*[1], *francesca del peschio*[1], *alessio mori*[1], ***roberto sirica***[1], *davide cino*[1], *maria simonetti*[1], *maria barbato*[1], *cosimo barbato*[1], *siriana de falco*[1], *dario fergola*[1], *cristina ramiro*[1], *maria rosaria fantuz*[1], *marika casillo*[1], *antonella di carlo*[1], *luisa circelli*[1], *giulia furino*[1], *rossana d'angelo*[1], *antonio fico*[1]

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Non-invasive prenatal testing (NIPT) using cfDNA is typically carried out to screen for common fetal chromosomal anomalies and sex chromosome aneuploidies (SCA), with the option to screen for a wider range of chromosomal aberrations (expanded/genome-wide NIPT) becoming increasingly available. This study describes the outcomes of pregnancies where NIPT indicated a rare autosomal aneuploidy (RAA) or a segmental aneuploidy (SA) greater than 7Mb. Data were collected from 51,140 consecutive singleton pregnancies referred for NIPT to the AMES laboratory for a 2-years period. Samples were sequenced using the VeriSeq™ NIPT Solution v2 (Illumina, Inc.) which allows two different screening menus: a standard NIPT menu for common autosomal trisomies and (SCA) and an expanded NIPT test menuthat includes the standard menu plus RAA and SA greater than 7 Mb.Classic trisomies and SCA were reported in 715 of the total (1.4%) cases: 379 trisomy 21 (0.74%);87 trisomy 18 (0.17%); 63 trisomy 13 (0.12%); 187 SCA (0.36%). Expanded NIPT was chosen by 31,293 (61%) of women with a total of 244 (0.8%) cases with additional findings: 118 (0.38%) RAAs, 104 (0.33%) SAs, and 22 (0.07%) cases with multiple aneuploidies. Diagnostic follow-up for 78% of RAA cases and 86% of SA cases: 6.5% of RAA and 22% of SA cases were of fetal origin, 90% were pathogenic. Confined placental mosaicism cannot be excluded. Of the 22 multiple aneuploidies, 12 (55%) were maternal malignancies. Clinical follow-up was available for 98% of cases and we are currently investigating the association of the reported aberrations with adverse perinatal outcomes.

Conflict of Interest: Luigia De Falco full time, giovanni savarese full time, pasquale savarese full time, monica ianniello full time, nadia petrillo full time, eloisa evangelista full time, raffaella ruggiero full time, teresa suero full time, mariasole bruno full time, rossella castiello full time, francesca del peschio full time, alessio mori full time, roberto sirica full time, davide cino full time, maria simonetti full time, maria barbato full time, cosimo barbato: None declared, siriana de falco full time, dario fergola full time, cristina ramiro full time, maria rosaria fantuz full time, marika casillo full time, antonella di carlo full time, luisa circelli full time, giulia furino full time, rossana d'angelo full time, antonio fico full time

P02.014.B Molecular autopsy for fetal structural anomaly - clinical outcomes of a multidisciplinary team approach

Elizabeth Wall[1], *Emily Petley*[1], *Fionnuala Mone*[2], *Samantha Doyle*[3], *Stephanie Allen*[4], *James Castleman*[5], *Tamas Marton*[6], *Denise Williams*[1]

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Background/Objectives: In the West Midlands clinical genetic service, perinatal deaths with a possible genetic cause are discussed in a monthly Perinatal Pathology Genetic Multidisciplinary Team (MDT) meeting, where possible diagnoses and appropriate genomic investigations are considered and recommended. The objective was to determine the outcomes of all cases presented in 2021.

Method: Cases were identified from MDT records and outcomes were recorded. All patients discussed at the meeting underwent a microarray and a post-mortem (PM) examination. Additional genomic testing included rapid exome sequencing, whole genome sequencing, single-gene testing or gene panels.

Results: One hundred and twenty three fetuses or babies born to 115 women were discussed in 12 MDT meetings in 2021. A genetic diagnosis was made in 22% (27/123). The diagnostic rate in cases with a clinically suspected genetic condition (where appropriate testing was complete) was 51% (27/53). The diagnostic yield was highest for cases with multiple anomalies, skeletal and neurological phenotypes and when a specific diagnosis was suspected. Of the diagnosed cases, 63% (17/27) were inherited and 37% (10/27) were de novo. PM examination was beneficial in 99% (122/123) cases, including for reclassification of a VUS in three cases.

Conclusions: Almost a quarter of cases discussed at our MDT meeting received a genetic diagnosis. In most cases, PM with MDT discussions provided detailed phenotyping, guided genomic testing, and enabled molecular findings to be interpreted. Molecular diagnosis enabled accurate recurrence risk counselling and appropriate family care. This MDT approach is recommended in routine practice for this group of patients.

Conflict of Interest: Elizabeth Wall: None declared, Emily Petley: None declared, Fionnuala Mone: None declared, Samantha Doyle: None declared, Stephanie Allen Birmingham Women's and Children's NHS Foundation Trust, James Castleman: None declared, Tamas Marton: None declared, Denise Williams: None declared

P02.015.C Non-invasive prenatal testing EQA - improving the detection of sex chromosome aneuploidies

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Background: External Quality Assessment (EQA) for non-invasive prenatal testing (NIPT) has been offered in collaboration between GenQA and EMQN since 2017. The additional assessment of NIPT for sex chromosome aneuploidies was introduced in 2020 to reflect the change in clinical practice. Results show the importance of EQA participation for non-invasive prenatal testing where the scope is rapidly evolving.

Methods: Two plasma samples (patient and/or artificial material) with corresponding clinical cases are provided for each EQA. Participants perform routine NIPT for common aneuploidies and submit clinical reports for assessment. Tailored performance feedback is provided, and a critical genotyping error is given where erroneous results are reported.

Results: EQA results highlight improved laboratory performance through educative feedback from EQA providers. Initial inclusion of an XYY case in 2020 saw nine laboratories (7.2%) receive a critical genotyping error as they failed to report the high chance result, where this was expected to be detected within the limitations of the test. The number of critical genotyping errors fell to almost half this initial figure (3.6%) when the same sample was re-issued in 2021.

Conclusions: Inclusion of NIPT for sex chromosome aneuploidies re-affirms the importance of EQA as a mechanism to independently measure the standard of laboratory testing, particularly where the scope of current testing expands. Initial results confirm improvement in both accuracy of testing, and reporting of test limitations as a consequence of tailored EQA feedback to participants.

Conflict of Interest: None declared

P02.016.D Expanding the mutational spectrum of the MPL gene: identification of two novel loss-of-function variants in a fetus with CAMT – Case study

Lejla Mulahasanovic[1;2], *Christine Froehlich*[1;2], *Susanna Hellmeister*[3], *Heinz Gabriel*[1;2], *Oliver Bartsch*[4;5], *Saskia Biskup*[1;2]

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Background/Objectives: Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare autosomal recessive disorder caused by biallelic loss-of-function variants in the *MPL* gene. The disorder affects the hematopoietic system and causes bone marrow failure, which results in severe thrombocytopenia at birth due to ineffective megakaryopoiesis. This can lead to aplastic anemia during the first years of life. Prenatal manifestations of CAMT, such as internal hydrocephalus and intracranial hemorrhage, have also been reported. In this case study we present the results of prenatal molecular genetic testing in a fetus (18 WG) with internal hydrocephalus, enlarged cisterna magna, suspected vermis aplasia and a white spot of the left ventricle.

Methods: Trio exome analysis of the fetus and both parents was performed using the NGS Illumina NovaSeq6000 system.

Results: We detected the maternally inherited pathogenic frameshift variant c.1040delC; p.Pro347His*fs**22 in *MPL* and a de novo deletion at 1p34.2, which encompasses the coding exons 1-6 of *MPL*, and the entire *TIE1* gene. Both variants likely result in a loss of the MPL protein, which is a known pathomechanism for CAMT. Although the deletion occurred de novo in the fetus, we were able to establish that it is derived from the paternal allele, based on the detection of a hemizygous maternal SNP within the deleted region at 1p34.2.

Conclusion: The variants are present in a compound-heterozygous state, and causative for CAMT, which is consistent with the fetal ultrasound findings. Overall, we identified two novel loss-of-function variants affecting the *MPL* gene and expanded the prenatal clinical spectrum of CAMT.

Conflict of Interest: Lejla Mulahasanovic Full time employment, Christine Froehlich Full, Susanna Hellmeister Full, Heinz Gabriel full, Oliver Bartsch full, Saskia Biskup full

P02.017.A Lethal congenital contracture syndrome type 3 in a Canadian First Nations population

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Background: The diagnosis of a genetic etiology for fetal akinesia deformation sequence (FADS) in the prenatal setting is challenging. It is especially difficult in patients from isolated populations in which genetic testing has been limited and the background allelic frequencies are unknown. Lethal congenital contracture syndromes type 3 (LCCS3; OMIM 611369) is a rare autosomal recessive condition caused by biallelic pathogenic variants in the *PIP5K1C* gene. There are only 10 cases of LCCS3 in the medical literature reported in 2 Israeli Bedouin kindreds (Narkis et al, 2007). We describe 3 cases sharing a novel homozygous variant in *PIP5K1C* in a Canadian First Nations population.

Methods: The index case is the fetus of a woman of Canadian First Nations descent who presented prenatally at 24 weeks gestation with fetal findings of multiple contractures. Clinical whole exome sequencing by duo analysis was conducted. Two additional cases from the same community were subsequently identified.

Results: The neonate was found to have multiple contractures, muscle atrophy, respiratory insufficiency, dysmorphic features and died at 11 days of age. Exome sequencing identified homozygous, likely pathogenic variants in PIP5K1C c.1292A>G, p.(Tyr431Cys). Two additional cases clinically identified shared a similar clinical course, and the same molecular cause.

Conclusions: The recurrent homozygous variant in the *PIP5K1C* gene and identification of three apparently unrelated cases from the same isolated community suggests the possibility of a founder effect in this population. These cases add to our understanding of an ultra rare genetic condition and provide personalized management in this underserviced population.

Conflict of Interest: None declared

P02.018.B Diagnostic yield of prenatal exome sequencing in Central Nervous System anomalies: a single-center experience and systematic literature review

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Background/Objectives: Prenatal exome sequencing (pES) has shown a significant incremental diagnostic yield over karyotype and chromosomal microarray analysis (CMA) in fetuses with structural anomalies. Optimized indications, detection rates in different fetal anomalies, and interpretation of variant pathogenicity are still under investigation. We aimed to assess the incremental diagnostic yield of trio-based pES after karyotype and CMA inconclusive results in Central Nervous System (CNS) anomalies.

Methods: Between January 2019 and December 2022, a cohort of 33 fetuses presenting isolated or non-isolated CNS anomalies was analyzed. In all cases karyotype and CMA were performed. In inconclusive cases, pES was offered. Trio-based pES was performed in 15 cases on genomic DNA extracted from fetal samplings and parental leukocytes. Library preparation and targeted enrichment were performed using the Twist Human Core Exome Kit and sequenced on the Illumina NovaSeq 6000 platform.

Results: In 5/15 cases (33%), pES disclosed likely pathogenic (LP) or pathogenic (P) variants in *ARID1A, BICD2, NFIA, RPGRIP1L* and *ZIC2* genes fitting the fetal phenotypes. In 6/15 (40%) cases, multiple Variants of Uncertain Significance (VUSs) were detected. One fetus carried both VUSs and LP variants, partially related to the phenotype. In three cases no variants were disclosed. Systematic literature review showed an incremental yield ranging from 19% to 57% in antenatal cohorts focused on CNS anomalies.[1-10]

Conclusion: In literature and in this cohort of fetuses, pES appears to have a high incremental diagnostic yield, supporting the proposal of pES earlier in the diagnostic route of CNS anomalies[1].

Ref.: [1]Yaron,2022;[2]Lei,2022,[3]de Koning,2021,[4]She,2021,[5]Heide, 2020,[6]Tan,2020,[7]Li,2020,
[8]Weitensteiner,2018,[9]Reches,2017,[10]Poirier,2015

Grant: none

Conflict of Interest: None declared

P02.019.C Prevalence of high-penetrant copy number variants in 7,732 low-risk pregnancies

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Introduction: The rate of clinically significant copy number variants (CNVs) in chromosomal microarray analysis in low-risk pregnancies is 1%. These results include CNVs with low and variable penetrance, while some patients are interested only in detection of high-penetrant variants. The objective of this study was to estimate the prevalence of high-penetrant copy number variants in a large cohort of low-risk pregnancies.

Methods: This retrospective study was performed using microarray results of pregnancies with normal ultrasound and maternal serum screening. All clinically significant (pathogenic and likely pathogenic) CNVs were recorded. Of these, only high-penetrant findings were selected. We excluded findings with low and medium penetrance, and CNVs with unknown clinical penetrance including: uniparental disomy of segments not related to known imprinted syndromes, mosaic aneuploidy of lower than 50%, and segmental mosaicism. The calculation was performed for the overall cohort, for women older and younger than 35 years, and after omission of non-invasive prenatal screening (NIPS) detectable findings (trisomies 13, 18 and 21).

Results: Clinically significant CNVs were detected in 118/7734 of cases (1:65, or 1.5%), and high-penetrant CNVs in 35/7734 of cases (1:221, or 0.45%). In woman aged ≥35 years the rate was 31/6264 (1:202, or 0.49%), vs. 4/2730 in woman aged<35 years (1:682, or 0.15%). Following omission of NIPS-detectable findings, the rate of high-penetrant CNVs was 24 (1:322, or 0.31%) in the whole cohort.

Discussion: The risk for high-penetrant CNVs in low-risk pregnancies is noteworthy, compared to the risk for miscarriage following invasive testing, even after normal NIPS results.

Conflict of Interest: None declared

P02.020.D External hydrocephalus as a prenatal feature of RASopathies

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Background/objectives: Brain malformations have been reported in RASopathies, including postnatal external hydrocephalus (EH), a nonobstructive form of cerebrospinal fluid accumulation in the subarachnoid space. Prenatal identification of EH is an extremely rare finding.

Methods: We report two fetuses detected with EH. Ultrasound Scan, echocardiography, and Magnetic Resonance Imaging (MRI) were performed. Karyotype, Chromosomal Microarray Analysis, and molecular investigation for RASopathies were requested on amniotic fluid.

Results: One fetus presented isolated mega-cisterna magna at 21 gestational weeks. Echocardiography showed pulmonary stenosis, and MRI at 27 weeks further showed EH, and corpus callosum and cerebellar vermis under the 10th centile. Molecular investigation detected the heterozygous de novo pathogenic variant c.923A>G p.(Asn308Ser) in *PTPN11* (MIM*176876). The other fetus, detected with ductus venosus agenesis, underwent echocardiography, showing dilated inferior vena cava. MRI at 27 weeks showed EH, corpus callosum and cerebellar vermis under the 10th centile. Molecular investigation identified the maternally inherited pathogenic heterozygous variant c.178G>A p.(Gly60Ser) in *PTPN11*. The prenatal detection of EH is challenging because few cases have been described, lacking a genetic characterization. We suggest considering this finding very carefully in prenatal diagnosis, alerting on the presence of additional features which may suggest a non-isolated finding.

Conclusion: Unexpected central nervous system anomalies, such as EH, could be detected in RASopathies. Noonan syndrome (NS) should be considered in the differential diagnosis of EH, investigating other evocative findings and considering molecular screening for mutations in NS related genes.

Grant References: none

Conflict of Interest: None declared

P02.021.A The evolving challenges of congenital titinopathies: late miscarriages, syndromic phenotypes, and "false" canonical exons

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Background: Titin (TTN) is the largest protein in the human body, expressed in both skeletal and cardiac muscles. The *TTN* gene contains 364 exons, that generate multiple isoforms. Titin truncating variants (*TTN*tv) cause a wide spectrum of recessive phenotypes.

Methods: We reviewed 97 published cases and collected 18 new cases of prenatal titinopathies carrying biallelic *TTN*tv in homozygosity or compound heterozygosity. Patients' DNA was analyzed using NGS panels or exome sequencing (ES); no other genetic alteration that could explain the phenotype was found.

Results: Cases with at least one variant in a metatranscript-only exon or in exon 359 display, on average, severe phenotypes with a high prevalence of arthrogryposis multiplex congenita (83%), fetal akinesia (54%), perinatal death (31%), and facial dysmorphisms (36%). Interestingly, we identified 4 cases with biallelic *TTN*tv in canonical exons, which are normally considered incompatible with life. Three of the patients died in uterus or after termination of pregnancy, while one of them displayed the first symptoms at 18 months and acquired independent walking at 23 months of age. He carries a *TTN*tv in exon 13, which, according to our data, has an average PSI (percentage splice in) of 70% in adult skeletal muscles.

Conclusion: Biallelic *TTN*tv can cause complex syndromic phenotypes. An in-depth study of isoforms and a reclassification of canonical exons are required to inform genotype-phenotype correlation.

Grant References
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Conflict of Interest: Maria Francesca Di Feo: None declared, Francesca Forzano: None declared, Angela F Brady: None declared, Maria Iascone: None declared, Patrizia D'Oria: None declared, Luigina Spaccini: None declared, Sergei A. Kurbatov: None declared, Elisa Giorgio: None declared, Guido C Casalis Cavalichini: None declared, Alfredo Brusco: None declared, Tania Attie-Bitach: None declared, Sheela Nampoothiri: None declared, Erin Ryan Erin Ryan is an employee of GeneDX, LLC, Michelle Morrow Michelle Morrow is an employee of GeneDX, LLC, Svetlana Gorokhova: None declared, Brigitte Chabrol: None declared, Manu Jokela: None declared, Bjarne Udd: None declared, Marco Savarese: None declared

P02.022.B Rapid non-invasive prenatal screening test for trisomy 21 based on digital droplet PCR

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Background: Non-invasive prenatal tests for the detection of fetal aneuploidies are based on the analysis of cell-free DNA (cfDNA) from plasma of pregnant women by next generation sequencing method. Compared to methods based only on the polymerase chain reaction (PCR), this is an expensive screening test. The development of alternative tests for routine genetic laboratories is therefore desirable.

Methods: We optimized the isolation of plasma cfDNA. Then we performed multiplex digital droplet PCR by detecting 16 amplicons from chromosome 21 and 16 amplicons from chromosome 18 as reference. Two fluorescently labeled lock nucleic acid probes were used for the detection of reaction products. The required accuracy was achieved by examining 12 chips from each patient using Stilla technology.

Results: We analyzed plasma cfDNA of 26 pregnant women with euploid pregnancies and 16 plasma samples from pregnancies with trisomy 21 to determine the cutoff level for sample classification. The test was validated on 30 plasma samples of pregnant patients with risk for trisomy 21 in the range from 1:4 to 1:801. Our results were in full agreement with the results of subsequent invasive diagnostic procedure. All test parameters (sensitivity, specificity, positive and negative predictive values) reached 100%.

Conclusions: High PPV, low cost and speed of analysis predetermine the method for implementation into the clinical workflow as a screening alternative offered to anxious patients having the risk for trisomy 21 before the confirmatory invasive procedure.

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Conflict of Interest: None declared

P02.023.C Case report: novel mutation in the MAGED2 gene causing transient antenatal Bartter syndrome

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Background: Antenatal Bartter's syndrome, the most severe form of Bartter's syndrome, is an inherited disorder, characterized by fetal polyuria, polyhydramnios and postnatal polyuria with persistent renal salt wasting. It requires close follow-up, in particular during the neonatal period, primarily because of prematurity. Mutations in the *MAGED2* gene, located on the X chromosome, have been recently detected in males with a transient form of antenatal Bartter syndrome or with idiopathic polyhydramnios. Here we report a healthy 39-year-old woman with two fetal death with hydramnios at 17 and 24 weeks, both fetuses were male. Additionally, her mother and grandmother also have history of fetal death. To determine the possible underlying genetic cause of fetal death family history, whole exome sequencing (WES) was perfomed.

Methods: WES was performed (Illumina Novaseq 6000®) and the resulting data was processed and analyzed with an in-house bioinformatics pipeline and Agilent Alissa Interpret® software.

Results: A novel loss-of-function mutation was identified, NM_177433.3:c.655_656delinsTA p.(Arg219*), in heterozygosity, in exon 4 of *MAGED*2 gene.

Conclusion: We report a carrier woman with a variant in *MAGED2* gene with two fetal losses, both males, and with a phenotype compatible with early-onset polyhydramnios caused by this gene. This diagnosis is important for clinical management, future pregnancy monitoring and parental counselling.

Conflict of Interest: Carolina Ribeiro CGC Genetics, Unilabs, marisa teixeira CGC Genetics, Unilabs, alexandra lopes CGC Genetics, Unilabs, joaquim sá CGC Genetics, Unilabs, rita cerqueira CGC Genetics, Unilabs

P02.024.D Prenatal presentation of Mowat-Wilson syndrome. Two cases report

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Objective: Mowat-Wilson syndrome (MWS) is a genetic disorder caused by *ZEB2* gene mutations, mainly characterized by moderate to severe intelectual disability, facial dysmorphism, epilepsy, Hirschsprung disease, corpus callosum anomalies and congenital heart defects. However, it is rarely diagnosed prenatally and therefore limited information is available on the prenatal phenotype. Here we report two new cases with prenatal malformations and confirmatory diagnosis, one prenatal and one postnatal.

Patient and methods

Case 1: prenatal ultrasound detection of complete agenesis of corpus callosum, single umbilical artery, mild bilateral pyelectasis, low grade urinary tract dilation and suspected hypospadias.

Case 2: critical pulmonary valve stenosis with intact septum, moderately hypoplastic right ventricle and increased nuchal translucency detected in prenatal untrasound.

NGS using Agilent in Illumina platforms. In-house pipeline, bioinformatic tools and HPOs. Sanger sequencing to confirm pathogenic variants.

Results: In both cases, de novo heterozygous variants have been identified in *ZEB2:*

c.1027C>T p.(Arg343Ter) previously described in MWS patients, but no prenatally. c.2447T>G (p.Leu816Ter) firstly described in this case.

Conclusions: Prenatal diagnosis of MWS is very challenging due to the variability of congenital anomalies observed as well as the few prenatal cases that have been reported. Among the varied MWS malformations detected to date, none of them are specific. Therefore, it is valuable to report the prenatal presentation of MWS to help clinicians raise their suspicion of MWS, when such characteristics are seen. Besides, prenatal clinical exome is a powerfull tool in the detection of genetic diseases with heterogeneous presentation.

Conflict of Interest: Evangelina Pestaña Full time, Carlos Ivan Rivera Full time, Begoña Adiego full time, Ana Maria Gonzalez-spinola Full time, Elena Van der Vorst full time, Alberta Belinchón Martínez full time

P02.025.A Fetal abnormalities leading to diagnosis of familial X-linked Osteopathia Striata with Cranial Sclerosis by whole exome sequencing

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A 31-year-old woman, with a medical history of epilepsy, was referred to a genetic clinic during her 30th week of pregnancy due to fetal macrosomia and polyhydramnios.

Pregnancy was uneventful and fetal chromosomal microarray showed a normal female. Under examination, patient showed facial dysmorphism with wide nasal bridge, hypertelorism, low set ears and frontal bossing. She reported her mother and sister had similar characteristics.

Fetal MRI demonstrated increased extra-axial spaces, skull-base thickening, occipital protrusion, frontal bossing and hypertelorism. Mother's brain MRI showed T2 hyper-intense signal in subcortical white matter, suggesting focal cortical dysplasia, and similar skull structure.

WES revealed fetal and mother heterozygote c.972delA loss of function (LOF) mutation in the AMER1 gene, an inhibitor of the canonic WNT signaling pathway. AMER1 LOF mutation expected to result in an upregulation of the WNT pathway and increased osteoblastic function.

AMER1 pathogenic variants cause X-linked Osteopathia Striata with Cranial Sclerosis (OS-CS). Females present with macrocephaly and characteristic facial features. Orofacial clefting, hearing loss and mild developmental delay was reported. Radiographic findings include cranial sclerosis, sclerosis of long bones, and metaphyseal striations. Males usually present with a severe phenotype including multiple- malformation syndrome, lethal in mid-to-late gestation or in the neonatal period.

Given the family history and a female fetus, the couple proceeded with the pregnancy, and the now three-month-old child is developing normally. Macrocephaly was reported.

The diagnosis of the hitherto unknown familial condition will lead to better surveillance and management, and prevention of future severely affected male offspring.

Conflict of Interest: None declared

P02.026.B ATXN3 (CAG)n triplet-primed PCR and hexadecaplex microsatellite marker panel for preimplantation genetic testing of spinocerebellar ataxia type 3 / Machado-Joseph disease

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Background/Objectives: Spinocerebellar ataxia type 3 / Machado-Joseph disease (SCA3/MJD) is an autosomal dominant neurodegenerative disorder caused by CAG-repeat expansion in *ATXN3*. Preimplantation genetic testing-monogenic (PGT-M) of SCA3/MJD should include reliable expansion-mutation detection coupled with high-risk allele determination using informative linked markers.

Methods: Triplet-primed PCR (TP-PCR) of *ATXN3* $(CAG)_n$ was evaluated on whole-genome-amplified single cells. An in silico search was performed to identify short tandem repeat (STR) markers within 1 Mb upstream and downstream of *ATXN3*. Candidate markers were screened for polymorphic potential on 16 anonymous DNAs. Shortlisted markers that could be co-amplified were simultaneously genotyped on 187 anonymous DNAs. Two couples underwent SCA3/MJD PGT-M combining *ATXN3* $(CAG)_n$ TP-PCR and linkage-based risk allele genotyping.

Results: In silico mining, filtering, and curation identified 139 STR markers. From these, 16 potentially highly polymorphic markers (8 upstream, 8 downstream) that could be multiplexed into a single-tube reaction were selected, and exhibited observed heterozygosities of 0.60-0.88 and 0.61-0.91 in Chinese and Caucasians, respectively. In both SCA3/MJD PGT-M cases, *ATXN3* $(CAG)_n$ TP-PCR results correlated tightly with linked marker analysis results for all embryos tested. Five of nine embryos were diagnosed as unaffected, one fresh double-embryo transfer was unsuccessful, while one frozen-thawed single-embryo transfer resulted in an unaffected live birth.

Conclusion: An *ATXN3* $(CAG)_n$ TP-PCR assay has been developed for robust expansion-mutation detection from whole-genome-amplified single cells, and a highly polymorphic hexadecaplex STR panel has been developed for rapid identification of informative markers linked to *ATXN3*. Both tools have been successfully applied to PGT-M of SCA3/MJD.

Conflict of Interest: Mulias Lian National University Hospital, Riho Taguchi: None declared, Mingjue Zhao: None declared, Gui Ping Phang National University of Singapore, Arnold Tan National University of Singapore, Caroline Lee National University of Singapore, Samuel Chong National University of Singapore

P02.027.C Single cell whole-genome sequencing in prenatal diagnosis

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The establishment of a non-invasive prenatal procedure is the holy grail of fetal genetic analysis, but the isolation and the reliable identification of individual fetal cells in maternal blood is very challenging, given their rarity.

Single-cell whole-genome sequencing offers an unprecedent opportunity to assess the genetic content of the isolated cell.

Here we present a multistep method for the identification, isolation and molecular characterization of individual circulating extravillous trophoblasts (cETVs), which has been validated in peripheral blood from 372 pregnant women. cEVTs were highly enriched prior to the identification and single cell sorting.

The single-cell DNA was whole genome amplified using *Ampli*1™WGA, which allows to obtain high-confidence CNV profiles with low-depth sequencing.

With this method, the genetic analysis from 131 women resulted in identification standard karyotyping following invasive prenatal diagnostic sampling of 16 cases with chromosomal abnormalities. Single cell analysis confirmed all these cases. Interestingly, in these cases there was a triploid Partial Hydatiform Mole (PHM) with a 69, XXY karyotype that best-fitted the underlying copy-number signal. Of the remaining 114 cases with normal fetal karyotype, 113 were correctly identified by single cell analysis. In one case a confined placental mosaicism for trisomy 16, not confirmed by karyotype on amniocytes, was revealed in cEVTs.

Our method results in the isolation of intact single fetal cells, which provide a source of unfragmented fetal DNA for the aneuploidies detection.

The above described findings suggest that our strategy may represent a great potential for a non-invasive comprehensive high-resolution fetal genomic profiling.

Conflict of Interest: Anna Doffini employee of Menarini SIlicon Biosystems, Ilaria Molinaro consultant for Menarini SIlicon Biosystems, rossana foti employee of Menarini SIlicon Biosystems, Chiara Mangano employee of Menarini SIlicon Biosystems, Claudio Forcato employee of Menarini Silicon Biosystems, Chiara Maranta employee of Menarini Silicon Biosystems, Emilia D. Giovannone employee of Menarini Silicon Biosystems, Genny Buson employee of Menarini Silicon Biosystems, Chiara Bolognesi employee of Menarini Silicon Biosystems, Rebecca Maiocchi consultant for Menarini Silicon Biosystems, Debora Lattuada: None declared, Enrico Ferrazzi: None declared, Paola Ricciardi-Castagnoli consultant for Menarini Silicon Biosystems, Thomas J. Musci employee of Menarini Silicon Biosystems, Francesca Romana Grati employee of Menarini Silicon Biosystems

P02.028.D First-trimester Cystic Hygroma as the prenatal presenting feature of ASCC1-related Spinal Muscular Atrophy Withith Congenital Bone Fractures

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Background/objectives: Fetal Cystic Hygroma is a lymphatic anomaly commonly detected at first-trimester ultrasound scans as increased nuchal translucency (NT) with septa. It is associated with aneuploidies, chromosomal microrearrangements, and monogenic disorders. A small fraction can be due to musculoskeletal anomalies. We report a fetus presenting cystic hygroma with biallelic pathogenic variants in *ASCC1* (MIM*614215), associated with Spinal Muscular Atrophy with Congenital Bone Fractures 2 (SMABF2, MIM#616867).

Methods: A couple was referred for scheduling the prenatal diagnosis of the paternal *ASCC1* (NM_001198799.3) c.1027C>T, p.(Arg343Ter) variant and the maternal 49kb 10q22 (GRCh37:10:73887835-73936961) deletion partially encompassing *ASCC1*, originally detected in their previous child with SMABF2. At 12 + 2 gestational weeks (GW), increased NT (>99th centile) with septa, interpreted as cystic hygroma, was noted. Chromosomal Microarray Analysis (CMA), and Clinical Exome Sequencing were performed after amniocentesis at 16 + 2 GW.

Results: The fetus harbored both the paternal *ASCC1* variant and the maternal deletion. Karyotype, CMA and ES did not identify known causes of cystic hygroma/increased nuchal translucency. The pregnancy was terminated at 19 GW. No signs of fetal akinesia or fractures were noted.

Conclusion: This is the first report of the association of SMABF2 with cystic hygroma/increased nuchal translucency, supported by the exclusion of other known causes. Fetal features of SMABF2 were absent at the time of termination of pregnancy. *ASCC1* and other causes of fetal musculoskeletal anomalies should be considered in the prenatal assessment of fetal neck fluid collections, even if apparently isolated.

Grant References: none

Conflict of Interest: None declared

P02.030.B The role of mosaicism ratio to reassess the positive predictive value (PPV) associated with the high risk NIPT results: experience on over 90,000 consecutive cases

Elena Corsetti[1], *Monica Faieta*[2], *Rossella Falcone*[2], *Sara Duca*[2], *Sara Giangiobbe*[1], *Chiara Cirioni*[1], *Clarissa Locci*[1], *Maria Ottavia Di Gregorio*[1], *Riccardo Giannico*[1], *Luca Forlani*[1], *Francesca Pizzuti*[2]

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Background/Objectives: Despite the recent improvements in sequencing chemistry and bioinformatics analysis, Non-invasive Prenatal Testing (NIPT) is still classified as a screening test due to the possibility of discrepancies between the obtained results and the fetal chromosomal asset. Using the massively parallel sequencing data from cell-free DNA screening is possible to calculate the mosaicism ratio (MR), expressed as the fraction of cfDNA affected by aneuploidy by the overall fetal fraction of the sample. The aim of this study is to define an improved positive predictive value (PPV) associated with the abnormal NIPT results, based on the MR.

Methods: The study is a retrospective analysis of data deriving from over 90000 pregnancies and generated using VeriSeq NIPT Solution v2. The high-risk results for common aneuploidies (T13-18-21), rare trisomies (RAR), copy number variation (CNV) and sex chromosome aneuploidies (SCA) has been classified within three MR ranges (low (< 0.4), intermediate (0.4-0.7) and high (> 0.7)) and for each the PPV has been estimated.

Results: A statistically significant increase in PPV was observed when the MR>0.7, compared to when the MR<0.7 (93.7% vs 49.6% $p = 4.75E\text{-}43$) for T13 and RAR followed by SCA, T18 and T21. No significant differences was observed for the CNVs.

Conclusion: The data of this study show that the MR can be used to identify a priori those results that are more likely to be discordant with the genetic status of the fetus. It can help the clinician improve understanding and interpretation of NIPT data, as well as genetic counselling management.

Conflict of Interest: Elena Corsetti Full time, Monica Faieta Full time, Rossella Falcone Full time, Sara Duca Full time, Sara Giangiobbe Full time, Chiara Cirioni Full time, Clarissa Locci Full time, Maria Ottavia Di Gregorio Full time, Riccardo Giannico Full time, Luca Forlani Full time, Francesca Pizzuti Full time

P02.031.C Prenatal diagnosis of Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome associated with a likely pathogenic variant in PIK3R2

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Background: *PIK3R2* encodes a subunit of PI3K enzyme which participates in the mTOR pathway. Gain-of-function variants in genes involved in this pathway lead to cell proliferation or reduced apoptosis resulting in diffuse or focal brain disorders. Megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) is a genetically heterogeneous syndrome, caused by deleterious variants in *PIK3R2, CCND2* or *AKT3*, with megalencephaly and cortical malformations as the main clinical findings.

Methods: We report a foetus from a nonconsanguineous couple presenting with macrocephaly in the 2nd-trimester ultrasound. Fetal MRI showed a bi-hemispheric malformation of cortical development with polymicrogyria (PMG), opercular abnormalities, ganglionic eminence enlargement, increased bi-hemispheric volume, and bilateral ventriculomegaly, without other major anomalies. The clinical picture suggested a mTORopathy.

Results: Whole exome sequencing (WES) detected a previously unreported heterozygous variant in *PIK3R2*: c.1669G>T, p.(Asp557Tyr), classified as of uncertain clinical significance and confirmed to be de novo after parental studies. This variant affects a highly conserved residue, where another pathogenic missense variant has previously been reported. Moreover, it is absent from controls, and multiple lines of computational evidence support its deleterious effect. It was thus reclassified to probably pathogenic. The same variant has subsequently been reported in the post-natal setting in a cohort of patients with epilepsy and PMG.

Conclusion: This case emphasizes the diagnostic power of neuroimaging techniques together with WES in the prenatal field. Establishing a molecular diagnosis was essential to counsel this couple adequately concerning prognosis and recurrence risk. This report of a prenatal phenotype contributes to establish this variant as disease-causing for MPPH.

Conflict of Interest: None declared

P03

Sensory Disorders (Eye, Ear, Pain)

P03.001.A USP48 as a regulatory gene involved in retinal ciliopathies

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Ciliopathies are a broad group of heterogeneous inherited disorders associated with dysfunction of the cilium, a ubiquitous microtubule-based organelle that translates extracellular stimuli into cellular responses. The retina is one of the most affected tissues by mutations in ciliary genes due to the highly specialised neurosensory cilium that photoreceptors display, also known as outer segment, where photoreception and phototransduction occurs. To date, mutations in more than 100 ciliary genes have been associated with retinal degeneration, accounting for almost 25% of inherited retinal dystrophy (IRD) cases. USP48 is a deubiquitinating enzyme whose role in the retina is still unexplored although previous reports indicate its relevance for neurosensory organs since dominant mutations in this gene are causative of hearing loss.

By means of several complementary biochemical assays – immunocytochemistry, co-immunoprecipitation, proteomics and western blotting – we describe that USP48 is a basal body-associated protein that interacts with ARL3 and UNC119a, causative of IRDs, using different domains and mechanisms.

Our results suggest that USP48 may act in the regulation of key ciliary proteins for photoreceptor function, the modulation of intracellular protein transport, and ciliary trafficking to the photoreceptor outer segment. To further understand the role of USP48 in retinal development and homeostasis, we are currently generating a *USP48* knockout isogenic line in induced pluripotent stem cells to produce retinal organoids and characterise the retinal and cilium alterations during the development and differentiation of the retina caused by USP48 absence.

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Conflict of Interest: None declared

P03.002.B A comprehensive genome wide association to functional study reveals the role of CNTNAP5 in glaucomatous neurodegeneration

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Background/Objectives: Primary angle closure glaucoma (PACG) is a major leading cause of blindness. In India, ~30% of people show a narrow iridocorneal-angle (<15°), but of these only 0.5-1% people develop PACG.

Methods: We conducted an haplotype-based age-agnostic model of progressive angle closure GWAS early-onset PACG patients (PACG:age ≤50years) compared with anatomically predisposed narrow-angle non-glaucomatous older individuals (PACS:age ≥60years). Further, we performed dual-luciferase assay for functional follow-up of risk variants of *CNTNAP5* and subsequently the role of *CNTNAP5* figured out in ocular development and morphology of the retinal nerve in zebrafish.

Results: In our GWAS cohort (PACG = 148;PACS = 92), we identified 13 SNPs of *CNTNAP5* that were associated with PACG. Subsequently, the prioritized SNP rs780010 of *CNTNAP5* was significantly ($P = 0.0024$) associated with higher cup-to-disc ratio, a clinical parameter correlated with glaucomatous neurodegeneration. We further validated the rs780010, in a replication cohort (PACG = 50;PACS = 39) and observed a significant association (OR = 2.30, $P = 0.012$).In Hi-C dataset, the associated genic region shows higher retinal neuronal expression of *CNTNAP5* with enhancer marks; these were subsequently validated using a dual-luciferase assay. Additionally, immunofluorescence analyses showed significant eye size reductions and retinal nerve thinning in zebrafish upon morpholino mediated knockdown of *CNTNAP5*.

Conclusions: Our GWAS results indicate a genomic association of the *CNTNAP5* with PACG. Further, post-GWAS functionalization led us to believe that *CNTNAP5* is an important player to perturb the development of the neural retina and thereby increasing the risk of PACG-associated vision loss.

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Conflict of Interest: None declared

P03.003.C Role of RBP4 in eye development: 7 novel families with ocular malformations and literature review

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Retinoic acid (RA) pathway plays a crucial role in both eye morphogenesis and visual cycle. Mono and biallelic pathogenic variants in *RBP4*, encoding a serum RA specific transporter, have been associated with variable ocular phenotypes. Although few families have been reported, recessive forms appear to be associated with retinal degeneration while dominant forms manifest through ocular development anomalies, mainly micro-anophthalmia and coloboma (MAC). Moreover, in dominant forms, maternal *RBP4* genetic status and content of diet during pregnancy appears to modify the risk of MAC occurrence and severity. To date, only 6 dominant and 6 recessive families have been described in the literature and our knowledge about *RBP4* genetic defects remains limited and needs further study.

Here we report 7 new families (11 patients) with isolated and syndromic MAC forms harbouring heterozygous *RBP4* (likely) pathogenic variants. For the first time, malformations that belong to the clinical spectrum of vitamin A deficiency are reported, providing a link with other RA disorders. We then confirm the existence of two distinct phenotypes depending on the nature of the variants and their mode of inheritance. Dominant forms, almost exclusively associated with missense variants, consist in ocular malformations, contrasting with the retinal degeneration constantly observed in recessive forms, thus probably involving different molecular mechanisms. Moreover, we add further evidence supporting the skewed inheritance and the role of maternal *RBP4* genetic status on the phenotypic severity in dominant forms. Finally, we show that biochemical analyses in patients could provide a biochemical signature crucial for classifying *RBP4* variants.

Conflict of Interest: None declared

P03.004.D Whole Genome Sequencing (WGS) for the molecular diagnosis of Hereditary Hearing Loss (HHL): the underestimated role of Structural Variants (SVs)

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Background: HHL is the most common sensory impairment, characterized by high genetic and clinical heterogeneity. Despite the implementation of high-throughput sequencing technologies for the molecular diagnosis of this disorder, many cases still remain unsolved, suggesting the presence of additional mechanisms beyond single nucleotide variants (SNVs) or small insertions/deletions (INDELs).

Methods: Data of five HHL trios, negative to SNP-array (for the identification of large copy number variants), whole exome sequencing and WGS (aimed at the identification of both SNVs/INDELs and intronic variants), were further analysed to search for SVs:

Quality control of WGS fastq files was carried out using FastQC (v0.11.9) and alignment to Human genome reference build 38 was performed using bwa-mem (v2.1);

SVs calling was carried out using Manta (v1.6.0) and Delly (v1.1.6). Deletions and duplications were then filtered using DeepSVFilter;

Annotation was performed using AnnotSV (v3.2.3).

Results: This approach identified a mean of 17 SVs (size < 50 Kb) within known HHL genes, potentially contributing to the patients' phenotype. The most interesting results include a ~27 Kb duplication within the *TSPEAR* gene identified in the proband and father of Family 1, both affected by nonsyndromic HHL, and a ~2 Kb micro-deletion in a regulatory region of *DCDC2* in the two probands of Family 2.

Conclusion: To date, the overall contribution of SVs in the etiopathogenesis of HHL is still underestimated. These data highlight the importance of testing for SVs in patients negative to prior genetic tests and highlight the power of WGS for the molecular diagnosis of HHL.

Conflict of Interest: None declared

P03.005.A Unveiling the genetic bases of Hereditary Hearing Loss (HHL): the application of a multistep diagnostic approach in a large Italian cohort

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Background: HHL diagnosis is extremely challenging, particularly considering the presence of peculiar scenarios, as dual molecular diagnoses and non-syndromic mimics (e.g. Usher syndrome type 2 due to variants within *ADGRV1* and *USH2A* genes).

Methods: After a deep clinical evaluation (to distinguish non-syndromic (NSHL) from syndromic (SHL) forms), a multistep molecular approach was applied to 102 Italian HHL patients, negative to *GJB2* mutations, as follows:

Multiplex Ligation Probe Amplification (MLPA) analysis of *STRC* in NSHL patients;

STRC Long-range PCR (LR-PCR) in patients carrying a heterozygous *STRC* deletion and an audiometric pattern suggestive of *STRC*-related deafness;

Whole-Exome Sequencing (WES) in negative patients and in SHL subjects.

Results: 82 apparently NSHL and 20 SHL patients were analysed. Regarding NSHL, MLPA/LR-PCR and WES provided an overall detection rate of 47.6%. Additionally, WES solved 55% of SHL cases. We identified four subjects displaying a dual molecular diagnosis and eight affected by non-syndromic mimics, five of them presenting Usher syndrome type 2 due to pathogenic variants in *ADGRV1* and *USH2A*. Considering this high prevalence, their carrier frequency in the Italian population was analysed exploiting an *in-house* database of Whole-Genome Sequencing data and resulted 2.6% for *ADGRV1* and 2.7% for *USH2A*. The latter resulted significantly higher than reported in literature (1:70).

Conclusions: The diagnostic yield of our multistep approach was 49%. Additionally, for the first time, the frequency of Usher syndrome type 2 healthy carriers in the Italian population was provided, representing the starting point for an effective implementation of diagnostic and preventive strategies.

Conflict of Interest: None declared

P03.006.B High molecular diagnostic rate in cases of retinitis pigmentosa and Usher syndrome using exome sequencing and a dedicated analytical pipeline

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Inherited retinal diseases (IRDs) are caused by mutations in more than 250 distinct genes. Retinitis pigmentosa (RP) is the most prevalent one and can also occur in conjunction with hearing loss, determining a different clinical entity, called Usher syndrome. This study aimed to determine the genetic landscape of pathogenic variants causing these conditions in a cohort of patients from Switzerland. All probands were recruited at the Eye Clinic of the Basel University Hospital and underwent a thorough ophthalmological evaluation. DNA was obtained from peripheral blood or saliva samples and analyzed by exome or Sanger sequencing, using an in silico bioinformatic pipeline combining published tools with software developed in-house.

We examined 104 probands with a diagnosis of RP (n = 88) or Usher syndrome (n = 16) and detected likely causative mutations in 84.6% of cases, a diagnostic rate that can be considered a very high compared to similar studies on European populations (average of 59.1%, 6 studies). The most prevalent disease-causing gene was *USH2A*, carrying pathogenic variants in 15 individuals, followed by *EYS* (n = 10), *RP1* (n = 8), *PRPF31* (n = 6), and *RPGR* (n = 4) genes. This distribution is very similar to that of other European cohort studies. The most prevalent inheritance pattern of mutations was autosomal recessive, accounting for 70% of all cases. Interestingly, we could detect three distinct variants that occurred in more than two unrelated probands: a frameshift insertion in *RP1* (n = 6), a large deletion in *EYS* (n = 3), and a small deletion in *CDHR1* (n = 3). These events are likely to represent founder mutations in Switzerland.

Conflict of Interest: None declared

P03.007.C Loss of function variants and locus heterogeneity in familial cholesteatoma

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Background: Cholesteatoma is a rare progressive disease of the middle ear. Most cases are sporadic, but some patients report a positive and extensive family history of cholesteatoma.

Methods: We performed an observational study of 21 individuals treated for cholesteatoma who were recruited from ten multiply-affected families. We used whole exome sequencing (WES) to identify functionally important gene variants segregating with cholesteatoma in the most distantly related participants from each family. These family studies were complemented with gene-level mutational burden analysis.

Results: Filtered data from pairs and trios of participants in each family revealed 398 rare, loss of function (LOF) variants co-segregating with cholesteatoma. We found LOF variants common to two (or more) families for six genes: *DENND2C*, *DNAH7*, *NBEAL1*, *NEB*, *PRRC2C*, and *SHC2*. The gene-level analysis of mutation-burden identified a significant burden for the genes in the DNAH gene family, which encode products involved in ciliary structure. Common pathways for the candidate genes included GTPase regulator activity, calcium ion binding, and degradation of the extracellular matrix.

Discussion and Conclusion: The number of candidate genes identified and the locus heterogeneity that we describe within and between affected families, suggest that the genetic architecture for familial cholesteatoma is complex. We are now conducting a WES genome-wide association study of 1114 sporadic cases and matched controls from the UK Biobank cohort to follow up on these findings. We will apply machine learning and pathway analysis to further elucidate the mechanisms of disease.

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P03.008.D Genetically refined and unsolved inherited corneal disease cohort offers opportunities for novel genomic discovery

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Background: Inherited corneal diseases (ICDs) are a group of phenotypically and genetically heterogeneous disorders that cause severe visual impairment. Advances in next generation sequencing have made it possible to identify the genetic cause of disease in approximately 75% of cases, however 25% of cases remain unsolved. Determining this 'missing heritability' is a major challenge.

Methods: Exome and/or genome sequencing was performed for all unsolved ICD cases, and whenever possible, patient-matched bulk RNA-Seq data was generated from affected corneal cells excised during corneal transplantation surgery. Exome and genome data was analysed using nf-core/sarek and GATK Best Practices. RNA-Seq data was analysed using pipelines for differential expression, alternative splicing, and pathway

enrichment. Gene burden approaches were adopted for specific ICD subtypes.

Results: A total of 230/1,100 ICD cases in our study cohort remain genetically unsolved, despite extensive screening for known ICD-associated genes. Most unsolved cases have been diagnosed with posterior polymorphous corneal dystrophy or Fuchs endothelial corneal dystrophy. We are now focused on identifying novel genetic causes of disease through extensive analysis of genomic data alongside integration with patient-matched transcriptomic datasets. Here we will present novel candidate ICD variants identified to date, in conjunction with relevant phenotypic information and segregation analysis.

Conclusion: Despite the unprecedented amount of available genomic, transcriptomic and proteomic data available, a significant proportion of ICDs remain unsolved. The novel candidate variants we have identified could be of future translational relevance for the ICD patient population.

Grant References: Fight for Sight PhD studentship: 5171/5172

Conflict of Interest: None declared

P03.009.A Knowledge on genetic cause and awareness of reproductive options in patients with inherited retinal dystrophies

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Background/Objectives: Inherited retinal dystrophies (IRDs) are a clinically heterogeneous group of monogenetic diseases leading to early onset blindness. We aimed to assess the patients' knowledge on the genetic cause of their IRD and its impact, as well as awareness of reproductive options.

Methods: An online questionnaire was set out among two IRD patient organizations. Questions included knowledge on genetic cause, risk of IRD for offspring and possibilities for reproductive options (e.g. invasive prenatal testing, preimplantation genetic testing (PGT)). We also listed all referrals for PGT for IRDs from 2008-2022.

Results: In total, 200 IRD patients (66% women, 33% in age category 21-40 yrs, 42% in age category 41-60 yrs) completed the questionnaire. Patients reported a known genetic cause in 78%. 22% of the patients reported to have visited a clinical geneticist. Of the patients with a known genetic cause, 7% was not aware of the risk of IRD for their offspring. Reproductive options were discussed by their doctor in 22% and 7%, respectively. Accordingly, patients were infrequently referred for PGT (only 2% of the current referrals). X-linked IRDs (RPGR, choroidemia, retinoschisis) were among the most frequent referrals for PGT, followed by cone disorders (achromatopsia).

Conclusion: Our data show the need to improve awareness and education on genetic cause and reproductive options for IRDs in both patients as well as clinicians. Eye care providers should refer parents timely for genetic counseling. These data will be used to define a strategy for effective genetic counselling of IRD patients.

Conflict of Interest: None declared

P03.010.B SpliceAI scores of USH2A variants are predictive of aberrant splicing in minigene assays

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Objectives: Variants that have an effect on pre-mRNA splicing are increasingly recognized as a relevant cause of *USH2A*-associated autosomal recessive retinitis pigmentosa and Usher syndrome type 2. Many splice site variants have been reported in public databases, but an effect on pre-mRNA splicing has only been verified for a subset of these variants. In this study, we aimed to extend the knowledge regarding splicing events by assessing a set of non-canonical, deep-intronic, exonic and branchpoint *USH2A* variants with a predicted effect on pre-mRNA splicing.

Methods: Seventeen non-canonical splice site variants, four deep-intronic variants, five exonic variants and two branchpoint variants were selected based on SpliceAI scores. *USH2A* constructs were generated and minigene splice assays were performed in HEK293T cells.

Results: An effect on pre-mRNA splicing was observed for 24 of the 28 variants, while four out of five exonic variants did not show deviations in splicing. Various events, such as pseudoexon inclusion, (dual) exon skipping and partial exon skipping were observed. Thirteen non-canonical splice site variants (76%), three deep-intronic variants (75%), none of the exonic variants (0%) and one branchpoint variant (50%) had a full effect on splicing.

Conclusion: SpliceAI scores are indicative of aberrant splicing in minigene splice assays. All non-canonical splice site variants and deep-intronic variants had an effect on splicing, as was reflected by generally higher SpliceAI scores. Interpretation of partial effects on splicing with respect to pathogenicity remains challenging and these variants were classified as variant of unknown significance.

Grant: Velux Stiftung

Conflict of Interest: None declared

P03.011.C Whole exome sequencing founds genetic association between optic neuropathy and retinal degeneration in patients with mixed phenotype

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Background/Objectives: Optic atrophy (OA) primarily directing towards retinal ganglion cells often results in retinal degeneration (RD), involving first peripheral visual field (retinitis pigmentosa, RP) and then, in the advanced stages, the central part of the retina, i.e. the macula (macular degeneration). The genetic landscape of causative mutations and genes greatly enlarged in the last decade suggesting common pathways. Therefore, molecular diagnosis is important for genetic counseling and treatment.

Methods: Ten patients expressing combined phenotype of optic neuropathy together with RD were selected from the clinical departments after full ocular examinations. Blood samples were collected from the probands, and genomic DNA was subjected to whole exome sequencing (WES) on NovaSeq 6000 platform. Variants in selected genes associated with glaucoma, OA, anterior-segment dysgenesis, and inherited retinal degeneration (IRD), were extracted from the WES data and filtered using systematic bioinformatics analysis and the American College of Medical Genetics and Genomics criteria.

Results: Potential pathogenic variants (PPVs) were found in 8 IRD-genes, including *HMCN1, RP1L1, PRPH2, NRL, CNGA3, CFB, C3, COL4A1*, and two genes associated with glaucoma, *OPTN* and *PITX2*. Nine patients carried PPV in at least one RetNet-gene. A rare PPV, *OPTN*-c.403G>T (p.Glu135Ter), was found in a proband presenting with myopia, cataract, typical signs of RP and optic disk excavation.

Conclusion: Genotype-phenotype correlation show a possible association between variants in IRD genes and optic neuropathy. Our findings enrich the phenotype spectrum of RetNet genes and provide clues for genetic screening in phenotypes including optic neuropathy.

References: RetNet, Richards et.al,2015

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Conflict of Interest: None declared

P03.012.D The p.C759F variant in USH2A is a pathogenic mutation: systematic literature review and meta-analysis of 524 genotypes

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Background: The p.C759F variant in the Usherin gene (*USH2A*) has been reported several times in the literature as the molecular cause of recessive retinitis pigmentosa, a form of retinal degeneration. However, its pathogenicity has been questioned once by the publication of two discordant genotypes from a single family.

Methods: To elucidate its role as a disease-causing mutation, we performed a meta-analysis on 524 individuals carrying this variant, according to data we collected by reviewing 341 research articles published in the last 22 years.

Results: We performed three independent statistical tests and ascertained with a very high degree of confidence that p.C759F is: i) enriched ~11x in patients with respect to healthy individuals (Chi-square = 718, $p\text{-value} = 3.45 \times 10^{-158}$); ii) enriched ~33x in patients compared to healthy individuals when in trans with a pathogenic mutation in *USH2A*, indicating it is a recessive mutation (Chi-square = 8233, $p\text{-value} < 5.0 \times 10^{-324}$); iii) enriched ~2400x in patients with respect to healthy individuals when in homozygosis (Chi-square = 47149, $p\text{-value} < 5 \times 10^{-324}$).

Conclusion: In summary, our results unambiguously confirm that p.C759F is a Mendelian recessive mutation, leading to retinal blindness.

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Conflict of Interest: None declared

P03.013.A Deciphering novel TCF4-driven molecular origins and mechanisms underlying a common triplet repeat expansion-mediated disease

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Background/Objectives: The predominant cause of Fuchs endothelial corneal dystrophy (FECD) is a CTG repeat expansion (CTG18.1) situated within an intron of the gene *TCF4*. Here, we use a primary patient-derived corneal endothelial cell (CEC) system to enhance our understanding of the multiple pathogenic processes, that are not mutually exclusive, underlying CTG18.1-mediated FECD and to identify novel patterns of *TCF4*-specific dysregulation.

Methods: We defined differential gene expression and alternative splice events using RNA-seq data from 15 biologically independent primary CEC lines with validating RNAScope experiments. To explore whether *TCF4* dysregulation, irrespective of CTG18.1 expansions, could cause FECD we interrogated exome data generated from our large genetically refined cohort of CTG18.1 expansion-negative FECD cases (n = 135).

Results: Analysis of the RNA-seq data and RNAScope experiments revealed subtle effects on *TCF4* expression, where a subset of exons are significantly dysregulated in expansion-positive CECs. Subsequently, we identified a significant enrichment (OR = 18.319; p = 0.00396) of rare and predicted deleterious (minor allele frequency<0.005; CADD>15) *TCF4* variants in CTG18.1 expansion-negative FECD cases compared to an internal control exome consortium dataset (n = 1,656). Interrogation of these variants suggests that, in rare instances, disruption of a specific subset of TCF4 isoforms may also confer CEC-specific disease independent of CTG18.1 expansions.

Conclusions: Our study supports the hypothesis that at least two distinct pathogenic mechanisms, RNA toxicity and *TCF4* isoform-specific dysregulation, underpin the pathophysiology of FECD. We anticipate these data will inform and guide the development of translational interventions for this common triplet-repeat mediated disease.

Grant References: Moorfield Eye Charity Springboard Award GR001337

Conflict of Interest: None declared

P03.014.B Repurposing drugs to treat SLC7A8 age-related hearing loss

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Background: Age-Related Hearing Loss (ARHL) is defined as bilateral sensorineural hearing loss characterized by a multifactorial etiology. It is a common condition, but no targeted therapies are available yet. Our published data (Espino Guarch et al.,2018) proved that *Slc7a8* knockout mice display ARHL and reported the presence of heterozygous pathogenic missense variants in ARHL patients. These considerations suggest *SLC7A8* as a possible therapeutic target to be further investigated.

Methods: The CRISPR/Cas9 technology was employed to create a reporter line expressing the luciferase gene under the transcriptional control of *SLC7A8* promoter in HEK293T cells. This line was used to select compounds able to increase the expression of luciferase, thus of *SLC7A8*. The GEOprofiles database (Barrett et al.,2013) was employed to identify putative *SLC7A8* expression regulators and select possible hit compounds.

Results: The reporter line was successfully generated. We selected and screened four compounds, detecting a significant increase in luciferase expression after treatment with compound1. Thus the dose-response curve for the compound was evaluated. We observed the highest expression fold change with low dosages, while higher concentrations caused cell death. Compound1 is an investigational anti-cancer drug and a chromatin remodeler. Interestingly, the protein targeted by the compound has already been associated with deafness, suggesting the possible appropriateness of the treatment for ARHL.

Conclusions: These results highlight the possible use of compound1 in the treatment of ARHL in *SLC7A8* mutated patients. Additional studies are in progress to better characterize *SLC7A8* activity and the compound efficacy.

Conflict of Interest: None declared

P03.015.C Involvement of GJD3 variants in familial Meniere disease and tectorial membrane attachment

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Background/Objectives: Meniere Disease (MD) is an inner ear disorder, characterised by episodic vertigo, sensorineural hearing loss (SHNL) and tinnitus. We studied involvement of connexins, proteins forming gap junctions by hemichannels in the sensory epithelia. Several variants in connexin genes are known to cause genetic SNHL.

Methods: We performed whole exome sequencing in 94 individuals from 70 different MD families. We calculated enrichment of missense variants in targeted connexins between familial MD and reference population frequencies. RT-qPCR and immunofluorescence were done in mice cochleae to identify expression of enriched candidate genes.

Results: An enrichment of missense variants in *GJD3* gene was found for Spanish population ($OR_{CSVS} = 4.22$[2.14-8.30], $FDR = 5.02 \times 10^{-4}$). We identified two *GJD3* missense variants, one synonymous variant and one downstream variant in 5 familial cases, segregating in 3 of these families. Moreover, they were also found in 7 sporadic MD. The protein model predicts the H175Y missense variant may change the interaction between connexons. We also revealed that the mouse ortholog *Gjd3* is expressed in the organ of Corti, particularly in the tectorial membrane.

Conclusion: We found *GJD3* variants could modify the channel's function and influence in the development of MD. In addition, we have observed for the first time the expression of *Gjd3* in mice tectorial membrane. Our results support previous findings suggesting genes encoding for proteins involved in the attachment of tectorial membrane and stereocilia are related with familial MD.

Grant references: ISCIII-PI20/1126; Andalusian Government EPIVERT-PI-0027-2020 and GEN4PHEN-PI-0266-2021; Horizon 2020 UNITI-848261; and the support of CuresWithinReach and the Knight Family.

Conflict of Interest: None declared

P03.018.B Identification of ADAMTS18 and SDK1 genes in patients with Meniere Disease with endolymphatic sac hypoplasia

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Background/Objectives: Meniere disease (MD) is an inner ear disorder defined by tinnitus and vertigo episodes associated with sensorineural hearing loss. Recently, two endotypes of MD were described considering the histopathology of endolymphatic sac (ES): hypoplastic and degenerative, each associated with a different clinical phenotype. Interestingly, hypoplastic patients showed higher frequency of familial MD. Our hypothesis is that hypoplastic endotype has a genetic background, and the objective is to identify genes associated with ES hypoplasia in MD.

Methods: WES was preformed from saliva of 42 hypoplastic MD patients, identified by angular trajectory of vestibular aqueduct (ATVA) in HR-CT. Data processing included calling for SNV/small-indels, Large-Structural (LSV) and Copy-Number (CNV) variation. SNV/indels were filtered by high/moderate impact prediction on protein function and rare AF<0.05 (gnomAD/gnomAD_nfe v3.1). Gene Burden Analysis (GBA) was carried out to reveal genes enriched in rare variants against reference population. Candidate orthologous genes expression was examined in mouse ear. CNV

and LSV were prioritized if they localized in high-constrain regions and showed pathogenic ACMG-score.

Results: SNV/indels GBA revealed 833 variants enriched genes. Prioritization based on higher number of variants/gene and more patients sharing variants highlights *SDK1* (OR = 3.69/3.89; 34.24% patients) and *ADAMTS18* (OR = 3.76/3.54; 34.14% patients) as candidate genes. Intense expression of *Adamts18* labelled in mouse spiral ganglion.

Conclusion: Rare variant enrichment in *ADAMTS18* and *SDK1* genes was found in MD patients with ES hypoplasia. More functional studies are needed to confirm the possible role of these genes in the pathophysiology of the disease.

Grant References: H2020-SC1-2019-848261; CECEU-PY20-00303(EPIMEN); Swiss Schmieder-Bohrisch Foundation.

Conflict of Interest: None declared

P03.019.C The genetic landscape of inherited retinal diseases in a cohort of Sardinian patients: distinctive findings from an isolated population

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Sardinian population can be considered a genetic isolate, due to its geographic location. The current Sardinia inhabitants' genome holds some signatures of a long history of isolation, which makes the population an ideal model to investigate genes and molecular mechanisms of disease. This peculiarity was confirmed in our study, where we explored the genetic background of Inherited Retinal Diseases (IRDs) in this population.

A total of 78 individuals from 58 different families were studied. Clinical, demographic and familiar data were collected for each patient, including three-generation pedigree, geographical origin, age of onset and presence of any systemic clinical manifestations. Whole Exome Sequencing (WES) and SNP-array analysis were performed in all individuals.

Overall, 70.5% (55/78) of patients were diagnosed with causative variants in 23 different genes. Besides having identified, in agreement with the literature, the *USH2A* and *ABCA4* genes as the main recurrent ones, our study revealed that novel variants in rarer genes are a trait of the Sardinian IRDs' patients. We detected a novel homozygous missense variant in the *CTSD* gene, suggesting new possible genotype-phenotype correlations. Four of our cases showed intragenic deletions, stressing the importance of a proper CNVs analysis. Moreover, WES analysis in large undiagnosed families led to the identification of novel candidate genes for IRDs.

This first study investigating IRDs in Sardinia highlights the uniqueness of the Sardinian genome and has important implications for diagnosis, prognosis, genetic counselling and therapeutic options for our IRDs' patients, whose molecular characterisation is mandatory to access new therapies and clinical trials.

Conflict of Interest: None declared

P03.020.D Exome sequencing high diagnostic yield in a broad spectrum of ocular disorders and its use in selecting patients for gene therapy

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Background: Retinopathies comprise a highly heterogenous group of clinical entities, with great phenotypic overlap and genetic background. These disorders lead to vision impairment or loss, with significant impact on the patient's and their family's quality of life. Since 2017, gene therapy is approved for patients with *RPE65* mutations causing Leber congenital amaurosis (LCA) or retinitis pigmentosa (RP). Revealing the genetic background of an ocular disease not only sets diagnosis and high-risk relative detection, but enables patient selection for gene therapy.

Methods: From 2018-2022, 38 patients with signs and/or family history of ocular disease were referred to our lab for genetic testing. Exome sequencing (ES) was performed on DNA extracted from peripheral blood: library preparation was performed with Clinical Exome (Sophia Genetics) or Whole Exome (Twist Bioscience) and sequenced on Illumina NextSeq-550 (Illumina). Bioinformatics analyses were conducted by SOPHiA DDM® bioinformatics pipelines.

Results: In 23/38 (60.53%) of cases definite genetic diagnosis was defined. Disease-causing mutations were found in 14 genes with most prevalent *ABCA4, RPE65* and *USH2A* related to RP, cone-rod dystrophy, Stargardt macular degeneration, LCA and Usher syndrome. Three patients were compound heterozygotes for *RPE65* mutations, qualifying for gene therapy.

Conclusion: ES permitted genetic diagnosis for ocular disease patients, enabling timely decisions for interventions and relative screening. Otherwise, single-gene testing would be an everlasting odyssey with questionable outcomes. Further, patients with *RPE65* mutations were eligible for gene therapy and revealing genetic background for others grants eligibility for future targeted therapies evolving from ongoing clinical trials.

Conflict of Interest: Georgia Christopoulou Full-time employment at Genotypos M.S.A., Full-time employment at Genotypos M.S.A., Stavros Bournazos Full-time employment at Genotypos M.S.A., Stavroula Samara Full-time employment at Genotypos M.S.A., Aikaterini Oikonomaki Full-time employment at Genotypos M.S.A., Ilias Georgalas: None declared, Tryfon Rotsos: None declared, Pantelis Constantoulakis Full-time employment at Genotypos M.S.A.

P03.021.A The co-occurrence of genetic variants in the TYR and OCA2 genes confers susceptibility to albinism

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Background/Objectives: The gaps in our knowledge of when/how genetic variants in different loci combine to give rise to human

phenotypes limit our understanding of the processes underlying the clinical and genetic heterogeneity of rare disorders. Here, we used albinism, an archetypal rare condition associated with hypopigmentation, as an exemplar for the study of genetic interactions.

Methods: We studied a cohort of 1,121 individuals with albinism. A "control" cohort of 29,451 individuals from the Genomics England 100,000 Genomes Project dataset was also analysed. We assumed a digenic model and utilised a genotype-based approach that focused on two prevalent albinism-related changes, *TYR*:c.1205G>A (p.Arg402Gln) and *OCA2*:c.1327G>A (p.Val443Ile). We hypothesised that when these two missense variants are both present in the heterozygous state, their interaction is driving the pathology observed in albinism.

Results: We found that when both *TYR*:c.1205G>A and *OCA2*:c.1327G>A are present in the heterozygous state, the probability of receiving a diagnosis of albinism is significantly increased (odds ratio 7.9; 95% confidence interval 4.4–13.2; p-value 4×10^{-9}). Further analyses in an independent cohort, the UK Biobank, supported this finding and highlighted that heterozygosity for the *TYR*:c.1205G>A and *OCA2*:c.1327G>A variant combination is associated with statistically significant alterations in visual acuity and central retinal thickness (traits that are considered albinism endophenotypes)

Conclusion: We have shown that dual heterozygosity for a *TYR* and an *OCA2* variant confers susceptibility to albinism. The outlined approach is likely to be relevant to the study of other rare disorders, and opens up new avenues for the investigation of oligogenic patterns.

Conflict of Interest: David Green: None declared, Vincent MIchaud: None declared, Eulalie Lasseaux: None declared, Claudio Plaisant: None declared, Tomas Fitzgerald: None declared, Ewan Birney Equity holder of Oxford Nanopore, Paid consultant to Oxford Nanopore and Dovetail; non-executive director of Genomics England., Graeme Black: None declared, Benoit Arveiler: None declared, Panagiotis Sergouniotis: None declared

P03.022.B Benchmarking of different splice prediction tools allow the identification of novel spliceogenic variants in inherited retinal dystrophies

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Background/Objectives: Variants affecting different pre-mRNA splicing mechanisms are responsible for multiple monogenic disorders. However, its prioritization and assessment remain challenging and demand the use of prediction tools, specifically for those variants located at non-canonical sites. Herein, we designed a strategy for the identification of likely spliceogenic variants in unsolved inherited retinal dystrophy (IRD) cases.

Methods: We benchmarked a total of thirteen splice prediction tools on a curated training dataset comprising 3637 rare genomic variants, of which 1535 have been classified in ClinVar and HGMD-pro as pathogenic or likely pathogenic. The optimal combination of tools was assessed using a validation cohort comprising 116 individuals with a rare disease harbouring 119 spliceogenic, disease-causing variants. A custom pipeline was developed and applied for the reanalysis of 228 unsolved IRD families (experimental cohort), of which 18 families underwent WGS, and the rest a custom gene panel. Functional validation of candidate variants was conducted when possible.

Results: The best-performing combination involved the SpliceAI and MaxEnt tools for analyzing all splice variant types and the BranchPoint tool for branchpoint variants. The application of our custom pipeline in the experimental cohort allowed the identification of 23 likely pathogenic spliceogenic variants, including synonymous, non-canonical, branchpoint, and deep-intronic variants that may contribute to explain the phenotype.

Conclusions: The developed pipeline contributed to an increase of up to 12% in the diagnostic yield of previously unsolved IRD patients reinforcing the importance of reanalysis strategies focused on identifying spliceogenic variants.

Grant References: ISCIII-ERDF/ESF (PI21/00244;FI19/00091), Andalusian-Government (PEER-0501-2019), F.Isabel Gemio/F.Cajasol (FGEMIO-2019-01), ISCIII-IMPaCT (IMP/0009)

Conflict of Interest: None declared

P03.023.C Evaluation of machine learning models for the detection of familial predisposition in Meniere's disease

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Background/Objectives: Machine learning (ML) is becoming more relevant in our daily lives, showing a great potential to recognize patterns and learn from them. Meniere disease (MD) is a rare inner ear disease characterized by episodes of vertigo, sensorineural hearing loss and tinnitus. The majority of diagnosed patients are sporadic, however, 8-9% show familial aggregation. This suggests a different genetic architecture that could be used for the detection of familial predisposition. This study aims to develop a ML-based tool capable of classifying MD patients into familial and sporadic, taking as input sequencing data.

Methods: Exomes sequencing data from 77 and 295 unrelated patients with familial and sporadic MD, respectively, were used. To identify the distinctive genes of these two cohorts, a non-synonymous variant enrichment analysis (MAF<0.1) was performed. Similarly, pathways and digenic combinations with high discriminatory potential were identified. The optimal input features were determined through recursive feature elimination with support

vector machine as base estimator. The different classification models were evaluated using 10-fold cross-validation.

Results: After comparing our data with Spanish and European reference populations, the algorithm developed classified familial and sporadic patients with high sensitivity and specificity when using a logistic regression model (AUC = 0.81±0.05). Preliminary results using this tool indicate that familial predisposition may exist in 20% of sporadic cases.

Conclusions: This study presents a ML-based tool capable of differentiating sporadic and familial MD patients using exome sequencing data. This could be applied to other rare diseases to facilitate genetic counseling.

Conflict of Interest: Pablo Román-Naranjo PRNV is supported by PY20-00303 Grant (EPIMEN)., Alba Escalera-Balsera: None declared, Alvaro Gallego-Martinez: None declared, Carmen Ayuso: None declared, Joaquín Dopazo: None declared, José María Millán: None declared, Miguel Ángel Moreno-Pelayo: None declared, Antonio Lopez-Escamez JALE has received funds from Instituto de Salud Carlos III (Grant# PI20-1126), CIBERER (Grant# PIT21_GCV21), Andalusian University, Research and Innovation Department (PY20-00303, EPIMEN), Andalusian Health Department (Grant# PI027-2020), Asociación Síndrome de Meniere España (ASMES) and Meniere's Society, UK.

P03.024.D Clinical and genetic aspects of Bardet-Biedl syndrome in adults in Norway

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Background and aims: Bardet-Biedl syndrome (BBS) is a rare non-motile ciliopathy affecting

multiple organ systems with main features including retinal dystrophy, obesity and renal dysfunction. Identifying disease-causing genes is important for a correct diagnosis and for personalized medicine. In this study, we aim to identify the genetic causes of BBS in adults in Norway and describe the phenotypes including ocular and oral findings in order to optimize the potential of personalized medicine.

Methods: Medical examination including height and weight (body mass index), eye and oral examinations. General blood tests and genetic analysis (exome and/or genome sequencing).

Results: A prospective study of 31 adults (>16 years of age) diagnosed with BBS (inclusion rate 67%). Mean age was 39.2 (SD 13.8) with 52% female. Preliminary data revealed genetic causal variants in some of the BBS genes: *BBS1* (8), *BBS5* (1), *MKKS* (1), *BBS7* (1), *BBS9* (2) and *BBS10* (7). One individual with features overlapping with McKusick-Kaufmann syndrome has variants in the *MKKS* gene. One participant had a genetic cause in the *CFAP410* gene. Statistical analysis on the dataset, including clinical evaluation, ocular and oral functions will be presented.

Conclusions: Preliminary results identified a probable or certain genetic cause in 95%. Findings will inform of specific needs that might be used for healthcare recommendations.

Conflict of Interest: Cecilie Rustad: None declared, Kristian Tveten: None declared, Ragnheidur Bragadottir: None declared, Hilde Nordgarden: None declared, Jeanette Ullmann Miller: None declared, Pamela Åsten: None declared, Shahrzad Arfa: None declared, Øystein Holla: None declared, Mina Susanne Weedon-Fekjær: None declared, Charlotte von der Lippe: None declared, Solrun Sigurdardottir Grant from the Olav Raagholt og Gerd Meidel Raagholts stiftelse

P03.025.A Mutational spectrum and clinical features of EYA1-related branchiootorenal syndrome

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Background: Branchiootorenal (BOR) syndrome is an autosomal dominant disorder characterized by the association of sensorineural, conductive, or mixed hearing loss with structural defects of the outer, middle, and inner ear, branchiogenic fistulas/cysts, and renal abnormalities. It is caused by mutations in the *EYA1*, *SIX1*, and *SIX5* genes. Point mutations and deletions in *EYA1* have been identified in approximately 40% of affected individuals.

Methods: Eight families who fulfilled the criteria for BOR syndrome were identified in this study. Patients and their family members were analyzed by WES, WGS, CNV, MLPA and Sanger sequencing. The mini-gene assay was performed to confirm the potential pathogenic effect of splice site variants.

Results: Two pathogenic nonsense variants, one pathogenic missense variant and three splice site variants were identified in six families. The mini gene assay confirmed the pathogenic character of the novel 82bp deletion (c.1051-80_1052del) affecting the *EYA1* intron 11 – exon 12 boundary. The heterozygous whole gene deletion was detected in two additional families. Analysis of the genotype-phenotype correlation revealed a high phenotypic variability between individual families. Moreover, we found a large intrafamilial variability in a large multiplex family with whole gene deletion identified in 11 affected members who suffered from different combinations of clinical manifestations including hearing impairment, microtia, preauricular and cervical fistulas, palate, facial nerve paresis and different degree of kidney abnormalities.

Conclusion: There is a great intrafamilial and interfamilial variability in the presence, severity and type of anomalies accompanying the *EYA1*-related BOR syndrome.

Grant References: APVV-20-0236, VEGA 1/0572/21, ITMS: 313021BZC9

Conflict of Interest: None declared

P03.026.B ERN-EYE Virtual Clinic for Rare Eye Diseases as a successful effort towards solving complex and rare ophthalmic conditions in Europe

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Background/Objectives: The European Reference Networks (ERNs) are virtual networks involving healthcare providers across Europe aimed to improve management of rare diseases. ERN-EYE is dedicated to Rare Eye Diseases (REDs) and covers 24 EU countries, to guarantee the best coverage of 900 REDs. One of the most important tools of the ERNs is a virtual clinic using the IT platform provided by the European Commission with a specific dataset. A referring clinician uploads patients' data to review by experts. The activities of this virtual clinic during 5 years (2017-2022) are presented.

Methods: The tools for the virtual clinic to solve RED cases in a meaningful time and provide education on a larger scale are described. Numbers of solved cases with illustrative examples are presented.

Results: Overall, 102 patients affected with REDs were referred to the virtual clinic. The patients were allocated into 4 major groups: retina (n = 58), anterior segment (n = 18), neuro-ophthalmology (n = 14) and paediatric ophthalmology (n = 12). Patients' data were discussed at the virtual meetings or through communication on IT platform. Nearly 70% of cases were solved (n = 70) and we observed higher solving success rate during virtual meetings.

Conclusion: Virtual clinic for rare eye conditions using telemedicine is an effective tool for the clinicians who encounter complex cases that require advice from other EU experts, and without a need for patient to travel across countries to seek for expertise. This is a secure clinical platform facilitating information sharing and education in REDs in the EU.

Grant References: European Union Grant (101085439-ERN-EYE 22-23).

Conflict of Interest: Monika Grudzinska Pechhacker part-time, In the past, member of Advisory Board, Novartis (2018), unrelated to this project, Francesco Rotolo full-time, Dorothée Leroux full-time, Amélie Gavard full time, Caroline Wernert-Iberg full time, Maximin Begin full time, Bernard Coupez full time, Petra Liskova full time, Daniel Böhringer full time, Frank G. Holz full time, Research grants and personal fees from Acucel, Allergan, Apellis, Bayer, Bioeq/Formycon, Roche/Genentech, Geuder, Heidelberg Engineering, ivericBio, Pixium Vision, Novartis and Zeiss; and personal fees from Alexion, Grayburg Vision, LinBioscience, Stealth BioTherapeutics, Aerie and Oxurion. All unrelated to this project, Michael Larsen full time, Steffen Hamann full time, Susana Noval full time, Dominique Bremond-Gignac full time, Birgit Lorenz full time, Elfride De Baere full time, none related to this project, Bart Leroy full time, Research Grants from GenSight Biologics, MeiraGTx-Janssen Pharmaceuticals, Novartis and ProQR Therapeutics. Unrelated to this study., GenSight Biologics (Paris, France), 4DMT (Emeryville, CA, USA), AAVantgardeBio (Milano, Italy), Akouos (Boston, MA, USA), Atsena Therapeutics (Durham, NC, USA), Bayer (Leverkusen, Germany), Biogen (Paris, France), IVERIC Bio (New-York, NY, USA), MeiraGTx (London, UK)-Janssen Pharmaceuticals (Raritan, NJ, USA), LookoutGTx, Novartis (Basel, Switzerkand), Opus Genetics (Raleigh, NC, USA), Oxurion (Leuven, Belgium), ProQR Therapeutics (Leiden, The Nederlands), Santen (Osaka, Japan), Spark Therapeutics (Philadelphia, PA, USA), REGENXBIO (Rockville, MD, USA), Vedere Bio (Cambridge, UK), ViGeneron (Planegg, Germany). Unrelated to this project., Hélène Dollfus full time, ERN-EYE was partly co-funded by the European Union within the framework of the Third Health Programme "ERN-2016 - Framework Partnership Agreement 2017-2021" and currently fully funded by the European Union under the grant agreement number 101085439 — ERN-EYE 22-23, Rhytm Therapeutics Inc. consultancy, unrelated to this project.

P03.027.C Comprehensive genetic testing in a cohort of 67 unrelated retinal dystrophy patients of Turkish and Eastern European descent

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Introduction:

Inherited retinal dystrophies (IRD) are a major cause of early vision loss, and feature progressive loss of photoreceptors and retinal dysfunction. Clinical variability is well-correlated with genetic heterogeneity, with more than 270 associated genes.

Materials & Methods:

67 unrelated patients diagnosed with IRD by a retina specialist between 2015-2022, were consulted by medical geneticists for syndromic features. 50 patients were analysed by WES with mtGenome analysis (75%), nine with clinical exome panel (13%), five with targeted single gene sequencing (7%) and three with WGS (5%). Nine available affected family members were also screened for familial variants.

Results: In 55 of 67 index patients, 50 pathogenic/likely pathogenic variants (P/LP) and 27 variants of unknown significance (VUS) were identified across 33 genes. The genes with the most frequent mutations were *ABCA4* (16/55), and *CRB1* (6/55). In two patients where WES revealed only one heterozygous *ABCA4* exonic variant, deep sequencing and familial segregation led to the identification of a non-coding *ABCA4* variant in trans. Two other patients with one heterozygous *ABCA4* and *TRPM1* variant, respectively, remain unsolved. Rare syndromic genotypes revealed by molecular studies were *MT-ATP6, CEP290, OTX2* and *SDCCAG8*.

Conclusions: We report the phenotypic and genomic landscape of 67 IRD families. Ongoing further studies include extended familial segregation analyses to reclassify VUS, deep sequencing in arIRD patients with a single heterozygous variant, and identity-by-descent analyses in phenotypically well-characterized arIRD families for novel gene identification.

Conflict of Interest: None declared

P03.028.D Four unique genetic variants account for 62.7% of pediatric retinal blindness in Chile

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Background/Objectives: Leber congenital amaurosis (LCA) and early-onset retinal dystrophy (EORD) are the leading causes of incurable blindness in childhood. Both the syndromic and non syndromic forms are characterized by a high degree of genetic and allelic heterogeneity. The aim of this study was to determine the genetic architecture of these disorders in Chile.

Methods: Sixty-seven individuals from 60 families initially seen for a severe visual dysfunction consistent with a provisional clinical diagnosis of LCA or EORD were analyzed using a custom panel of 212 IRD genes, which included all genes causing nonsyndromic and syndromic LCA/EORD and differential diagnoses. Confirmation of variants and segregation with the disease were performed using Sanger sequencing.

Results: Pathogenic variants were identified in 64 cases, leading to a detection rate of 95.5%. 17 genes and 126 variants, of which 32 were unique, were involved. Homozygosity was observed in two-thirds of the families, supporting high inbreeding in the Chilean population. *CRB1*, *LCA5* and *RDH12* accounted for the vast majority of the cases (72%). *CRB1* was the most frequent of them (43.75%). Four unique variants (*CRB1* p.Cys948Tyr and p.Ser1049-Aspfs*40, *RDH12* p.Leu99Ile and *LCA5* p.Glu415*) two of which resulted from a founder effect, accounted for 62.7% of all disease alleles.

Conclusion: This study disclose a high degree of inbreeding in the Chilean families affected with pediatric retinal blindness, resulting in a very limited repertoire of mutations. This observation suggests mutation-specific detection of the four major disease-causing variants (62.7% of the cases) as a first-tier analysis in Chilean patients.

Conflict of Interest: None declared

P03.029.A Genomic landscape of 311 patients with ocular developmental diseases in Spain based on NGS data

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Introduction: Ocular developmental diseases (ODDs) encompass a wide range of heterogeneous and overlapping phenotypes, whose molecular characterization remains challenging. Here, we aimed to broaden the genetic profiling of ODDs using different NGS approaches.

Methods: We performed different NGS approaches in a cohort of 311 cases, divided into six phenotypic sub-groups: isolated microphthalmia/anophthalmia, coloboma, congenital cataracts, anterior segment disorders, and overlapping panocular presentations. First, up to 460 genes were analyzed in custom panels. As a second-line, an in-house network-based algorithm was used to prioritize potential candidate genes in WES/WGS analysis.

Results: Overall, 37% (115/311) of cases ODDs cases were fully characterized with 108 SNVs and 14 SVs, ranging from 21% of coloboma cases to 42% of cataract or panocular cases. A total of 55 genes were involved, with the top 5 genes being *PAX6, GJA8, CYP1B1*, *CRYAA* and *CHD7*. 23% of patients were partially characterized as carrying monoallelic variants in phenotype-related recessive genes, or VUS in a presumed new phenotypic association or potential new ODD genes. WGS is helping to identify SVs that were previously overlooked, non-canonical splicing and non-coding variants in deep-intronic, 5' UTR and other regulatory regions, which are being functionally assayed.

Discussion: This study tackles the missing heritability of ODDs using different cutting-edge sequencing approaches. Gene-targeted panels represent a cost-effective tool for diagnosing ODDs, but WGS analysis improves the diagnosis of complex cases thanks to prioritizing overlooked variants in non-coding regions.

Grants: Spanish Health Institute Carlos III (PI17_01164 and PI20_00851).

Conflict of Interest: None declared

P03.030.B A new family with an autosomal dominant transmission of GJB6 non-syndromic deafness

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Pathogenic variants in human connexins can give rise to multiple diseases, also known as connexinopathies. Variants in the genes *GJB2* (encoding connexin 26) and *GJB6* (encoding connexin 30) are mainly linked to an autosomal recessive form of non-syndromic hearing loss. Autosomal dominant pathogenic variants in the *GJB6* gene that cause two different pathologies are also described in literature. The first is known as Clouston syndrome, which is characterised by a triad of nail dystrophy, alopecia and palmoplantar hyperkeratosis. The other is associated with a very rare case of non-syndromic hearing loss that has been reported only once in a family in Italy, [Grifa et al. 1999]. The phenotypes in this family vary from mild hearing loss to profound sensorineural deafness.

We report the second family with non-syndromic dominant sensorineural bilateral hearing loss across five generations, involving 12 individuals. Genetic testing identified a probably pathogenic heterozygous missense variant, c.173C>G, p.(Pro58Arg) in *GJB6*. The first clinical signs of hearing loss reported in this family vary in age, with some members presenting hearing loss from birth, and others reporting hearing loss only once they have reached adulthood.

Our finding seems to reinforce compelling arguments that implicate *GJB6* in a very rare form of non-syndromic deafness.

Conflict of Interest: None declared

P03.031.C Novel mutations in BLOC-1 and BLOC-2 HPS genes in patients with non-syndromic ocular albinism: using NGS to improve diagnosis and follow-up

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Background: Hermansky–Pudlak syndrome (HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism (OCA) or ocular albinism (OA), a bleeding diathesis, and, in specific subtypes, organ involvement (pulmonary fibrosis, granulomatous colitis, or immunodeficiency). To date, 11 HPS subtypes (HPS-1 to HPS-11) have been molecularly identified.

Methods: performed trio exome sequencing in paediatric patients diagnosed with OCA/OA at Meyer Children's Hospital, Florence.

Results: In seven paediatric patients the apparently isolated OCA/OA we unexpectedly identified pathogenic variants in HPS-associated genes. Six were novel variants in BLOC1S5, HPS3, BLOC1S3, HPS6 and three were previously deported changes in HPS6 and HPS5. All the novel variants were classified as pathogenic and most of them are frameshift, as previously reported. All patients showed an apparently isolated ocular phenotype with no organ involvement. Only one of two siblings with variants in BLOC1S5 developed a pilocytic astrocytoma, deemed to be unrelated to HPS.

Conclusions: Our results reinforce genotype-phenotype correlations for BLOC-1 and BLOC-2 deficiency, improving also the recognition of mild phenotypes. Reporting novel variants especially in BLOC1S5 and BLOC1S3 is important given the small number of patients described in the literature. Also, exome sequencing allowed reaching a molecular diagnosis that was more accurate than the clinical one and starting specific hematologic follow-up.

Conflict of Interest: None declared

P03.032.D Identification of Gap junction protein alpha 8 (GJA8) variants in eleven families with microphthalmia and cataract: further genotype-phenotype correlation

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Introduction: *Gap junction protein alpha 8* (*GJA8*), encodes a connexin protein (Cx50) involved in the formation of gap junctions important for lens development and ocular growth. *GJA8* variants, initially linked to isolated cataracts/lens anomalies, have recently been implicated in other ocular phenotypes including microphthalmia, coloboma, glaucoma and anterior segment disorders, primarily in combination with cataract.

Methods: We identified *GJA8* variants in individuals with developmental eye disorders, including cataract and microphthalmia in the UK, Spain, France and USA, using whole exome/genome sequencing or customized NGS panels of ocular development genes.

Results: We report *GJA8* variants in 15 individuals from 11 new families with developmental eye disorders. In seven individuals with microphthalmia and cataracts, four previously reported pathogenic variants were identified: c.196T>G;p.(Tyr66Asp), c.226C>T; p.(Arg76Cys), c.263C>T; p.(Pro88Leu) and c.281G>A; p.(Gly94Glu) [NM_005267.5]. In one family, three affected individuals harboured the variant c.592C>T; p.Arg198Trp: two individuals manifested microphthalmia, microcornea and congenital cataract, and one manifested microcornea with cataract. Additionally, one individual with microphthalmia and sclerocornea had a variant c.280G>C; p.Gly94Arg and 4 individuals with isolated cataract had the following variants: c.200A>G p.Asp67Gly, c.116C>T; p.Thr39Met, and 1q21 microdeletions (2 individuals). Several codons described in this study have been previously implicated, showing hotspots.

Conclusion: Our findings support the frequent contribution and importance of *GJA8* variants in developmental eye disorders and add to genotype-phenotype correlation. Furthermore, we highlight clustering of these variants within the gene, which may ultimately further our understanding of pathogenesis and future therapeutic options.

Funding: Baillie Gifford Microphthalmia, Anophthalmia, Coloboma Support; National Institutes of Health

Conflict of Interest: None declared

P03.033.C The mutational spectrum of RPE65 and associated phenotypes in the Saudi Arabian population

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Background: Disease-causing variants in RPE65 are associated with early onset retinopathy, but some patients have mild retinopathy with near normal visual acuity. This study reports the genotypes and describes the associated retinal phenotypes in the Saudi population.

Methods: A retrospective case series. Data extracted from the clinical records included the genotypes, visual acuity, and the phenotype on retinal imaging.

Results: Forty-seven patients from 35 families were identified. Except for one patient who was heterozygous for two RPE65 variants, all patients had homozygous RPE65 alleles. Fifteen variants were identified: missense ($n = 10$) including two novel alleles, and loss of function ($n = 5$). The visual acuity range was from 20/30 to light perception. Most patients had retinal vascular attenuation and outer retinal alterations, with one patient manifesting with fundus albipunctatus phenotype.

Conclusion: High rate of homozygosity for RPE65 alleles is caused by familial consanguinity in the population. The recurrent variant p.(Arg91Trp) causes severe retinal degeneration in most patients, but one had a milder fundus albipunctatus-like phenotype, which could be due to disease modifiers. Characterisation of the therapeutic window for patients with mild retinopathy is needed before commencing treatment.

Grant references: None.

Conflict of Interest: Rola Ba-Abbad King Khaled Eye Specialist Hospital, Genetic testing was partially funded by Novartis., Majeedah AlOtaibi King Khaled Eye Specialist Hospital

P04

Internal Organs & Endocrinology (Lung, Kidney, Liver, Gastrointestinal)

P04.001.A The role of CLDN variants in the development of calcium-based kidney stones

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Claudins (CLDNs) are a large transmembrane protein family that are found in the tight junctions of epithelial cells. They form various kinds of ion-specific pores and barriers that regulate the paracellular exchange of calcium and other ions in the nephron. Certain *CLDN* variants such as those in *CLDN16* have been well documented as a monogenic cause of kidney stones while other *CLDN* variants have been associated with kidney stones in genome-wide association studies. The Gupta laboratory recruited a cohort of children and adults with recurrent kidney stones and sequenced their *CLDN* genes, identifying 13 rare or novel variants in *CLDN* genes in the cohort.

Preliminary data of one of the *CLDN8* variants (CLDN8 A94V) shows disrupted localization to the tight junctions that results in a leakier tight junction compared to the wildtype protein. Another variant (CLDN8 M97T) shows normal localization but still results in a leakier tight junction compared to the wildtype protein. By contrast, two *CLDN4* variants show no difference in protein localization or cell layer permeability compared to wildtype.

CLDN8 impedes the paracellular flow of cations such as calcium in the distal nephron. Variants in *CLDN8* could be a polygenic risk factor for the development of kidney stones. This study will aid in the prediction of kidney stone recurrence based on genotype, especially in younger patients who lack the typical environmental risk factors for stones and hopefully pave the way for more targeted interventions.

This work was supported by a grant from The Kidney Foundation of Canada.

Conflict of Interest: None declared

P04.002.B A novel non-recurrent CNV deletion involving TBX4 causes congenital alveolar dysplasia in newborn

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Background: Congenital alveolar dysplasia (CAD) is one of lethal lung developmental disorders (LLDDs) in neonates characterized by acute respiratory distress and frequent refractory pulmonary hypertension. While largely genetically undefined, cases of CAD resulting from *FGF10* abnormalities have been discovered as have affected neonates with heterozygous SNVs or CNVs involving *TBX4*. The identified recurrent CNVs at 17q23.1q23.2 are flanked by LCRs and encompass *TBX4* and *TBX2*. In mice, depletion of *Tbx4* and *Tbx2* leads to hypoplastic lungs.

Methods: Using trio-genome sequencing (GS) and histopathological evaluation, we analyzed a family with a newborn with clinically suspected LLDD.

Results: A male proband was born from uncomplicated pregnancy in good condition and without any structural malformations. From the first hours of life he suffered from severe respiratory insufficiency which was not amenable to treatment and he died at 28th day of life. GS in an infant revealed a novel ~1.07 Mb heterozygous non-recurrent CNV deletion at 17q23.2 (chr17:61,451,736-62,521,987, hg38), encompassing *TBX4* but leaving *TBX2* intact. The deletion arose de novo on the paternal chromosome. Histopathological evaluation demonstrated lobular maldevelopment with lung growth arrest along the spectrum of CAD, pulmonary arterial hypertrophy, and superimposed acute alveolar inflammation.

Conclusion: This is the first report of a patient with CAD and de novo heterozygous 17q23.2 CNV involving *TBX4* but leaving *TBX2* intact. While *Tbx4* and *Tbx2* are essential in regulating mouse lung organogenesis, our results suggest that perturbation of *TBX4*, but not *TBX2*, could produce a severe lung phenotypes in humans.

Grants: National Science Centre, Poland, 2019/35/D/NZ5/02896

Conflict of Interest: None declared

P04.003.C Challenges of clinical variant interpretation for endocrine disorders in a UK diagnostic laboratory

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Background/Objectives: Genetic testing has become an integral part of disease management within the UK's National Health Service (NHS). In 2018 NHS England launched the Genomic Medicine Service (GMS) to coordinate genetic testing through seven Genomic Laboratory Hubs (GLHs) with the aim of providing equitable access to genomic testing across England. Using endocrine disorders as an example, here we demonstrate how the use of nationally agreed clinical eligibility criteria, structured phenotype data, automated application of PanelApp virtual gene panels, and implementation of ACGS guidelines, are all necessary to deliver equitable service with fast and accurate variant interpretation for heterogeneous disorders.

Methods: Targeted sequencing analysis of the coding regions of genes by next generation sequencing, were performed on patient samples. Data was then processed with an in-house bioinformatics pipeline, candidate variants were classified using ACGS guidelines and confirmed via Sanger sequencing.

Results: By integrating curated genotype and phenotype data, we have upgraded and downgraded our reports from variant of uncertain significance to likely pathogenic and downgraded pathogenic to variant of uncertain significance.

Conclusion: Variant interpretation for endocrine disorders is generally difficult as clinical expression and disease severity may vary among affected individuals due to the presence of genetic heterogeneity. In order to overcome these challenges and keep up with high number of samples we receive on a daily basis, we implement standardized guidelines and in some cases conduct MDT meetings with the referring clinicians to enable discussion of variant classification in accordance with the patient's clinical phenotype.

Grant References: Not Applicable

Conflict of Interest: None declared

P04.004.D Effects of lipid-lowering drugs on improving kidney function: A drug target Mendelian randomization study

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Background/Objectives: Chronic kidney disease (CKD) frequently co-occurs with cardiovascular diseases, hypertension, and diabetes, but few comorbid condition specific therapies exist. Using drug-target Mendelian randomization (MR), we investigated existing treatments for these conditions as potential treatments for improving kidney function.

Methods: Genetic variants to proxy the effects of statins, ezetimibe, and alirocumab/evolocumab (lipid-lowering drugs); angiotensin-converting enzyme inhibitors, β-blockers, and calcium channel blockers (antihypertensive drugs); and metformin (antidiabetic drug) and their effect on low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP), and HbA_{1c} (respective targets for each drug class) were obtained from published genome wide association studies (GWASs). Variant-outcome effect on creatinine and cystatin-C based estimated glomerular filtration rate (eGFRcrea and eGFRcys, respectively) and blood urea nitrogen (BUN), were also obtained from GWAS. Two-sample MR analysis was conducted using R (version-4.2.1).

Results: Each 1SD lowering in LDL-C caused by ***HMGCR*** variants, proxying the effect of statins, **was associated with 0.01SD higher eGFR** (*eGFRcrea* $\beta = 0.012$, *95% CI* $= 0.007\text{-}0.018$, $p = 0.0003$; *eGFRcys* $\beta = 0.012$, *95% CI* $= 0.002\text{-}0.022$, $p = 0.017$), and **0.01SD lower BUN** ($\beta = -0.010$, *95% CI* $= -0.020\text{-}0.000020$, $p = 0.05$), with consistent findings from sensitivity analysis and no bias due to pleiotropy ($P_{Egger} = 0.22\text{-}0.70$). Findings for other lipid-lowering drugs remained inconclusive. Similarly, we found limited evidence for the causal role of antihypertensive or antidiabetic drugs in improving kidney function.

Conclusion: The genetically proxied LDL-C lowering effect of statins in improving kidney function provide insights into a potential drug target for future trials to address the treatment of CKD and comorbidities.

Conflict of Interest: Schyler Bennett: None declared, Venexia Walker: None declared, Jie Zheng: None declared, Ben Brumpton: None declared, Kristian Hveem: None declared, Anna Köttgen: None declared, Bjørn Olav Åsvold: None declared, Tom Gaunt T.G. receives funding from Biogen for unrelated research., Humaira Rasheed: None declared

P04.005.A Genetic landscape of growth hormone deficiency in Portuguese patients

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Background/Objectives: Growth Hormone (GH) is produced by the pituitary gland and is the main regulator of growth during childhood. GH deficiency (GHD), in the absence of treatment, results in growth failure and severe short stature. Many GHD cases that were initially classified as idiopathic are becoming recognized to be genetic in origin, with variants identified in genes involved in the pituitary development and/or function. Our aim was to determine the genetic basis of a cohort of 150 Portuguese patients with idiopathic GHD.

Methods: We performed Whole Exome Sequencing (WES), followed by bioinformatic analyses using standard pipelines. Genetic variants were filtered in 505 genes, all biologically related to the GH axis, pituitary development, and/or pituitary-related disorders. This panel included 46 genes already known to be associated with GHD. Putatively pathogenic variants were selected by filtering non-synonymous variants located in the exons and intron-exon boundaries, with an allele frequency below 0.1% in gnomAD, absent in a Portuguese healthy cohort ($n = 50$), and with a CADD score equal or greater than 20.

Results: We identified 641 variants, including 69 variants in 25 GHD-related genes, and 572 variants in 259 additional genes. *CEP290*, *LRP2*, *VPS13B*, *HERC2*, *CDH23*, and *GLI2* were the most frequently implicated genes. Missense variants were the most frequent type.

Conclusion: This WES analysis of Portuguese GHD patients identified 641 potentially pathogenic variants and top mutated genes that can be potential pathogenic candidates in this disorder.

Grant References: Portuguese Foundation for Science and Technology (UI/BD/151021/2021), and Sidra Medicine (SDR200059).

Conflict of Interest: None declared

P04.006.B High Frequency of MEFV Disease-Causing Variants in Children with Very-Early-Onset Inflammatory Bowel Disease

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Objectives: Biological similarities between inflammatory bowel disease (IBD) and familial Mediterranean fever (FMF) have been described in humans and animal models suggesting a possible common genetic basis. FMF is caused by variants in the *MEFV* gene which encodes pyrin, an immune regulator. The aim of the study was to investigate the carrier rate of disease-causing *MEFV* variants in children of different ethnicities diagnosed with very-early-onset IBD (VEO-IBD).

Methods: The cohort included 23 children less than 6 years old clinically diagnosed with VEO-IBD. All had undergone whole exome sequencing that was negative for candidate IBD gene variants. The exomes were evaluated for *MEFV* monoallelic and biallelic disease- causing variants. Findings were compared to exome sequencing data of 250 probands with suspected monogenic diseases other than IBD.

Results: Of the 23 children diagnosed with VEO-IBD, 12 (52%) were carriers of at least one *MEFV* disease-causing variant. The most frequent variants identified were p.M694V and p.E148Q (42% each). In the control group, 41/250 patients (16.4%) were found to be carriers ($P < 0.00003$). On comparison by ethnicity, the allelic frequency of *MEFV* variants was found to be higher across the VEO-IBD group in 13 of 14 ethnicities compared to the control group.

Conclusion: This study showed that the rate of *MEFV* variants in patients with VEO-IBD was threefold higher than in individuals without IBD. The clinical importance of this finding is yet to be defined. We suggest that disease-causing variants in the *MEFV* gene should sought in cases of VEO-IBD.

Conflict of Interest: None declared

P04.007.C The clinical use of exome sequencing to diagnose PCD patients

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Background: Primary ciliary dyskinesia (PCD) is a heterogeneous disorder of dysfunctional motile cilia. PCD is characterized by severe recurrent otosinopulmonary infections, male infertility due to abnormal spermatozoa motility, situs inversus totalis, complex situs abnormalities such as situs ambiguous (heterotaxy) and associated congenital heart disease. Identification of ultra-structural defects in the cilia based on electron microscopy has been the traditional test to confirm the diagnosis, but this approach is no longer the sole "gold standard". The clinical use of exome sequencing (ES) has increased the diagnostic yield in patients with heterotaxy PCD.

Methods: ES data of > 146 proband (and when available their parents) were analyzed for a panel of 148 heterotaxy PCD related genes from 2019 until now at the Center of Medical Genetics in Ghent. Variant classification was based on ACMG/AMP and ACGS guidelines.

Results: We identified multiple class 4 or 5 variants explaining the clinical presentation in 21% of the cases. The majority (97%) of the cases have an autosomal recessive inheritance. In our cohort, 70% of the class 4 or 5 variants were loss-of-function variants and DNAH11 was the most affected gene. Furthermore, we reported variants of unknown significance (class 3) in an additional 6% of the probands. Hence more genetic testing is required to solve these cases (panel updates, phenotypic spectrum, and familial segregation).

Conclusion: With a diagnostic yield between 21-27%, we confirm that ES can be used as a second "golden standard" for the diagnosis of heterotaxy PCD.

Conflict of Interest: None declared

P04.008.D Whole-exome sequencing and variant spectrum in children with suspected inherited renal tubular disorder: the east India tubulopathy gene study

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Background: Inherited tubulopathies are a heterogeneous group of genetic disorders. Data in Indian population is sparse.

Methods: Children (< 18 years) with suspected tubular disorders were recruited for molecular testing through Whole-exome sequencing (WES). Multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing were done when required.

Results: There were 77 index cases (male =73%). Median age at diagnosis was 48 months (IQR 18.5 to 108 months). At recruitment, the number of children in each clinical group was as follows: distal renal tubular acidosis (dRTA) = 25; Bartter syndrome = 18; isolated hypophosphatemic rickets (HP) = 6; proximal tubular dysfunction (pTD) = 12; nephrogenic diabetes insipidus (NDI) = 6; kidney stone/nephrocalcinosis (NC) = 6; others = 4. We detected 55 (24 novel) P/LP variants, providing genetic diagnoses in 54 children (70%). The diagnostic yield of WES was highest for NDI (100%), followed by HP (83%; all X-linked HP), Bartter syndrome (78%), pTD (75%), dRTA (64%), and NC (33%). Molecular testing had a definite impact on clinical management in 24 (31%) children. This included revising clinical diagnosis among 14 children (26% of those with a confirmed genetic diagnosis and 18% of the overall cohort), detection of previously unrecognized co-morbidities among 8 children (sensorineural deafness $n = 5$, hemolytic anemia $n = 2$, and dental changes $n = 1$) and facilitating specific medical treatment for 7 children (primary hyperoxaluria $n = 1$, cystinosis $n = 4$, tyrosinemia $n = 2$).

Conclusion: Our study generated useful data regarding inherited tubulopathies in the Indian children.

Conflict of Interest: None declared

P04.009.A Integrated multi-omics approaches uncover the landscape of gastric cancer in patients with diabetes mellitus

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Background: The co-occurrence of Diabetes Mellitus (DM) and cancer is known for years, but the complex interplay between DM, anti-diabetic therapy and the increased cancer risk is still unclear. Herein, we used integrated multi-omics analysis of genomic, transcriptomic and proteomic data to uncover mechanisms that characterize gastric cancer (GC) in DM patients.

Methods: Forty-two GC samples from patients with DM (n = 21) and without DM (n = 21), presenting similar clinico-pathological features were retrospectively selected. Genomic, transcriptomic and proteomic analysis were performed using NGS or and Liquid Chromatography Analysis - Mass Spectrometry. Differential copy number variants (DCNV), differential expressed genes (DEG) and differentially expressed proteins (DEP) were extracted with specific bioinformatic pipelines and used for integrated for *multi-omics* Gene Ontology analysis.

Results: Genomic, transcriptomic and proteomic analyses revealed 1962 DCNV, 745 DEG and 370 DEP. The overall somatic molecular GC profile in patients with DM was specific, when confounding clinical and pathological variables (gender, histological type, microsatellite instability) were removed. *Multi-omics* analysis identified 2592/3077 differential altered molecules between GCs from patients with and without DM, related to similar biological terms, and pinpointing 147 enriched biological terms. Fundamental cellular metabolic processes, such as transmembrane transporter activity of ions and energy production, targeted mainly by CNVs, characterized DM GC, namely high intracellular Ca^{2+}, which were reminiscent of diabetic pancreas behavior.

Conclusion: GC arising in DM patients under anti-diabetic therapy is a different clinical entity characterized by specific

genetic defects, and transcriptional and proteomic programs that recapitulate the behavior of pancreas.

Conflict of Interest: None declared

P04.010.B Genetic evaluation of five patients with ROHHAD-NET

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Rapid-onset obesity, hypothalamic dysfunction, hypoventilation, autonomic dysregulation and neuro-endocrine tumors (ROHHAD-NET) is a very rare condition of unknown etiology. Multiple potential etiologies have been proposed, including a genetic or auto-immune cause. Thus far, by whole exome sequencing, a genetic cause was not detected. We identified five patients suspected for ROHHAD(-NET), of whom two developed a tumor, a ganglioneuroma and a ganglion cell tumor. We performed germline whole genome sequencing (WGS) for five patients, including four patient-parent trios, and optical genome mapping (OGM) for two patients to explore the (non-) coding regions and genomic structural variants (SVs).

WGS data were aligned to build GRCh38 and variant calling was performed using GATK and annotated with Ensemble Variant Effect Predictor v105. Rare (GnomAD population frequency <0.1%) and possibly pathogenic (CADD ≥15 and/or SpliceAI ≥0.5) single nucleotide variants and small insertions/deletions were selected. SV detection was performed for the WGS data using Manta and GRIDSS and for the OGM data using the rare variant pipeline included in the Bionano Solve package. SVs affecting coding or regulatory regions and absent from population databases were selected. We explored a de novo and autosomal recessive scenario but did not identify candidate variants in a recurrently affected gene locus in two or more patients.

In conclusion, by comprehensive genome wide data analysis we did not identify a probable genetic cause for ROHHAD-NET. These data do not support a genetic etiology for ROHHAD-NET, which strengthens the auto-immune hypothesis.

Funding: Childhood Cancer-free Foundation

Conflict of Interest: None declared

P04.011.C Biallelic variants in the calpain regulatory subunit CAPNS1 cause pulmonary arterial hypertension

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Background: Pulmonary arterial hypertension (PAH) is a multifactorial, progressive and often fatal disease. Although monogenic causes of PAH have been identified, in the majority of patients the disease etiology remains unclear. Here we describe two (unrelated) consanguine families presenting with PAH and their genetic and molecular work-up.

Methods: Exome sequencing was performed, as well as analysis of whole blood RNA, calpain protein expression, and RNA sequencing on lung tissue of one affected patient. The frequency of pathogenic variants in *CAPNS1* was also investigated in genome data of a cohort of over 1000 unresolved PAH patients.

Results: We identified two unique, biallelic variants, in canonical splice sites of *CAPNS1*, which encodes the regulatory subunit of the calpain holoenzyme. The calpain holoenzyme is involved in pulmonary vascular development and increased levels of calpain have been reported in pulmonary arterial smooth muscle cells. The identified variants co-segregated with PAH in both families. The two variants lead to loss-of-function (LoF) due to demonstrated aberrant splicing and resulted in the complete absence of the CAPNS1 protein in affected patients. In the cohort of 1000 unresolved PAH patients no other biallelic *CAPNS1* LoF variants were identified.

Conclusion: We are the first to demonstrate that biallelic, LoF variants in *CAPNS1* can cause PAH. The screening of *CAPNS1* in patients affected with PAH, especially with suspected autosomal recessive inheritance, should be considered. The study highlights the involvement of the calpain pathway in human PAH, pointing towards calpain inhibitors as potential novel therapeutic targets.

Conflict of Interest: Alex Postma: None declared, Christina Rapp: None declared, k Knoflach: None declared, Alexander Volk: None declared, Johannes Lemke: None declared, m Ackermann: None declared, Nicolas Regamey: None declared, Philipp Latzin: None declared, l celant: None declared, s jansen: None declared, hj bogaard Dutch Cardiovascular Alliance (Grant CVON2017-4: DOLPHIN-GENESIS), a ilgun: None declared, Marielle Alders: None declared, k van Spaendonck-Zwarts: None declared, Danny Jonigk: None declared, Christoph Klein: None declared, Stefan Gräf: None declared, Christian Kubisch: None declared, arjan houweling Dutch Cardiovascular Alliance (Grant CVON2017-4: DOLPHIN-GENESIS), Matthias Griese chILD-EU (FP7, No. 305653)

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P04.012.D A phenome-wide association study of idiopathic pulmonary fibrosis reveals complex genetic relationships with age-related diseases

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Background: Idiopathic Pulmonary Fibrosis (IPF) is a disease of progressive lung scarring. Disease mechanisms underlying IPF and associated comorbidities, or other fibrotic traits, are poorly understood.

Aims: Investigate overlapping genetic signatures across IPF and related diseases through genomic methods using large-scale public data.

Methods: A Phenome-Wide Association Study (PheWAS) was performed to identify traits associated with 20 IPF genetic risk variants using public databases *Phenoscanner* and *OpenGWAS*. Trait associations were defined using a significance threshold

($p < 1 \times 10^{-5}$). Overlapping signals were further defined through colocalisation analyses (*coloc*).

Diseases, or disease biomarkers, associated with IPF risk variants in PheWAS analyses were investigated for broad genetic relationships through genetic correlation analyses. Genetic correlation scores (r_g) were estimated with a recent IPF GWAS (N = 24,589) using LD Score Regression (*LDSC*) (significance threshold: $p < 0.05$).

Results: Traits meeting significance criteria in the PheWAS investigation included cancers (ovarian, prostate, lung, breast), chronic obstructive pulmonary disease (COPD), telomere length, and biomarkers of liver disease and diabetes. These traits were prioritised for further analyses.

Signals associated with increased IPF risk (beta:0.149, $p = 4.9 \times 10^{-32}$), reduced telomere length (beta:-0.067, $p = 5.6 \times 10^{-220}$), and decreased ovarian cancer risk (beta:-0.095, $p = 1.76 \times 10^{-11}$) colocalised at a telomerase (*TERT*) variant, rs7725218.

Positive broad genetic correlations with IPF were identified with primary biliary cholangitis (PBC) (r_g:0.1380, $p = 0.04$) and body mass index (r_g:0.1259, $p < 0.01$). Negative correlations were shown with prostate cancer (r_g:-0.1055, $p = 0.02$), COPD (r_g:-0.2074, $p < 0.01$), and telomere length (r_g:-0.3065, $p < 0.01$).

Conclusions: These results indicate an overlap of genetic signatures across IPF and multiple diseases, suggesting complex interplay of disease mechanisms.

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P04.013.A Stratification of Non-Alcoholic Fatty Liver Disease (NAFLD) patients using Exome Sequencing analysis and metabolomics profiles

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Background/Objectives: Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease with a global prevalence of approximately 25%. It includes a wide range of liver damage including simple steatosis (non-alcoholic fatty liver; NAFL) and non-alcoholic steatohepatitis (NASH). The aim of this study was to identify the genetic and metabolic factors underlying the progression from NAFL to NASH.

Methods: We performed Whole Exome Sequencing (WES) and untargeted metabolomics analyses on 154 patients with biopsy-proven NAFLD enrolled at the Liver Unit of the Department of Medical Sciences, University of Turin. NASH diagnosis (liver steatosis >5%; lobular inflammation and hepatocyte ballooning) was made on 108 participants, the rest of individuals had a simple steatosis.

Results: Variants (SNVs) obtained by WES analysis were prioritized based on frequency (MAX AF<1%) and ACMG annotation (pathogenic, likely pathogenic and VUS). We calculated a score for each gene weighting the variants according to severity. Logistic regression models were fitted to distinguish between NASH and NAFL, splitting the dataset in training (70%) and test set (30%) using a 10-times repeated k-fold validation (k = 10). The model including genetic scores showed an improvement in the stratification performance (AUC = 0.61) over one based on age, gender and BMI (AUC = 0.57). Metabolomics analyses highlighted 15 metabolites, that added to the covariates and genetic score showed an AUC of 0.71.

Conclusion: This integrated approach could be useful to clarify how the genetics and metabolomics could improve NAFLD patients' stratification.

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Conflict of Interest: None declared

P04.014.B Bi-allelic REN mutations and undetectable plasma renin activity in a patient with progressive chronic kidney disease

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Renin-expressing cells and functioning renin are thought to be essential for embryonic kidney development, as demonstrated by cases of bi-allelic loss-of-function REN mutations leading to renal tubular dysgenesis.

We identified a patient with a combination of a truncating mutation p.W10X in the signal peptide of preprorenin and a missense mutation p.P113L in the mature peptide of renin. The p.W10X mutation abolishes proteosynthesis of preprorenin. The p.P113L mutation replaces an evolutionary conserved leucine residue and affects the site regulating enzymatic activity of prorenin and renin. This genetic constellation results in loss of clinically detectable plasma renin activity. The patient had a normal birth and postnatal period. During her childhood and teenage years, she suffered from hypotension and frequent presyncope, as well as other characteristic findings of hyporeninism, including hyperkalemia, acidemia, and hyperuricemia. The patient also suffers from slowly progressive chronic kidney disease.

Bi-allelic mutations affecting REN function do not always result in classic renal tubular dysgenesis. These findings raise questions as to the role of prorenin and renin in renin-expressing cells functionality and embryonic kidney development.

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P04.015.C Novel variants in the PKD1 and PKD2 genes in German patients with autosomal dominant polycystic kidney disease

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is a potentially fatal genetic kidney disease, accounting for a significant proportion of end-stage renal disease. Most ADPKD patients carry a causative sequence variant in the PKD1 or PKD2 gene. The molecular diagnosis of ADPKD is hampered by high allelic heterogeneity and the presence of six highly homologous PKD1 pseudogenes.

Methods: Over a 36-month period, DNA from 119 individuals with suspected ADPKD was analyzed as part of routine diagnostic testing. Detection of variants was performed by targeted next-generation sequencing of ADPKD genes and, in case of PKD1, also by long-range PCR followed by Sanger sequencing. Copy number variations were detected using NGS data and multiplex ligation-dependent probe amplification (MLPA).

Results: A total of 73 variants were identified in 119 patients, corresponding to a detection rate of 61.3%. 65 variants were identified in PKD1 (89.0%) and 8 in PKD2 (11.0%). These include 39 novel variants detected in PKD1 and one novel variant in PKD2. 40 variants (1 missense, 12 nonsense, 19 frameshift, 5 splice site, and 3 large deletions) were classified as pathogenic according to ACMG guidelines. An additional 12 variants were rated as likely pathogenic (9 missense, 2 splice site, and 1 in-frame deletion). The remaining 21 variants (20 missense and 1 in-frame deletion) were classified as variants of uncertain significance.

Conclusions: Our results highlight the high allelic heterogeneity of PKD1 and PKD2 variants. The discovery of 40 previously undescribed variants has significantly expanded the spectrum of known ADPKD causative variants.

Conflict of Interest: Ralf Zarbock MVZ Martinsried, Karin Mayer MVZ Martinsried, Manuela Scholz MVZ Martinsried, Julia Philippou-Massier MVZ Martinsried, Imma Rost MVZ Martinsried, Konstanze Hörtnagel MVZ Martinsried

P04.016.D Pathophysiological basis of the dominant transmission in NPHS2-associated podocytopathy

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Background: Mutations of *NPHS2* are the most frequent causes of autosomal recessive podocytopathies. We formerly showed that the oligomers of the encoded podocin reduces the distance between the neighbouring nephrin molecules, i.e. the size of the glomerular pore. Two families with autosomal dominant FSGS were found to carry a de novo heterozygous *NPHS2* last-exon mutation affecting the second oligomerization site (p.L330Pfs*17[c.989_992delTGTC] and p.L330Vfs*15[c.988_989delCT]). We aimed to determine the effect of wild-type (wt) podocin on the nephrin-nephrin distance in the presence of these podocin mutants.

Methods: Two nephrin constructs tagged with FRET pairs were transiently coexpressed with podocin variant(s) in HEK293 cells. FRET was measured between the two nephrin constructs in living cells. The fluorescence decay was measured by time-correlated single photon counting.

Results: The dominant and the wt podocin variants strongly colocalized. In accordance with our former results, the wt podocin increased the FRET efficiency, i.e. reduced the distance between the nephrin constructs. This effect was abolished when any of the two dominant *NPHS2* variants was coexpressed with wt podocin (V5-wt+HA-wt vs V5-wt+HA-L330Pfs*17: $p = 4.1 \times 10^{-4}$, V5-wt +HA-wt vs V5-wt+HA-L330Vfs*15: $p = 4.0 \times 10^{-4}$).

Conclusions: Wild-type podocin is unable to reduce the distance between nephrin molecules in the presence of any of the two de novo podocin variants, suggesting an altered heterooligomerization. It explains the dominant negative effect of these podocin mutants and thus the observed exceptional dominant transmission of *NPHS2*-associated glomerulopathy.

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Conflict of Interest: None declared

P04.017.A Socioeconomic and environmental risk assessment of chronic obstructive pulmonary disease across smoking behaviors and populations

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Background: Smoking is the leading risk factor for chronic obstructive pulmonary disease (COPD), yet many people who never smoke develop COPD. We hypothesize that considering socioeconomic and environmental factors beyond smoking can better predict and stratify risk of COPD.

Methods: We constructed the COPD Socioeconomic and Environmental Risk Score (SERS) adjusted by smoking behaviors in a longitudinal cohort analysis from the UK Biobank. We tested the ability of SERS to predict incident COPD in current, previous, and never smokers of European and non-European ancestries compared to a composite polygenic risk score (PGS). We tested

associations using Cox proportional-hazard models and assessed predictive performance using Harrell's C-index.

Findings: SERS (C-index = 0.770, 95%CI 0.756-0.784) was significantly more predictive of COPD than smoking status (C-index = 0.738, 95%CI 0.724-0.752), pack-years (C-index = 0.742, 95%CI 0.727-0.756), or PGS (C-index = 0.663, 95%CI 0.649-0.678). Compared to the remaining population, individuals in the highest deciles of SERS and PGS had hazard ratios (HR) = 7.24 (95%CI 6.51-8.05, P < 0.0001) and 1.69 (95%CI 1.51-1.89, P < 0.0001) for COPD. Never smokers in the highest decile of SERS were more likely to develop COPD than previous and current smokers in the lowest decile with HR = 4.95 (95%CI 1.56-15.69, $P = 6.65 \times 10^{-3}$) and 2.92 (95%CI 1.51-5.61, $P = 1.38 \times 10^{-3}$), respectively. In general, prediction accuracy of SERS was lower in non-European compared to European populations.

Interpretation: Socioeconomic and environmental factors beyond smoking can predict and stratify COPD risk. They can help identify non-smokers with significantly higher risk of COPD than certain smokers. Risk screening for COPD should consider non-traditional socioeconomic and environmental factors.

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P04.018.B Medullary sponge kidney: an unsolved enigma and its association with distal renal tubular acidosis

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Background: Medullary sponge kidney (MSK) is a description of radiographic features. MSK has an association with distal renal tubular acidosis (dRTA). However, the pathogenesis remains unclear.

Methods and results: We report three patients with acidosis and MSK, in whom primary dRTA is confirmed by genetic analyses of *SLC4A1* and *ATP6V1B1* genes. Furthermore, an extensive genetics first approach using the 100,000 Genomes Project dataset showed that many patients with medullary sponge kidney related phenotypes are genetically tested with a gene panel not containing the dRTA genes.

Conclusion: Our cases highlight that the radiological description of MSK is not a straightforward disease or entity. Therefore, when MSK is mentioned in a case file, one should consider broader medullary phenotypes amongst which a diagnosis of dRTA as primary genetic cause for secondary medullary imaging abnormalities. In order to facilitate the diagnostic process, dRTA genes should be added to genepanels for renal cystic disease

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Keywords: Medullary sponge kidney, distal renal tubular acidosis, medullary cystic kidney disease, nephrocalcinosis, genetic testing, nephrogenetics.

Conflict of Interest: None declared

P04.019.C Value of exome sequencing "first" for autosomal dominant polycystic kidney disease

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Background and Aims: Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common Mendelian kidney diseases, that can progress to end-stage kidney failure. Pathological variants in PKD1 or PKD2 genes are found in about 78% and 15% respectively. The sequencing of PKD1 by short read sequencing technics with exome capture such as Exome sequencing (ES) has been describe as technically challenging given 6 pseudogenes with more than 98% homology in PKD1 exonic regions 1 to 33.

Method: ES was performed in 684 unrelated adult patients with kidney disease from the department of Nephrology of Sorbonne University, Paris, France. Genomic DNA was extracted and exonic coding regions (37 megabases) were enriched with the Twist Human Core Exome kit, and paired-end sequenced on NextSeq500 (Illumina) machine.

Results: Of the 684 patients, 143 had renal cysts. Pathogenic or probably pathogenic variations in PKD1, PKD2 have been identified in 26 patients. In this cohort, 18.2% of the 17 pathogenic variants are reported in PKD1, and 14 (82%) are included in exons 1 to 33. In 5 of the 26 patients diagnosed (19%), meaningful additional genetic were found, falling either in CFTR, DHCR7, HFE, F8, or ACTA2 gene.

Conclusion: ES highlights PKD1 and PKD2 pathogenic variants detection without false positive. This strategy allowed us to provide appropriate genetic counseling (CFTR, DHCR7), as management of yet unexpected additional genetic diseases that can affect ADPKD phenotype. ES appears effective for PKD1/PKD2 variant detection, providing additional information for ADPKD management in 20% of cases.

Conflict of Interest: None declared

P04.020.D Mitochondrial nephropathy: MT-TL1 m.3243A>G mitochondrial DNA variant detected from whole exome sequencing can explain cases of adult unknown nephropathies

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Background and Aims: We investigated the incidence of the mtDNA pathogenic variant *MT-TL1* m.3243A>G in the CKD Sorbonne University, Paris, France N = 1197, whole exomes). The prevalence of m.3243A>G has been estimated ranging from 0.08% to up to 0.25 % in different population.

Method: WES has been used as a first-tier exploration of adult's nephropathies of unknown origin and we etrieved m.3243A>G variation in blood DNA and then determined the mtDNA heteroplasmic level respective from urine sample or kidney tissue when available with an orthogonal method.

Results: *MT-TL1* m.3243A>G pathogenic variant was detected in 1,7% of the patients (20 patients over 1197 patients). An orthogonal method confirmed the presence of m.3243A>G variant

in 10 patients supporting the possibility to diagnose from blood DNA analysed by WES. In all cases, the presence of m.3243A>G had major clinical implications for the patients and their families: living related donor aborted for two patients, a molecular diagnosis in three patients with unknown nephropathies and in three patients with histological diagnosis of focal segmental glomerulosclerosis.

Conclusion: This population-based study reinforces the place of WES as first-tier exploration for adult patients with chronic kidney disease in whom phenotypes are often poor and/or atypical. The mitochondrial genome analysis with the same assay allowed the detection of yet unsuspected *MT-TL1* m.3243A>G variant in 1,7% of patients suggesting enrichment compared to current prevalence observed in other population. Our data suggest that the entity "mitochondrial nephropathy" can represent a significant part of adult unknown nephropathies.

Conflict of Interest: None declared

P04.021.A Differential gene expression in asymptomatic and mild SARS-CoV-2 infected individuals

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Background/Objectives: COVID-19 is a disease caused by the SARS-CoV-2 virus which has become a global health issue. The infection can develop from asymptomatic, or mild symptoms, to severe forms that can end up in death. This wide phenotypic variability could be due to genetic factors, playing a main role in the infective process. Our objective is to analyze the expression of genes related to virus pathogenesis and host immune response in mild/or asymptomatic COVID-19 cases.

Methods: The expression of 9 genes (*ACE2, TFRC, IRF9, CCL5, IFI6, IFNG, TGFB1, IL1B* and *OAS1*) was determined in upper airways samples from 97 SARS-CoV-2 infected individuals and 30 healthy controls by RT-qPCR.

Results: A higher expression of *TFRC, IRF9, CCL5, IFI6, TGFB1, IL1B, IFNG* and *OAS1* genes ($p < 0.005$) was observed in individuals infected with SARS-CoV-2 when compared to controls. Consequently, Principal Component Analysis (PCA) showed that all the genes were grouped in the first component, excepting *IL1B* and *ACE2* which were grouped in the second component. Moreover, *ACE2* was significantly correlated to *IL1B* ($p = 0.044$) and *IFNG* ($p = 0.001$). Higher levels of expression of *IFNG* associated with high viral loads ($p = 0.031$) in infected individuals.

Conclusion: Higher expressions levels of genes related to antiviral activity and inflammatory pathways are found in mild/or asymptomatic COVID-19 cases. Overexpression of *IRF9, IFI6* and *OAS1* genes indicates that they may exert a protective effect in the studied individuals. However, *ACE2* and *IL1B* expression does not appear to be altered in mild/or asymptomatic SARS-CoV-2 infections.

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Conflict of Interest: None declared

P04.022.B The penetrance of monogenic diabetes variants in a diverse hospital-based biobank

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Maturity Onset Diabetes of the young (MODY) is a monogenic form of diabetes that is characterized by an autosomal dominant inheritance. Patients have early-onset diabetes, a strong family history of diabetes, and often demonstrate extra-pancreatic symptoms. Variants in *GCK, HNF1A* and *HNF4A* account for more than 80% of all MODY diagnoses. Research in European ancestry (EA) cohorts has demonstrated that in population settings, *HNF1A* and *HNF4A* variants are less penetrant as compared to their penetrance in proband ascertained cohorts. Here, we examine the penetrance of MODY variants in a diverse hospital-based biobank, Bio*Me*, using exome sequencing data of 7,775 individuals of African American ancestry (AA), 10,144 individuals of EA and 11,081 individuals of Hispanic-Latino Ancestry (HA) .

We identified 11 AA, 15 EA, and 12 HA carriers of MODY variants that are defined as "pathogenic" or "likely pathogenic" by the American College of Medical Genetics and Genomics (ACMG) guidelines. Twenty-five individuals carried a variant in *HNF1A*, 9 in *GCK*, and 4 in *HNF4A*. Carriers of these variations tended to more often have a T2D diagnosis compared to non-carriers; i.e. 55% in AA carriers compared to 29% in non-carriers, 27% in EA carriers compared to 10% in non-carriers, and 42% in HA carriers compared to 31% in non-carriers. Only two carriers had been diagnosed with T2D before the age of 40.

For the first time, our study expands on the penetrance work by incorporating unselected, non-European ancestry populations with the goal of informing disease risk in these populations.

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Conflict of Interest: None declared

P04.023.C Molecular spectrum of genetic anomalies in pediatric patients with early onset of renal cystic disease

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Background/Objectives: renal cystic disease (RCD) includes autosomal dominant and recessive polycystic kidney disease (ADPKD and ARPKD), ciliopathies, *HNF1B*-nephropathy, and kidney/urinary tract congenital anomalies (CAKUT). We describe a cohort of pediatric RCD patients and provide genotype-phenotype correlations.

Methods: Patients with early findings of hyperechogenicity/ renal cysts were retrospectively recruited from the Pediatric Nephrology Unit (Turin University Hospital, Italy). Clinical exome sequencing-CES was performed at the Immunogenetics and Transplant Biology Unit.

Results: Among the 65 recruited patients (mean age 8±5.9 years), 39 (60%) had causative variants, 18 (27,7%) carried variants of uncertain significance (VUS) and 8 tested negative. Twenty-three patients (n = 23/40, 57,5%) with ADPKD clinical suspicion obtained a molecular diagnosis (20 *PKD1*, 2 *PKD2* and one *HNF1B* variants), while 14 carried VUS in coherent genes. Seven patients with ARPKD suspicion had biallelic *PKHD1* variants and one had two *PKD1* variants. Four (44.4%) of 9 CAKUT cases were solved with variants in *HNF1B* (n = 3) and *PAX2* (n = 1). Two (n = 2/3, 66.7%) patients with ciliopathy carried biallelic *BBS10* variants. Three TSC cases were confirmed, and two medullary cystic disease cases were negative. Interestingly, among 9 patients with multiple variants, 6 had two variants in *PKD1*, 2 carried coexisting variants in *PKD1*/*PKHD1*, and one with a biallelic variant in *BBS10* had an additional *PKD2*.

Conclusion: Whit a time- and cost-effective diagnostic approach CES identified a definitive molecular cause in 60% and a likely one in 28% of the patients, revealed two clinical phenocopies and found 9 patients with multiple variants, potentially associated with a more severe phenotype.

Conflict of Interest: None declared

P04.024.D The genetic landscape of HHT in Israel - Data summary from the national HHT center

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Objective: Due to wide clinical variability and age-related penetrance, many patients with HHT remain undiagnosed. Little is known about the epidemiology of HHT in Israel, a country with a unique and diverse demographics. We aim to describe the genetic landscape among families with HHT in Israel.

Methods: A retrospective study. All patients who attended the genetic clinic at the Israeli national HHT center between 2017-2021 were included. Data were retrieved from patients' medical charts.

Results: 146 patients, from 75 seemingly unrelated families attended the HHT genetics clinic.130 patients (89%) were genetically tested for HHT; 65 probands have undergone diagnostic tests and 65 had cascade testing. Disease-causing variants (DVs) were detected in 52 (80%) probands; DVs were found in ACVRL1 (65.2%), ENG (30.4%), and SMAD4 (4.4%) genes. Three DVs accounted for HHT in 54% of families: The ACVRL1 c.1120C>T variant was identified in 40% of families, all of them ashkenazi jews. The ACVRL1 c.1189G>A variant was found in 4% of families. The ENG c.511C>T variant was present in 10% of families. Altogether we detected 23 different DVs; Of them, 10 have never been reported. Only 36.5% of the genetically diagnosed probands fulfilled three Curaçao clinical diagnostic criteria; 60% fulfilled 2 clinical criteria only.

Conclusion: We report on recurrent HHT DVs in the local population. In addition, we highlight the limitations of the Curaçao criteria. Our study adds to the knowledge of the genetic landscape and should be extended to further delineate the epidemiology and the optimal diagnostic approach.

Conflict of Interest: None declared

P04.025.A Development of a new cellular model to evaluate the clinical impact of PKD1 variants exploiting base editors

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Background/Objectives: Polycystic Kidney Disease (PKD) is the most common single-gene disorder leading to kidney failure. Almost 80% of cases are attributed to germline variants in *PKD1*, a significant portion of which is classified as "variant of unknown significance" (VUS). Therefore, a proper experimental model to study the functional impact of different genetic lesions is needed to confirm their pathogenicity.

Methods: The HEK293T cell line was transfected with a sgRNA targeting exon 15 of *PKD1* to generate homozygous or heterozygous mutants, which were functionally characterized to identify suitable readouts. Heterozygous clones were then used to introduce different *PKD1* additional variants, using base editors (BE).

Results: In starving conditions, *PKD1*-/- exon 15 cells were characterized by increased resistance to cell death and a disrupted autophagic pathway. Furthermore, RNA sequencing data indicated that double knock-out cell lines display an upregulation of genes involved in proliferative pathways and epithelial-mesenchymal transition compared to +/+ and +/- clones.

We then transfected *PKD1*+/− exon 15 cells with a sgRNA and BE to introduce a specific variant in exon 42, generating a "double hit" cell line which represents a more likely real-life situation. Functional assessment of the variant's pathogenicity is currently ongoing.

Conclusion: We generated a new PKD cellular model that can be easily exploited to reproduce some of the VUS variants identified in our cohort of patients. Results obtained allowed to identify selective read-outs to be used for a rapid screening to assess the phenotypic impact of specific *PKD1* variants.

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P04.026.B Molecular signature of multiple hepatocellular adenomas from a patient harboring a germline variant in HNF1A: the role of somatic mutations

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Background: Hepatocellular adenomas (HAs) are benign tumors that can develop in patients harboring germline *HNF1A* variants. In

this study, 6 HAs and non-neoplastic hepatic parenchyma were sampled from the liver of a 17-year-old male affected by diabetes and hepatic adenomatosis needing transplantation. The patient carried a pathogenic germline *HNF1A* variant.

Materials/methods: DNA and RNA were extracted from each sample. Clinical exome sequencing was performed, data aligned for germline and somatic variant calling and analysis restricted to a panel of tumor-associated genes. Variants were validated with Sanger sequencing, ddPCR and MLPA. Transcriptomic profiles of each sample were obtained by RNAseq. Expression data were validated by RT-PCR, WB, and histological staining.

Results: HAs appeared genetically heterogenous with a second somatic variant in *HNF1A* in 4 lesions, in *ARID1A* in 1 lesion and no additional variants in the remaining HA and non-neoplastic parenchyma. Variants were confirmed by complementary sequencing approaches.

In HAs carrying a second *HNF1A* variant, RNAseq data showed an upmodulation of fatty acid synthesis and mTOR pathway while angiogenesis was downmodulated. Contrariwise, in the HA harboring the *ARID1A* variant, angiogenesis was upmodulated while fatty acid metabolism was downmodulated. Notably, *NOTCH4* was specifically upmodulated in this tumor. Independently of the second mutation, immune response was downmodulated.

Conclusions: this work provides a molecular characterization of multiple HAs from a patient with germline *HNF1A* variant. Their transcriptional profiles depend on genetic background as different somatic variants influence different biological pathways. Of note, an *ARID1A* somatic variant promotes angiogenesis and a more aggressive phenotype.

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Conflict of Interest: None declared

P04.027.C Interstitial lung disease and subcutaneous edema as predominant phenotypes in patients with biallelic variants of THSD1

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Background: THSD1 (thrombospondin type-1 domain-containing protein 1) is a transmembrane protein that belongs to the family of Thrombospondin proteins. Heterozygous pathogenic variants in *THSD1* are known to cause intracranial berry aneurysm (OMIM 618734, autosomal dominant inheritance). Moreover, biallelic variants in *THSD1* were recently listed in OMIM to cause Lymphatic malformation 13 (OMIM 620244, autosomal recessive inheritance). Three reports are published about premature infants with nonimmune hydrops fetalis, cardiac defects and hemangiomas. However, childhood interstitial lung disease (chILD) and lymphedema did not dominate the phenotype of previously published patients.

Methods: Detailed clinical characterization of six children with genetically confirmed homozygous frameshift variants in *THSD1* identified by whole-exome sequencing (WES) or whole-genome sequencing (WGS).

Results: A homozygous variant *THSD1*(NM_018676.4):c.1627_1630del p.(Lys543Valfs*2) was identified in six prematurely born children now aged 1y, 2y, 11y, 12y, 18y, and 20y respectively. All patients had early-stage nonimmune hydrops fetalis, hemangioma, pleural effusion, and ascites, which appear to regress over the further course of time. In children and adolescents, affected individuals show pronounced subcutaneous edema of the face, extremities or genitals. Two of the six patients were diagnosed with predominantly chronic chILD, one of them with CT-morphological signs of early lung fibrosis.

Conclusion: We expand the number of individuals described with biallelic variants in *THSD1*, their clinical spectrum, and highlight clinical variability in affected patients, with symptoms such as chILD and pronounced subcutaneous edema.

Conflict of Interest: None declared

P04.028.D MYH9: a promising partner of filtrin, a protein member of the kidney slit diaphragm

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Background/Objectives: Filtrin, is a member of the slit diaphragm (SD) complex. Findings of our group suggest that NEPH3 acts as a modifier gene for thin basement membrane nephropathy (TBMN). In TBMN patients categorized as severe or mild, we detected the NEPH3-p.V353M. Co-immunoprecipitations showed an increase in the interaction of NEPH3-p.V353M with NPHS1-wt and with NEPH3-wt or NEPH3-p.V353M itself.

Methods: This study aims to find filtrin partners. We performed immunoprecipitation experiments, immunofluorescence experiments in human differentiated podocytes and on mouse kidneys and immune electron microscopy on human kidneys.

Results: Filtrin interacts with Myh9. This interaction was confirmed with immunoprecipitations and MS. Immunofluorescence experiments in human differentiated podocytes and mouse kidneys demonstrated co-distribution of Neph3 and Myh9 to the foot processes. Immune-gold staining on human kidneys demonstrated that both Neph3 and Myh9 are localized in the immediate proximity of slit diaphragms.

Conclusions: This study provides evidence of direct or indirect interaction of Neph3 with Myh9; a protein implicated in the development of FSGS and other rare diseases.

Conflict of Interest: Charalambos Stefanou Full time, Collaborator, Myrtani Pieri Full time, Konstantinos Voskarides Full time, Gregory Papagregoriou Full time, Niovi Santama Full time, Constantinos Deltas Full time, PI: The CY-Biobank project receives funding from the European Union's Horizon 2020 research and innovation program, under grant agreement No. 857122/The CY-Biobank project receives funding from the Government of the Republic of Cyprus through the Deputy Ministry of Research,

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P04.029.A Monogenic disorders leading to solid organ transplantation:the experience with 447 pediatric patients from a single Italian center

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Background: While only a small percentage of children with genetic disorders undergo organ transplantation, up to 80% of transplanted children has a genetic condition. Every year in Italy ~148 children undergo transplantation and more remain in the waiting list. We describe a cohort of pediatric patients affected by internal organ disorders studied with NGS with the aim to collect data for improving diagnostic yield.

Methods: We recruited patients with internal organ pathology that underwent Clinical Exome Sequencing (CES) from 2018 to 2022. The analysis was based on in-silico gene panels requested by pediatric nephrologists, cardiologists, or gastroenterologists.

Results: 447 patients were recruited: 206 females, 241 males, average age 8 years. 247 (55%) had kidney, 107 (24%) liver, 69 (28%) heart, and 24 (25%) pancreas involvement. Overall, diagnostic rate (DR) was 28%, varying according to the organ (32% for kidney, 25% for liver/pancreas, 28% for heart). In 16% of cases variants of unknown significance (VUS) were identified. Higher diagnostic yield was observed for diseases with a well-known genetic base and isolated organ involvement, such as polycystic kidney disease (DR = 27/32;91%), while a lower rate was observed for complex phenotypes (DR = 2/21;10%).

Discussion: The diagnostic yield of NGS for transplant-related disorders in our cohort was 28%, consistent with DR for adult cardiac (25%), hepatic (25%), or renal disorders (40%). Since DR for pediatric syndromic disease reaches up to 55%, we speculate that our DR might be increased by familiar segregation of VUS and considering extra-organ symptoms through deep-phenotyping-driven CES re-analysis.

Conflict of Interest: None declared

P04.030.B Familial hyperprolactinemia caused by homozygous PRLR mutation

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Background/Objectives: Hyperprolactinemia is usually an acquired condition. Typical clinical manifestations include hypogonadism, infertility, and galactorrhea. Rarely, hyperprolactinemia has been attributed to a variant in the prolactin receptor encoded by PRLR, presenting as agalactia, without hypogonadism, infertility, or galactorrhea. We now delineate the clinical phenotype and the genetic basis of marked hyperprolactinemia found in several females of a kindred of Jewish Indian decent.

Methods: Clinical, laboratory and imaging assessment of patients; Whole exome sequencing of the proband. Variant segregation analysis by Sanger sequencing.

Results: Four females of reproductive age with elevated prolactin levels (X6-10 of the upper limit of norm), harbored a homozygous PRLR (NM_000949.7; c.1750del; p.Glu584AsnfsTer49) frameshift variant, affecting the intracellular domain. All four had regular ovulatory cycles and no galactorrhea. Ethylene glycol precipitation and pituitary Magnetic Resonance Imaging revealed no evidence of macroprolactin or pituitary adenoma, respectively. Three homozygous females conceived spontaneously. Of three who gave birth, only one nursed without difficulties, while 2 reported 'lack of milk' and did not have breast engorgement after stopping breastfeeding.

Conclusion: We describe a novel homozygous PRLR p.Glu584AsnfsTer49 variant predicted to affect the intracellular domain of the PRLR, resulting in significant, apparently asymptomatic hyperprolactinemia among homozygous females in their reproductive age, with a possible mild lactation difficulty. We suggest that the hyperprolactinemia might compensate for the lower activity or stability of the PRLR.

Grants: The Morris Kahn Family Foundation.

Conflict of Interest: None declared

P04.031.C Evaluating the causal relationships between urate, systolic blood pressure and kidney function: a bidirectional two-sample Mendelian Randomization study

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Background: Kidney function and blood pressure are highly interrelated and decline in kidney function may be caused by or result from hypertension. Associations between hyperuricemia, hypertension, and chronic kidney disease (CKD) have been reported in observational studies. However, causal inference between these three traits is challenging due to potentially bidirectional relationships. Therefore, we applied Mendelian randomization (MR), to assess the causal relationships between urate, systolic blood pressure (SBP) and estimated glomerular filtration rate (eGFR).

Methods: We applied pairwise univariable MR, by using genetic associations with urate and SBP from UK Biobank and genetic associations with eGFR from CKDGen. We performed multivariable MR (MVMR) to assess the independent effects of urate and SBP on eGFR. All MR results were reported in SD-unit change in outcome per SD-unit change in exposure.

Results: Univariate MR analysis suggested a causal effect of eGFR on urate (β = -1.039; 95%CI: -1.612~-0.466; P = 3.787e-04). However, the causal effect of urate on eGFR was biased by pleiotropy. There was evidence of a causal effect of SBP on eGFR (β = -0.003; 95%CI: -0.011~-0.005; P = 3.787e-04), while little evidence of a causal effect of eGFR on SBP. Finally, there was strong evidence of a casual effect of urate on SBP (β = 0.067; 95%

CI: 0.041~0.092; P = 2.764e-7). MVMR results suggested the causal effects of urate and SBP on eGFR were independent.

Conclusion: Our results provide evidence of the unidirectional causal effects of urate on SBP, SBP on eGFR and eGFR on urate.

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Conflict of Interest: Haotian Tang full-time student, This project was supported by the MRC Integrative Epidemiology Unit (MC_UU_00011/4)., Venexia Walker full-time, This project was supported by the MRC Integrative Epidemiology Unit (MC_UU_00011/4)., Tom Gaunt full-time, This project was supported by the MRC Integrative Epidemiology Unit (MC_UU_00011/4).

P04.032.D Congenital Hypopituitarism mutation spectrum revealed by whole exome sequencing

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Background/Objectives: Congenital hypopituitarism(CH) refers to deficiency of one or more hormones produced by the pituitary and is characterized with highly variable phenotype, including shorth stature, evolving hormone deficiency, various degree from the different pituitary cell lineages. Especially in some of them with complex phenotype neither the clinical phenotype nor the treatment response could be explained. Unravelling the genetic etiology of the disease is important for the timely diagnosis in children,their treatment and outcome

Methods: 14 unrelated children with cardinal symptom short stature were diagnosed with CH by auxology, functional hormonal dynamic tests, MRI of the hypothalamic-hypophyseal unit etc. DNA from blood samples was isolated and whole exome sequencing(WES) was performed on Novaseq 6000, Illumina platform. Former molecular studies were negative in most of them(candidate gene approach).

Results: A variety of mutations in genes *SEMA3A, IGFALS, GLI3, TXNRD2, TSC2, ASXL1, PAPPA2, NF1, COL9A3, GHR, SCN5A* and *MACF1* were revealed. In 3/14 (21%) patients pathogenic or likely pathogenic genetic variants were found with substantial diagnostic contribution. In 3 patients new rare variants were described, not found in public databases. In four patients two mutations were found in combination(*TXNRD2* and *TSC2*; *PAPPA2* and *SETD2; NF1* and *HBB*; *COL9A3* and *TSHR*), explaining more complex phenotypes.

Conclusions: WES is a powerful tool for detecting mutations in patients with congenital pituitary developmental malformations, complex phenotype and variable hormonal deficiencies, negative in former molecular studies. Therefore in such patients the WES would be in favour for an earlier molecular diagnosis, subsequent personalized treatment and genetic counselling in affected families.

Grant References: MES:D-132/14.06.2022,D01-395/18.12.2020, D01-278-14.12.2022,D01-302/17.12.2021,D01-165/28.07.2022

Conflict of Interest: None declared

P04.033.A Applying personalized medicine- the endo-genetic clinic

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Background: The rapid advancement in genetics has led to a paradigm change in endocrinology, expanding our knowledge and unveiling monogenic etiologies in multiple endocrine disorders. Making an accurate diagnosis enables tailored treatment and family genetic counselling.

Objective: To evaluate patients with suspected monogenic endocrine disorders in a specialized clinic.

Methods: Our endo-genetic clinic was founded in 2018. The consultations are performed mostly by one physician with a double board certification in endocrinology and medical genetics. We described over 300 patients evaluated in the clinic, distribution of referred disorders, different characteristics and the yield of genetic work-up.

Results: Over 300 patients were evaluated at the clinic, 44% adults and 56% pediatric patients. The most common reason for referral of adult patients was monogenic diabetes (35% of adult cases and 23% of all referrals) whereas short stature was the most common pediatric referral (31% of cases and 19% of all referrals). Other indications included calcium and bone metabolism disorders (12%), adrenal abnormalities (9%), disorders of sexual development (8%), obesity (6%), recurrent hypoglycemia (4%), hypogonadotropic hypogonadism (3%) and more rare cases of multi-pituitary hormone deficiency, thyroid abnormalities etc. A diagnosis was made in 35% of pediatric patients and 26% of adult cases (total 32%). Variants of uncertain significance were found in 6%. 23% of children and 43% of adults did not complete the recommended tests.

Conclusions: An endo-genetic specialized clinic is an excellent example of personalized medicine. In many cases, the evaluation, work-up and analysis influence endocrine recommendations, treatment, follow-up, and family consultation.

Conflict of Interest: None declared

P04.034.B The genetic regulation of the gastric transcriptome is associated with metabolic and obesity-related traits and diseases

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Background/Objectives: The stomach is part of the upper gastrointestinal tract and secrets acid and enzymes which play a key role in metabolism and microbial defence. We analyzed the transcriptome and genetic regulatory profile of two distinct stomach sections, antrum and corpus, to identify tissue-specific gene expression and tissue-specific quantitative traits loci (eQTL) with the aim to characterize potential gene expression regulatory

mechanisms associated with metabolic phenotypes and related diseases.

Methods: We performed bulk RNA sequencing of gastric antrum and corpus mucosa from 431 healthy individuals. Genome-wide SNP genotyping was conducted with blood samples. Different statistical genetics approaches and bioinformatics tools were used for transcriptome profiling, eQTL identification, partitioning heritability, TWAS, and downstream analyses.

Results: The transcriptome profiling highlights the heterogeneity of gene expression in the stomach. We identified enriched pathways revealing distinct physiology. Furthermore, we found an enrichment of the SNP-based heritability of metabolic and obesity-related diseases by considering section-specific highly specifically expressed genes. In particular, we could prioritize candidate effector genes for multiple metabolic traits, like *RAB27B* being a regulator of weight and body composition.

Conclusion: Our findings show that the two sections of the stomach antrum and corpus vary in their transcriptome and genetic regulatory profile pointing out physiological differences which are mostly related to metabolism. Moreover, our findings shed light on the influence of the genetic regulation of the gastric transcriptome on metabolic and obesity-related traits and diseases.

Grant References: German Research Foundation (DFG).

Conflict of Interest: None declared

P04.035.C Identification of rare pathogenic variants associated with pulmonary fibrosis susceptibility through whole genome sequencing

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Background: Previous genetic studies on pulmonary fibrosis (PF) highlight a pathogenic role for common and rare variants. We aimed to identify rare pathogenic variants and associated enriched biological pathways through the analysis of whole genome sequencing data from two independent cohorts of PF patients.

Methods: Four pathogenic variant categories were defined according to predicted variant consequences plus CADD scores: loss of function variants, missense variants, protein altering variants, and protein truncating variants. Synonymous variants were included as a control group. Gene burden testing based on $P < 2.5 \times 10^{-6}$ for rare variants (allele frequency < 0.1%) was performed through Testing Rare vAriants using Public Data (TRAPD) workflow in 507 PF patients from PROFILE cohort and 451 PF patients from GE100KGP cohort against 76,156 control participants from gnomAD database. Overrepresentation analysis of gene ontology terms and gene concept network analysis were conducted to relate the significant genes to their potential functional categories (corrected $P < 0.05$).

Results: 119 significant genes with pathogenic variants shared between the two cohorts were identified, 95 genes included missense variants and 116 genes included protein altering variants. Enriched functional categories for the 119 genes included "actin filament-based process", "ATP-dependent activity", "extracellular matrix structural constituent" and "myosin complex".

Conclusions: Rare variants were identified in a number of new genes associated with PF and highlight functional roles in cell contraction, metabolism and extracellular matrix synthesis.

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Conflict of Interest: None declared

P04.036.D A novel GATA6 mutation in neonatal diabetes mellitus: from molecular diagnosis to targeted therapy

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Background: Neonatal diabetes mellitus (NDM) is a rare condition characterized by hyperglycemia presenting in the first months of life. We present a case of a newborn with a birth weight of 1,000 g (gestational age 32 weeks) and congenital heart defects. Glucose at day 1 was 271 mg/dl with mild ketonemia. C-peptide was undetectable in 3 different samples. Insulin therapy was started at a dose of 0.04/0.08 IU/kg/h. At 20 days of life NT-proBNP was 14789 (ref. value <320). Fecal elastase was <50 mg/g/feces (ref values>200). NT-proBNP was consistent with heart condition; fecal elastase, along with undetectable endogenous insulin secretion, was indicative of pancreas agenesis/hypolplasia. Pancreas was not visible on ultrasound and nuclear magnetic resonance.

Methods: The clinical diagnosis of NDM prompted a screening for genes associated with this condition. Clinical exome sequencing of the proband and his parents was performed on genomic DNA by using the NovaSeq6000 platform (Illumina). Genes involved in NDM were filtered out for analysis.

Results: Genetic analysis revealed a novel de novo mutation in GATA6 gene (c.1502C>G, p.Ser501Ter). The mutation is predicted to be pathogenic, resulting in the introduction of a premature stop codon. The presence of a GATA6 mutation was consistent with pancreatic imaging and laboratory results and prompted replacement therapy with pancreatic enzymes in order to improve body weight gain.

Conclusions: This report highlights the importance of screening GATA6 gene in patients with congenital cardiac defects and pancreatic agenesis and it emphasizes the importance of genetic counselling in these patients.

Conflict of Interest: None declared

P05

Skeletal, Connective Tissue, Ectodermal and Skin Disorders

P05.002.B Transcriptomic meta-analysis characterizes shared molecular mechanisms between Psoriasis and Obesity

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Background/Objectives: Despite the abundance of epidemiological evidence for the high comorbid rate between Psoriasis and Obesity, systematic approaches on common inflammatory mechanisms have not been adequately explored. Here, we performed a transcriptomic meta-analysis of publicly available RNA-sequencing datasets to unveil putative mechanisms that are postulated to exacerbate both diseases and establish the inflammatory circuit.

Methods: We considered all publicly available total RNA-sequencing datasets between adult Psoriasis and Obesity patients in comparison to healthy controls. We performed two late-stage, disease-specific meta-analyses and explored their commonalities via gene set enrichment analysis. We further investigated shared co-expression patterns through consensus co-expression network analysis (cWGCNA) on paired-end datasets to unravel conserved co-expression modules derived from the expression matrices of both comorbid diseases.

Results: Our systematic search identified 4 psoriatic (cases = 76, controls = 75) and 5 metabolically healthy obese (cases = 76, controls = 55) datasets. Single-gene meta-analyses revealed significant overlaps between up- ($n = 170$, $P = 6.07 \times 10^{-65}$) and down-regulated ($n = 49$, $P = 7.1 \times 10^{-7}$) transcripts, associated with the increased T cell response as well as their polarization to the pathogenic T helper (Th) 17 subtype. Our cWGCNA approach disentangled eleven consensus correlated modules that incorporated the majority of shared deregulated genes, associated with either the differentiation of leukocytes or metabolic pathways with a similar direction pattern in both comorbid diseases.

Discussion: Our novel findings through whole transcriptomic analyses characterize the inflammatory commonalities between Psoriasis and Obesity implying the assessment of several expression profiles that could serve as putative comorbid disease progression biomarkers, as well as elucidate the clinical efficacy of Th17-inhibiting therapies in obese psoriatic patients.

Conflict of Interest: None declared

P05.004.D Genetic testing of 76 families with palmoplantar keratoderma reveals a monogenic cause in more than 78 %

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Background/Objectives: Hereditary palmoplantar keratoderma (PPK) is a rare disorder characterized by hyperkeratinisation of palms and soles. The patient group is very heterogeneous with either isolated skin disease or PPK as part of syndromes. Numerous genes are related to PPK contributing to the diversity of the disease. Few studies have performed genetic testing on clinical well-described patient groups. We have established a large cohort of patients from Denmark and will present the results of genetic testing.

Methods: We recruited patients and affected family members in 2016-2023. Deep phenotyping and genetic testing was performed. The test strategy was exome/genome-based in-silico panel or targeted Sanger sequencing, when clinical relevant. Variants were interpreted using the ACMG guidelines.

Results: We established a cohort of currently 139 patients from 76 families. The type of PPK among 'probands' were punctate 55 % (n = 42/76), diffuse 34 % (n = 26/76), focal 7 % (n = 5/76), and striate 4 %. A genetic cause was identified in >78% of the families. *AAGAB* was the major cause of punctate PPK (n = 37/42 families). Among the non-punctate types, disease-causing variants were identified in different genes including: *DSG1, DSP, KRT9*, KRT1, *AQP5, KRT16, LORICRIN, ABCA12, COL7A1, CARD14*, *DST*.

Conclusion: Our results emerge the hereditary nature of the disease. It supports the value of genetic testing of patients with PPK in order to confirm a diagnosis and distinguish the different sub-types.

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Conflict of Interest: None declared

P05.005.A The exocyst component Exoc6B associated with spondyloepimetaphyseal dysplasia with joint laxity type 3 (SEMDJL3) is required for primary ciliogenesis and chondrogenic but not osteoblast differentiation in vitro

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Background/Objectives: Biallelic pathogenic variants in *EXOC6B*, a component of the exocyst molecular tether are linked to SEMDJL3 (OMIM #618395). Little is known about Exoc6b. Further the pathomechanisms underlying SEMDJL3 remain obscure. We investigated the role of Exoc6b during osteoblast and chondrogenic differentiation in vitro.

Methods: shRNA lentiviral knockdown, reverse-transcription quantitative PCR, immunocytochemistry and immunoblotting.

Results: Exoc6b localizes at the basal body and periciliary region in C3H10T1/2 mesenchymal stem cells (MSCs) and ATDC5 pre-chondrocytes. Its depletion in MSCs and pre-chondrocytes, impeded primary ciliogenesis. Notably this effect was more severe in the latter. Silencing *Exoc6b* in MSCs did not affect osteogenesis and Hedgehog activation. In contrast, its inhibition in prechondrocytes deregulated chondrocyte differentiation. It led to elevated expression of *Col2a1* and *Ihh* that promote chondrocyte proliferation and *Col10a1*, a marker of chondrocyte hypertrophy. Expression of *Axin2*, a canonical Wnt pathway modulator, which stimulates chondrocyte hypertrophy was repressed. Extracellular matrix (ECM) mineralization and levels of *Mmp13* and *Adamts4* that are terminal hypertrophy markers and involved in ECM remodelling were markedly suppressed. In *Exoc6b* silenced prechondrocytes the expression of *Bglap*, a major bone matrix constituent was significantly downregulated during chondrocyte differentiation, suggesting a blockade of transdifferentiation to osteoblast fate that normally occurs for a subset of hypertrophic chondrocytes.

Conclusion: Our findings uncover a lineage specific requirement of Exoc6b in primary ciliogenesis and skeletal differentiation and provide initial insights into the molecular basis of SEMDJL3.

Grant references: Projects # ECR/2016/00145 and 2020-0107/CMB/ADHOC-BMS from SERB and ICMR, respectively, Government of India awarded to Priyanka Upadhyai.

Conflict of Interest: None declared

P05.006.B Comprehensive approach focusing on the molecular profile of a rare genodermatosis: Epidermodysplasia Verruciformis

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Epidermodysplasia Verruciformis (EV) (OMIM:226400, OMIM:618231, OMIM:618267), characterized by predisposition to HPV infections is an exceedingly rare genodermatosis. To date, approximately 30 biallelic *EVER1*(*TMC6*), *EVER2*(*TMC8*) and *CIB1* variants have been associated with EV. Generalized recurrent warts typically appear during early childhood, particularly on sun exposed areas, and evolve into non-melanoma skin cancers in 30-70% affected individuals due to persistent HPV infections. Here we report 6 affected individuals born to 5 consanguineous families with severe clinical manifestations of EV diagnosed at earlier ages.

Clinical and pathological findings in addition to the oncological follow-up of the affected individuals were detailed and the whole coding regions with exon-intron boundaries of *EVER1*, *EVER2* and *CIB1* were analyzed by Sanger sequencing.

Intriguingly, all individuals presented with multiple cutaneous squamous and basal cell carcinoma in various sun exposed regions requiring numerous surgeries even enucleation, without known underlying immunodeficiency. We identified a total of five pathogenic variants (3 *EVER2*, 2 *EVER1*) which four of them are novel and majority of the affected individuals harbored splice-site variants. Of these one of them carried a novel heterozygous *EVER2* splice-site variant; further studies for the detection of the second variant on the trans allele and RNA expression are ongoing. Additionally, the most severely affected individual in our cohort, had an in-frame deletion predicted to be decreasing the stability of transmembrane protein EVER2.

This study further expands the clinical and mutational spectrum of EV and demonstrates that there isn't a predominant gene among these three to be prioritized in the molecular etiology.

Conflict of Interest: None declared

P05.007.C Evident variants of LTBP3 causing dental anomalies with skeletal dysplasia in three Egyptian families: dental management of one patient

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Background/Objectives: Dental anomaly with short stature (DASS) (OMIM# 601216) is an autosomal recessive inherited syndrome with heterogenous phenotypes. The cardinal signs of the disorder are amelogenesis imperfecta (AI), hypodontia, and skeletal dysplasia with platyspondyly as well. The LTBP3 candidate gene is widely expressed in ameloblasts, bones and large blood vessels. The pathogenic LTBP3 variant causes enamel loss, short stature and cardiac problems. We present six new patients from three unrelated families (four females and two males) with dental anomalies and short stature with the dental management performed to one patient.

Methods: The six patients suffered of discolored teeth with tendency to be short and cardiac problems. Family 1 had four affected siblings, while family 2 and 3 had one affected offspring each. Whole exome sequencing (WES) was done.

Results: The six patients had DASS as disorder. The detected LTBP3 variants revealed two novel frameshift variants and one nonsense mutation previously reported. The frameshift variants were c.3545_3546dup p.(Gly1183ArgfsTer43)(family 1) and c.2001_2002delCC p.(His668Profs Ter122) (family 3). The nonsense mutation was c.2886C>Gp.(Tyr962Ter)(family 2). Root canal treatments and full mouth rehabilitation were the dental management offered to one patient to restore esthetical functional and psychological problems.

Conclusion: Amelogenesis imperfecta (AI) is an evident phenotype in many inherited disorders. The alteration of LTBP3 gene predispose the variable dental anomalies associated with the syndrome, as well as the skeletal dysplasia. The proper diagnosis reveals an optimum dental management strategy.

Grant References: The work was funded with the STDF project no: 33458

Conflict of Interest: Nehal Hassib full, consultant, rasha elhossini full, collaborator, Heba Mustafa full, collaborator, Mohamad Abdelhamid full, principal investigator

P05.008.D Diaphragmatic Hernia in a newborn with COL1A1 associated classical Ehlers-Danlos syndrome

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Diaphragmatic rupture is a rare complication of classical Ehlers-Danlos Syndrome (cEDS). There has been no report of diaphragmatic hernia in cEDS affected newborns. In this case report, we present a male born by spontaneous vertex delivery to a mother affected with cEDS. Ultrasound scan was performed to monitor growth and a ventricular septal defect six days before birth, at which time there was no evidence of diaphragmatic hernia. After delivery, signs of severe respiratory distress developed, and review of a chest radiograph made a diagnosis of diaphragmatic hernia. On day three of life, surgeons identified and repaired a small posterolateral diaphragmatic defect. The patient made a good recovery despite complication with chylothorax. There was no suggestion of pulmonary hypoplasia after repair. The heterozygous pathogenic familial mutation *COL1A1* c.934C>T;p.(Arg312Cys) was confirmed with bidirectional sequencing in the infant. *COL1A1* variants can be associated with Arthrochalasia EDS and Osteogenesis Imperfecta, which has been rarely associated with diaphragmatic hernia in infancy. There were no clinical signs of osteogenesis imperfecta in this family. The familial mutation affects the highly conserved arginine residue within the Gly-X-Y triplet motif of the type-I collagen protein. This variant has been reported in multiple families as a rare cause of autosomal dominant cEDS. We present a detailed report of this patient journey, radiology images and review the literature on diaphragmatic hernia and rupture in classical EDS. This family offers a keen illustration of the phenotypic and genetic overlap in the inherited connective tissue disorders.

Conflict of Interest: None declared

P05.009.A Mechanical strain stimulates the cytonuclear shuttling of the desmin intermediate protein in skeletal muscle cells

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Background/Objectives: Living cells are constantly exposed to mechanical stimuli, which plays a crucial role in maintaining tissue homeostasis through cellular proliferation, migration, tissue repair, altered metabolism, and even stem cell differentiation and maturation. Desmin, a muscle-specific intermediate filament protein, transmits mechanical stress in muscle tissue in conjunction with LINC complexes. Recent studies suggest that desmin may also serve as a signaling platform in mechanotransduction pathways. Mutations in desmin or desmin chaperones can lead to pathological conditions such as myofibrillar myopathy, arrhythmogenic cardiomyopathy, and dilated cardiomyopathy.

Methods: To determine the impact of mechanical strain on the nuclear localization of desmin, human immortalized skeletal muscle cells (myoblasts and myotubes) were subjected to uniaxial stretching for 5 hours.

Results: A quantitative statistical analysis of the colocalization of two channels (DAPI and desmin) showed that there was a significant increase in the nuclear import of desmin in correlation with mechanical strain.

Conclusion: The observation of nuclear accumulation of desmin and higher colocalization signals between desmin and DAPI in the group subjected to strain suggests that desmin may play a strain-dependent regulatory role through mechanisms that are yet to be understood.

Grant References: EMBO Scientific Exchange (Grant 9337)

Conflict of Interest: None declared

P05.010.B The natural history of classical Ehlers Danlos Syndrome in adults and children

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Background/Objectives: Classical Ehlers-Danlos Syndrome (cEDS) is a rare type of EDS defined by cardinal features of skin hyperextensibility, joint hypermobility and atrophic scarring. The National EDS Service in the United Kingdom sees individuals with rare types of EDS, and currently has data for 140 individuals (100 adults, 40 children) within the London service. The aim of this report is to provide an update on the natural history of cEDS across a large cohort.

Methods: Individuals with a clinical and molecular diagnosis of cEDS will be asked for consent to enter their clinical and genetic information into a research database. Data of natural history of cEDS will be collected and presented.

Results/Discussion: This report presents a large cohort of cEDS patients, male: female 64:76, ages 3-88 years (mean age 38 years). As well as data regarding typical features of cEDS, this cohort presents data including cardiovascular involvement and pregnancy complications. This report presents specialist clinical experience of a large cohort of individuals with a broad age range. This will help to improve understanding of the natural history of cEDS over a lifetime, improving management and patient quality of life.

Conflict of Interest: Chloe Angwin Grants received: Imperial College London Biomedical Research Council funding, Niamh Wilkinson Grants received: Imperial College London Biomedical Research Council funding, Duncan Baker: None declared, Juliette Harris: None declared, Michael Pope: None declared, Ravinder Sehra: None declared, Neeti Ghali Grants received: Imperial College London Biomedical Research Council funding, Fleur Van Dijk Grants received: Imperial College London Biomedical Research Council funding, principal investigator

P05.011.C Two novel variants of the MBTPS1 gene in Czech family with autosomal recessive skeletal dysplasia, growth restriction and congenital cataracts

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Background: Only eight patients with biallelic pathogenic/likely pathogenic variants in *MBTPS1* gene have been described to date. Six of them mainly present with Kondo-Fu type SED (spondyloepiphyseal dysplasia), remaining two are described to have presenile cataract, alopecia, oral muscular disorder and psoriasis-like phenotype (CAOD). Most of the affected individuals show growth restriction, SED and cataracts. Herein we present two affected siblings with novel variants in *MBTPS1* gene.

Methods: Exome sequencing was performed in the older affected child and both healthy parents. Confirmation of the identified variants and segregation analysis were done using Sanger sequencing.

Results: The proband, a 7-year-old girl, suffered from prenatal growth failure, and her growth remains restricted. She has joint hypermobility, irregular ossification of femoral and tibial epiphyses and patella, bilateral congenital cataracts, and dysmorphic face with large protruding ears. Her brother, aged 4 months, was noted to have diaphragmatic hernia and hypotonia, in addition to poor growth. Due to his low age, dysmorphic features could not be reliably assessed. Two novel likely pathogenic variants in the *MBTPS1* gene (NM_003791.4) c.163G>A and c.1022C>G in trans position were identified in both children. The first variant is predicted to impact splicing, while the second variant, p.(Pro341Arg) affects a very conserved amino acid in the peptidase domain.

Conclusion: The presented case extends the phenotypic spectrum of pathogenic variants in *MBTPS1* and confirms that these can be linked to growth restriction as well as congenital cataracts.

Grant References: Supported by MH CZ – DRO, University Hospital Motol, Prague, Czech Republic 00064203

Conflict of Interest: Veronika Oppeltová Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Czech Republic, Marketa Vlckova Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Czech Republic, Marcela Malíková Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Czech Republic, Marek Havelka: None declared, Anna Křepelová Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Czech Republic, Carlo Rivolta IOB - Institute of

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P05.012.D Clinical exome sequencing in diagnostics of collagenopathies identified novel pathogenic DNA variant in SMAD3 gene

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Hereditary connective tissue disorders assigned also as collagenopathies in the majority arise due to the dysfunction of collagen molecules. In general, collagenopathies can be divided into main groups, skeletal (osteochondrodysplasias) and soft connective tissue dysplasias. The development and usage of different sequencing approaches, such as clinical exome (CES) or whole exome (WES) platforms bring new possibilities of genetic diagnostics into routine practice.

In our study, we present the analysis of 37 patients with diagnosed collagenopathy using a clinical exome sequencing platform and a specific virtual gene panel of 80 genes, collected based on HPO database. Analyzed patients were divided into 7 phenotypic groups based on their personal history, exactly group of Marfan syndrome (29.7 % samples), unclassified collagenopathies (21.6 %), Ehlers-Danlos syndrome (13.5 %), Marfan-like syndromes (10.8 %), Stickler syndrome (10.8 %), osteogenesis imperfecta (10.8 %) and hereditary exostoses (2.7 %). Altogether, we have detected pathogenic and potentially pathogenic DNA variants in 37.8 % ($n = 14$) of the samples. Identified causal DNA variants associated with the phenotype were identified in the COL1A1, COL11A1, FGFR3, FKBP14 (compound heterozygote status) and SMAD3 genes. In the case of the proband, carrying DNA variant c.206+2T>G in the SMAD3 gene, we have identified a novel, previously unpublished DNA variant.

Finally, determining the clinical causality of pathogenic DNA variants, we have been able to additionally determine the diagnosis in 37.5 % of patients within the group of unclassified collagenopathies, which can lead to the more precise, genomic based, personalized medical care.

Conflict of Interest: None declared

P05.013.A Phacomatosis pigmentovascularis caused by postzygotic mosaicism in GNAQ

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Phacomatosis pigmentovascularis is a rare genodermatosis characterized by the co-occurrence of widespread capillary malformations and dermal pigmentary disorders associated with a variety of other cutaneous or extracutaneous manifestations. Activating somatic variants in *GNA11* and *GNAQ* have been identified in patients with phacomatosis pigmentovascularis and predominantly reported in Asian or Asian-related populations. Here, we present a 41-years-old caucasian woman with a congenital bilateral melanosis bulbi, limb hypertrophy, naevus flammeus of the affected leg as well as dermal blue naevi. NGS-based diagnostic revealed the heterozygous missense variant c.548G>A, p.(Arg183Gln) in *GNAQ* classified as likely pathogenic. The variant has been detected in skin samples of naevus flammeus and blue naevi, but not in blood or unaffected skin samples indicating postzygotic mosaicism. *GNAQ* encodes a GTPase-activating protein involved in the MAPK/ERK signaling pathway that plays a crucial role in cell proliferation and differentiation as well as tumorigenesis. As *GNAQ* variants are also associated with uveal melanoma an ophthalmological and dermatological surveillance program is recommended in these patients.

Conflict of Interest: None declared

P05.014.B Spondyloepiphyseal dysplasia with congenital joint dislocations in nine Indian patients

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Background: Spondyloepiphyseal dysplasia with congenital joint dislocations (SEDCJD, #MIM 143095), is an autosomal recessive chondrodysplasia, characterised by congenital joint dislocations, short stature, kyphosis, progressive vertebral defects, platyspondyly and club foot. It is caused by biallelic pathogenic variations in *CHST3* (carbohydrate sulfotransferase 3) gene resulting in the reduction of sulfotransferase activity.

Methods: Nine individuals with SEDCJD were evaluated. We performed Sanger sequencing of *CHST3* gene in eight and exome sequencing in one individual.

Results: Seven male and two female patients with SEDCJD from five unrelated families of Indian origin are described here. Consanguinity was noted in four families. The age at examination of patients ranged from 3 years to 15 years. The main presenting clinical features were joint dislocations [9/9; elbow joint dislocation (7/9), hip dislocation (5/9)], short stature (9/9), kyphosis (8/9) and club foot (3/9). Radiographical features included vertebral defects (9/9), distal bifid humeri (5/9), flattened femoral epiphysis (9/9), supernumerary carpal bones (7/9), coxa vara (6/9) and large epiphysis (9/9).

Five homozygous variants c.500A>G;p.(His167Arg), c.688G>A;p.(Glu230Lys), c.976dupG;p.(Asp326GlyfsTer186), c.1165G>C;p.(Ala389Pro) and c.431G>A;p.(Gly144Ser) were identified in *CHST3* gene (NM_004273.5), of which four were novel and one was known. These variants were absent in gnomAD and in our in-house dataset of 2609 exomes and were predicted to affect protein function. All these variants are located on sulfotransferase domain of CHST3 protein and were classified as likely pathogenic.

Conclusion: We describe clinical, radiological and molecular findings of nine Indian individuals with SEDCJD from five Indian families.

Grant reference ID: SB/SO/HS/005/2014 (Department of Science and Technology, India)

Conflict of Interest: None declared

P05.015.C Genotype-phenotype correlations in collagenopathies

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Background: Collagenopathies are congenital diseases with variable symptomatology and heterogeneous genetic background, associated with abnormal collagen - important component of the connective tissue in skin/tendon/cornea/bones, building also the support of internal organs and blood vessels. Some of them had distinct clinical course, while others ambiguous or oligosymptomatic. The aim of the study was to determine the molecular basis and genotype-phenotype correlations in selected osteoarticular collagenopathies: osteogenesis imperfecta (OI), Ehlers-Danlos syndrome (EDS), Bethlem/Ullrich myopathy, spondyloepiphyseal dysplasia (SED).

Patients and Methods: The study included 21 pediatric patients with clinical diagnosis of OI and 25 - EDS hypermobile/visceral. Diagnostic approach was based on connective-tissue NGS panel.

Results: Molecular background was successfully established in 20/21 OI patients (*COL1A1*, novel: c.1299+1G>A, c.1688del, c.833G>C; *COL1A2*, novel: c.983G>C, c.2009G>T, c.776G>A), but only in 9/25 patients with EDS suspicion: 3 had Marfan disease (*FBN1*, novel: c.4598A>G), 1 EDS visceral (*COL3A1*), 1 X-linked valvular dysplasia (*FLNA*, novel: c.869-2A>G) and 3 EDS hypermobile (*COL5A1*, novel: c.3622C>A; *COL11A1*, novel: c.3677C>G). Among patients routinely tested due to arthrogryposis/myopathy we identified 3 with Ullrich myopathy (*COL6A1*), 1 with Bethlem myopathy (*COL6A3*), 4 with SED (*COL1A1*, novel: c.2999C>T; *COL2A1*, *COL9A1*) and 1 with Schmid metaphyseal chondrodysplasia (*COL10A1*, novel: c.1995dup)

Conclusion: Targeted NGS testing is an effective tool in diagnostics of collagenopathies. While the clinical diagnosis of OI could be confirmed genetically in most cases, the genetic basis in EDS hypermobile has been established only in some, with surprising diagnosis of Marfan with no evident heart/eyes involvement.

Grant references: Supported by CMHI project S182/2019.

Conflict of Interest: None declared

P05.016.D Birk-Barrel syndrome – difficult way to the diagnosis of the ultra-rare disease

Dorota Piekutowska-Abramczuk[1], *Maria Jędrzejowska*[2;3;4], *Agnieszka Madej-Pilarczyk*[2], *Elżbieta Ciara*[1], *Dorota Jurkiewicz*[1], *Marlena Młynek*[1], *Paulina Halat-Wolska*[1], *Krystyna Chrzanowska*[1]

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Background: Birk-Barel syndrome is a rare, genetic disorder characterized by congenital hypotonia, developmental delay, intellectual disability and dysmorphic features. Some patients have cleft palate, joint contractures and scoliosis. Feeding problems are frequent and respiratory failure may develop. Underlying genetic defect includes mutations in the *KCNK9* gene: maternally inherited (genomic imprinting) or de novo.

Patients and Methods: We present 2 brothers: 4.5y (P1) and 2y (P2) with hypotonia, psychomotor delay, poor speech, joint contractures and face dysmorphia. Both required tube feeding and night invasive ventilation. P2 was operated due to cleft palate. Diagnostic approach aiming to explain the genetic background included MS-MLPA, aCGH, neuromuscular NGS panel and whole exome sequencing (WES).

Results: Initially Prader-Willi syndrome, congenital myotonic dystrophy and chromosomal aberrations were excluded. Analysis of 211 neuromuscular genes (P1) revealed no (likely) pathogenic variant related to the observed phenotype. Further WES revealed the known *KCNK9* variant c.706G>C p.(Gly236Arg), reported in Birk-Barel syndrome. Sanger sequencing confirmed the *KCNK9* variant in the P1 and P2 and in their asymptomatic mother. Therefore the diagnosis of Birk-Barrel is fully justified.

Conclusion: Targeted NGS testing is not always sufficient to obtain the unequivocal diagnosis of ultra-rare disease. Selection of causative molecular variants is complex and requires critical approach, including careful genotype-phenotype analysis in patient and in the members of the whole family.

Grant references: Supported by CMHI project S182/2019.

Conflict of Interest: None declared

P05.017.A Heterozygous variants in both the N- and C-terminal domains of IHH lead to defective secretion, confirming the pathogenicity of these variants in patients with short stature and/or brachydactyly

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Introduction: Indian-hedgehog (IHH) plays a crucial role in endochondral bone development. It is synthesized as a precursor which is translocated to the endoplasmic reticulum and autocleaved into the active-secreted N-terminal peptide (IHH-N) and a C-terminal peptide (IHH-C), critical for self-cleavage.

In humans, IHH-N variants have been associated with Brachydactyly type A1 (AD) and Acrocapitofemoral dysplasia (AR), only three of which have been functionally studied. However, heterozygous *IHH* variants, majority classified as variants of unknown significance (VUS) are being increasingly identified, by NGS, in individuals with short stature and/or brachydactyly (Vasques et al., 2018; Sentchordi-Montané et al., 2020).

Aim: To functionally test *IHH* VUS variants and to determine whether IHH-C variants are pathogenic.

Methods: Nine *IHH* variants (4 IHH-N, 5 IHH-C) observed in patients with short stature and/or brachydactyly, a positive control and two Wobble variants were introduced into the pCMV6-IHH construct by site-directed mutagenesis and transiently transfected

into HEK293T cells. Cultured media and cell lysates were collected and the different IHH peptides were analyzed by western blot.

Results: Surprisingly, all 9 mutants showed reduced IHH-N secretion (<50%) and decreased intracellular stability of both, IHH-N and IHH-C peptides compared to wildtype. Wobble variants behaved similarly to wildtype.

Conclusions: All 9 *IHH* variants can now be re-classified as pathogenic. Reduced stability and impaired secretion are likely to be the underlying disease mechanism. Importantly, our study provides the first insight into functional consequences of C-terminal variants and highlights the importance of functional studies to confirm the pathogenicity of VUS.

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Conflict of Interest: None declared

P05.018.B Non-syndromic cleft palate only – identifying potential disease causing genes through targeted sequencing

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Non-syndromic cleft palate only (nsCPO) is one of the most common malformations, has a multifactorial etiology and a heritability of >90%. So far, only 10 common risk loci for nsCPO have been identified. Based on epidemiological observations it can be assumed that rare variants in genes with high penetrance also have an impact on the development of nsCPO. The overall goal of our work is to identify such genes. In a previous study, we searched for de novo copy number variants in exome sequencing data of nsCPO trios and generated a list of 104 potential nsCPO candidate genes.

The aim of the study presented here was to further prioritize and identify the best candidate genes among the preselected genes.

After an extensive literature search, we selected 13 genes for targeted sequencing in 167 individuals with nsCPO and 275 unaffected controls. Sequencing was performed using single-molecule Molecular Inversion Probes (smMIPs) on an Illumina NovaSeq platform, and variant calling was performed using GATK UnifiedGenotyper. Variants were filtered for a CADD score >15 and allele frequency <0.0005.

Sequencing revealed 90 variants in the 167 affected individuals. After stringent quality control and filtering, nine variants in seven prioritized candidate genes remained. One of these nine variants is a truncating mutation in *ADGRL2*, a gene encoding a protein suspected to interact with CDH1, a known risk factor for orofacial clefting. Another variant was identified in *DLX1*, which is supported as a gene involved in palatogenesis in mice.

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P05.020.D New insights from five cases of Spondylodysplastic Ehlers Danlos Syndrome

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Background: Spondylodyplastic type Ehlers Danlos syndrome (spEDS) is an autosomal recessive disorder characterised by short stature, muscular hypotonia, bowing of the long bones, skin hyperextensibility and pes planus. The condition is caused by bialleleic pathogenic variants in *B4GALT7*, *B3GALT6* or *SLC39A13*.

The combination of a connective tissue and skeletal phenotype brings many of these patients to the attention of the Paediatric and Genetics services for investigation and diagnosis. In some cases, the skeletal phenotype predominates. This can range from an antenatal appearance of a severe skeletal dysplasia, leading to second trimester termination to a postnatal progressive kyphoscoliosis.

Case presentation: We report five patients from three families presenting with spEDS with a focus on clinical presentation and radiological findings. In three of these patients, variants in *B3GALT6* confirmed the diagnosis with clinical features in keeping with previously reported cases. The presence of multiple haemangiomas however, is a novel finding and may expand the phenotype in this condition. In two siblings, a variant in *SCL39A13* is thought to be causative. Interestingly, the maternal carrier displayed mild features raising the possibility of heterozygous phenotypic expression.

Discussion: These cases highlight the distinctive features that set apart the more prevalent *B3GALT6* spEDS phenotype from the rarer cases of *SLC39A13* in the literature. The small number of *SLC39A13*- related spEDS makes variant interpretation challenging due to a milder phenotype and subsequent uncertainty when applying phenotypic weight to ACMG guidelines.

The multisystem involvement of spEDS necessitates a multidisciplinary approach to treatment and ongoing management.

Conflict of Interest: None declared

P05.021.A A novel skeletal dysplasia caused by a pathogenic variant in UXS1

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Background/Objectives: The protein backbone in heavily glycosylated proteoglycans is bound to glycosaminoglycans through a tetrasaccharide linker. *UXS1* encodes UDP-glucuronate decarboxylase 1, which catalyzes synthesis of UDP-xylose, the donor of the first building block in the linker.

Defects of other enzymes involved in formation of the tetrasaccharide linker cause so-called linkeropathies, characterized

by short stature, radio-ulnar synostosis, decreased bone density, congenital contractures, dislocations and more.

In zebrafish UXS1 activity is essential for production and organization of skeletal extracellular matrix.

Methods: Whole exome sequencing was performed in a family where father and son presented with a skeletal dysplasia. Wt and mutant UXS1 enzymes were recombinantly expressed in E. coli and purified. Enzyme activity was evaluated by LC- MS/MS. In vivo effects were studied by metabolomics and western blotting of blood samples from affected individuals compared to controls, including an unaffected relative.

Results: A likely pathogenic heterozygous *UXS1* variant NM_001253875.1 c.557T>A p.(Ile186Asn) in the son, was de novo in the father.

Radiographic changes in the son included short long bones, accelerated skeletal maturity, normal epiphysis and cupping of metaphysis, especially in the lower extremities, findings in keeping with the *UXS1* mutant zebrafish phenotype.

The variant located in the enzyme dimer interphase is predicted to disrupt interactions and reduce enzyme activity. Purified wt UXS1 enzyme, in contrast to the mutant form, was able to convert UDP-glucoronate to UDP-xylose.

Conclusion: This is the first report of a *UXS1* variant causing a skeletal dysplasia in humans.

Conflict of Interest: None declared

P05.022.B STAT1 binds to CAD and periodontitis risk SNP rs10757278, causing inhibition of lncRNA CDKN2B-AS1 expression, which activates transcription of collagen genes in cells of the extracellular matrix

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Background: Long noncoding RNAs are modulators of gene expression due to the capacity to interact with DNA, RNA and proteins. lncRNA CDKN2B-AS1 is a significant genetic susceptibility locus for cardiovascular disease and is associated with other pathologies, including periodontitis. We aimed to identify gene networks that regulate and transmit CDKN2B-AS1 activity in gingival fibroblasts.

Methods: We computationally screened SNPs for allele-specific changes of predicted transcription factor binding sites that were in strong linkage disequilibrium (r^2>0.8) with shared associations of CAD and periodontitis (Transcription Factor Affinity Prediction tool). Allele-specific transcription factor binding was determined (Antibody Electrophoretic Mobility Shift Assay) and activity of regulatory elements was quantified (500bp dual-luciferase reporter assays) in gingival fibroblasts. CDKN2B-AS1 transcripts levels were downregulated (locked nucleic acids oligonucleotides) in gingival fibroblasts and, following RNA-sequencing, we performed gene set enrichment analysis (go, hallmark, reactome, tmod).

Results: We validated rs10757278 locating to a STAT1 binding site ($P = 0.005$) as a biological functional SNP. rs10757278-G reduced STAT1 binding by 12.8% and increased luciferase activity 1.7-fold compared to rs10757278-A ($P = 0.0056$). Collagen chain trimerization (M27812) showed strongest upregulation ($p = 1.2 \times 10^{-7}$). Strongest upregulated gene was *LINC00536* ($\log_2FC = 7.2$, $p < 4.4 \times 10^{-9}$).

Conclusion: We validated functional effects of rs10757278 on STAT1 binding in gingival fibroblasts, previously reported in lymphoblastoid cell lines, implying upstream inflammatory signals mediate CDKN2B-AS1 expression. Our results also validated previous observations in kidney cells, which showed that CDKN2B-AS1 directly contacted collagen genes. CDKN2B-AS1 represses collagen expression, which may have a significant role in the etiology of CAD and periodontitis.

Grant References: SCHA 1582/14-1

Conflict of Interest: Weiwei Shi full, Jia-Hui Song full, Henrik Dommisch full, January Weiner full, Arne Schäfer full, principal investigator

P05.023.C When the phenotype is more severe than expected: coexistence of X-linked and dominant ichthyosis in an African patient

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Background/Objectives: The most common types of ichthyosis are autosomal dominant or ichthyosis vulgaris and X-linked recessive ichthyosis. Clinical forms of these diseases are quite indistinguishable. Ichthyosis vulgaris is caused by heterozygous mutations in the *FLG* gene encoding filaggrin. For the X-linked recessive form, mainly males are affected and the majority of the cases are caused by complete deletion of the steroid sulfatase gene (*STS*) on chromosome Xp22.3. Other cases result from partial deletion or point mutations of the *STS* gene. Co-segregation of both ichthyosis types has been described previously in several populations, but to the best of our knowledge not in African population. Filaggrin defects seem to synergize with deficient activity of steroid-sulfatase causing exacerbation of the clinical features.

Methods: We present an African patient with a very severe phenotype of ichthyosis. Meticulous phenotyping with molecular confirmation of both X-linked recessive and autosomal form of ichthyosis was obtained.

Results: Accurate clinical examination and skin biopsy led to clinical diagnosis of severe ichthyosis. Subsequently gene panel analysis by exome seqencing confirmed a hemizygote intragentic deletion of *STS* in co-existence with a heterozyguous likely pathogenic variant in *FLG*.

Conclusion: Ichthyosis vulgaris and X-linked recessive ichthyosis are well known and clinically similar, with variable severity. We describe the first African patient with confirmed coexistence of both ichthyosis forms, causing severe exacerbation of the clinical features, with impotant mental health implications.

References

Alaverdian DA et al. Metab Pers Ther. 2019
Wang X et al. Med Genet. 2018

Grants

Conflict of Interest: None declared

P05.024.D Establishing an objective clinical spectrum, genotype-phenotype correlations and CRMP1 as a modifier in the Ellis-van Creveld syndrome: the first systematic review of EVC and EVC2-associated conditions

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Background/Objectives: The Ellis-van Creveld (EVC) syndrome is an autosomal recessive skeletal ciliopathy that was first identified in the Old Order Amish. Since its discovery, two causal genes have been identified, *EVC* and *EVC2*, showing that several cases were misdiagnosed and were in fact other entities. Nevertheless, there has not been any adequate phenotypic characterization of molecularly defined EVC syndrome so far.

Methods: We performed a systematic review of case reports of EVC syndrome with molecular confirmation of pathogenic variants in *EVC* or *EVC2*. Demographic, genetic and clinical information of patients was assessed.

Results: We reviewed 725 papers and obtained 54 case reports/series that met the inclusion criteria, with a total subject sample of 310. Of these, 190 had biallelic variants, while 28 were affected carriers of monoallelic variants. Our analysis revealed new phenotypes that have not been classically linked to the syndrome, and others which have but that are very rare. Monoallelic symptomatic forms had less expressivity, and biallelic cases were milder if associated with *EVC* and/or missense variants. Finally, we identified *CRMP1*, a gene whose coding region partially overlaps with *EVC*, as a potential dosage-dependent genetic modifier of the severity of the EVC syndrome.

Conclusion: We provided the first objective clinical characterization of molecularly-defined EVC syndrome and identified the first associated genetic modifier, *CRMP1*, which had not been prior implicated in human disease.

Conflict of Interest: Jorge Diogo Da Silva Full-time employee of Centro Hospitalar Universitário de Santo António., Ana Rita Soares Full-time employee of Centro Hospitalar Universitário de Santo António., Ana Maria Fortuna Full-time employee of Centro Hospitalar Universitário de Santo António., Natalia Tkachenko Full-time employee of Centro Hospitalar Universitário de Santo António.

P05.025.A Partial clinical response to Secukinumab in paediatric patient with c.386C>T/p.Thr123Met homozygous missense mutation in IL36RN gene caused to Acrodermatitis Continua of Hallopeau

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Background/Objectives: Acrodermatitis Continua of Hallopeau (ACH) is a rare, chronic, inflammatory disease that usually presents with sterile pustules on the fingers or toes. It is considered to be a variant of pustular psoriasis. First findings about genetic background of this disease was related with *IL1RN* gene variants. It usually affects middle-aged women, because of the rarity of ACH in paediatric population there is no standardized guidelines for treatment. This case report aimed to correlate genetic background and treatment.

Methods: Genomic DNA was isolated from peripheral blood samples. Whole exome sequencing was performed in proband, targeted specifically disease related genes (*IL36RN, CARD14, AP1S3, TNIP1, SERPINA3, IL1RN*). Sanger sequencing was performed for confirmation in probands detected mutation and first-degree relatives.

Results: Patient was offspring of cousins. In proband, a c.368C>T/p.Thr123Met homozygous missense loss-of-function mutation was detected in exon 5 of *IL36RN* gene, whereas the parents were heterozygous and her 23-year-old sister was wild type for the same genetic variant. This mutation defined as variant of uncertain significance according to the ACMG classification due to low clinical evidence rate.

Conclusion: A 13-year-old female patient, diagnosed ACH eight years ago, had multiple different therapies and for the last one year she have had partial clinical response with Secukinumab, an IL-17 monoclonal antibody. Even if IL-17 associated with IL-36 cytokine pathway, it is not a targeted therapy for our patient because it is a quite common cytokine in many inflammatory diseases. Genetic consultation was provided to the family about this genetic condition.

Grant References: None

Conflict of Interest: None declared

P05.026.B Next-generation sequencing-based panel testing identifies heterogeneous genetic etiologies of short stature

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Background: Identifying the molecular etiology for short stature (defined as height 2 or more standard deviations below the mean for age and sex) can guide treatment, allow early screening and supportive therapy for associated features, and inform familial recurrence. We retrospectively assessed the utility of NGS multigene panel testing for individuals with short stature and provided an overview of the positive genetic findings identified in this population.

Methods: Clinical reports for 744 patients with an indication of short stature who underwent panel testing at Blueprint Genetics were examined. Testing included both sequence and copy number variant (CNV) analyses of NGS data from validated clinical exome assays, including established non-coding variants. A positive result was defined as the identification of pathogenic or likely pathogenic variant(s) consistent with the patient's phenotype and known associated disease inheritance.

Results: A positive result was reported in 10.9% of patients (n = 81/744) in 27 genes. Most frequent positive results were associated with autosomal dominant RASopathies (*PTPN11*, *CBL*, *HRAS*, *KRAS*, *LZRT1*, *RIT1*) (n = 32) and autosomal dominant *FGFR3*-related conditions (n = 10). CNVs accounted for 1.4% of the positive results, including five deletions involving *SHOX* gene. One patient received a positive result through identification of a non-coding variant in *GHR* gene.

Conclusion: Nearly 11% of patients in this cohort received a positive result, including a non-coding variant and CNVs, demonstrating the benefit of including robust CNV analysis and targeting disease-associated intronic variants in multi-gene panel testing for individuals with suspected monogenic short stature.

Conflict of Interest: Johanna Huusko Blueprint Genetics, a Quest Diagnostics company, Alicia Scocchia Blueprint Genetics, a Quest Diagnostics company, Kaisa Kyöstilä Blueprint Genetics, a Quest Diagnostics company, Lili Ramirez Blueprint Genetics, a Quest Diagnostics company, Liisa Pelttari Blueprint Genetics, a Quest Diagnostics company, Satu Valo Blueprint Genetics, a Quest

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P05.027.C Vascular EDS in adulthood: an overview of clinical and molecular features of 151 individuals

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Background/Objectives: Vascular Ehlers-Danlos syndrome (vEDS) is a rare heritable connective tissue disorder, characterised by arterial and hollow organ fragility due to pathogenic heterozygous *COL3A1* variants. Descriptions of molecular and clinical features, including natural disease course follow-up, of large vEDS cohorts are important for improved diagnosis and disease management.

Methods: Adults with a diagnosis of vEDS were identified through the London EDS Service, with DNA analysis completed prior to study commencement, between the years 2004 and 2021. Major events were defined as symptomatic clinical events that required specialist management or intervention, that occurred spontaneously or would not have occurred to the same degree in an unaffected individual.

Results: 151 adults (89:62, female:male, 86 index cases, 31 families) with a clinical and molecular diagnosis of vEDS were identified. Median age at diagnosis was 33 years (interquartile range, IQR 23-46). We identified 87 different *COL3A1* variants in this population and formed six distinct classifications. Missense substitutions and splice site variants accounted for 80% of all variants, 56% of which were triple-helical glycine substitutions. Major clinical events were observed in 50% of individuals; vascular events were predominant (38%) followed by gastrointestinal events (16%), with first events occurring at a median age of 33 years (IQR 26-41).

Conclusion: This cohort provides unique insight into clinical and molecular characteristics of vEDS in adults and aids more informed diagnosis, counselling, and management of the condition by furthering understanding of established genotype-phenotype associations. This data builds upon previous vEDS cohorts and emphasises the importance of future (inter)national collaborations.

Conflict of Interest: Niamh Wilkinson Imperial College London Biomedical Research Council funding, Katherine Von-Klemperer: None declared, Elena Cervi: None declared, Duncan Baker: None declared, Chloe Angwin Imperial College London Biomedical Research Council funding, Juliette Harris: None declared, Michael Pope: None declared, Ravinder Sehra: None declared, Neeti Ghali Imperial College London Biomedical Research Council funding, Fleur Van Dijk Imperial College London Biomedical Research Council funding

P05.028.D A diagnosis of Vascular EDS in childhood: clinical and molecular features of 60 individuals

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Background/Objectives: Diagnoses of Vascular Ehlers-Danlos syndrome (vEDS) in childhood are primarily made due to positive family history, but can occur following major arterial or intestinal events. This research aims to capture comprehensive descriptions of clinical and molecular features of this cohort, including major clinical events, and identify drivers of vEDS diagnosis in childhood to address deficits in literature in this young population group.

Methods: Individuals diagnosed with vEDS in childhood (<18 years), confirmed by identification of a (likely) pathogenic *COL3A1* variant, were identified through the National EDS Service in London. DNA analysis was completed prior to study commencement, between the years 2004 and 2021.

Results: 60 individuals (30:30 female:male, n = 22 index) with a diagnosis of vEDS in childhood were identified. Median age at diagnosis was 7 years (interquartile range, IQR 3-12). Diagnosis was predominantly driven by family history of vEDS (63%). A total of 10 major clinical events in childhood (4 vascular, 6 gastrointestinal) were recorded in 9 individuals. First events occurred at a median age of 9 years (IQR 0-12). In individuals without a positive family history or major event, easy/excessive bruising was the most common reason for testing (82%).

Conclusion: This cohort provides additional details of the clinical and molecular characteristics of individuals with vEDS and contributes new insight into drivers of diagnosis in individuals diagnosed in childhood, especially in those where testing was initiated due to presence of minor diagnostic criteria. This data establishes the foundation for future (inter)national collaborations, essential for improvement in diagnosis and management.

Conflict of Interest: Niamh Wilkinson Imperial College London Biomedical Research Council funding, Elena Cervi: None declared, Bart Wagner: None declared, Katherine Von-Klemperer: None declared, Toby Andrew: None declared, Neeti Ghali Imperial College London Biomedical Research Council funding, Fleur Van Dijk Imperial College London Biomedical Research Council funding

P05.029.A SUPT7L loss of function variants cause a developmental disorder with generalized lipodystrophy

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Nadja Ehmke[1], Dominik Seelow[1;4], Melanie FRADIN[6;7], Uwe Kornak[1;2;8], Björn Fischer-Zirnsak[1;2]

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Background: Various disorders are known which comprise lipodystrophy as a partial or generalized feature, leading to a progeroid appearance of the affected individuals. The previously described disease genes play a role in several pathways affecting nuclear function, lipid biogenesis, DNA repair and transcriptional regulation. We identified variants in *SUPT7L*, which encodes a component of the transcriptional coactivator complex STAGA, as the molecular cause of a multisystem condition with progeroid features and generalized lipodystrophy.

Methods: We investigated the variants in proband's dermal fibroblasts by transcriptome sequencing, immunofluorescence and immunoblotting. Using CRISPR/Cas9, a *SUPT7L* knockout HeLa cell line was generated to further validate the findings from the primary cell line.

Results: The proband presented with severe global developmental delay, short stature, neonatal teeth and reduced subcutaneous fat tissue, resulting in an overall progeroid appearance. A missense and a frameshift variant in *SUPT7L* were identified a compound heterozygous state. The predicted missense variant caused alternative splicing which leads to a frameshift and results in the absence of SUPT7L protein in fibroblasts. Moreover, altered expression of genes involved in DNA repair was detected. We investigated this process and showed an increased rate of DNA double-strand breaks in proband-derived fibroblasts. This effect was also confirmed in the *SUPT7L* knockout cell line. Transient overexpression of wildtype *SUPT7L* normalized these findings and thus rescued the cellular phenotype.

Conclusion: Here, we present an individual with a severe multisystem condition caused by pathogenic *SUPT7L* variants and propose *SUPT7L* as a novel candidate gene for progeroid phenotypes with generalized lipodystrophy.

Conflict of Interest: None declared

P05.030.B Mosaic Gorlin syndrome with ophthalmological developmental defects and a mosaic SMO variant

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Background/objective: A 46-year-old individual with multiple basal cell carcinomas (BCC) was diagnosed with her first BCC at age 32. She had several eye anomalies: microphtalmia, and microcornea of the left eye, iriscoloboma of the right eye and binocular congenital ptosis. Gorlin syndrome (GS) was suspected, but previous genetic evaluation with sequencing and copy number analysis of *PTCH1* and *SUFU* and RNA analysis of *PTCH1* was normal. There were no palmar pits, cardiac fibromas, macrocephaly or jaw cysts. One abdominal hypopigmented skin lesion. Skin biopsies from two BCCs were analyzed due to suspected mosaicism.

Methods: Sequencing analysis (NGS) of *PTCH1*, *PTCH2*, *SUFU* and *SMO* genes in DNA from tissue and of *SMO* variant in DNA from blood.

Results: In DNA from two separate BCC the *SMO* variant c.1234C>T, p.(Leu412Phe) was detected (NM_005631.5). Analysis from blood was negative.

Conclusions: The *SMO* variant has been described earlier in Happle-Tinschert syndrome (HTS), Curry-Jones syndrome (CJS) and segmental GS. No ophthalmological changes have been described in GS caused by the mosaic *SMO* variant except retinal detachment. Due to the overlap between HTS, CJS and GS we support the suggestion that the conditions can be seen as a continuous spectrum caused by mosaic activating variants in genes in the sonic hedgehog pathway. We propose that ophthalmological developmental defects in combination with multiple BCCs can be seen in mosaic GS caused by mosaic *SMO* variant.

Conflict of Interest: Cecilie Rustad: None declared, Wenche Sjursen: None declared, Josephine Prener Holtan co-PI ReSa study, funded by Fondsstiftelsen, OUH, Yes, speaker - Novartis, Yes, Advisory board: Janssen pharmaceutical by Johnson and Johnson (XLRP) and Novartis (luxturna). Consultant PYC therapeutics, Anvor Rossow: None declared, Charlotte von der Lippe: None declared, Kristin Halvorsen Hortemo Yes, speaker at symposia about skin cancer in 2022 supported by Abbvie., Yes, Advisory Board for Sun Pharma 2022.

P05.032.D Homozygous mutations in KIF22 are responsible for a milder form of spondyloepimetaphyseal dysplasia with joint laxity, leptodactylic type

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Dominant mutations in *KIF22*, encoding a kinesin-like protein, are responsible for spondyloepimetaphyseal dysplasia with joint laxity, leptodactilic type (Lepto-SEMDJL). It is characterized by short stature, flat face, generalized joint laxity with multiple dislocations, and progressive scoliosis and limb deformity.

By targeted gene sequencing, we identified an autosomal recessive *KIF22* variant (c.146G>A [p.49Arg>Gln]) in three patients from three unrelated families presenting with clinical features similar to those of patients carrying a dominant *KIF22* variant (c.443C>T or c.446G>A), although the spinal involvement appeared later and was less severe in patients with a recessive variant. Relatives harboring the c.146G>A variant at the heterozygous state were asymptomatic.

Recessive *KIF22* variant affected a conserved residue located in the active site and potentially destabilized ATP binding. RT-PCR and western-blot analyses demonstrated that both dominant and recessive *KIF22* variants do not affect *KIF22* mRNA and protein

expression in patient fibroblasts compared to controls. As lepto-SEMDJL presents phenotypic overlap with chondrodysplasias with multiple dislocations (CMD), disorders due to defective proteoglycan biosynthesis, we then analysed proteoglycan synthesis in patient skin fibroblasts. Compared to controls, DMMB assay showed a significant reduction of total sulfated proteoglycan content in culture medium but not in the cell layer and immunofluorescence demonstrated a strong reduction of staining for chondroitin sulfates but not for heparan sulfates, similarly in patients with recessive or with dominant *KIF22* variants.

These data identify a new recessive *KIF22* pathogenic variant, link for the first time *KIF22* pathogenic variants to altered proteoglycan biosynthesis, and place the Lepto-SEMDJL in the CMD spectrum.

Conflict of Interest: None declared

P05.033.A How mosaic level influence the phenotype – lesson form a family study of monozygotic twins discordant for metachondromatosis due to a novel postzygotic PTPN11 mutation

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Background/Objectives: Genetic mosaicism is of a great interest due to its role in human disease. Monozygotic twins (MZTs) arising from a single zygote are considered as genetically identical, and any differences likely to be caused by postzygotic events. Thus, phenotypically discordant MZTs offer a unique opportunity to study genotype-phenotype correlation.

Methods: Using whole exome sequencing (WES) and NGS-based deep amplicon sequencing we analyzed three-generation family with metachondromatosis (MC) starting from a pair of MZTs discordant for MC. For MZTs WES was performed on DNA samples purified from hair follicles.

Results: In MC discordant MZTs we identified a novel postzygotic p.(Gln175His) *PTPN11* variant. Interestingly, significant differences in mosaic ratio was observed between twins and within different tissue types within one individual. In DNA extracted from hair follicles, in affected twin the p.(Gln175His) VAF was nearly constitutional 45%, while in unaffected twin VAF was 12%. On blood of MZTs low-level mosaicism was identified in both samples (affected twin VAF 5% and unaffected twin VAF 2%, respectively). In further family study we confirmed constitutional (VAF 50%) p.(Gln175His) variant in all affected individuals.

Conclusion: Our results indicate that the phenotypic manifestation of p.(Gln175His) in examined family clearly depends on mutational load. From the typical MC in constitutional carriers of p.(Gln175His) variant, through mild phenotype in high-mosaic level (VAF 45% in affected twin), to normal phenotype in low-mosaic level carrier (VAF 12% in unaffected twin).

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Conflict of Interest: None declared

P05.034.B Systematic search for microRNA regulated genes with roles in the etiology of periodontitis

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Aims: Several array-based miRNA expression studies indicated increased transcript levels of of hsa-mir-130a-3p, -142-3p, -144-3p, -144-5p, -223-3p, -17-5p and -30e-5p in the inflamed gingiva of periodontitis patients compared to healthy controls. We aimed to determine direct target genes and signaling pathways that are regulated by these miRNAs.

Material and Methods: We transfected a miRNA mimic (mirVana) of each miRNA into human primary gingival fibroblasts cultured from 3 different donors. RNA-Sequencing was performed after 24 hours. Differential gene expression was analysed using DESeq2. Gene set enrichment analysis was performed with the data sets tmod, reactome, hallmark, Go and MSigDB. miRNA inhibition was validated on the transcript and protein level using quantitative RT-PCR and reporter genes for hsa-mir-130a-3p.

Results: Gene-set enrichment demonstrated significant roles for cell cycle regulation of hsa-miR-130a-3p ($P_{adj} = 5 \times 10^{-15}$), miRNA-144-3p and -5p ($P_{adj} = 4 \times 10^{-40}$ and $P_{adj} = 4 \times 10^{-6}$), miR-17-5p ($P_{adj} = 9.5 \times 10^{-23}$) and miR-30e-5p ($P_{adj} = 8.2 \times 10^{-18}$). Additionally, significant roles in cytokine signaling was shown for miR-130a-3p ($P_{adj} = 5 \times 10^{-15}$), miR-223-3p ($P_{adj} = 2.4 \times 10^{-7}$) and miR-142-3p ($P_{adj} = 5 \times 10^{-11}$). The gene *WASL*, *ENPP5*, *MANBAL*, *IDH1* showed the most significant and strongest downregulation after has-miR-142-3p, -17-5p, -223-3p and -30e-5p transfection, respectively. Moreover, hsa-miR-130a-3p, -144-3p and -144-5p regulated the periodontitis risk genes *CPEB1*, *ABCA1* and *ATP6V1C1*, respectively. Moreover, we found that all analyzed miRNAs significantly increased the gene *MET*, which participates in wound healing as well as tissue regeneration and remodeling.

Conclusion: Gene set enriched emphasized the significance of cytokine signaling and the regulation of cell proliferation in the etiology of periodontitis and add evidence for *CPEB1*, *ABCA1* and *ATP6V1C1* as susceptibility genes of periodontitis

Conflict of Interest: Luyang Zheng full, Avneesh Chopra: None declared, January Weiner full, Henrik Dommisch full, Arne Schäfer full, OX/BER research partnership (OX-BER 1 STEM1)

P05.035.C Pathogenic LZTR1 variants are frequent among individuals with isolated café-au-lait macules

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Background/Objectives: Cafè-au-lait macules (CaLMs) are hyperpigmented skin lesions commonly found in the general population. CaLMs with well-defined borders are detected in individuals with neurofibromatosis type 1 (NF1), Legius syndrome and Noonan syndrome. Pathogenic variants in *LZTR1* (Leucine Zipper Like Transcription Regulator 1, MIM*600574), a tumor suppressor

gene encoding a substrate receptor for the cullin 3-RING ubiquitin ligase complex involved in the polyubiquitination of RAS GTPases, have been identified in individuals with schwannomatosis, as well as in patients with dominant and recessive forms of Noonan syndrome. We report on a cohort of patients showing CaLMs and pathogenic variants in *LZTR1*.

Methods: Individuals with CaLMs, with negative *NF1/SPRED1* molecular testing and in which a pathogenic/likely pathogenic variant in *LZTR1* was found were included. Clinical assessment was performed by both dermatologists and geneticists, including a physical examination and evaluation of clinical records.

Results: We report on 32 pediatric and adult individuals (mean age 23.5 years) from 20 independent families showing CaLMs and pathogenic variants in *LZTR1*. In most of them, CaLMs were 6 or more, with regular borders. No individuals showed schwannomas or other neoplasms. The detected variants were predominantly truncating, indicating a loss of function behaviour (9 frameshift, 5 splicing, 1 in-frame deletion, 1 nonsense).

Conclusion: This study further extends the phenotypic variability associated with pathogenic variants of *LZTR1*, which in addition to conferring susceptibility to schwannomatosis and causing dominant and recessive Noonan syndrome, may be present in individuals with multiple CaLMs in absence of any other clinical manifestations.

Grant References: none

Conflict of Interest: None declared

P05.036.D Single-cell based co-expression networks to prioritize candidate genes for non-syndromic cleft lip with or without cleft palate

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Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common birth defects and has a multifactorial etiology. More than 45 genetic risk loci have been identified by genome-wide association studies (GWAS), and these loci include more than 400 positional candidate genes. Due to the developmental time point of nsCL/P development and scarcity of functional data, prioritization of causal genes at these loci has been challenging. Recent research has suggested that the candidate genes *Irf6* and *Tfap2a* are part of a regulatory network in which *Irf6* influences the gene expression of *Tfap2a* and potentially further nsCL/P genes. Using single-cell RNA sequencing (scRNA-seq) data from the embryonic mouse face, we recently showed that this co-expression is particularly present in the periderm, ectodermal surface and basal cells at mouse embryonic day E11.5. We therefore hypothesize that co-expression analyses of candidate genes in single-cell data of embryonic tissue will help to prioritize candidate genes from GWAS loci. We will now extend our work to systematically investigate co-expression of all nsCL/P candidate genes using scRNA-seq data from human (Xu et al. 2022 bioRxiv) and mouse (Li et al. 2019 Development) embryos. We will construct co-expression networks with high-dimensional weighted gene co-expression analysis (hdWGCNA; Morabito et al. 2022 bioRxiv). These co-expression networks will advance our understanding of causal genes at nsCL/P GWAS loci and will give insights into their potential genetic interactions.

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P05.037.A Variants in EFCAB7 underlie nonsydromic postaxial polydactyly

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Polydactyly is the most common limb malformation that occurs in 1.6-10.6 per one thousand live births, with incidence varying with ancestry. The underlying gene has been identified, for many of the ~100 syndromes that include polydactyly. While for the more common form, nonsydromic polydactyly, eleven candidate genes have been reported. We investigated the underlying genetic cause of autosomal recessive nonsyndromic postaxial polydactyly in four consanguineous Pakistani families. Some family members with postaxial polydactyly also presented with syndactyly, camptodactyly, or clinodactyly. Analysis of the exome sequence data revealed three novel homozygous frameshift deletions in *EFCAB7*: [c.830delG; p.(Gly277Valfs*5); in two families and [c.829delG; p.(Gly277Valfs*5)]; and [c.1350_1351delGA;p.(N451Ffs*2)] each in one family. Sanger sequencing confirmed that these variants segregated with postaxial polydactyly, i.e., homozygous in the two to three members with postaxial polydactyly and heterozygous or wild type in the unaffected members. EFCAB7 displays expression in the skeletal muscle and on the cellular level in cilia. IQCE-EFCAB7 and EVC-EVC2 are part of the heterotetramer EvC complex, which is a positive regulator of the Hedgehog pathway, that plays a key role in limb formation. Depletion of either *EFCAB7* or *IQCE* inhibits induction of *Gli1*, a direct Hedgehog target gene. Variants in *IQCE* and *GLI1* have been shown to cause nonsyndromic postaxial polydactyly, while variants in *EVC* and *EVC2* underlie Ellis van Creveld and Weyers syndromes, which include postaxial polydactyly as a phenotype. This is the first report of the involvement of *EFCAB7* in limb anomalies.

Conflict of Interest: None declared

P05.038.B isolated median alveolar cleft: mild manifestation of familial frontorhiny with homozygous ALX3 pathogenic variant

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Background: Midline facial clefts are one of the symptoms in frontonasal dysplasias (FND). Frontorhiny (MIM#136760) is a distinctive presentation of FND caused by recessive pathogenic

variants in the *ALX3* homeobox gene. Frontorhiny is characterized by ocular hypertelorism, wide and short nasal ridge, bifid nasal tip, broad columella, widely separated nares, long and wide philtrum and V-shaped hairline, with ptosis and midline dermoid cysts in some patients. *ALX3* is essential for normal facial development.

Methods: Here, in the present study, we have investigated three siblings in a consanguineous family and found an homozygous *ALX3* pathogenic variant with exome sequencing.

Results: Both sisters exhibited classical features of frontorhiny including bifid nasal tip, long philtrum with prominent bilateral swellings and unilateral ptosis. The youngest girl having a more severe phenotype, this led to a medical genetics consultation. Exome sequencing revealed a homozygous nonsense variant in *ALX3* inherited from both parents consistent with the clinical diagnosis of frontorhiny. The couple later had a third child with isolated median alveolar cleft and no other craniofacial malformation, this boy is also homozygous for the *ALX3* pathogenic variant.

Conclusion: This is the first report of major intrafamilial variability with expansion of *ALX3*-related FND phenotype to a milder form with isolated alveolar cleft. Patients with isolated midline notch of the upper alveolus (albeit rare) may have a mild phenotype of frontorhiny, with a 25 % risk for the parents to have another affected child with more visible median craniofacial defects requiring specialized surgical management.

Conflict of Interest: None declared

P05.039.C Analysis of a non-lethal biallelic frameshift mutation in ZMPSTE24 reveals utilization of alternative translation initiation codons

Lukas Kaufmann[1], *Johannes Pilic*[2], *Lisa Auinger*[3], *Anna-Lena Mayer*[1], *Jasmin Blatterer*[1], *Johann Semmler-Bruckner*[4], *Safdar Abbas*[5], *Khurram Rehman*[6], *Muhammad Ayaz*[7], *Wolfgang Graier*[2;8], *Roland Malli*[2;8], *Erwin Petek*[1], *Klaus Wagner*[1], *Ali Al Kaissi*[9], *Muzammil Ahmad Khan*[5], *Christian Windpassinger*[1;10]

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Background/Objectives: Restrictive dermopathy is a lethal condition caused by biallelic loss-of-function mutations in *ZMPSTE24*, whereas mutations preserving residual enzymatic activity of the ZMPSTE24 protein lead to the relatively milder mandibuloacral dysplasia with type B lipodystrophy (MADB) phenotype. Remarkably we identified a homozygous, presumably loss-of-function mutation in *ZMPSTE24* [c.28_29insA, p.(Leu10-Tyrfs*37)] in two consanguineous Pakistani families segregating MADB. In this study we performed functional analysis to clarify how lethal consequences are prevented in affected individuals.

Methods: Colocalization experiments in human AD-293 cells (Agilent) using different *ZMPSTE24* expression constructs were conducted to examine alternative translation initiation at potential N-terminal start codons. High-resolution fluorescence microscopy was performed with an array confocal laser scanning microscope (Axiovert 200 M, Zeiss).

Results: Expression experiments proved utilization of two previously unknown, alternative N-terminal translation initiation sites in mutated *ZMPSTE24*, resulting in almost full-length, ER-located proteins. Remarkably, one of these alternative start codons is newly formed at the insertion site. The second translation initiation site corresponds to the methionine codon at amino acid position 13 of the wild type protein.

Conclusion: To the best of our knowledge, this is the first study reporting an N-terminal frameshift mutation creating a novel, physiologically relevant translation initiation site downstream of the canonical start codon, preventing complete loss of protein function and the associated lethal consequences. Beyond that, our results point to incorrect variant interpretation and in-silico prediction of N-terminal frameshift mutations, since the formation of potential, novel start codons at the mutation sites is not considered in current variant interpretation algorithms.

Conflict of Interest: None declared

P05.040.D Neuroimaging features of MOPDII in ten patients with PCNT mutation: A Tertiary Centre Experience

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Background/Objectives:

Microcephalic osteodysplastic primordial dwarfism (MOPD) type II, the most common type of microcephalic primordial dwarfism is characterized by intrauterine growth restriction, severe proportionate short stature, microcephaly, and increased risk for cerebrovascular disease (1). In this study, we aimed to delineate both vascular and nonvascular neuroimaging findings of a group of MOPDII patients with PCNT mutation.

Methods: The study included ten patients from nine unrelated consanguineous families from Turkey. All patients were subjected to medical history taking, three generations pedigree construction, clinical and neurologic examination, and anthropometric measurements. Cranial magnetic resonance imaging (MRI) findings were retrospectively evaluated.

Results: Of 10 patients, while six displayed the same corpus callosum dysgenesis pattern with a thin trunk (body) and underdeveloped rostrum, four patients had moyamoya disease along with both acute and chronic ischemic lesions. Of four patients with moyamoya disease, a simplified gyral pattern with microcephaly was detected in three of them. Interestingly one patient had severe shallow dental root. Hippocampal sclerosis and ventriculomegaly were among the other neuroimaging findings.

Conclusion: Structural brain abnormalities in addition to cerebrovascular arteriopathy further expand the neuroimaging features of MOPDII. This may be overlooked and complicate the course with a wide range of clinical manifestations due to hemodynamic instability.

References:

1.Waich S, Janecke AR, Parson W, Greber-Platzer S, Müller T, Huber LA, et al. Novel PCNT variants in MOPDII with attenuated growth restriction and pachygyria. Clinical genetics. 2020;98(3):282-7.

Conflict of Interest: Akcahan Akalin Hacettepe University, rahşan göçmen Hacettepe University, Pelin Simsek-Kiper Hacettepe University, ekim taşkıran Hacettepe University, yasemin alanay Acıbadem University, Vildan Göknur Haliloglu Hacettepe

University, Gulen Eda Utine Hacettepe University, koray boduroglu Hacettepe University

P05.041.A Whole genome sequencing as a diagnostic tool in oligodontia

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Background/Objectives: Oligodontia, i.e. the missing of more than six permanent teeth, is a common congenital anomaly. Although monogenetic causes have been identified, the majority of cases remains unsolved. We tested the use of whole genome sequencing (WGS) as a diagnostic tool in non-syndromic oligodontia.

Methods: Phenotypic data of four index patients was collected and WGS performed. Candidate variants were segregated in all available family members.

Results: We identified three heterozygous pathogenic variants in known oligodontia genes: a de novo one-exon deletion in *PAX9*, a *PITX2* final-exon frameshift variant in a patient and his affected daughter and a *WNT10A* variant in a patient and his healthy mother. Interestingly, *PITX2* variants cause Axenfeld-Rieger syndrome 1 (RIEG1), a congenital syndrome featuring severe ocular malformation and oligodontia. In contrast, in our family initially isolated oligodontia was suspected and a mild ocular phenotype only diagnosed upon targeted ophthalmologic examination prompted by our findings. In the third patient, the maternally inherited heterozygous variant in *WNT10A* - alone not sufficient to cause oligodontia - was identified together with a 2.1kb deletion of a non-coding potentially regulatory element in *FGF7*. In the fourth patient we identified a potential splice site variant of uncertain significance in *PTH1R*.

Conclusion: Using WGS we identified three single nucleotide and two structural variants of interest in four families with oligodontia. We thus conclude that WGS is a powerful tool in the diagnostics of oligodontia. Additionally, we note that final exon frameshift variants in *PITX2* cause a predominantly dental phenotype rather than classical RIEG1.

Conflict of Interest: None declared

P05.043.C A functional analysis of pathogenic variants in NIPAL4 in patients with congenital ichthyosis

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Background: Autosomal recessive congenital ichthyosis (ARCI) is a group of rare heterogeneous cornification disorders characterised by skin scaling and erythroderma. ARCI refers to harlequin ichthyosis and the lamellar ichthyosis/nonbullous congenital ichthyosiform erythroderma phenotypic spectrum. The aim of this study was to add to the understanding of structural consequences of *NIPAL4* variants and the genotype/phenotype correlation.

Methods: SeqMan Ultra was used to analyse 196 ARCI patient samples from Central Europe for mutations in *NIPAL4*. Various in silico tools were used to predict pathogenicity and analyse protein structure.

Results: Pathogenic or likely pathogenic variants were found in around 11% of patients. Most patients had been diagnosed with lamellar ichthyosis, few of them with congenital ichthyosiform erythroderma. Scales were mostly small and fair or light brown in colour, texture was more superficial, and the scales did not form prominent ridges. Erythema was mild or not seen. Palmoplantar keratoderma was frequent, sometimes presenting with hyperlinearity. Crystal structures are not available; however, NIPAL4 isoform 1, but less so isoform 2, showed similarities with a drug transporter from *Starkeya novella* previously described with an outward-facing transmembrane topology. In contrast, NIPAL4 was predicted to have nine transmembrane domains, in line with 2D predictions. Most pathogenic variants were located in transmembrane regions and supposed to alter secondary structures and transport properties of NIPAL4.

Conclusion: This study further highlighted the genotype/phenotype correlation in ARCI. It revealed structural consequences of *NIPAL4* variants and paves the way to a functional understanding of the role of NIPAL4 deficiency.

Conflict of Interest: None declared

P05.046.B Homozygeous patogenic variants in the LTBP1 gene detected in two patients diagnosed with cutis laxa type, IIE

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Background/Objectives: Latent transforming growth factor b (TGFb)-binding proteins (LTBPs) are essential components of the extracellular matrix. Recently premature truncating mutations in the *LTBP1* gene have been reported in eight patients with connective tissue involvement, craniofacial and skeletal dysmorphology, and heart defects. Here we describe two more cutis laxa, type IIE patients molecularly diagnosed with LTBP1 mutations. This is the second report on cutis laxa patients carrying LTBP1 mutations.

Case-1: A 5 year-old boy was referred to our clinic due to short stature and craniosynostosis. He was born to consanguineous parents at term. He was operated for inguinal hernia. His height was 85,7 cm(-5 SDS), weight 13,5 kg(-2,2 SDS). Physical examination showed a coarse face, proptosis, brachydactyly, cutis laxa. Whole exome sequencing (WES) analysis was performed and a novel homozygous c.3152delG mutation was detected in *LTBP1* gene.

Case-2: A 2,5 year-old girl referred to our clinic for neurodevelopmental delay. She was born to non-consanguineous parents at term. She had an operation due to atrial septal defect and inguinal hernia. Her height was 66 cm(-6 SDS), weight 6,7kg(-5,2 SDS). Physical examination showed hypotonia, brachydactyly and cutis laxa. A next generation sequencing panel revealed a novel

homozygous c.2418+2T>C mutation in *LTBP1* gene, which is responsible for Cutis laxa,type IIE.

Conclusion: This study contributed to the expansion of the literature by adding two new patients to the cutis laxa syndromes. Using a targeted gene panel shortens the diagnostic process in patients with genetically heterogeneous diseases.

Conflict of Interest: None declared

P05.047.C Variability between Jeune Asphyxiating Thoracic Dystrophy and Short-Rib Polydactyly Type III linked to DYNC2H1: Phenotypic, Genotypic Review and searching for a Modifier Gene

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Jeune Asphyxiating Thoracic Dysplasia (JATD) and Short-Rib Polydactyly Syndrome Type III (SRP3) are autosomal recessive conditions taking part of the skeletal ciliopathies group. Both disorders share common skeletal features including short ribs, trident-shaped pelvis, and possible polydactyly, or bowing of femora.

However, SRP3 is characterized by early antenatal detection with severe skeletal manifestations and various visceral malformations, and is considered at the severe end of the clinical spectrum. The gene *DYNC2H1* is causative for both phenotypes and encodes for a primary cilia protein essential for ciliary intraflagellar transport.

Our aim was to study the phenotype of SRP3 and JATD individuals with *DYNC2H1* variants and to further analyze our ciliome data to identify additional variants arguing in favor of modifier gene(s).

We included eighteen JATD and six SRP3. We highlighted some SRP3 specificities (spike metaphyses, polydactyly, severe visceral malformations). No clear genotype-phenotype correlation was outlined. Therefore, we decided to seek variant(s) in other ciliopathy gene(s) that could have a modifier effect on the phenotype.

Bioinformatic analyses with different selection criteria on all variants were performed. The method aimed to compare the number of patients of each group carrying at least one variant of the selection in the analyzed groups of genes. The comparison was statistically evaluated with LRT tests and the p values were corrected for multiple comparisons (Bonferroni method). Although not significant, these analyses revealed promising results in *TMEM2* with variants found in the JATD group only. These preliminary results are currently further explored with an expanded cohort of patients.

Conflict of Interest: None declared

P05.049.A A targeted NGS panel with a 20% diagnostic yield in Short Stature reveals the contribution of digenic inheritance in growth disorders disclosing novel potential gene interactions

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Short stature is one of the most common endocrinological conditions of childhood that occur isolated or in presence of complex phenotypes. To date monogenic causes have been identified in more than 200 mendelian syndromes and skeletal dysplasia.

The diagnostic yield of a 106-gene NGS panel was evaluated in a cohort of 302 patients with idiopathic and syndromic short stature. Pathogenic/Likely pathogenic (P/LP) variants were identified in 61 patients representing the 20% of cases. Twenty-nine genes were mutated in at least one patient and 12 genes in more than one, namely *ACAN, KISSIR, GNAS, NF1, SLC26A2, GLI2, PTPN11, GNRHR, SOS, HEXS1, NPR2, COMP*.

Interestingly 9 patients carried a VUS in addition to the P/LP variant and three patients carried 2 pathogenic variants suggesting digenic inheritance involving the tested genes. Among these, patient #1006 carried variants in *ACAN* and *FBN1*. From the IPA Ingenuity database, we extracted a predictive indirect gene interaction *ACAN-FBN1* mediated by FN1 a dimeric glycoprotein of the cartilage extracellular matrix. Patient # 2553 carried variants in *SOS1* and *RAF1*, both belonging to the RAS-MAPK pathway. Patient # 1698 carried pathogenic variants in *SHOX* and *COMP* suggestive of interaction between these two chondrogenesis proteins.

In conclusion, digenic/oligogenic inheritance should be taken into account in deciphering the genetic basis of some forms of short stature, also considering those VUS, that in presence of a P/LP variants are usually underestimated. Besides the relevance for the diagnosis, this might lead to highlight novel interactions involved in the complex process of growth.

Conflict of Interest: None declared

P05.050.B Rapid turnover skeletal disease from in-frame tandem duplication of exons 4-9 in TNFRSF11A encoding RANK

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Mendelian disorders featuring rapid bone turnover result from: i) RANK activation due to in-frame 12, 15, 18, or 27-bp duplication in exon 1 of *TNFRSF11A* encoding RANK's signal peptide and ii) juvenile Paget's disease due to deactivating mutations in *TNFRSF11B* encoding osteoprotegerin (OPG) or a single heterozygous missense mutation in SP7 encoding osterix (OSX). We report rapid turnover skeletal disease in a mother and fetus due to a novel duplication of *TNFRSF11A*. A 38-year-old nonconsanguineous woman wore hearing aids at age 15 months. Brittle teeth were noted at age 3 years. Loss of adult dentition began at age 9 years and she was edentulous at 27 years. Bone scintigraphy showed increased uptake in the long bones, and CT revealed hypoplastic ossicles and semicircular canals, and absent cochleas. Radiographic survey revealed a normal skull, but thickened cortices of tubular bones. Serum alkaline and acid phosphatase were elevated. At age 14 years, she had normal stature, slight knock-knee deformity, and had fractured major limb bones. Calcitonin and bisphosphonate therapy were given. At age 30 years, a prominent forehead, deep-set eyes, and anterior tibial bowing were apparent. Family history was negative for deafness or fractures. Exonic Sanger sequencing was negative for both *TNFRSF11A/B*. However, exon copy number analysis showed 3 copies of RANK exons 4-9. Long-range sequencing delineated an in-frame tandem duplication predicting a RANK fusion protein of one extracellular RANKL-binding domain combined with double intracellular activation domains. Studies are underway to report the mechanism for this unique disorder featuring constitutive RANK activation.

Conflict of Interest: None declared

P05.051.C Remarkably mild clinical presentation of classical Ehlers-Danlos syndrome (cEDS) in a three generation family due to a novel COL5A2 glycine substitution

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cEDS is caused by variants in *COL5A1* (70%), *COL5A2* (13%) and rarely in *COL1A1* or other genes (<10%). Most *COL5A1* defects represent mutations generating a premature termination codon leading to haploinsufficiency. In contrast, nearly all *COL5A2* variants represent structural missense or in-frame exon-skipping splice mutations, resulting in the production of mutant α2(V)-chains. Despite the absence of genotype-phenotype correlations, *COL5A2* variants often lead to a more severe cEDS phenotype.

Index patient is an eight year old boy showing some characteristic features suspicious for cEDS as generalized joint hypermobility (Beighton score 8/9), soft velvety skin, easy bruising and umbilical hernia. Additionally, a present wrist and thumb sign, pes valgus, scapulae alatae, hypotonia and motor developmental delay as well as subtle facial dysmorphic features were obvious.

Genetic analysis identified a novel *COL5A2* missense variant p.(Gly582Val) in the triple helix domain of the pro-α2(V) chain, classified as likely pathogenic. The mother and maternal grandmother also carry the variant. The grandmother has a Beighton score of 6/9, congenital hip dysplasia, flat foot, high arched palate, soft skin, umbilical hernia, striae and piezogenic papules. The mother is similarly affected.

None of our p.(Gly582Val) carriers fulfill the obligate criteria suggestive for cEDS: skin hyperextensibility and atrophic scarring. Clinical descriptions of cEDS cases with *COL5A2* glycine substitutions are rare with isolated cases lacking the combined major criterion 1. Publishing detailed genotype-phenotype characterizations will contribute to reconsider future updates of the EDS nosology concerning the significance of skin hyperextensibility/atrophic scarring to avoid underdiagnoses by strict application the current criteria.

Conflict of Interest: Karin Mayer MVZ Martinsried GmbH, Medicover Genetics, Martinsried, scientific advisory board Deutsche Ehlers-Danlos Initiative e.V., Monika Cohen MVZ Martinsried GmbH, Medicover Genetics, Martinsried, Lisa Peterson MVZ Martinsried GmbH, Medicover Genetics, Martinsried, Konstanze Hörtnagel MVZ Martinsried GmbH, Medicover Genetics, Martinsried

P05.052.D Delineating the clinical and molecular spectrum of congenital limb defects in Indian subcontinent using genomic approaches

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Background/Objectives: Limb reduction defects range in severity from missing fingers to partial or complete absence of a limb. The genetic etiology for approximately one third of limb reduction defects remains unidentified till date. The objective of the study was to explore the genomic variants involved in the pathogenesis of congenital limb defects (CLD): radial ray defect (RRD), and split-hand/foot malformation (SHFM).

Methods: We evaluated 106 individuals with CLD from unrelated families from India (from 2017-2022). Targeted Sanger sequencing was carried out for known syndromes. Genomic evaluation was performed using chromosomal microarray (CMA) and whole exome sequencing (WES).

Results: In our cohort of 106 individuals [RRD (n = 68) and SHFM (n = 38)], molecular diagnosis was established in 31 subjects (29%). Diagnostic yield of genomic testing was 21% (14/68) in RRD and 45% (17/38) in SHFM. The yield of CMA was 16% (4/25) in SHFM and 22% (5/23) in RRD, whereas diagnostic yield of WES was 33% (10/30) in SHFM and 19% (5/38) in RRD. A total of 22 novel variants including rare pathogenic variants in *TBX3*, *CEP57* and a probable founder variant in *RBM8A* were identified.

Conclusion: Though limb defects are commonly encountered; the overall yield of genomic evaluation remains low. We provide an update in delineation of causative genes/genomic loci for CLD in clinical practice. Our cohort illustrates the utility of genome-wide testing, especially singleton WES in diagnosis of unclassified RRD and SHFM.

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Conflict of Interest: None declared

P05.053.A Genotype-phenotype correlation analysis in patients with generalized pustular psoriasis

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Background: Generalized pustular psoriasis (GPP) is a rare, severe psoriatic subtype with a strong genetic component. More often it occurs in episodes than continuously, and typical trigger factors are changes in e.g. medication, infections, surgery, Besides GPP, affected individuals can also suffer from concomitant psoriatic subtypes. Bi-allelic variants in *IL36RN* and *MPO* have been identified as the main genetic risk factors and the presence of variants is associated with a younger age of onset. Other genes (*CARD14*, *SERPINA3*, *AP1S3*) are known as rarely affected disease genes. To assess correlation of typical disease features/ concomitant disease with the presence of disease variants, we performed subphenotype analyses in 72 patients.

Methods: Whole exomes were analysed for variants in five disease genes. Differences in stratified groups were determined using Fisher's exact test.

Results: The average age of onset was 32.2. ± 22.83 years. *IL36RN* variants were detected in 20 of 72 patients (28%), *MPO* variants in 15 of 72 (21%), while variants in *CARD14*, *SERPINA3* and *AP1S3* were rarer (4%, 3%, 4%, respectively). In 61-68 individuals, we had clinical data for course of disease, palmoplantar pustulosis, plaque psoriasis and joint affection. By performing subphenotype analyses for carriers of *IL36RN* or *MPO* variants, we did not find any significant correlation.

Conclusion: Our patient group might be underpowered to detect significant correlations, suggesting to increase sample sizes and to collaborate with other groups. Evidence for correlation of genetic factors with concomitant diseases will be important in therapeutic decision-making of this severe entity.

Conflict of Interest: None declared

P05.054.B A TBX4 variant in congenital patellar dislocation, an ultra-rare disorder with unknown genetic etiology

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Congenital anomalies of the patella can be present in several disorders and a number of genes have been associated to variable phenotypes. Among them, in congenital patellar dislocation (CPD; %169000) no causative genes have been recognized. The genetics of patella anomalies reflects the complexity of associated developmental processes.

The molecular diagnosis is crucial to define the type of patellar defect and allow a personalized treatment. Despite progresses in understanding the underlying developmental processes, many patients remain undiagnosed.

We applied WES to the molecular diagnosis of a large kindred with CPD. The proband showed bilateral patellar dislocation, with internally rotated knee valgus, severe hypoplasia and external dislocation of the patella, his father and a brother exhibited an overlapping phenotype, while his sister and the paternal aunt showed a milder phenotype with patellar dislocations in adolescence after sport-related trauma. Other signs or dysmorphisms were not present, and males were more severely affected than females, as reported for CPD.

WES allowed the identification of a novel heterozygous frameshift mutation (c.735delT) in the *TBX4* gene in all affected family members. *TBX4* encodes for a T-box transcription factor that plays a critical role in the developmental regulation of hindlimbs during embryogenesis. Heterozygous pathogenic variants in *TBX4* are associated with ischio-coxo-podo-patellar syndrome (ICPPS, #147891), an ultra-rare condition characterized by patellar aplasia/ hypoplasia, often accompanied by abnormalities of the pelvis and femur. According to our results this form of CPD may be considered the mild end of ICPPS.

Grant: LINEA2 from Department of Health Sciences/UNIMI to Miozzo M.

Conflict of Interest: None declared

P05.055.C Dual molecular diagnosis in patients with skeletal dysplasia - data from tertiary genetic center

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Introduction: We conducted a retrospective analysis of data from skeletal dysplasia database. Database contains next generation sequencing and clinical data of 168 consecutive patients with different skeletal dysplasia syndromes. Goal of the present study was to determine the frequency of patients with more than one molecular diagnosis (genetic comorbidity).

Materials and Methods: Next generation sequencing was performed for all patients.

Results: Molecular diagnosis was established for 102 out of 168 patients (60,71%). Among these patients, six patients (3,57 %) had been diagnosed with dual diagnoses. The results are presented in the table.

Patient	Clinical findings	Diagnosis 1 (skeletal displasia)	Diagnosis 2 (other diagnosis)
1	asymptomatic for osteopetrosis in 16-year-old	Osteopetrosis, autosomal-dominant (*CLCN7*)	Stargardt disease (*ABCA4* biallelic)
2	asymptomatic for Alport syndrome in 5-year-old	Mandibulofacial dysostosis Guion-Almeida type (*EFTUD2*)	Alport syndrome, autosomal dominant (*COL4A3*)
3	Osteogenesis imperfecta type XI unexpressed in the fetus	Osteogenesis imperfecta type II (*COL1A2*)	Osteogenesis imperfecta type XI (*FKBP10* biallelic)
4	complex phenotype of both diseases	Mandibulofacial dysostosis Guion-Almeida type (*EFTUD2*)	Rett syndrome (*MECP2* in girl)

Table a. continued

Patient	Clinical findings	Diagnosis 1 (skeletal displasia)	Diagnosis 2 (other diagnosis)
5	complex phenotype of both diseases	Joubert syndrome type 6 (*TMEM67* biallelic)	Williams-Beuren syndrome (7q11.23 microdeletion)
6	complex phenotype of both diseases	Split-foot malformation with mesoaxial polydactyly (*MAP3K20* biallelic)	Deafness autosomal recessive type 1A (*GJB2* biallelic)

Conclusion: In the present study, dual diagnosis was present in 3,57% patients with skeletal dysplasia. Genetic comorbidity among patients with skeletal dysplasia is not negligible and it should be considered in patients with complex phenotypes.

Conflict of Interest: Marija Mijovic full time, Goran Cuturilo full time, Jelena Ruml Stojanovic full time, Aleksandra Miletic full time, Brankica Bosankic full time, Hristina Petrovic full time, Bojana Vasic full time, Nadja Vukasinovic full time

P05.056.D National French retrospective cohort of 22 individuals with kyphoscoliotic Ehlers-Danlos syndrome: emphasis on vascular involvement

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Background: kyphoscoliotic Ehlers-Danlos syndrome (kEDS, MIM 225400 and 614557) is caused by biallelic pathogenic variants in *PLOD1* (lysylhydroxylase 1) or *FKBP14* (FK506-binding peptidyl-prolyl cis-trans isomerase). kEDS associates muscle hypotonia, joint hypermobility, kyphoscoliosis and tissue fragility. Our study aimed at identifying follow-up recommendations for kEDS, especially for cardiovascular management.

Methods: our cohort was recruited through the French network of competence centers for EDS. Inclusion criterium was a molecularly-confirmed kEDS diagnosis. Data were collected thanks to a questionnaire filled in by referring physicians. This study was approved by our local ethical committee.

Results: we recruited 22 individuals (10 males; 12 females), aged 3 to 64 years. Pathogenic variants were identified in *PLOD1* in 15 cases and *FKBP14* in 7. Every individual displayed joint hypermobility. 17 had congenital hypotonia; 16 developed kyphoscoliosis and 9 underwent arthrodesis from 8 to 15 years. 3 adults presented osteoporosis, but no individual had fracture before adolescence. 1 individual had spontaneous eye globe rupture. 3 presented neonatal intraventricular cerebral hemorrhages. Only 2 individuals displayed early varicose veins and 1 a mild mitral and aortic valvulopathy. 7 individuals had arterial abnormalities: 2 dysplastic arteries (hepatic and vertebral) and 5 spontaneous dissections (mesenteric, vertebral, subrenal abdominal aorta and external iliac) from 16 to 60 years.

Conclusion: our study further supports vascular fragility in kEDS (23% of individuals), starting as early as adolescence. Regular noninvasive vascular explorations should be performed, probably since early age. We also underline the importance of specific assessments, including musculoskeletal complications, osteoporosis risk and eye globe fragility.

Conflict of Interest: None declared

P06

Cardiovascular Disorders

P06.001.A Comparison of knock-in mouse and hiPSC-based models of arrhythmogenic cardiomyopathy carrying the DSG2 p.Q558* mutation

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Background: Arrhythmogenic cardiomyopathy (ACM) is one of the most common inherited cardiomyopathies, characterized by progressive myocardial fibro-fatty replacement, ventricular arrhythmias and sudden cardiac death. Among the known disease genes, those encoding for the desmosomal proteins plakophilin-2

(PKP2), desmoplakin (DSP), and desmoglein-2 (DSG2) are most commonly mutated. The pathogenesis of ACM remains to be elucidated and therefore the establishment of reliable disease models is crucial.

Objective: We aim to generate and characterize the phenotype of novel hiPSC-derived cardiomyocytes (hiPS-CMs) and knock-in (KI) mice carrying the *DSG2* p.Q558* mutation.

Methods and results: hiPSCs were obtained from epithelial renal cells of an ACM patient and subsequently differentiated into hiPS-CMs, together with their isogenic controls generated by prime editing. In parallel, CRISPR/Cas9 was used to obtain both heterozygous and homozygous KI mouse models, which were all viable and fertile.

All our models showed reduced expression of DSG2 gene and deregulation of components of cardiac mechanical and electrical junctions. RNA-seq suggested alterations in genes involved in electrical transmission and in fibrosis. Moreover, the homozygous KI mice displayed an abnormally large left atrium and, under forced endurance training, left ventricular hypertrophy.

Conclusions: We provided a preliminary phenotypic characterization of novel models for ACM harboring the p.Q558* mutation in DSG2. Further analyses, such as electrophysiology studies on hiPS-CMs and histological analyses on mouse hearts, will help evaluating the presence of typical ACM features in these models and discovering the underlining pathogenic mechanisms.

Funding: DCVA2017-2018ARENA-PRIME (Dutch Heart Foundation); Foundation "De Drie Lichten" in The Netherlands.

Conflict of Interest: None declared

P06.002.B Genetically elevated abdominal fat and non-fasting triglycerides: implications for cardiovascular risk

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Background: Elevated fasting triglycerides (TG) are a risk factor for cardiovascular disease (CVD). However, non-fasting TG, which reflect the presence of remnant cholesterol, may have additional influences on CVD risk. Recently, we showed that abdominal obesity is associated with increased postprandial TG after a high-fat meal, independent of fasting TG.

Objective: We aimed to investigate the impact of abdominal obesity on non-fasting TG and the implications of elevated non-fasting TG for CVD risk.

Methods: We utilized data from the UK Biobank (application number 32683), including non-fasting (<8 hours since last meal) and fasting (≥8 hours of fasting) TG for 389,507 and 15,436 individuals, respectively. A genetic risk score for waist-hip ratio adjusted for BMI (WHR_{adjBMI}) was used to determine the effect of abdominal obesity on non-fasting and fasting TG. The association between non-fasting and fasting TG and incident CVD was assessed by Cox regression.

Results: The WHR_{adjBMI} genetic risk score had a greater effect on non-fasting (beta = 0.0073 sd/allele) than fasting TG (beta = 0.0059 sd/allele) ($P_{difference} = 0.042$). Each 1 mmol/l increase in non-fasting and fasting TG levels was associated with a 1.15-fold (95%CI 1.13 - 1.16) and 1.12-fold (95%CI 1.06 - 1.19) increase in CVD risk, respectively, adjusting for age, sex, hypertriglyceridemia treatment, and assessment center.

Conclusion: Genetic predisposition to abdominal obesity increases non-fasting TG more than fasting. The association between non-fasting TG and risk of CVD is stronger than that of fasting TG.

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Danish Diabetes Academy

Conflict of Interest: None declared

P06.003.C Angiographic and clinical findings in a cohort of patients with spontaneous coronary artery dissection and vascular Ehlers-Danlos syndrome

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Background: Spontaneous coronary artery dissection (SCAD) is an uncommon cause of acute myocardial infarction (MI) often affecting middle-aged women. It has a different pathophysiology to atherosclerotic MI and requires different evaluation and treatment. In several SCAD cases, there may be a rare underlying inherited connective tissue disorder. Vascular EDS (vEDS) appears to be one of the most common of these rare diseases. vEDS is characterised by vascular and hollow organ fragility and caused by pathogenic variants in *COL3A1*. We report on the presentation, angiographic and extra-cardiac imaging findings, and management of SCAD in a cohort with molecularly confirmed vEDS and compare to SCAD cases without a known underlying genetic aetiology.

Methods: Patient data was collected from the UK National EDS service and the SCAD registry. Individuals with a positive *COL3A1* molecular analysis report and angiographic findings confirming SCAD were included in the SCAD-vEDS cohort. Age and sex-matched controls with SCAD but no diagnosis of vEDS were used for the angiographic analysis (SCAD-nonvEDS).

Results: Data from a total of 7 patients were analysed. The age range of individuals with confirmed vEDS and SCAD was between 29-50 years with a male to female ratio of 1:2.5. There were no clear differences in location, nature (multi-vessel, multi-segment) and severity between SCAD-vEDS and SCAD-nonvEDS patients.

Conclusions: A diagnosis of vEDS predisposes to SCAD occurrence. There are no differences between angiographic findings in individuals with SCAD and vEDS versus no known heritable connective tissue diagnosis. Larger numbers through international collaboration will help validate these observations.

Conflict of Interest: None declared

P06.004.D Genetic variants predisposing to cardiac hypertrophy in sudden cardiac death cases with myocardial fibrosis and hypertension

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Background/Objectives: Sudden cardiac death (SCD) is an important mortality cause in Western countries. One common underlying pathology of SCD is hypertrophic cardiomyopathy (HCM), which usually causes accumulation of fibrotic tissue in the myocardium. Our objective was to identify new candidate variants contributing to hypertensive HCM in SCD victims.

Methods: We did whole exome sequencing from FFPE myocardial tissue samples from 96 SCD cases being part of the FinGesture study cohort (N = 5869). All cases included in the study had hypertensive HCM and myocardial fibrosis as main pathological finding at autopsy. We searched for rare variants with minor allele frequency <0.005, estimated as pathogenic, and present in ≥3 cases. Odds ratios were calculated using geographically matched controls (Finrisk dataset). We checked if the identified variants had associations with cardiac/hypertensive diseases in the Finnish National Genetic Study (FinnGen) dataset.

Results: We found 45 variants significantly associated with hypertensive HCM and SCD. Thirty-nine are missense variants, two splice-site variants, two frameshift deletions, and two in-frame deletions. Twenty-four variants are estimated to be highly pathogenic and associated with cardiac/hypertensive diseases in the FinnGen dataset. Including a frameshift deletion located in *DHRS7C*, encoding for short chain dehydrogenase/reductase 7C. This protein is highly expressed in the heart and localized in the endo/sarcoplasmic reticulum in cardiomyocytes.

Conclusions: This study reveals new genetic variants that could contribute to SCD in association with hypertensive HCM and myocardial fibrosis.

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P06.005.A Integrated systems genetics approach in human aortic smooth muscle cells identifies causal genes and networks in coronary artery disease

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Background: More than 300 loci are associated with coronary artery disease (CAD). Vascular smooth muscle cells (SMCs) play critical roles in CAD pathogenesis; therefore, we hypothesized that a subset of the CAD loci is associated with cellular and molecular phenotypes of SMCs.

Methods: We isolated SMCs from the ascending aortas of 151 heart transplant donors with diverse genetic ancestries and cultured them in quiescent and proliferative conditions. We measured the extent of migration, proliferation, calcification, and gene expression in quiescent and proliferative SMCs. We identified the quantitative trait loci (QTL) associated with cellular phenotypes, mRNA expression and splicing, and circular RNA expression. We colocalized the CAD loci with cellular and molecular QTLs.

Results: We identified 79 CAD loci associated with migration, proliferation, or calcification. 84 and 164 CAD loci were associated with mRNA expression and splicing, respectively. One sex-biased CAD locus colocalized with the sex-biased expression of TERF2IP. The most significant CAD locus, 9p21, regulated the splicing of CDKN2B-AS1 in proliferative SMCs. We also predicted MIA3 and SNHG18 as effectors transcripts in the 1q41 and 5p15 loci and showed the impact on proliferation in vitro. Further, MIA3 protein was significantly reduced in SMCs in thin fibrous caps of late-stage atherosclerotic plaques compared to early fibroatheroma with thick and protective fibrous caps in mice and humans. Finally, we identified three CAD loci associated with a circRNA transcript but not an mRNA transcript.

Conclusion: Our results predicted candidate causal genes affecting various atherosclerosis-relevant SMC phenotypes that modulate the genetic risk for CAD.

Conflict of Interest: Mete Civelek University of Virginia, Leducq Foundation Global Network of Excellence National Institutes of Health Coulter Foundation

P06.006.B Novel genetic variants associated with sudden cardiac death due to primary myocardial fibrosis

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Objective: Post-mortem investigations in SCD victims reveal that myocardial fibrosis is a common finding, as more than 90% of the SCD victims have fibrotic accumulation in the myocardium. Our aim was to identify novel candidate genes and variants associated with the presence of myocardial fibrosis in sudden cardiac death (SCD) victims.

Methods Whole exome sequencing was performed in 127 victims of SCD associated with nonischemic cardiomyopathy with primary myocardial fibrosis as the only pathological finding in autopsy. We sought rare variants (minor allele frequency <0.005) estimated to be pathogenic and present in three or more cases. Geographically matched controls were used in statistical analyses. A computational approach was used to identify protein interactions for identified genes in cardiomyocytes. Associations of the identified variants with cardiac disease endpoints were investigated in the Finnish national genetic study (FinnGen) dataset.

Results: We identified 21 missense and one nonsense variant. Heart enhanced protein interactions were identified in 16 genes. Four missense variants were highly likely to be pathogenic,

significantly associated with SCD and primary myocardial fibrosis and were also associated with cardiac diseases in Finnish population. These variants locate in cartilage acidic protein 1 (*CRATC1*), calpain 1 (*CAPN1*), unc-45 myosin chaperone A and B (*UNC45A* and *UNC45B*).

Conclusions: We identified novel variants and candidate genes predisposing to SCD associated with primary myocardial fibrosis. These variants and genes contribute to regulation of extracellular matrix production and cardiomyocyte function.

Grant References: Academy of Finland (349331, 333284, 333349), The Finnish Foundation for Cardiovascular Research, Orion Research Foundation.

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P06.007.C Can Metformin reduce AAA risk? A Mendelian randomisation study

Katie Saxby[1], *Frank Dudbridge*[2;3], *Tanmoy Roychowdhury*[4;5], *Derek Klarin*[6;7], *Greg Jones*[8], *Philip Tsao*[7;9;10], *Scott Damrauer*[11;12;13], *Matthew Bown*[1;2], *Christopher Nelson*[1;2]

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Background/Objectives: An abdominal aortic aneurysm (AAA) is a swelling of the main artery in the body estimated to affect 0.92% of adults (aged 30-79) worldwide. Rupture is often fatal and surgical intervention may be offered if the risk of rupture is high. There is no treatment to prevent AAA or to slow aneurysm growth aside from dietary and lifestyle recommendations. Metformin, a drug prescribed to treat type 2 diabetes, has previously been associated with a potential reduction in AAA disease risk but no causal link has been shown. Here we investigate the causal link between Metformin and AAA risk through Mendelian randomisation (MR).

Methods: We conducted a two-sample MR analysis using genetic variants associated with gene expression of five Metformin drug targets that also show a genetic association with decreased glycated haemoglobin (HbA1c) levels. Effect sizes are obtained from within UK Biobank for HbA1c, and within AAAgen for AAA risk, a multi-ancestry meta-GWAS analysis of 39,221 cases and 1,086,107 controls.

Results: We identified statistically significant evidence of a causal association between a genetic proxy for Metformin action and a decrease in AAA risk, OR = 0.58 (95%CI: 0.37-0.90 p = 0.015). We estimate that on average a one standard deviation decrease in HbA1c, measured via Metformin gene targets, reduces AAA risk by over 40%.

Discussion: Metformin use in those at increased risk of AAA may reduce incidence of disease. Clinical trials are required to assess the efficacy of Metformin in reducing disease risk.

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P06.008.D Deep phenotyping and comprehensive genomic studies in human congenital heart defects

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Background/Objectives: Congenital heart defects (CHD) affect 2.3 to 9.3 per 1000 live births resulting in mortality (30%) and morbidity (10%). Genetic heterogeneity and environmental factors complicate the diagnosis. This study investigates deep phenotyping and genomic analyses in CHD to improve the diagnosis and management.

Methods: Families affected with CHD are recruited in this study with an informed consent. Perinatal evaluation along with detailed examination of heart is carried out in abortus referred for autopsy. Postnatal evaluation with echocardiography is performed in neonates and children. Appropriate genomic studies are performed in them.

Results: We recruited 37 families (23 fetuses and 14 neonates/children; 20 females and 17 males) with CHD. Gestational age of the fetuses ranges from 13-22 weeks. There are children from day

one to 18 years of age. A chromosomal abnormality is detected in nine individuals (9/25, 36%) (aneuploidy-3 and unbalanced chromosomal rearrangement-6) through chromosomal microarray/karyotype. Monosomy 19 is identified in an abortus.

Singleton/trio-exome sequencing is performed in 17 families. A monogenic disorder is noted in four (4/12) families [MAP3K7 (cardiospondylocarpofacial syndrome), DNAH5 (ciliary dyskinesia, primary, 3, with or without situs inversus), FBN1 (Marfan syndrome) and LIFR (Stuve-Wiedemann syndrome)]. Two families (2/12) have variants of uncertain significance in CHD7 (CHARGE syndrome) and TBX3 (Ulnar-mammary syndrome) each, inherited from an unaffected parent. Remaining families are non-diagnostic.

Conclusion: Overall, we attained diagnosis in 15/22 (68%) families. Further analysis is underway for remaining families. This data represents the preliminary results of an ongoing study.

Grant References: Department of Biotechnology, India (BT/PR40007/MED/97/490/2020).

Conflict of Interest: Shalini Nayak Full time, Principal investigator - Department of Biotechnology, Government of India (BT/PR40007/MED/97/490/2020), Subrahmanya Vasishta: None declared, Akhila Vasudeva Full time, Akkatai S Teli Full time, Anju Shukla Full time, Dhanya Lakshmi Narayanan Full time, Siddaramappa J Patil Full time, Leslie Edward S Lewis Full time, Suneel C Mundkur Full time, Rama Rao Damerla Full time, Co-investigator - Department of Biotechnology, Government of India (BT/PR40007/MED/97/490/2020), Gunjan Banga Co-investigator - Department of Biotechnology, Government of India (BT/PR40007/MED/97/490/2020), Katta Girisha Full time, Co-investigator - Department of Biotechnology, Government of India (BT/PR40007/MED/97/490/2020)

P06.009.A Somatic mutations reveal clonal cell populations in atherosclerotic plaques

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In recent years, the potential involvement of clonal cell populations in atherosclerosis has emerged. Evidence from independent research groups unambiguously showed that smooth muscle cells clonally expand in experimental atherosclerosis, and clonal hematopoiesis of indeterminate potential (CHIP) was identified as a novel independent risk factor for atherosclerotic cardiovascular disease. However, whether clonal cell populations contribute to human atherosclerotic lesions remains elusive.

In this study, we performed deep whole-exome sequencing of 32 segments from 14 carotid plaques of patients undergoing carotid endatherectomy. We unveiled a landscape of somatic mutations confined to plaque tissue (*i.e.*, not detected in patient-matched buffy coats). Based on variant allele frequencies, we estimated that individual locally expanded clones contributed with up to 15% of plaque segment cell content. In addition, seven patients were CHIP carriers and in several of these patients, hematopoietic clones comprised over 30% of the cell population of plaque segments.

Taken together, we provide evidence that somatic mutations and clonal cell populations (expanded locally or invading from the circulation) are inherent features of atherosclerosis.

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Conflict of Interest: None declared

P06.010.B Comparing the drug target effects profile of Cholesteryl ester transfer protein (CETP) between East-Asian and European ancestries

Diana Dunca[1], *sandesh chopade*[2], *maria gordillo-maranon*[1], *aroon hingorani*[1], *Karoline Kuchenbäcker*[1], *Chris Finan*[1], *Amand Schmidt*[1;3]

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Background: Inhibition of cholesteryl ester transfer protein (CETP) has been originally evaluated for raising HDL-C for efficacy against coronary heart disease (CHD). Drug target Mendelian randomization (MR) studies have shown that CETP inhibition reduces CHD risk in Europeans, but not in East-Asians, questioning the value of pharmacological CETP inhibition in these subjects. Here, we compared drug target MR effects of CETP inhibition in Europeans and East-Asians, focusing on cardiometabolic efficacy outcomes and relevant safety outcomes.

Methods: We used ancestry-specific GWAS summary statistics for Europeans (n = 1,320,016) and East-Asians (n = 146,492) from the Global Lipids Genetics Consortium. Colocalization was employed to determine potential trans-ancestry signals between *CETP* variants for HDL-C and LDL-C. Subsequently, drug target MR was used to estimate ancestry-specific effects of on-target *CETP* inhibition on 11 plasma biomarkers and 15 cardiometabolic and non-cardiometabolic outcomes between East-Asians and Europeans. Differences between ancestries were evaluated using interaction tests, applying a multiplicity corrected alpha of 1.9×10^{-3}.

Results There was strong support (posterior probability: 99.8%) of a shared causal *CETP* variant affecting HDL-C in both populations. Given the absence of an LDL-C signal in East-Asians, similar colocalization was not observed for LDL-C. Employing drug target MR weighted by HDL-C, we found that lower CETP had more pronounced decreasing effects in Europeans on LDL-C, LP[a], systolic blood pressure and pulse pressure (interaction p-values $> 1.9 \times 10^{-3}$). Lower CETP protected against CHD, angina, and heart failure in both ancestries.

Conclusion: In conclusion, CETP-inhibition is anticipated to prevent cardiovascular disease in both European and East-Asian populations.

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P06.011.C Complete loss of the atrial natriuretic peptide-converting enzyme CORIN leads to LA-CHAF syndrome: left atrial cardiomyopathy, hypertension, arrhythmia and fibrosis

Hagit Baris Feldman[1;2], *Hofit Gadot*[1], *David Zahler*[2;3], *Adi Mory*[1], *Galit Aviram*[4], *Emil Elhanan*[1;5], *Gabriella Sheffer*[6], *Ilana Goldiner*[6], *Yam Amir*[1;2], ***Alina Kurolap***[1], *Jacob N. Ablin*[2;7]

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Background: The *CORIN* gene encodes for the atrial natriuretic peptide (ANP)-converting enzyme, critical for ANP activation and blood pressure homeostasis. *Corin-/-* mice exhibit hypertension and cardiomyopathy, yet congenital complete CORIN-deficiency has not been observed in humans to date. We describe two siblings with *CORIN* loss-of-function, presenting with left atrial cardiomyopathy, hypertension, arrhythmia and fibrosis (LA-CHAF). This provided us with the unique opportunity to study this signaling pathway and clinical implications of CORIN loss.

Methods: Both siblings were genetically evaluated by exome sequencing. Follow-up assays were performed on plasma collected from Patient 1 compared to healthy controls, and individuals with hypertension or atrial fibrillation. Plasma levels of CORIN, NT-proANP, and the fibrosis biomarkers PICP and TIMP-1, were measured. To assess whether plasma from a CORIN-deficient patient fails to induce cGMP production and regulate ENaC levels, we incubated HEK293 cells with plasma-supplemented culture medium and measured the downstream products.

Results: Both siblings are homozygous for the *CORIN* c.684dupG variant, resulting in no observable plasma CORIN and NT-proANP levels. HEK293 cells incubated with patient plasma produced cGMP levels comparable to healthy controls; however, they exhibited higher levels of β-ENaC. PICP, but not TIMP-1, is elevated in the patient's plasma.

Conclusions: Our finding show that complete CORIN loss leads to LA-CHAF syndrome, highlighting the importance of CORIN for normal ANP activity, as well as for cardiovascular and left atrial health. These findings suggest that increasing soluble CORIN may have therapeutic potential for cardiovascular disease patients who do not respond to other treatments.

Conflict of Interest: None declared

P06.012.D Polygenic risk in familial dilated cardiomyopathy

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Background: Dilated Cardiomyopathy (DCM) is characterised by left ventricular dilation associated with systolic dysfunction and is a common cause of heart failure. Currently, pathogenic variants are identified in 20-40% of patients with familial DCM while most patients have an unknown cause. Here, we aimed to assess polygenic contribution to familial DCM and to assess polygenic inheritance of common DCM co-morbidities.

Methods: Polygenic risk scores (PRS) for indexed left ventricular end-systolic volume, (DCM-PRS), were calculated in two cohorts of familial DCM patients (Cohort_1 = 76 probands and Cohort_2 = 124 probands). Additionally, PRS was calculated for co-morbidities including hypertension, obesity, Type 1 and Type 2 Diabetes, and alcohol use disorder. PRS results were distributed against a healthy control population (n = 3,831).

Results: Cohort_1 had a significantly higher DCM-PRS than the healthy controls (64th percentile median; $P < 0.001$). These findings were replicated in Cohort_2 (59th percentile median; $P < 0.01$). Further, in both Cohorts, the mean DCM-PRS in probands with pathogenic/likely pathogenic variants was not significantly different from controls ($P = 0.128$; $P = 0.184$), while those without a clear monogenic cause had a significantly higher PRS ($P < 0.001$; $P = 0.025$). Finally, co-morbidities hypertension and Type 2 Diabetes appear to have an association with polygenic inheritance in DCM patients.

Conclusion: The results presented here suggest that the collective effects of common variants may be a key determinant of DCM in a subset of families, particularly in those in whom a causative rare variant has not been identified. Additionally, this data suggests that some common DCM co-morbidities may be influenced by polygenic inheritance.

Conflict of Interest: None declared

P06.013.A Identification of medically important genomic variants related to cardiovascular phenotypes in "healthy" adults

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Clinical exome sequencing can potentially detect incidental and secondary findings (SFs) unrelated to the indication for ordering the sequencing but of medical value for patient care.

As pathogenic variants in genes associated with cardiovascular phenotype carry a risk of sudden death, reporting identified variants associated with these genes is critical for the patients and their family members.

We aimed to examine the detection rate of medically important genomic secondary findings related to cardiovascular phenotype in 952 parents of probands undergoing clinical trio exome sequencing testing due to various pediatric diseases.

Methods: Cardiovascular disease-related SFs were actively searched for in the parents as part of ES data analysis performed in Beilinson hospital between 06/2019 and 12/2022. Variants were filtered by frequency, mode of inheritance, ClinVar classification, presence in local disease-causing variant databases, and protein-truncating effect.

Results: Cardiovascular disease-related SFs were found in 19/952 individuals (2%), including 6 truncating variants in *TTN* gene, 2 *TTR* variants and other variants in 10 additional genes related to different types of cardiomyopathies and channelopathies.

Reporting pathogenic variants in "healthy" individuals raises medical and ethical dilemmas. Presymptomatic testing, difficulty in reaching a consensus on disease severity, lack of information regarding disease penetrance, potential medical implications for children already born, medico-legal aspects, and stigmatization are only part of the complexity. Nevertheless, since the first presenting symptom of these disorders might be a life-threatening event, the advantages of reporting these variants and referring the patients and their family members to cardiological follow-up might overweight the disadvantages.

Conflict of Interest: None declared

P06.014.B Sexual dimorphism in SMAD3 mutation carriers

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Objective: Individuals harbouring *SMAD3* mutations are at-risk for aneurysms/dissections throughout the arterial tree. Based on prior reports of sex differences in thoracic aortic aneurysm/dissection, we investigated whether there is sexual dimorphism for vascular events in *SMAD3* mutation carriers.

Methods: We analysed two large pedigrees with ≥ 82 individuals segregating missense mutations in the MH2 domain of *SMAD3*. The included mutation-carriers (n = 50) were subcategorized according to sex, the presence/absence of vascular lesions and the localisation of vascular involvement. For the latter, we distinguished two categories: (1) aneurysm/dissection without involvement of the aortic root/ascending aorta, (2) aneurysm/dissection affecting the aortic root/ascending aorta. We coded, using the same methodology, an independent cohort of 77 *SMAD3* mutation-carriers described by Schepers et al. and assessed the sex distribution of individuals included in the LOVD (*SMAD3*) database.

Results: In our two pedigrees, 11/29 (38%) mutation-carrying females had no vascular involvement, whereas all 21 mutation-carrying males did ($p = 0.001$). Of the 18 females with vascular involvement, 6 (33%) had vascular involvement without ascending or root aneurysm; only one of the 21 males (5%) did ($p = 0.02$). The Schepers cohort included 53 classifiable individuals: 6/21 females had vascular disease sparing the root/ascending aorta while only 1/21 male did ($p = 0.07$). The LOVD database included an overrepresentation of males ($p = 0.023$).

Conclusions: (1) non-penetrance is more common in women, (2) thoracic echocardiography in at-risk females is not as reassuring as in males in terms of risk of a) vasculopathy in other locations and b) being a transmitting female if mutation status is unknown.

Conflict of Interest: None declared

P06.015.C Study of differential expressed genes and allele specific expression in abdominal aortic aneurysm

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Background/Objectives: Abdominal aortic aneurysm (AAA) is characterized by a local progressive dilatation of the aorta on its abdominal segment that might eventually rupture. We aimed to characterize gene expression profiles of aortic AAA tissue of different diameters and types, focusing on inflammatory and atherosclerotic AAA.

Methods: We used STAR and RSEM to perform alignment and quantification of the RNAseq data of aortic tissue samples from 97 AAA patients. Additionally, to quantify allele specific expression (ASE) and study the expression differences within each individual, we used phASER on 12 individuals that had available genotype.

Results: Among the 76 differential expressed genes (DEGs) between aneurysms of different diameters, we found relevant genes for immune response (*ARID5A*, *CCL2*) and cell-cycle regulation (*DUSP8*, *LMNA*). Four genes were differentially expressed between atherosclerotic and inflammatory aneurysms (*AL3584722*, *CILP*, *C2orf49*, and *SLITRK4*). On average, each sample exhibited ASE for 530 genes (FDR<0.05) and 22 of these were shared with the DEGs identified by diameter. These genes, such as *CCDC9* or *CCL2* are being investigated for their potential causal role in aneurysm diameter development.

Conclusion: Our results found DEGs between different sizes and types of aneurysms. Most of these DEGs have been associated with AAA development in previous case-control studies. Additional ASE results allow the discovery of potential causal genes for AAA progression.

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Conflict of Interest: Gerard Temprano-Sagrera: None declared, Olga Peypoch Collaborator in the PID2019-109844RB-I00 grant that finances the project., Jaume Dilmé Collaborator in the PID2019-109844RB-I00 grant that finances the project., Begoña Soto-Carricas: None declared, Laura Calsina-Juscafresa Collaborator in the PID2019-109844RB-I00 grant that finances the project., Lluís Nieto: None declared, José Román Escudero: None declared, Mercedes Camacho: None declared, Maria Sabater-Lleal Principal investigator of the PID2019-109844RB-I00 grant that finances the project., Ana Viñuela: None declared

P06.016.D genetic causes of syndromic and non-syndromic forms of monogenic obesity: a study in 37 consanguineous families from pakistan

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Background: Recessive mutations in known obesity genes explained around 30% of the cases of severe obesity in populations with high prevalence of inbreeding, such as Pakistani population, which represents a unique opportunity to identify novel genes or variants. Recent studies suggest how polygenic background (polygenic risk scores, PRS) could modifies the penetrance of monogenic variants, demonstrating an interplay between both.

Methods: Here we assess rare variants in 41 obesity-associated genes in 34 consanguineous families form Pakistan, which comprise 37 affected children with early onset of severe obesity.

Families were classified as extreme, non-extreme or syndromic obesity based on clinical information. Whole genome sequencing (WGS) was performed for all members of the family (i.e. probands, father, mother, and healthy siblings).

Results: Based on phenotype-first approach, we identified 14, 15 and eight families with extreme, non-extreme and syndromic forms respectively. WGS revealed eight different pathogenic or likely pathogenic mutations in *LEP, LEPR, BBS10, BBS9* and *TTC8* genes. Three different variants in *LEPR*, *LEP* and *BBS9* genes were novel (c.2396-2A>G, c.-29 + 1G>C and c.113-1G>A, respectively). PRS were highest in individuals classified as extreme compared to the other phenotypic groups. This diagnostic yield of 36% and 75% extreme and syndromic forms respectively.

Conclusions: Phenotype-first approach and genotype screening with WGS results in an interesting combination to identify potential mutations in genes that cosegregated with phenotypes. We highlight the importance of combine phenotype-clinical information together with genetics to better understand the underlying bases of obesity and discover new genes, variants and identify pathways for new treatments.

Conflict of Interest: None declared

P06.017.A Expanding the evidence of a semi-dominant inheritancein GDF2 associated with Pulmonary Arterial Hypertension

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Background: Pulmonary arterial hypertension (PAH) sometimes co-exists with hereditary hemorrhagic telangiectasia (HHT). Despite being clinically diagnosable according to Curaçao criteria, HHT can be difficult to diagnose due to its clinically heterogenicity and highly overlapping with PAH. Genetic analysis of the associated genes can help to confirm or discard the presumptive diagnosis.

Methods: As part of the clinical routine and to establish a genetic diagnosis, we have analyzed a cohort of 103 patients with PAH through a customized Next Generation Sequencing (NGS) gene panel and whole exome sequencing (WES).

Results: In 2021, we published a homozygous missense variant in *GDF2* in a pediatric patient diagnosed with PAH associated with HHT and a missense variant along with a heterozygous deletion in another idiopathic PAH patient (compound heterozygous inheritance). In order to establish variant segregation, we analyzed all available family members. In both cases, parents were carriers for the variants, but neither was affected. Two years later we have detected for the first time a homozygous variant in an adult patient with suspected PVOD. In addition, we have detected an indel in heterozygosity in two twins affected with IPAH whose mother is heterozygous but healthy.

Conclusions: In total, five patients with variants in *GDF2* were identified, two in homozygous state, two in heterozygous state and one compound heterozygote. Our results expand, once again, the clinical spectrum and the inheritance pattern associated with *GDF2* pathogenic variants suggesting incomplete penetrance with variable expressivity with a semi-dominant pattern of inheritance.

Grants: PI21/01593, FCHP unrestricted grant.

Conflict of Interest: None declared

P06.018.B Single-cell dissection of the immune response after a myocardial infarction

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Background: The immune response plays a pivotal role in initiating cardiac repair and remodeling in both the acute and chronic phase after ST-Elevated myocardial infarction (STEMI). However, imbalance in the immune response can lead to excess scar tissue formation resulting in ischemic heart failure.

Methods: To dissect the longitudinal character of the immune response in STEMI patients over time, we performed single-cell RNA-sequencing (scRNA-seq) on a total of 95,995 peripheral blood mononuclear cells (PBMCs) collected from 38 STEMI patients at hospital admission, 24 hours (acute phase) and 6-8 weeks (chronic phase) after STEMI. Additionally, comparison to healthy controls was performed using previously generated scRNA-seq data on 33,878 PBMCs from 38 age- and sex-matched healthy controls from the Dutch population cohort Lifelines DEEP.

Results: Compared to healthy controls, we observed relatively more classical monocytes and less CD56dim natural killer cells in STEMI patients at admission, and these differences remained until 24 hours after STEMI. The monocytes showed the largest gene expression profile changes in STEMI patients compared to healthy controls, and in STEMI patients over time. This was associated with cytokine signaling in STEMI patients versus healthy controls, and during the acute phase of the disease. During the chronic phase, genes related to toll like receptors (TLR) signaling became more active.

Conclusions: Our analyses indicate monocytes are central players in the inflammatory response after STEMI, and that different immunological changes occur over time. These findings contribute to dissect the immune response after STEMI and aid as guidance for further therapeutic studies.

Conflict of Interest: None declared

P06.020.D De novo missense variants in RRAGC as a cause of an early-onset mTORopathy

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Background: Ras-related GTP-binding protein C (RagC), a subunit of the Rag heterodimer encoded by the *RRAGC* gene, is central to the regulation of Mechanistic target of rapamycin complex 1 (mTORC1). The activation of Rag heterodimer depends on the binding of GTP in response to the abundance of amino acids and RagC thus influences cell growth in response to nutrient homeostasis. *RRAGC* variants have previously been implicated in early-onset cardiomyopathy, with only a single case described so far.

Methods: In-depth phenotyping and trio exome sequencing was performed on individuals with fatal cardiomyopathy. Patient-derived skin fibroblasts as well as a HEK293 cell model were used to study the effects of *RRAGC* variants on the mTORC1 pathway.

Results: De novo missense variants in *RRAGC* were identified by exome sequencing in three infants who suffered from fatal dilated cardiomyopathy, hepatopathy and brain abnormalities. All reported variants were previously reported as occurring somatically in follicular lymphoma in the context of increased mTORC1 activation. Performed functional studies revealed effects of *RRAGC* variants on cell size and p70S6k (ribosomal protein S6 kinase 1) signaling. Patient-derived fibroblast additionally showed disturbed TFEB (transcription factor EB) signaling and altered subcellular TFEB localization. Findings were confirmed for all three *RRAGC* variants in a HEK293 cell model.

Conclusion: Our study establishes de novo missense variants in *RRAGC* as a cause of an early-onset fatal mTORopathy with involvement of heart, brain and liver. Functional studies show a constitutive over-activation of the mTORC1 pathway for all reported *RRAGC* missense variants.

Conflict of Interest: None declared

P06.021.A Genetic testing in severe structural congenital heart disease in Southern Alberta, Canada

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Introduction: Recent evidence has suggested the percent of patients with severe structural congenital heart disease (CHD) who carry an identifiable genetic cause may be around 17%. It remains incompletely understood how this proportion may differ among different classes of CHD, and what optimal genetic testing practices might be.

Methods: Our regional cross-sectional study analyzed 581 patients born between 2010 – 2020, inclusive, with severe CHD, defined as requiring surgery within 12 months of life. Cases were ascertained from a regional cardiac database. Cardiac defects were stratified based on the classification presented in the CDC's 2007 National Birth Defects Prevention Study. Genetic testing results were classified as abnormal if they were considered by a clinical geneticist likely to be causative. We excluded 68 patients with any aneuploidy, along with 13 further patients with insufficient records for a final study cohort of 500 patients.

Results: Genetic testing was completed on 248 of 500 (49.6%) patients, including both isolated and syndromic cases. 15% (38/248) had a diagnostic genetic result, which we refer to as the yield. Septal defects, right and left ventricular outflow tract obstruction and atrioventricular septal defects had yields of 17% (7/41), 15% (5/34), 14% (11/76), and 13% (2/15), respectively. Molecular genetic testing overall carried a considerably higher yield of 34% (18/53) compared with 10.9% for cytogenetic testing (26/239).

Discussion: This study contributes to our understanding of genetic testing practices and yields across different groupings of severe CHD. Further work will be needed to characterize any differences across these subgroups.

Conflict of Interest: None declared

P06.022.B Retrospective reclassification of variants in a cohort of patients with hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most common hereditary heart disease, mainly caused by pathogenic variants in sarcomere genes. Since the development of Next-generation sequencing (NGS) technologies, variants of uncertain significance (VUS) have been increased, resulting accurate classification a great challenge, especially in variants classified before ACMG criteria. The aim of the study was to assess the rate of reclassified variants in our cohort of 1316 HCM patients previously sequenced by Sanger or NGS.

We reassessed and reclassified all different previously identified candidate variants, following current ACMG-AMP criteria. After reclassification, those patients who had been partially sequenced by Sanger sequencing and did not carry a clearly pathogenic variant were re-sequenced using NGS.

We have identified 319 different variants in 487 carriers. Initially, 58.6% of these variants were classified as pathogenic or likely pathogenic. However, after retesting, only 41.1% were included in this category. On the other hand, 40.8% and 0.6% were classified previously as VUS and likely benign, respectively, but then the percentages were 54.5% and 4.4%. Therefore, the change of classification occurred in 22% of the variants when the current ACMG-AMP criteria were applied (54 variants were reclassified to VUS (76%), 13 to likely benign (18.3%) and 4 to pathogenic (5.6%)). After this, 282 patients were carriers of at least one pathogenic variant. Finally, we have identified 6 new carriers of a pathogenic variant in 33 re-sequenced patients (18% efficiency).

A periodic re-evaluation of variants is necessary, leading to a change of classification in a significant proportion of cases.

PI18/00719

Conflict of Interest: None declared

P06.023.C PMEPA1 mutation found in 3 Japanese families with systemic connective tissue disorders

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Several genes relating to transforming growth factor-beta (TGF-beta) signaling have been identified to be responsible for systemic connective tissue disorders such as Marfan and Loeys-Dietz syndrome (MFS/LDS). To identify new genes responsible for these diseases, exome sequencing was performed in 304 patients with MFS/LDS-like features and young aortopathy without pathogenic gene variation in known MFS/LDS genes. Patients with family history of skeletal and/or aortic features resembling MFS/LDS were identified to have a mutation in PMEPA1. Proband was a 43y tall male with funnel chest, arachnodactyly and a history of osteosarcoma. His mother and two children showed tall stature, skeletal and/or aortic phenotypes. The identified PMEPA1 variant (c.624_625insC, p.S209Qfs3*) is co-segregated with similar stature and deformities in this family members. Also, a 33y female in unrelated family, showing skeletal and aortic phenotypes, revealed to have the same mutation of PMEPA1. In addition, a 34y male in another unrelated family, showing tall stature, skeletal phenotypes with mitral valve prolapse and family history of abdominal aortic aneurysm, revealed to have the same PMEPA1 mutation. All these individuals with PMEPA1 mutation in 3 families showed MFS/LDS habitus and some had aortic diseases, co-segregated with these phenotypes. Furthermore, this particular variant was independently identified in three familial thoracic aortic aneurysms and dissections (FTAAD) pedigrees of European ancestry. Since PMEPA1 is known to suppress TGF-beta signals and promote progression of many types of cancer, PMEPA1 gene is thought to play an important role in regulation of TGF-beta signaling, causing MFS/LDS-like features as well as cancer progression.

Conflict of Interest: None declared

P06.025.A Exome-based case-control study identifies NOTCH1 variants as the major monogenic cause of CHD

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Background/Objectives: Congenital heart defects (CHDs) are the most common malformation amongst newborns. Its genetic basis is still not fully elucidated. Identifying and characterizing variants in disease-associated genes helps to increase diagnostic yields and improves genotype-phenotype correlation, risk stratification and the clinical management of patients.

Methods: In gene level enrichment testing of an exome-based case-control study (3907 CHD cases and 5157 controls using BWA tool, GATK V4.1, Hail 0.2.7 and VEP V104), *NOTCH1* showed the most robust enrichment. To further validate and expand this result, we performed association tests (Fisher´s exact) for specific domains and posttranslational modification sites in NOTCH1. The functional effects of representative variants were analyzed using a luciferase reporter assay in HEK293T-cells.

Results: *NOTCH1* variants were found in 34 cases accounting for 0.87% of the studied cohort. Besides truncating variants ($p = 1.3e\text{-}6$), alterations in disulfide-bonds in NOTCH1 were significantly enriched ($p = 0.0015$). 79% of the identified cases displayed non-syndromic CHD and 56% showed conotruncal defects, suggesting this might be the primary resulting abnormality. De novo occurrence of the *NOTCH1* variant could be proven in seven families. Functional analysis showed a complete impairment of the signaling capability of the tested nonsense variants and moderately reduced capabilities for missense variants.

Conclusion: Our study helps to strengthen the association of *NOTCH1* as the most common monogenic cause of CHD by assigning additional causally associated variants. The observed phenotypic spectrum consists primarily of conotruncal defects, with most cases showing non-syndromic CHD.

Grant References: **P**e**R**s**O**nalized Genomics for **C**ong**E**nital H**E**art **D**isease (PROCEED)

Conflict of Interest: None declared

P06.027.C Monogenic and polygenic predisposition to thrombophilia is associated with higher risk of peripheral artery disease

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Background: Recent GWAS studies have reported significant associations between loci including genes involved in thrombosis and peripheral artery disease (PAD). However, the respective contribution of monogenic vs polygenic thrombophilia to PAD remains unclear.

Study design: To address this question, we analyzed clinical and genetic data from 469,814 individuals (45-69 years old) from the UK Biobank. Exome sequencing and imputed genotyping array data were used to identify individuals with monogenic (F5 [rs6025] and F2 variants [rs1799963]) or polygenic thrombophilia (venous thromboembolism polygenic risk score [PRS-VTE] using 248 variants excluding SNPs defining F5 and F2 mutation).

Results: Monogenic F5 and F2 genetic variants were found with a higher cumulative frequency in 699 PAD cases (7.4%, $N_{cases} = 9{,}504$) compared to 38,348 in the control group (6.6%, $N_{controls} = 460{,}310$) ($P_{chi\text{-}square} = 0.003$). Carriers of these variants were significantly more likely to experience a PAD event (OR: 1.13 [95% CI: 1.04-1.22]) compared to non-carriers. In contrast, these variants were not associated with coronary artery disease (CAD) ($P = 0.72$) nor cerebrovascular disease (CVD) ($P = 0.58$). The PRS-VTE was also associated with PAD (OR: 1.05 [95% CI: 1.03-1.04], per 1SD increase in PRS).

Conclusions: Both monogenic and polygenic thrombophilia are associated with an increased risk of PAD. This study points to a particular benefit of anti-coagulants in preventing or treating PAD in a subset of patients genetically predisposed to thrombophilia.

Conflict of Interest: Katerina Trajanoska: None declared, Vincent Mooser Has received honoraria from DalGene, Has received shares from MedeLoop

P06.028.D Genetically raised dietary antioxidants and cardio-respiratory health

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Background: Observational studies of raised dietary antioxidants suggest a beneficial effect on health but the results from interventional studies generally show no effect. There are no robust studies targetting people exposed to high levels of environmental oxidants where any effects of raised antioxidants might plausibly be stronger.

Objectives: We used Mendelian randomization to explore whether raised serum antioxidants are associated with markers of cardio-respiratory health. Using continuous markers of cardio-respiratory health provided sufficient statistical precision to test for effect modification by exposure to cigarette smoke, air pollution and poor diet.

Methods: Outcome data on forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and the pulse wave arterial stiffness index was derived from UK Biobank participants. We identified single-nucleotide polymorphisms (SNPs) associated with serum ascorbate (vitamin C), retinol (vitamin A), and β-carotene from external data sources. We performed two sample Mendelian randomization analysis using exposure beta coefficients from the literature and outcome coefficients from the UK Biobank.

Results: 317,754 participants were included. We found no consistent relationship between genetically raised serum antioxidant levels and cardio-respiratory health measures (serum ascorbate $p = 0.57$, retinol $p = 0.41$, and β-carotene $p = 0.44$). There was no evidence of effect modification by levels of environmental oxidants.

Conclusions: Our findings support interventional studies showing no causal relationship between dietary antioxidants and cardio-respiratory disease outcomes. Further, our results do not support interventions to increase serum levels of ascorbate, retinol, or β-carotene in people exposed to high levels of environmental oxidants (Wellcome Grant ID: 209207/Z/17/Z).

Conflict of Interest: Azam Saied NHS, Laura Horsfall Full, Wellcome Trust Grants: 209207/Z/17/Z and 225195/Z/22/Z

P06.030.B Genetic uptake in Loeys-dietz syndrome genes is highest in spontaneous coronary artery dissection patients with extra-coronary arterial involvement

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Spontaneous coronary artery dissection (SCAD) is the prime cause of acute myocardial infarction in women below the age of 50 as well as in pregnant or postpartum mothers. To date, its genetic etiology is relatively poorly studied. We recently reported that rare variants in the known Loeys-Dietz syndrome (LDS) genes are significantly enriched in SCAD patients as compared to the general population (4.5% versus 1.5%). We aimed to validate our prior findings in an independent cohort of SCAD patients ($N = 99$) using haloplex-based gene panel sequencing of the coding regions and exon/intron boundaries of TGFB2/3, SMAD2/3 and TGFBR1/2. Only one novel heterozygous missense variant in SMAD2 (variant of unknown significance, 0.5% of alleles) was identified in the replication cohort, revealing a significantly lower uptake than what was observed in the discovery cohort (Chi2 $p = 0.02$). Both patient groups were compared with respect to the proportion of patients exhibiting connective tissue disease manifestations, fibromuscular dysplasia, extra-coronary arterial involvement, a positive family history and hypertension by means of Chi2 statistics, but no meaningful differences could be observed. Upon phenotypic comparison of the pooled variant-positive to variant-negative patients, extra-coronary artery manifestations were found to be more common in SCAD patients with extra-coronary arterial manifestations (Chi2 $p = 0.04$). Additional SCAD patients are being recruited and genetically tested to confirm this finding. Taken together, our findings suggest that the genetic uptake in LDS genes may differ between discrete SCAD endophenotypes.

Conflict of Interest: None declared

P06.031.C Exome sequencing to identify causative variants of blood clot dysfunction

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Background/Objectives: The establishment of a stable blood clot is an essential part of haemostasis and requires the conversion of fibrinogen to fibrin. Congenital fibrinogen disorders (CFDs) are a heterogenous group of disorders caused by variants in the fibrinogen gene cluster (*FGB*, *FGA* and *FGG*) that affect the quantity and/or quality of circulating fibrinogen. We aim to continue our efforts identifying and characterising variants causing CFDs.

Methods: Whole exome sequencing (WES) is used to identify causative variants in the coding regions and intron-exon junctions of *FGA, FGB* and *FGG* of patient samples. Variants are assessed for pathogenicity in silico and supported by laboratory work when necessary. Our investigations can be complemented with functional analysis of the fibrin clots formed from patient samples to support our findings.

Results: So far from 192 exomes, we have identified the causative fibrinogen gene variant in 155 individuals (79% of cases) and identified 34 pathogenic variants not previously reported.

Conclusion: WES allows for the identification of the causative variant in most individuals with a CFD however it offers low sensitivity for detecting structural variants and coverage is restricted to coding regions only. Overcoming these limitations is necessary as after WES several individuals have no identifiable causal variant despite having a clear clinical phenotype. Of additional interest is that patients with the same causative variant can have different clinical manifestations with further research required to understand how genetics plays a role in this heterogeneity.

Grant References: The Swiss National Science Foundation and the iGE3 PhD salary award

Conflict of Interest: None declared

P06.032.D Output and challenges in the revaluation of variants of uncertain clinical significance in hypertrophic and dilated cardiomyopathy

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Background: Next generation sequencing identifies variants of unknown clinical significance (VUS), which may become disease-causing when later reassessed. Our aim was to evaluate the output and the challenges encountered by reviewing VUS identified between 2016-2022 in patients with isolated hypertrophic (HCM) or dilated cardiomyopathy (DCM).

Method: A total of 68 VUS identified in 52 unrelated patients (22 DCM and 30 HCM) were reassessed according to the ACMG guidelines**. Eleven patients had more than one VUS (21%). VUS reported in patients who also had a LP/P variant during the first assessment (15/68) were also included. The reanalysis was performed by 6 different biologists. We furthermore reevaluated the genes according to the updated ClinGen assessment.

Results: Two variants in well-known sarcomeric genes, *MYPBC3* and *TNNI3* present in 2 HCM patients, were upgraded to likely pathogenic. One patient with a very severe phenotype also carried a pathogenic variant in the same gene (*MYBPC3*). Three variants were downgraded to likely benign. The other 63 variants remained VUS. Nine VUS (13%) were in genes which are not anymore considered to be evidence-based for CMD or CMH respectively.

Discussion: Our reassessment showed a total of 7% (5/68) reclassification, with 3% (2/68) being upgraded. The ACMG's criterion which allowed to upgrade them was PS3 (well-established functional studies). A major challenge is the high prevalence of rare variants (PM2) which does not allow to easily downgrade VUS to likely benign. Current efforts to improve variant interpretation might help to reduce the number of VUS and therefore minimizing uncertainty.

Conflict of Interest: None declared

P06.033.C Characteristics and cardiovascular outcomes of individuals with familial hypercholesterolaemia: A comparative study of individuals in a primary care dataset and a specialist register

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Background: It is unknown whether there are significant gaps in clinical outcomes between individuals with clinical FH in primary care, and those who have been referred to lipid clinics and under specialist management. We evaluated cardiovascular disease(CVD) outcomes in individuals with FH diagnoses in primary care compared with individuals in a specialist FH register.

Methods: We analysed electronic records of 4,311 adults with FH-diagnoses in their primary care records (from the UK Clinical Practice Research Datalink) and 2,290 adults with FH in the Simon Broome (SB) register, all free of CVD at baseline, and with linked secondary care records from 2007 to 2020. Incidence rates of composite and subtypes of CVD, and all-cause mortality rates were determined in both FH cohorts.

Results: At the time of FH-diagnosis, individuals in primary care were older (48.5years vs 40.6years), had lower levels of LDL-cholesterol (4.63mmol/l vs 7.11mmol/l), had higher prevalence of hypertension (22.3%-vs-11.2%), type 2 diabetes (6.1%-vs-1.1%), chronic kidney disease (4.9%-vs-0.5%). Primary care subjects had similar incidence rates (per 100,000 person-years) of composite CVD as the SB subjects (18.83 [16.07-19.77] vs 16.40 [15.25-17.64]), but significantly higher rates of coronary intervention procedures. All-cause-mortality rate (per 100,000 person-years) was higher in primary care FH-subjects compared to those in the SB register (9.54 [8.31-10.96] vs 3.76 [3.25-4.35]).

Conclusion: Individuals with FH-diagnoses in primary care have similar CVD incidence but worse mortality outcomes than patients with FH managed in specialist lipid clinics. This underscores the need for earlier recognition and optimal management of FH in primary care.

Conflict of Interest: Barbara Iyen: None declared, Ralph Akyea: None declared, Steve Humphries Grants from British Heart Foundation during the conduct of the study, Joe Kai: None declared, Nadeem Qureshi: None declared

P06.034.B Enhancing DCM Diagnosis through systematic CNVs Analysis: Results from a Large Cohort Study

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Background/Objectives: Dilated cardiomyopathy (DCM) is one of the most common cardiomyopathies, with a prevalence of 1/250-1,000. Despite significant advances in genetic diagnostic technologies, the yield of DCM studies is still less than 40% and the contribution of copy number variants (CNVs) to the etiology of DCM is currently unknown. This study aims to determine the percentage of disease-causing CNVs identified in a large cohort of DCM cases.

Methods: This is a retrospective study in which the presence of CNVs was evaluated in a cohort of individuals with DCM using next-generation sequencing (NGS). NGS data filtering and classification were performed using a custom pipeline. CNVs were detected using a read-depth approach.

Results: Of the 6,512 consecutive independent DCM probands sequenced by NGS we were able to evaluate CNVs in 89% (n = 5,839). A pathogenic (P) or likely-pathogenic (LP) CNV was identified in 67 patients (diagnostic yield of 1.02%). CNVs were confirmed by an orthogonal molecular technique in 52 patients (27 Sanger, 22 MLPA, 2 amplicon, and 1 dPCR). The highest number of CNVs was identified in DMD (n = 40), followed by TTN and LMNA. Only two of these patients had other variants potentially associated with DCM: a LP variant in MYH7 and a homozygous pathogenic variant in ALMS1.

Conclusion: In our DCM cohort, 1.02% of patients harbored P/LP CNVs. These results suggest that systematic CNV analysis in NGS studies improves the yield of genetic testing in DCM diagnosis.

Conflict of Interest: Iria Gómez Díaz Full-time, Ivonne Cárdenas Reyes Full-time, Luis De la Higuera Romero Full-time, Laura Cazón Full-time, Rosalía Peteiro Full-time, Marlene Perez Barbeito Full-time, Maria Sanchez Full-time, Anahi Sanluis Verdes Full-time, Guillermo Smith Ramos Full-time, Emilia Maneiro Full-time, Paula Rebolo Full-time, Paula Velez Full-time, Diego Cabrera Argaña Full-time, Xusto Fernandez Full-time, Almudena Amor Full-time, María Valverde Full-time, Soledad García Hernández Full-time, Martin Ortiz Genga Part-time, Juan Pablo Ochoa Full-time

P06.035.C Striking phenotypical differences between Ipo8 knock-out mouse models on different genetic backgrounds explored by RNA-sequencing

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IPO8 encodes importin-8, a ubiquitously expressed nuclear transport receptor of the importin-β family. Importin-8 translocates cargoes such as proteins and RNAs from the cytosol to the nucleus. We recently identified bi-allelic loss-of-function *IPO8* variants causing a syndromic form of thoracic aortic aneurysm (TAA). An Ipo8 knock-out (Ipo8-/-) mouse on a C57Bl/6N genetic background displayed root and ascending aortic aneurysms from 8 weeks of age onwards. Surprisingly, the identical Ipo8 knock-out on an Sv129 genetic background did not show any aneurysm development.

C57Bl/6N Ipo8-/- and WT, and Sv129 Ipo8-/- and WT (N = 8 per group) were sacrificed at 16 weeks. The aortic root and ascending aorta were isolated and RNA was extracted to perform RNA-sequencing

RNA-sequencing data analysis of the four groups was performed, surprisingly pointing out that the largest variation between the groups was due to the genetic background, and not due to the mutation itself. Further analysis pointed towards contributions of TGF-β signalling and cytokine-cytokine interaction to the remarkable phenotypic difference. Notably, these dysregulated pathways have been linked to TAA development before. The insights obtained in our models will further inform the controversial debate of what is TAA-driving or a secondary event

Based on the striking divergent cardiovascular phenotype of the C57Bl/6N and Sv129 Ipo8-/- strains, RNA-sequencing of aortic root and ascending aorta showed interesting differentially expressed pathways and genes, which will be investigated further. Moreover, our model emphasizes the importance of mouse genetic backgrounds in disease modelling

FWO fellowship 11J3123N

Conflict of Interest: None declared

P06.036.D An optimised protocol for isolation of single cells from mouse aorta for single-cell RNA sequencing

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Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful technique to provide insights into disease mechanisms. Pathomechanistic interrogation of aortic pathologies, due to the involvement of a heterogeneous group of cell types, especially benefits from the in-depth information that scRNA-seq provides. A critical step in the scRNA-seq protocol is the preparation of a single-cell suspension that reflects the native tissue composition. We optimized a protocol to isolate high-quality single cells from mouse aortic root and ascending aorta tissue samples. Different enzymes, concentrations, and incubation times are compared. Our results demonstrated that using a mix of 1.8 U/ml liberase, 1.4 U/ml elastase and 550 U/ml of DNase I during 1.25h is an optimal way to preserve live cells while allowing the full digestion of the tissue. The suspension is then centrifuged twice at 300g for 5 minutes. Adding 1.5% BSA is crucial to maintain viability and minimise cell loss during washing steps. Finally, the suspension is filtered through a 35 µm strainer. Cell number and viability are measured with an automated cell counter using AO/PI staining. We obtained a single-cell suspension, with minimal cell debris and clumping, of over 25,000-40,000 cells per aorta segment and a viability greater than 90%. The protocol was also tested in the Fbn1C1041G/+ mouse model (~Marfan syndrome), yielding over 50,000-60,000 cells with a cell viability of more than 90%. Overall, this protocol delivers a good number of highly viable cells from murine aortic root and ascending aorta samples for scRNA-seq experiments.

Grant reference: FWO fellowship 11I2423N

Conflict of Interest: None declared

P06.037.A Investigation of Molecular Pathways Effective in Vascular Calcification in Chronic Renal Insufficiency Patients at the Tissue Level

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Objective: Chronic renal insufficiency (CRI) is a public problem effecting cardiovascular well being and life expectancy. Vascular calcification (VC), is mineralization of vascular tree with the loss of arterial and venous flexibility. As normal end result of aging

process, VC causes vascular stiffness increasing morbidity and mortality.

Material and Methods: Herein we investigated RUNX2 and IL-6/STAT3 signalling pathways through hsa-miR-30a-5p, hsa-miR-223-3p and downstream members of these pathways using RNA PCR technique. From the waste vascular tissue of 37 patients collected during renal transplantation operation expression levels of target miRNA and genes were analyzed with qRT-PCR at the Biorad Real time PCR platform.

Results: There were 8 females and 29 male patients with an average age of 36 +/− 14 years. For normalization of expression levels hsa-mir-145-5p was used as internal control which is expressed at vascular tissue at physiological condition.

Conclusion: We have detected that Hsa-miR-223-3p and Hsa-miR-30a-5p expressions were increased in vascular tissue of chronic renal failure patients effecting osteoblastic differentiation at the vascular tissue.

Conflict of Interest: None declared

P06.038.B Genetics of a rare and complexe congenital heart defect: the congenitally corrected transposition of the great arteries

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Congenitally corrected transposition of the great arteries (CCTGA), a complex and rare congenital heart disease, is laterality defect. The aim of this study is to understand the mechanisms and genes that control the alignment of cardiac chambers, and lead to the CCTGA. We analysed à cohort of 43 trios (41 sporadic cases and 2 familial cases) of non syndromic CCTGA by next generation sequencing analysis (whole-genome and whole-exome sequencing). Variant selection criteria were: rare (<1%), damaging (using SIFT and PolyPhen), the gene is known to play a role in heart morphogenesis. Under the hypothesis of Mendelian model with de novo genomic alterations, no major gene could be identified. Under the hypothesis of a complex model of inheritance with incomplete penetrance, a total gene set of 156 genes matched each of our selection criteria. The highly heterogeneous combinations of susceptibility rare variants, mostly inherited from the healthy mother and father respectively, functionally converge to give rise to the CCTGA phenotype. To prove that a given allele combination is associated with the CCTGA phenotype, we showed a highly significant enrichment of rare variants in cases, any given deleterious variant combination within the CCTGA gene set being specific to the affected individual. A replication of these results was obtained on another cohort of CCTGA simplex cases with statistical significance ($p = 0.04$, OR 1.5). These data contradict a monogenic mode of inheritance, favoring a polygenic origin of the disease where parents harbour a genetic predisposition (susceptibility alleles) and transmit this genetic risk to their offspring.

Conflict of Interest: None declared

P06.039.C Dose-effect of celiprolol in mice modelling vEDS

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Background/Objectives: Patients with vascular Ehlers-Danlos syndrome (vEDS) caused by pathogenic variants in the *COL3A1* gene are at increased risk of arterial events such as dissections and/or ruptures. In addition to preventive and emergency surgical interventions, the most important therapeutic modality is the use of antihypertensive drugs. Clinical and long-term observational studies showed the reduction of arterial events upon treatment with the beta-blocker celiprolol. Previously, we have demonstrated that celiprolol increases the reduced aortic rupture force in mice modelling vEDS. Recently, two other groups have reported conflicting results on survival of celiprolol-treated mice using different mouse vEDS models and celiprolol doses. Here, we therefore analysed the effects of different doses of celiprolol in our (i.e. the same) mouse vEDS model.

Methods: Four-week-old heterozygous *Col3a1*^{m1Lsmi} and wild-type mice of both sexes were treated with the three formerly studied doses of celiprolol for four weeks. Following treatment, plasma levels of celiprolol were measured using LC-MS/MS. The survival of animals per treatment group was also analysed.

Results: Mortality was clearly higher in heterozygous mice treated with higher doses of celiprolol compared to heterozygous mice treated with lower doses and wild-type mice. Furthermore, a trend towards higher plasma concentrations upon higher doses of celiprolol was observed.

Conclusions: There is currently no clinical consensus regarding the medical treatment of vEDS. Thus, it is important to explore the reason for the controversial results concerning the efficacy of celiprolol in mouse models of vEDS. Our results may help to clarify the clinical relevance of celiprolol in vEDS patients.

Conflict of Interest: None declared

P06.040.D FBN1 mutation profile in Korean patients with TAAD, : Data from AMC Hereditary Thoracic Aortic Aneurysm Registry

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Background: Marfan syndrome(MFS), caused by mutations in fibrillin-1(*FBN1*), is a relatively common hereditary connective tissue disorder. The thoracic aortic aneurysms and acute aortic dissections(TAAD) are the leading causes of mortality in MFS patients. This study investigated the spectrum of FBN1 variants in Korean patients with TAAD, and the genotype–phenotype correlations.

Methods: The study subject were enrolled from the Asan medical center(AMC) Hereditary Thoracic Aortic Aneurysm Registry between October 2016 and December 2021. MFS was diagnosed based on Ghent-criteria, clinical manifestations, and detection of *FBN1* mutations using Sanger sequencing, and Next-Generation Sequencing(NGS).

Results: Total 302 patients(M:F = 203:99) were enrolled in AMC Hereditary Thoracic Aortic Aneurysm Registry and family members(N = 70) were also performed FBN1 genetic test. Pathogenic variants(PV) of *FBN1* were detected in 50.3%(N = 152, M:F = 93:59). Most frequent type of PV was null variant(46.7%,

N = 71), followed by other missesnse variant(28.9%, N = 44) and cysteine changed missense variant(27.0%, N = 41). 102 patients with PV underwent cardiovascular surgery. In familial study, 20 familial memebers(28.6%) with PV underwent cardiovascular surgery. There were 5 pediatric patients, who underwent cardiovascular surgery. In these cases, 3 variants were detected in exon 30-32 and 2 variants were in intron 46-47.

Conclusion: This study shows distributions of the genetic alterations of FBN1 in TAAD patients. Not only null variants, but also missense mutations with Cysteine alteration are the causes of the cardiovascular phenotype. TAAD in pediatric patients is caused by variants of specific-loci of the FBN1. This can be helpful for genetic counseling and preventive surgery in families with Marfan syndrome.

Conflict of Interest: Sunghee Min Asan medical center, Suk Jung Choo Asan medical center, Eul Ju Seo Asan Medical Center

P07

Metabolic and Mitochondrial Disorders

P07.001.A Novel BOLA3 and WARS2 variation cause potentially lethal autosomal recessive hypertrophic cardiomyopathy – expanding the molecular and phenotypic spectrum

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Background: Hypertrophic cardiomyopathy is a relatively common manifestation of mitochondrial disorders. The exact relation between affected gene contributing to mitochondrial disorder and development of hypertrophic cardiomyopathy remains unclear. Biallelic disruption of *BOLA3* is associated with multiple mitochondrial dysfunction syndrome type 2 with hyperglycinemia (MMDS2, OMIM #614299). *WARS2* deleterious variants are associated with two clinical entities: neurodevelopmental disorder, mitochondrial, with abnormal movements and lactic acidosis, with or without seizures (NEMLAS, #617710) and parkinsonism-dystonia 3, childhood-onset (PKDYS3, #619738); hypertrophic cardiomyopathy was not previously associated with *WARS2*-dependent disorders.

Material and methods: Here we report two cases of mitochondrial hypertyrophic cardiomyopathies identified by whole exome sequencing. Both patients were identified by the search for non-sarcomeric, monogenic causes of hypertrophic cardiomyopathy. Patient 1 (male) presented early hypertrophic cardiomyopathy, leukoencephalopathy with epilepsy, and hyperglycinemia. Family history was positive to early death in older brother. Patient 2 (female) presented mild developmental delay, severe lactic acidosis and hypertrophic cardiomyopathy, and was molecularly diagnosed post mortem.

Results: Patient 1 was compound heterozygous for in trans *BOLA3* gene variants: c.170-10T>A and c.11G>A (p.Trp4*). Patient 2 was compound heterozygous for in trans *WARS2* gene variants: c.298_300del (p.Leu100del) and c.949A>G (p.Ile317Val).

Conclusions: Both variants in *BOLA3* c.170-10T>A and c.11G>A (p.Trp4*) were not reported to date, hence this study expands the molecular spectrum of *BOLA3*-associated multiple mitochondrial dysfunctions syndrome 2 with hyperglycinemia. Since hypertrophic cardiomyopathy was not previously described in patients with biallelic *WARS2* defect, this study broads the clinical manifestation of *WARS2*-dependent disorder.

Conflict of Interest: None declared

P07.002.B DTYMK deficiency causes cardiomyopathy and genomic incorporation of modified nucleotides

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We previously described DTYMK deficiency as the cause of a lethal neurodegenerative disease (Vanoevelen et el. (2021) *Acta Neuropathalogica* 143(2):245-262). DTYMK encodes the dTMPK (deoxythymidylate monophosphate kinase) enzyme which catalyzes the penultimate step in the biosynthesis of dTTP.

In this study we characterize the heart phenotype that associates the neurodegenerative aspect and shed light on the potential compensatory mechanism which allows to sustain life into the first phases of life.

We use a *dtymk* knockout zebrafish model to study the functional effect of DTYMK deficiency on cardiac structure and function. Cardiac edema progressively develops in *dtymk -/-* embryos from 3dpf onwards, leading to lethality at 4-5dpf. Cardiac structure is severely affected, leading to a significantly reduced heart rate and cardiac dysfunction. We studied cardiac morphology in detail using confocal microscopy and document functional cardiac parameters (heart rate, fractional shortening and ejection fraction) in wildtype and *dtymk -/-* embryos.

Secondly, we performed transcriptome analysis to unravel the potential compensatory mechanism. The biosynthetic pathway for dTTP is completely blocked by DTYMK-deficiency. However, both the patients and the zebrafish model develop to some extent, albeit with many problems, there must be an alternative source of dTTP. We have performed RNASeq of wildtype and *dtymk -/-* embryo's at 3dpf. Pathway analysis revealed several candidate compensatory genes in the pyrimidine biosynthesis pathway. These targets are currently being validated.

This study will shed light on the enigmatic compensatory mechanism and its functional consequence for cardiac function and provide leads for future treatment options.

Conflict of Interest: None declared

P07.003.C X-linked creatine transporter deficiency (SLC6A8): An underdiagnosed disease in females with intellectual disability

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Background: Creatine transporter deficiency (CreaTD) caused by pathogenic variants in *SLC6A8* is the second most common cause of X-linked intellectual disability. Symptoms include intellectual disability, epilepsy, and behavioral disorders and are caused by reduced cerebral creatine levels. Targeted treatment with oral supplementation is available, however the treatment efficacy is still being investigated. There are clinical and theoretical indications that heterozygous females with CreaTD respond better to supplementation treatment than hemizygous males. Unfortunately, heterozygous females with CreaTD often have more subtle and uncharacteristic clinical and biochemical phenotypes, rendering diagnosis more difficult.

Method and results: We report a new female case who presented with learning disabilities and seizures. After determining the diagnosis with molecular genetic testing confirmed by proton magnetic resonance spectroscopy (^{1}H-MRS), the patient was treated with supplementation treatment including creatine, arginine, and glycine. After 28 months of treatment, the patient showed prominent clinical improvement and increased creatine levels in the brain. Furthermore, we provide a review of the 32 female cases reported in the current literature including a description of phenotypes, genotypes, diagnostic approaches, and effects of supplementation treatment.

Conclusion: We find that supplementation treatment should be tested in heterozygous female patients with CreaTD, and a prospective treatment underlines the importance of diagnosing these patients. Since CreaTD has variable expressivity, the diagnosis should be suspected in a broad clinical spectrum of female patients and can only be made by molecular genetic testing. ^{1}H-MRS of cerebral creatine levels is valuable when confirming the diagnosis, especially when assessing variants of unknown significance.

Conflict of Interest: Malene Mejdahl Nielsen Medical Doctor at Department of Growth and Reproduction, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, Esben Thade Petersen Danish Research Centre for Magnetic Resonance, Centre for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark Section for Magnetic Resonance, Department of Health Technology, Technical University of Denmark, Kgs. Lyngby, Denmark, Christina Dühring Fenger Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre, Denmark Amplexa Genetics, Odense, Denmark, Mette Cathrine Ørngreen Center for Inherited Metabolic Diseases, Departments of Paediatrics and Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, Hartwig R. Siebner Danish Research Centre for Magnetic Resonance, Centre for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark Department of Neurology, Copenhagen University Hospital Bispebjerg and Frederiksberg, Copenhagen Denmark Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark, Hartwig R. Siebner holds a 5-year professorship in precision medicine at the Faculty of Health Sciences and Medicine, University of Copenhagen which is sponsored by the Lundbeck Foundation (Grant Nr. R186-2015-2138), Hartwig R. Siebner has received honoraria as speaker from Sanofi Genzyme, Denmark, Lundbeck AS, Denmark, and Novartis, Denmark, as consultant from Sanofi Genzyme, Denmark, Lophora, Denmark, and Lundbeck AS, Denmark, and as editor-in-chief (Neuroimage Clinical) and senior editor (NeuroImage) from Elsevier Publishers, Amsterdam, The Netherlands., Hartwig R. Siebner has received royalties as book editor from Springer Publishers, Stuttgart, Germany and from Gyldendal Publishers, Copenhagen, Denmark., Vincent Oltman Boer Danish Research Centre for Magnetic Resonance, Centre for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark, Michal Považan Danish Research Centre for Magnetic Resonance, Centre for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark, NIH grant R01 EB016089, Allan Lund Center for Inherited Metabolic Diseases, Departments of Paediatrics and Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark, Sabine Grønborg Center for Inherited Metabolic Diseases, Departments of Paediatrics and Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, Trine Bjørg Hammer Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre, Denmark

P07.005.A Genotype predicting therapeutic response in Phenylketonuria (PKU): National implementation of the Sapropterin NICE guidance

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Phenylketonuria (PKU) is a recessive metabolic disorder where phenylalanine hydroxylase (PAH) gene variants result in reduced enzyme activity. Subsequent raised blood phenylalanine can cause irreversible brain damage if untreated. NICE guidance (2021) recommended Sapropterin treatment for a subset of responding patients (age ≤21 and during pregnancy) to reduce PKU symptoms and need for a phenylalanine-restricted diet; NHSE has since extended access to all PKU patients. Sapropterin mimics the cofactor tetrahydrobiopterin (BH4) boosting residual PAH activity. Genotype is a predictor of therapeutic response, with expanding evidence in literature/databases (BioPKU).

Sapropterin responsiveness testing has been commissioned for England to address the backlog of 2000 untested patients. All PKU patients undergo genetic testing (R283) to predict treatment responsiveness; genotype and evidence is communicated to clinicians through tailored genomic reports.

Since November 2021, SWGLH has received >1200 requests from PKU patients with a biochemical diagnosis. To date, of the 867 patients reported, 11.6% of genotypes were reported as likely responsive (treatment advised), 45.1% likely unresponsive (treatment not recommended), and 42.3% unclear response (biochemical response testing may be appropriate). 1.5% of referrals (20 patients) only had one PAH variant identified; whole genome sequencing (WGS) is ongoing to determine intronic/regulatory PAH variants or rare BH4 defects. This project aims to give a

complete picture of the variant spectrum associated with PKU in England, and a better understanding of therapeutic response for these patients.

We present the genotype spectrum of this cohort and predicted therapeutic response, and case studies illustrating the impact from a patient's perspective.

Conflict of Interest: None declared

P07.006.B Whole exome sequencing revealed the genetic spectrum of monogenic diabetes mellitus in affected Bulgarian children

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Background: Monogenic diabetes (MD) comprises an increasing part of diabetes mellitus (DM), hypothesized to reach 7% of the total diabetic population. Among Bulgarian children and adolescents with DM, we assume about 2-3% frequency of MD, which are currently classified as Type 1 DM and are treated as such. In this study, we aimed to establish an accurate molecular diagnosis of this condition, which will lead to improved patient care and implementing the algorithm for DNA analysis in our patients.

Materials and methods: Whole Exome sequencing (WES) was applied for molecular diagnosis of monogenic diabetes in 15 children suspicious for MD - negative for T1DM antibodies or with syndromic manifestations.

Results: In 4 children (27%) mutations in *GCK* (MODY2 diabetes) were discovered (c.238G>T, c.821A>G, c.863+1G>A and c.1092C>A). In 2 cases *ABCC8* mutations (responsible for Hyperinsulinemic hypoglycemia) were established (c.275G>A and c.4661G>T). In 3 relatives *AGPAT2* mutation c.268delC put the diagnosis Berardineli lipodystrophy, corresponding to the clinical phenotype. In other 2 patients, we found *PPARG* mutation (c.491G>A) and new homozygote mutation in *PRIM1* (c.638+36C>G), responsible for dwarfism-immunodeficiency-lipodystrophy syndrome.

Discussion: The accurate diagnosis of MD is necessary to clarify the etiology and explain other accompanying signs, guide the most appropriate treatment, predict the clinical course, family genetic studies and prenatal diagnosis.

Conclusion: Our study is the first one in Bulgaria, revealing the molecular spectrum of MD. We detected pathogenic mutations and put genetic diagnosis in 73% of our cohort.

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Conflict of Interest: None declared

P07.007.C Prenatal diagnosis of mucopolysaccharidosis type I on hepatosplenomegaly and coarse features

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Background: Mucopolysaccharidosis type I (MPS1) is a rare (1 in 200,000 births) lysosomal disease linked to pathogenic variants in *IDUA* gene (autosomal recessive). *IDUA* codes for the α-L-iduronidase enzyme and its deficit affects lysosomal storage of dermatan sulfate and heparan sulfate. Clinical features are variable, ranging from a severe form (Hurler type) with onset before 1 year, to an attenuated form with later onset (Hurler-Scheie and Scheie types).

Case: We describe here the first description, to our knowledge, of a prenatal visceral presentation of MPS1. This was the second pregnancy of a non-consanguineous couple. At 24 gestational weeks, a hepatosplenomegaly and a long philtrum were noted on ultrasound. No hydrops was described. Chromosomal Microarray Analysis and exome sequencing were performed on amniotic fluid. Two pathogenic variants (compound heterozygosity) in *IDUA* were identified. Enzymatic analysis on cultured amniocytes confirmed diagnosis of MPS1 (deficient activity of α-L-iduronidase enzyme). The couple then opted for a medical termination of pregnancy.

Conclusion: Although the combination of hepatosplenomegaly and coarse facial features is highly suggestive of lysosomal disease, these signs tend to occur in early childhood in individuals with Hurler syndrome. This visceral involvement has never been reported antenatally in the literature. Reported prenatal diagnoses of Hurler syndrome are almost all family history screenings and a few cases of hydrops. This case also illustrates the growing impact of genome-wide studies in pregnancy timing to make informed decisions in the face of signs that are not necessarily specific.

Conflict of Interest: None declared

P07.009.A Integration of genomics and metabolomics for characterization of IEMs: A success story from a developing country

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Background/ Objectives: Inborn errors of metabolism are a heterogeneous group of rare inherited metabolic disorders. Early identification and treatment are crucial since many IEMs are treatable if diagnose. The objective is to elucidate undefined

metabolic disorders causing intellectual disability by integration of high-throughput analytical and Next Generation Sequencing approaches.

Methods: Advanced analytical techniques were used for detailed metabolite analyses and Next generation Whole exome sequencing was used for genetic analysis followed by Sanger DNA sequencing.

Results: We describe two consanguineous families from the developing country Pakistan. First family had two adult cases of GAMT deficiency that presented with a history of global developmental delay, cognitive impairments, excessive drooling, behavioural abnormalities, contractures and apparent bone deformities initially presumed to be the reason for abnormal gait. Exome sequencing reveals a homozygous nonsense variant in *GAMT*: c.134G>A (p.Trp45*) and an abnormal level of guanidino acetic acid and creatine using metabolomics approaches.

Second family has two affected individuals of Juvenile Paget disease and one affected individual also has Sterol disorder (Sitosterolemia). Affected individuals had facial dysmorphism, skeletal deformities, deafness, presence of blue sclera, short-stature, etc. Exome sequencing identified that female has pathogenic variants in two genes as: *ABCG5* p.Ser73Pro and *TNFRSF11B* p.Trp74Cys and in the index patient only one pathogenic variant, *TNFRSF11B* p.Trp74Cys was identified.

Conclusion: An effective method for finding confirmed disease-causing variations is the integrated analysis of metabolomics (GC-MS, HPLC-MS, MS-MS) and genomics (WES, WGS).

Grants references

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Conflict of Interest: None declared

P07.010.B The impact of GCK-MODY on procreation, pregnancy and fetal development

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Background: Maturity Onset Diabetes of the Young (MODY) monogenic diabetes accounts for up to 6.5% of all diabetes cases. The presence of causative variant in the glucokinase (GCK) gene is responsible, for 60-80% of cases of monogenic diabetes and 2-6% of all cases of gestational diabetes. Many pregnant women with GCK-MODY are misdiagnosed and treated as type 1 or type 2 diabetes, which may increase obstetric complications or be associated with inappropriate diabetes management. The aim of this study was to assess the impact of MODY on procreation, pregnancy and fetal development.

Methods: 78 pregnancies in 33 patients with genetically confirmed GCK-MODY were examined for obstetric and perinatal outcomes. Medical data were analysed: type of genetic variant, treatment, fertility, pregnancy complications, type of delivery and child development based on a questionnaire. Mutation status was known in 64 cases. Clinical outcomes were compared between affected (n = 33) and unaffected (n = 31) offspring.

Results: The most common pathogenic variant found in the *GCK* gene was *c.1151C>T* (15,2%). 33.3% of patients had difficulties with insulin dosing due to frequent hypoglycemia. 6.4% of pregnancies ended in miscarriage. 6.76% of pregnancies were delivered by cesarean section, including one due to fetal distress. One case was diagnosed with right aortic arch in prenatal sonography.

Conclusions: Recognizing the correct type of diabetes is crucial, especially for GCK-MODY, which involves early initiation of insulin therapy. Making correct diagnosis makes it possible to individualize treatment and reduce potential obstetric complications.

Conflict of Interest: None declared

P07.011.C Alteration of microRNAs from urinary extracellular vesicles during the course of Fabry nephropathy

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Background: Early initiation of treatment is essential for successful management of Fabry disease, but sensitive and noninvasive biomarkers of Fabry nephropathy are lacking. Urinary extracellular vesicles (uEVs) represent a promising source of biomarkers of kidney involvement. Among them, microRNAs (miRNAs) are important post-transcriptional regulators of gene expression implicated in the pathogenesis of various kidney diseases. We aimed to identify uEV-derived miRNAs involved in the development and/or progression of Fabry nephropathy.

Methods: Genetically confirmed Fabry patients and matched controls were included. RNA was extracted from uEVs by size exclusion chromatography. Using miRNA urine exosome panels, 87 miRNAs were determined in a discovery cohort. Individual qPCRs were performed on a validation cohort that included chronological samples collected over the past decade.

Results: miR-21-5p and miR-222-3p were upregulated in patients with stable renal function and in patients with progressive nephropathy compared with control subjects. In addition, miR-10b-5p, miR-30a-5p, and miR-204-5p were down-regulated in patients with progressive nephropathy compared with control subjects, however, only miR-204-5p expression significantly decreased in the chronological samples. Functional enrichment analysis revealed that dysregulated miRNAs are associated with various pathophysiological pathways in the kidney.

Conclusion: The miRNA cargo in uEVs changes with the course of Fabry nephropathy. Therefore, they might be potential biomarkers for early identification of patients with different clinical course of Fabry nephropathy and may provide a new option to prevent or attenuate its progression.

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Conflict of Interest: Tina Levstek Travel and accommodation funding from Takeda., bojan vujkovac Speakers' fees and consultancy honoraria from Sanofi-Genzyme, Takeda, Amicus, Chiesi, and Greenovation Biotech GmbH., Member of the EU Advisory Board of Fabry Registry sponsored by Sanofi Genzyme, andreja cokan vujkovac Speakers' fees from Sanofi-Genzyme and Takeda., Travela and accommodation funding from Sanofi-Genzyme and Takeda., Katarina Trebusak Podkrajsek Takeda, Sanofi, Speakers' fees from Takeda., Travel and accommodation funding from Sanofi Genzyme.

P07.012.D Bardet-Biedl Syndrome (BBS) gene in obesity and neuronal aberrations: BBS4 mediates endoplasmic reticulum stress activation

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Background: Bardet-Biedl Syndrome (BBS) is a ciliopathy syndrome associated with at least 24 BBS genes. Obesity and neuronal abnormalities are key features of BBS, both related to cellular stress conditions. The etiology of BBS obesity was initially related to central mechanisms, partly attributed to hypothalamic dysregulation of appetite and satiety. However, emerging evidence from our and others work indicates that BBS obesity is caused also by peripheral/ adipose tissue dysregulation, with both ciliary and non-ciliary related mechanisms. BBS genes are involved in cellular stress during early stages of adipocytes and retinal development, mediating BBS retinitis pigmentosa and obesity.

Methods: BBS4-silenced (SiBBS4) adipocytes (*m3T3-F442A*) and neuroblastoma (*hSH-SY5Y*) cell lines were studied for cell proliferation, differentiation and endoplasmic reticulum (ER) stress response using molecular, biochemical, and immunohistochemistry methodologies.

Results: SiBBS4 pre-adipocytes presented accelerated proliferation and differentiation rates and augmented fat accumulation. ER stress levels were augmented at early differentiation stages in parallel to BBS4 expression levels. SiBBS4 neurons and pre-adipocytes cells presented significant reduction in phospho-IRE1α and failed nuclear translocation of activated ER stress transcriptional factors. Apoptosis markers were up-regulated upon BBS4 depletion and ER-stress induction.

Conclusion: BBS4 affects neurogenesis and adipogenesis at early differentiation stages. BBS4 depletion results in reduced responsiveness to ER stress, partly explained by failure to translocate activated ER transcription factors to the nucleus, consequently leading to increased predisposition to early apoptosis. Thus, BBS4 affects obesity and neuronal anomalies through dysregulation of nuclear transport under ER stress response in neuronal and adipocyte cells during early differentiation days.

Conflict of Interest: avital horwitz patent, RUTH BIRK patent

P07.013.A Biallelic variants in MRPL49 cause combined oxidative phosphorylation deficiency characterised by pleiotropic clinical presentations

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Combined oxidative phosphorylation deficiency (COXPD) is a rare clinically and genetically heterogeneous disorder. Biallelic variants in over 50 genes have been associated with variable phenotypes consistent with defects in mitochondrial oxidative phosphorylation. Pathogenic variants in genes encoding multiple members of the mitochondrial ribosomal large and small subunits have been associated with COXPD and varied clinical presentations.

Exome and genome sequencing were undertaken on affected and unaffected individuals to identify new genes/variants responsible for COXPD in multiple families. Respiratory chain complex and proteomic analysis of fibroblasts from two affected individuals were performed. Yeast and human cell assays of mitochondrial function were undertaken to identify the effects of the disease associated variants. Fluorescent immunolabeling with high-resolution confocal imaging was performed to determine localisation in neurosensory cells.

Six unrelated families were ascertained with presentations of Perrault syndrome (primary ovarian insufficiency and sensorineural hearing loss, SNHL) to severe childhood onset of developmental delay, leukodystrophy, hypoglycaemia and seizures. Biallelic variants (homozygous missense variants in five families and compound heterozygosity for a loss of function and missense variant) in *MRPL49* were identified. Substitutions at residues 88 and 92 were identified in multiple families, implicating a potential mutational hotspot. A suite of functional assays applied to yeast and primary patient cell lines confirmed that the disease-associated variants disrupted mitochondrial function. MRPL49 localised to the murine inner ear consistent with the SNHL phenotype in affected individuals.

We describe a novel form of COXPD expanding the genetic heterogeneity of this rare, complex monogenic disorder.

Conflict of Interest: None declared

P07.014.B Genetic interaction between triglyceride levels and the risk of type 2 diabetes through time in the Spanish general population

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Background/Objectives: High levels of triglycerides have been linked to insulin resistance and type 2 diabetes (T2D). Triglyceride levels can interact with genetic variations in relation to T2D development. We had previously selected 97 SNPs and genes from data obtained by exome sequencing in a case-control study. Our aim was to study the interaction between these SNPs, T2D and triglyceride levels through time.

Methods: Around 2000 adults (Di@bet.es study, a general population study focused in T2D prevalence in Spain, with a 7.5 years follow up) were studied. The SNPs were analysed by amplicon-based NGS. Mixed generalized linear-model were used for statistical analysis.

Results: Significant interaction between triglycerides and 4 SNPs in T2D development through time were found: rs2286372 (*CACNA2D4* gene, adjusted p-value $<10^{-16}$), rs17149965 (*SCIN*, adjusted p-value 0.03), rs11088818 (*ANKRD30BP2*, adjusted p-value $<10^{-16}$) and rs397746628 (*PDIA6* and *ATP6V1C2*, adjusted p-value $<10^{-16}$). *CACNA2D4, SCIN and ATP6V1C2* are related with ion transport, which can have an important role in T2D. *PDIA6* is related with T2D through its role in the inhibition of endoplasmic reticulum stress.

Conclusion: Triglycerides play an important role in T2D risk and their interaction with different genes through time. We have identified genetic variants involved in T2D, their interaction with triglyceride and time and their modification of the T2D risk depending on triglyceride levels, a parameter used in routine clinical practice.

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P07.016.D Gyrate atrophy of the choroid and retina: novel insights in phenotype, diagnosis, and treatment outcomes

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Background: Gyrate atrophy of the choroid and retina (GACR) is an inborn error of metabolism caused by biallelic pathogenic variants in *OAT*. This leads to hyperornithinemia and secondary creatine deficiency. Clinically, patients with GACR develop chorioretinal atrophy in childhood, leading to severe visual impairment.

Current treatment is aimed at lowering plasma ornithine. However, treatment greatly affects quality of life and compliance is variable. In addition, it remains unclear whether lowering plasma ornithine leads to slowing down disease progression.

Methods: Following PRISMA guidelines, a systematic review was conducted. PubMed and Embase were searched for studies related to therapy in GACR. The patient registry was set up simultaneously, with inclusion after informed consent.

Results: The review included 33 studies, mostly case reports (n = 27). Treatments applied included dietary protein restriction, pyridoxine, and lysine, which lowered plasma ornithine to variable degrees.

15 patients are included in the Gyrate Atrophy Registry (11-57 years). Age at diagnosis varied from 0 to 35 years. Ten different pathogenic variants were identified, of which one is carried by all patients with Dutch heritage. Mean plasma ornithine at inclusion was 650 µmol/L. All patients that underwent MR spectroscopy showed cerebral creatine deficiency. 40% of patients exhibited fat free mass index <P10.

Conclusion: Current treatment modalities play a role in lowering plasma ornithine, but the long-term benefit is unclear. Our patient registry will contribute to the increasing knowledge on GACR, while simultaneously providing additional insight in the effect of current treatment.

Grant references: Metakids and Amsterdam Gastroenterology, Endocrinology & Metabolism (AGEM).

Conflict of Interest: None declared

P07.017.A Lysine reduction therapy improves neurocognitive outcomes in pyridoxine-dependent epilepsy

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Background and objectives: Pyridoxine-dependent epilepsy (PDE-ALDH7A1) is a developmental epileptic encephalopathy characterized by seizure improvement after pyridoxine supplementation. Adjunct lysine reduction therapies (LRT) reduce the accumulation of putative neurotoxic metabolites. Our objective was to examine the association between LRT and cognitive outcomes.

Methods: Participants were recruited from within the International Registry for Patients with Pyridoxine-Dependent Epilepsy 2014-2021. The primary outcome was standardized developmental test scores associated with overall cognitive ability. The relationship between test scores and treatment was analyzed with multivariable linear regression using a mixed-effects model.

Results: A total of 112 test scores from 60 participants were available. On average, treatment with pyridoxine and LRT was associated with a non-significant increase of 6.9 points (95% CI -2.7 to 16.5) on developmental testing compared to treatment with pyridoxine alone. For the sub-analysis, a total of 14 developmental testing scores were available from 8 participants. On average, treatment with pyridoxine and LRT in the first six months of life was associated with a significant increase of 21.9 points (95% CI 1.7 to 42.0) on developmental testing.

Discussion: Pyridoxine and LRT at any age was associated with mild improvement in developmental testing and treatment in

early infancy was associated with a clinically significant increase in developmental test scores (evidence level IV). These results provide insight into the mechanism of intellectual and developmental disability in PDE-ALDH7A1, emphasizing the importance of treatment in early infancy with both pyridoxine and LRT. This neurometabolic disease meets the newborn screening criteria.

Conflict of Interest: None declared

P07.018.B Investigation of branched-chain amino acid transaminase-1 (BCAT1) as a novel candidate gene and metabolic etiology of pediatric neurodegeneration

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Background: Pediatric neurodegenerative disease is a heterogeneous category of disorders characterized by the progressive loss of neurologic function that affects approximately 0.6 out of 1,000 live births. Mitochondrial dysfunction and alterations in branched-chain amino acid (BCAA) metabolism have been linked to disease pathogenesis.

Methods: Human peripheral blood mononuclear cell (PBMC)-derived monocytes were isolated from patients prior to GM-CSF differentiation and activation of M1-polarized macrophages. RNAi-mediated knockdown of BCAT1 was performed in neural progenitor cells (NPC) via lentiviral transduction. NPCs were differentiated into neurons, astrocytes and oligodendrocytes.

Results: In an undiagnosed patient cohort, we previously discovered a rare case exhibiting a neurodegenerative phenotype with biallelic missense mutations in branched-chain amino acid transaminase-1 (BCAT1), an enzyme in the BCAA catabolic pathway that facilitates reversible deamination. Our study in patient-derived activated M1 macrophages confirmed the variants disrupted protein and cellular function with a significant decrease (57.6%; $p = 0.013$) in BCAT1 and IL6 when compared to age/sex-matched controls. NPC lentiviral transduction of BCAT1 RNAi displayed a similar decrease in BCAT1 mRNA and protein expression. Following NPC differentiation, we assessed protein and mRNA levels and cell morphology over a 15-day period and observed differences in BCAT1 neurons cultured at varying densities in comparison to scrambled controls.

Conclusions: We provide one of the first investigations into the contribution of BCAT1 in pediatric neurodegeneration with significant implications for defining mitochondrial dysfunction disease mechanisms. Techniques involving integrated genomic, transcriptomic, and metabolomic analysis should be further utilized in gene discovery and the development of effective therapeutic targets.

Conflict of Interest: None declared

P07.019.C Evaluation of monogenic variants and polygenic risks in determining MODY phenotype using whole genome sequencing

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Background: At least 14 genes have been known to cause Maturity-onset diabetes of the young (MODY). Despite the availability of genetic testing only a fraction of clinically suspected cases is usually confirmed. It is also not clear to what extent type 2 diabetes (T2D) related polygenic inheritance may be responsible for the determination of MODY phenotype.

Methods: We applied whole genome sequencing (WGS) with 30x coverage to identify the pathogenic variants and evaluate the impact of the T2D polygenic risk score (PRS) distribution in the cohort of 77 suspected MODY patients and their family members.

Results: We were able to identify and confirm causative mutations (including novel variants) in 21 patients. The identified allelic variants correspond to the MODY2/*GCK*, MODY3/*HNF1a* and MODY8/*CEL* genes. For another 24 individuals, using the WGS data we identified 35 potentially pathogenic variants in MODY and T2D-related genes. From identified variants, 13 (*PAX4, GCK, MADD, IGF1, GIPR, GCKR*) have not been previously reported and inheritance patterns in the corresponding families are currently being validated. We also present a potential impact of increased T2D PRS scores on the early onset of diabetes in our cohort.

Conclusion: In this study, we have confirmed a genetic cause for a number of MODY cases and have identified several novel pathogenic and potentially pathogenic mutations. We demonstrate that WGS not only identifies monogenic variants in a large proportion of patients but also allows to estimate the extent of polygenic risk as the potential cause of early diabetes.

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Conflict of Interest: None declared

P07.021.A Pathogenic variants of the coenzyme A biosynthesis-associated enzyme phosphopantothenoylcysteine decarboxylase cause autosomal-recessive dilated cardiomyopathy

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Coenzyme A (CoA) is an essential cofactor involved in a range of metabolic pathways including the activation of long-chain fatty acids for catabolism. In humans, it involves five enzymatic steps catalyzed by four enzymes: pantothenate kinase (PANK), 4′-phosphopantothenoylcysteine synthetase (PPCS), phosphopantothenoylcysteine decarboxylase (PPCDC), and CoA synthase (COASY). Defects in three of four genes involved in the canonical CoA synthesis, except *PPCDC*, have been described, two related to neurodegeneration with brain iron accumulation (NBIA), and one associated with a cardiac phenotype.

Patients were two sisters (P1 and P2) from a nonconsanguineous family with lactic acidosis (HP:0003128), urinary excretion of dicarboxylic acids (HP:0003215), increased levels of long-chain acylcarnitine in plasma samples (HP:0045045), and dilated

cardiomyopathy (HP:0001644). Both sisters died in the first four month of life. Whole exome sequencing was performed on P2. To validate the selected candidate variants, a functional genomics analysis was performed to study CoA levels and mitochondrial function in P2-derived fibroblasts. A yeast model was also performed to analyze the pathogenicity of the variants.

We have identified biallelic variants in *PPCDC*: p.Thr53Pro and p.Ala95Val. P2-derived fibroblasts showed an absence of PPCDC protein, nearly 50% reductions in CoA levels and defects in mitochondrial respiration. Functional studies performed in yeast suggest these mutations to be functionally relevant.

We have described a new, ultra-rare severe inborn error of metabolism due to pathogenic variants of *PPCDC* with the support of the generation of specific disease models to verify the pathogenicity of new variants and to establish a genotype-phenotype relationship.

PI19/01155; BFU2017-82574-P; B2017/BMD3721

Conflict of Interest: None declared

P07.022.B 16p11.2 distal BP2-BP3 deletions and SH2B1 variants are associated with a subtype of obesity with accelerated metabolic disease

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Background/Objectives: Obesity and type 2 diabetes (T2D) are prevalent, heterogeneous conditions associated with significant morbidity. Identification of subgroups of individuals whose metabolic disease is driven by shared etiologies can inform precision medicine approaches.

Methods: Through phenome-wide association scans (PheWAS) and body mass index (BMI)-matched control analyses, we assessed the metabolic consequences of 16p11.2 BP2-BP3 deletions ($N = 184$) and mutations in encompassed *SH2B1* ($N = 94$) in the UK, Estonian, and DECIPHER biobanks.

Results: Deletion prevalence in population biobanks was ~10-fold lower than in clinically-ascertained DECIPHER. PheWAS identified twenty-three traits associated with the deletion in UK Biobank, replicating associations with BMI ($\beta = 3.9 kg/m^2$; $p = 1.3 \times 10^{-10}$) and cognitive ability (β=-2.2 points; $p = 8.6 \times 10^{-6}$). Compared to BMI-matched controls, deletion carriers presented early-onset obesity (DEL = 41%; ctrl = 23%; $p = 0.003$) and T2D (DEL = 38%; ctrl = 14%; $p = 1.8 \times 10^{-6}$) which was difficult to treat despite higher medication and replicated in the Estonian Biobank. Renal (e.g., cystatin C; $p = 6.0 \times 10^{-14}$), hepatic (e.g., alkaline phosphatase; $p = 1.9 \times 10^{-4}$), and inflammation (e.g., C-reactive protein; $p = 0.015$) biomarkers were elevated, suggesting increased rate of diabetic co-morbidities among carriers. Conversely, carriers were not at increased risk for hypertension ($p = 0.451$), cardiovascular diseases ($p = 0.351$), or dyslipidemia ($p = 0.916$), despite a significantly altered serum lipid profile. Suggestive of a causative role for *SH2B1* haploinsufficiency, the proportion of diabetics was higher among carriers of loss-of-function variants ($p = 0.035$) and Mendelian randomization suggests that decreased *SH2B1* expression increases T2D risk ($p = 8.1 \times 10^{-6}$).

Conclusion: We identify a subgroup of individuals with early and complex obesity and T2D, in whom early and targeted treatments may be particularly effective at reducing risk of long-term complications.

Conflict of Interest: None declared

P07.023.C Inborn errors of metabolism: solving old cases by combination of transcriptomic analysis and long-read sequencing

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Inborn errors of metabolism (IEM) are a large group of rare diseases most of which have a diagnostic biochemical biomarker. In most cases, between 30 and 50% of the patients remain genetically unsolved. Here we describe the application of stratified multi-omics layers to solve the most elusive cases.

Cases with biochemical or clinical diagnosis of hyperphenylalaninemia, glycogenosis, peroxisomal disorder, maple syrup urine disease or glucose brain transport were included. They were detected in the Spanish neonatal screening program or by targeted metabolomics analysis after clinical suspicion. In all cases DNA genetic analysis was inconclusive, i.e., one pathogenic variant in autosomal recessive defects or no variants were found. To increase the diagnosis rate transcriptional analysis by RNA-Seq (when possible) in combination with gene targeted long-read sequencing (TLRS) using Oxford Nanopore technology (MinION) was conducted. Functional genomic studies to determine the clinical significance of the detected variants were also applied.

Transcriptomic analysis made evident down regulation, mono-allelic expression or presence of aberrant transcripts in one specific gene related to the disease. TLRS of a specific genomic region (3 Mb) containing the potential affected gene and its annotated *cis* regulatory elements (CRE) was done. The results allowed the identification of inversions, duplications, transposable elements (i.e., LINE_L1, SVA_F) or methylation defects in the candidate genes being able to conclude the diagnosis.

IEM are an excellent example to demonstrate the successful combination of different multi-omics layers to solve the most elusive cases.

PI19/01155.

Conflict of Interest: None declared

P07.025.A Can transcriptomics be used to diagnose rare mitochondrial diseases?

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Background/Objective: Mitochondrial diseases are rare neuromuscular heterogenetic disorders that are often not easy to diagnose since they are phenotypically and genetically diverse. We explored the diagnostic potential of full transcriptome analysis on metabolic systems and pathways.

Methods: The full transcriptomes from 10 patients were analysed on a DNB-SEQ platform. The patients' samples were classified into two groups: 8 patients in Group A (peripheral blood leucocytes) and 2 patients in Group B (muscle tissue). Dr. TOM was used to characterise differentially expressed genes and understand their role in biological pathways.

Results: In Group A: *CMPK2* and *SCO2* were down-regulated whilst *FAM210B*, *ALAS2*, and *SLC25A397* were up-regulated. *ALAS2* and *SLC25A39* functioned in the haem biosynthetic process whilst *FAM210B* regulated erythrocyte differentiation. In Group B: *ACSL1, PDK4, PDP1, SLC16A1, SLC19A2* were down-regulated whilst *FASN* and *TKT* were up-regulated. *ACSL1, SLC16A1 and FASN* were associated with the lipid metabolic process. *PDK4* and *PDP1* were involved in the regulation of pyruvate dehydrogenase complex activity. *SLC19A2* encoded a thiamine transporter protein. *TKT* is involved in the glyceraldehyde-3-phosphate biosynthetic process.

Conclusion: Transcriptomics proved to be a useful tool to identify perturbed gene expression transcripts in the patients. The expression profiles acted as biological signatures. They helped to prioritise mitochondrial function target genes and understand their role on biological pathways and the development of the clinical phenotype. The candidate disease genes could be used to extend the research to family trios.

Grant Reference: Malta Government Scholarship Scheme

Conflict of Interest: None declared

P07.026.B Diagnostic yield of panel-based genetic testing in a German cohort of early onset obesity patients

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Background/Objectives: Monogenic obesity (MO) is a rare genetic condition manifested by early-onset less than 5 years in affected individuals. To date, 9 genes are well known to cause MO with most of the mutations targeting the leptin-melanocortin signaling pathway. Here, we examined a large cohort of German patients with early-onset obesity to establish the genetic diagnosis and treatment options

Methods:454 subjects with early-onset obesity were ascertained from the center for Rare Endocrine Diseases, Ulm, Germany. These cases were searched for the genetic variants in the MO genes (*LEP*, *LEPR*, *MC4R*, *SIM1*, *KSR2*, *POMC*, *PCSK1*, *NTRK2*, and *MRAP2*). Additionally, MLPA was also employed to search for the CNV variation of the *SH2B1 locus*

Results: Out of 454 cases, we identified bi- or monoallelic variants in 176 cases. Panel testing identified 23 variants of class 4 and 11 of class 5 according to the ACMG guidelines. In addition, 42 class 3 variants were observed. We also detected patients with 11 potential risk variants of obesity. In our cohort, most of the mutations were found in the following genes accordingly: *MCR4* (36 cases); *LEPR* (33 cases); *KRS2* (29 cases); *PCSK1* (29 cases); *POMC* (14 cases); *LEP* (12 cases); *NTRK2* (12 cases); *MRAP2* (7 cases) and *SIM1* (1 case). Moreover, MLPA allowed the identification of 3 cases with microdeletion of 16p11.2 locus including *SH2B1*

Conclusion: Our findings compellingly substantiate the utility of panel testing for the identification of rare variants in MO in order to potentially increase the number of patients eligible for precision medicine for obesity

Conflict of Interest: None declared

P07.028.D Hypopituitarism as a new sign of Mitochondrial complex IV deficiency nuclear type 4

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Background: Mitochondrial complex IV deficiency nuclear type 4 (MC4DN4) is an ultra-rare disorder caused by biallelic pathogenic variants in *SCO1*, which plays an important role in cytochrome C oxidase assembly.

Only six affected individuals have been reported in the literature thus far. They presented with severe encephalopathy, hepatopathy, lactate acidosis and hypertrophic cardiomyopathy. All had an early age of onset and fatal outcome within the first months of life, except for one.

Methods: We conducted a retrospective review of patients with MC4DN4 diagnosed and followed at Meyer Children's Hospital. Our aim was to describe their phenotype.

Results: We found two unrelated families with three affected individuals aged 10, 13 and 34 years, with homozygous novel missense *SCO1* variants. All patients exhibited psychomotor regression, progressive encephalopathy with absent speech and paresis, epilepsy, hepatopathy, and lactate acidosis, but no cardiomyopathy. Interestingly, hypopituitarism with short stature and tendency to obesity was diagnosed in all three patients during childhood. To the best of our knowledge, hypopituitarism has not been previously described in MC4DN4. Chromosomal microarray and exome sequencing excluded additional genetic causes of hypopituitarism.

Conclusions: Our report shows that patients with MC4DN4 can survive beyond infancy and suggests that hypopituitarism may be a newly recognized manifestation of the disorder.

Conflict of Interest: None declared

P07.029.A Organoids as a model to study altered lipid metabolism and its implications in pathology

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Introduction: Lipids are biomolecules with key cellular roles in diverse functions such as maintaining the integrity of the cellular membranes or contributing to the energy homeostasis. However, altered lipid homeostasis, by excess of lipid synthesis or external uptake or by decrease removal, they have been shown to contribute to the development of different pathologies.

Materials and Methods: We have used hepatic organoids derived from patient and control individuals from diseases associated to unbalanced lipid metabolism to characterize their intracellular lipid content. Additionally, we also performed transcriptomic analysis to expose differential expressed genes, which could either explain or be the consequence of the aforementioned results.

Results: Lipidomic and transcriptomic study in liver organoids derived from patients with alpha-1 antitrypsin deficiency and Niemann Pick type B revealed alterations in lipid homeostasis that contribute to the progression of both diseases. Lipidoma showed a dramatic increase in triglycerides and cholesterol esters, and gene expression study showed differentially expressed genes in patients and control liver organoids that are involved in a variety of biological processes such as oxidative phosphorylation, peroxisomal metabolism and lipid metabolism.. .

Conclusions: The combination of different "omic" techniques, in this experiment transcriptomic and metabolomic studies, provide complementary points of view and contribute to elucidate and better understanding of the pathophysiology mechanisms underlying diseases.

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Conflict of Interest: None declared

P07.030.B Neuropathological hallmarks of antenatal mitochondrial diseases with a corpus callosum defect

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Corpus callosum defects are frequent congenital cerebral disorders caused by mutations in more than 300 genes. These include genes implicated in corpus callosum development or function, as well as genes essential for mitochondrial physiology. However, in utero corpus callosum anomalies rarely raise a suspicion of mitochondrial disease and are characterized by a very large clinical heterogeneity. Here, we report a detailed pathological and neuro-histopathological investigation of 9 fetuses from 4 unrelated families with prenatal onset of corpus callosum anomalies, sometimes associated with other cerebral or extracerebral defects. Next generation sequencing allowed the identification of novel pathogenic variants in 3 different nuclear genes previously reported in mitochondrial diseases: *TIMMDC1*, encoding a complex I assembly factor never involved before in corpus callosum defect; *MRPS22*, a protein of the small mitoribosomal subunit, and *EARS2*, the mitochondrial tRNA-glutamyl synthetase. The present report describes the antenatal histopathological findings in mitochondrial diseases and expands the genetic spectrum of antenatal corpus callosum anomalies establishing OXPHOS function as an important factor for corpus callosum biogenesis. We propose that, when observed, antenatal corpus callosum anomalies should raise suspicion of mitochondrial disease and prenatal genetic counseling should be considered.

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Conflict of Interest: None declared

P07.031.C Exome reanalysis in adults with a 'metabolic phenotype' increased diagnostic yield with 25%

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Introduction: An UMD consortium study included 24 adult patients suspected to have an inherited metabolic disorder (IMD) who received diagnostic care but remained undiagnosed. This study aims to establish a diagnosis by reanalysis of exome data in combination with integrated genomic and metabolic analysis.

Methods: Inclusion criteria were adults with a clinical or biochemical profile compatible with IMD and no diagnosis after standard of care genetic and metabolic work-up. Patients underwent deep phenotyping, exome data were reanalyzed and untargeted metabolomics was performed. For each patient the complete dataset was analyzed and interpreted to identify the underlying cause of disease.

Results: Six out of 24 patients (25%) were diagnosed. Two brothers presenting with splenomegaly, increased lysosphingomyelin-509 and foamy macrophages were suspected to have Niemann Pick C, however, no molecular diagnosis was found. Reanalysis showed two homozygous variants in *APOE* explaining the phenotype with familial lipemic splenomegaly as diagnosis. An adult male had a multitude of physical complaints. No leads were found in diagnostic care and exome reanalysis showed a short-tandem repeat expansion in *CNBP* resulting in a myotonic dystrophy type II diagnosis. Combined exome reanalysis of three family members, sharing seizures and abnormal brain imaging as part of their phenotype, lead to the detection of a likely pathogenic variant in *EGFR*.

Conclusions: Exome reanalysis in an adult cohort resulted in a 25% diagnostic yield. This study highlights the importance of deep phenotyping in relation to variant interpretation in a multidisciplinary setting involving an IMD specialist, neurologist, clinical and molecular geneticist.

Conflict of Interest: None declared

P07.032.D The molecular and clinical characteristics of very long-chain acyl-COA dehydrogenase deficiency in king saud medical city a tertiary center experience

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Background/Objectives: To identify and analyze the clinical, biochemical, and molecular genetic data, correlation of prevalent variants, and outcomes of very Long-Chain acyl-Coa dehydrogenase (VLCAD) deficiency.

Methods: A retrospective observational cross-sectional analysis was conducted on patients with VLCAD deficiency confirmed by molecular genetic testing at (the Genetic/Metabolic Section), King Saud Medical City (KSMC), Riyadh, Saudi Arabia, from 2016 to 2023. Demographic, clinical, biochemical and molecular genetic data were abstracted from electronic hospital records.

Results: A total of 14 children had confirmed VLCAD deficiency. The mean age was 5.6 days at presentation. Clinically of the 14 patients, 10 (72%) presented with Rhabdomyolysis, Hepatomegaly in 9 (64%), cardiomyopathy in 7 (50%) and 7 (50%) with Hypoglycemia. Out of 14, three variants were detected: C.1310A>C p.(Glu437Ala) in 2 (14%), c.134C>A p.(Ser45*) in 6 (43%) and C.65C>A:p.S22X in 6 (43%). 12(86%) patients are alive, while 2(14%) are deceased. Furthermore, no significant relationship between genotype and survival ($P = 0.719$).

Conclusion: Patients mainly presented in the neonatal period either by newborn screening or clinically with metabolic decompensation and biochemical abnormalities. The onset of clinical findings, abnormal metabolic screening and molecular genetic testing, have led to timely interventions and satisfactory outcomes; in our cohort, variant c.1310A presented an excellent prognosis; moreover, in contrast to other studies, variants c.65 and c.134 had been associated with poor clinical outcomes. Indeed presented early with metabolic decompensation but had an overall improved outcome comparatively. Further long-term prospective longitudinal studies would be invaluable to understand the disease and how different variants reveal prognostic outcomes.

Conflict of Interest: None declared

P07.033.A Newborn diagnosis of pyridoxine-dependent epilepsy through a combined genetic approach

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Background: Neonatal encephalopathy is a complex disease with a long range of symptoms. Despite the multi-factorial causes, a significant proportion is related to a genetic factor. Here we report a newborn patient presenting neonatal encephalopathy with hypotonia, aciduria and hyperlactacidemia. To determine the possible underlying genetic cause, whole exome sequencing (WES) and a real-time quantitative PCR (qPCR) studies were perfomed and the combined results helped to achieve a diagnosis.

Methods: WES was performed (Illumina Novaseq 6000®) and the resulting data was processed and analyzed with an in-house bioinformatics pipelin and Agilent Alissa Interpret® software. A qPCR amplification study (Rotor-Gene Q, QIAGEN) was performed to detect duplications or deletions at target regions of the *ALDH7A1* gene.

Results: WES was initially conducted and the result identified the variant NM_001182.5:c.1507G>A p.(Gly503Ser), in apparent homozygosity, in exon 17 of the gene *ALDH7A1*. Additionally, to confirm a possible duplication in the 5q23.2 region, a qPCR for pyridoxine-dependend epilepsy was performed and, even though it excluded the duplication, it revealed the presence of the deletion c.(?_1490-1)_(*1_?)del, encompassing exons 17 and 18 of *ALDH7A1* gene, which indicated that the previously identified missense variant was actually in hemizygosity rather than homozygosity.

Conclusion: Here we report a newborn diagnosed with pyridoxine-dependent epilepsy (MIM 266100) through a combined genetic study.This rapid genetic identification is essential for a prompt treatment and an improved long-term health. Additionaly it emphasizes the importance of utilizing multiple genetic testing or more comprehensive methods to enhance the family's genetic counseling.

Conflict of Interest: None declared

P07.034.B Investigating causal associations between non-alcoholic fatty liver disease and coronary artery disease

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NAFLD is a chronic liver disease with established genetic and lifestyle risk factors commonly viewed as a hepatic manifestation of the metabolic syndrome. Coronary artery disease (CAD) is a leading cause of morbidity and mortality in NAFLD patients, however there is conflicting evidence around the causal association between CAD and NAFLD. Therefore, we investigated whether genetic NAFLD and CAD are causally associated traits. We first performed a GWAS meta-analysis for NAFLD in 10,137 cases and 702,250 controls from 4 large European datasets, UK Biobank, the eMERGE Network, the FinnGen cohort and EPoS consortium. We identified eight genome-wide significant (GWS; $P < 5.0e{-}08$) NAFLD risk loci. We found that 3 of these loci (TRIB1, TM6SF2 and APOE) colocalized with GWS CAD loci, previously identified within the CARDIoGRAMplucC4D consortium. There was a positive genetic correlation between NAFLD and CAD in the linkage disequilibrium score regression [$rg = 0.46$ ($SE = 0.05$), $P = 6.7e\text{-}18$]. Subsequently, we investigated causality of this correlation by bidirectional Mendelian randomisation (MR) analysis. There was no significant casual effect of NAFLD on CAD [IVW, $\beta = -0.02$, $SE = 0.09$, $P = 0.81$] or vice-versa [IVW, $\beta = 0.01$, $SE = 0.01$, $P = 0.29$]. This finding is suggestive of a mediatory effect of other factors, like blood lipids, that would explain the observed correlation. We conclude that our study demonstrates partial colocalization between NAFLD and CAD loci, with no evidence to support a direct causal relationship between the two conditions. We will further explore the potential mediatory effect of blood lipids via multivariable MR analysis.

Conflict of Interest: None declared

P07.035.C Application of proteomics for interpretation of NGS sequencing data in rare diseases

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There are more than 7000 rare disorders, and the number is still growing thanks to the whole exome and genome sequencing. These technologies help to uncover the molecular basis of many undiagnosed diseases, but the findings are frequently not easy to interpret and have to be complemented by functional studies. Recently, high throughput omic approaches, such as transcriptomics and proteomics, have also been implemented into the investigations of variant pathogenicity or discovery of potential candidate genes.

We have applied quantitative proteomics approach to several cases of rare diseases. One of the projects aimed to characterize the p.L394P alpha-galactosidase A (AGAL) variant that was identified in individuals with end-stage kidney disease by a screening program for Fabry disease. The proteomics showed that the presence of the mutated protein induces ER stress and unfolded protein response, specifically the ATF6 branch. Another application aimed to decipher the unusual phenotype of a female patient with intellectual impairment, severe hypercholesterolemia and very low copper level that is heterozygous for a pathogenic mutation in *OTC* gene. Whole exome sequencing detected that the patient is also compound heterozygous for variants in *AAGAB* gene. AAGAB controls the assembly of adaptor protein complexes that mediate sorting of cargo proteins in the endomembrane system, and the proteomics showed that the AP1 and AP2 proteins are significantly decreased in the patient´s fibroblasts.

Quantitive proteomics can significantly strengthen the evidence needed to confirm pathogenicity of the investigated variants and provide valuable information about the pathological mechanisms underlying the disease.

Conflict of Interest: None declared

P07.036.D Impact of birth weight on the relationship between obesity and type 2 diabetes

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Background/Objectives: Observational studies have suggested that the relationship between obesity and the risk of type 2 diabetes may be influenced by birth weight. We aimed to examine this hypothesis using data from 219,817 individuals of European-ancestry in the UK Biobank (application ID:32683).

Methods: We used a weighted polygenic score comprising 173 birth weight-increasing loci to determine participants' birth weight and measured their BMI at baseline. During a median follow-up of 12.9 years, 10,744 individuals developed incident type 2 diabetes. We employed Cox-regression analyses to examine the interaction between the birth weight polygenic score and obesity on the risk of type 2 diabetes.

Results: We found a significant negative interaction between the polygenic score and BMI with respect to the incidence of type 2 diabetes ($P = 0.019$), indicating that a lower birth weight score was associated with a stronger association between BMI and diabetes risk. Specifically, we found that obesity was associated with a 10.34-fold (95%CI 8.87, 12.05), 9.30-fold (95%CI 8.51, 10.17), or 7.42-fold (95%CI 6.38, 8.64) higher risk of type 2 diabetes in individuals with low, intermediate, or high polygenic score for birth weight, respectively, compared to individuals with normal weight in the same birth weight score category.

Conclusion: Birth weight may modify the association between obesity and the risk of type 2 diabetes. Our findings emphasize the importance of preventing excess weight gain, particularly in individuals with a low birth weight, in reducing the risk of type 2 diabetes.

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Conflict of Interest: None declared

P07.037.A Chromosome 20p11.2 deletions cause congenital hyperinsulinism via likely disruption of a FOXA2 control region

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Background: Persistent congenital hyperinsulinism is a rare, genetically heterogeneous condition characterised by dysregulated insulin secretion leading to life-threatening hypoglycaemia. For up to 50% of affected individuals, screening of the known hyperinsulinism genes does not identify a disease-causing variant. Large deletions have previously been used to identify novel regulatory regions causing hyperinsulinism.

Methods: We searched genome-wide for novel large (>1Mb) deletions in 1106 individuals with hyperinsulinism of unknown cause. Deletions were called with SavvyCNV using off-target reads from targeted next-generation sequencing data generated during routine diagnostic testing. Deletions were confirmed and fine-mapped by genome sequencing or ddPCR. Analysis of public human islet ATAC-seq and HI-C data was used to identify regulatory regions.

Results: We identified large overlapping heterozygous deletions in five individuals (range 3-8Mb) spanning chromosome 20p11.2. The deletions had arisen de novo in four probands. The deletions were not present in internal controls, the UK Biobank or the GnomAD SV database. The minimal deleted region shared between the five individuals was 2.4Mb. We identified a putative non-coding control region of *FOXA2* within this region. Heterozygous loss-of-function coding variants in *FOXA2* have previously been reported as a rare cause of hyperinsulinism.

Conclusion: We have shown that chromosome 20p11.2 deletions are a novel cause of hyperinsulinism. Our data suggests that these deletions may cause disease by dysregulating the pancreatic beta-cell transcription factor, *FOXA2*. These findings provide new insights into the regulation of *FOXA2* in the beta-cell and improve knowledge of the genetic mechanisms of hyperinsulinism.

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Jayne Houghton: None declared, Jasmin Hopkins: None declared, Michael Weedon: None declared, Vrinda Saraff: None declared, Melanie Kershaw: None declared, Engela Honey: None declared, Nuala Murphy: None declared, Amanda Ackermann: None declared, Dinesh Giri: None declared, Ana Tangari Saredo: None declared, Indi Banerjee: None declared, Khalid Hussain: None declared, Nick Owens: None declared, Sarah Flanagan PI on Wellcome Trust (223187/Z/21/Z)

P07.038.B The relationship between depression and type 2 diabetes in presence of adiposity effect

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Introduction: Depression is a common co-morbidity of type 2 diabetes (T2D). Here we assessed the causal relationships and shared genetics between them.

Material and Methods: We applied two-sample bi-directional Mendelian randomization (MR) to assess causality between T2D and depression. We investigated potential mediation effects using two-step MR. To identify shared genetics, we performed 1) GWAS, separately, and 2) multi-phenotype GWAS (MP-GWAS) of T2D (cases = 19,344, controls = 463,641) and depression, using two depression definitions– major depressive disorder (MDD, cases = 5,262, controls = 86,275) and self-reported depressive symptoms (n = 153,079) in UK biobank. We analyzed expression quantitative trait loci (eQTL) data from public databases to identify target genes in relevant tissues.

Results: MR demonstrated significant causal effect of depression on T2D (OR = 1.26[1.11-1.44], $p = 5.5 \times 10^{-4}$), but not in the reverse direction. Mediation analysis indicated that 36.5% [12.4-57.6%, $p = 0.0499$] of the effect from depression to T2D was mediated by BMI. GWAS of T2D and depressive symptoms did not identify shared loci. MP-GWAS identified seven shared loci mapped to *TCF7L2, CDKAL1, IGF2BP2, SPRY2, CCND2-AS1, IRS1, CDKN2B-AS1*. MDD was insignificant in both GWAS and MP-GWAS. Most MP-GWAS loci had an eQTL including SNPs implicating the cell cycle gene *CCND2* in pancreatic islets and brain, and insulin signaling gene *IRS1* in adipose tissue, suggesting a multi-tissue and pleiotropic underlying mechanism.

Conclusion: Our results highlight the need to prevent T2D at the onset of depressive symptoms, and to maintain a healthy weight in the context of its effect on depression and T2D co-morbidity.

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Conflict of Interest: None declared

P07.039.C PMPCA-related disease: novel patient with moderate to severe phenotype and two novel variants

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Background: The *PMPCA* gene encodes the α-subunit of mitochondrial processing peptidase (α-MPP), an enzyme responsible for cleavage of nuclear-encoded mitochondrial precursor proteins after their import into mitochondria. Mutations in this gene have been described in app. 25 patients with non-progressive or slow-progressing cerebellar ataxia with variable age of onset and severity. Typical finding was cerebellar atrophy and Leigh-like striatum changes in severe cases.

Methods: We report a patient ascertained using WES. Fibroblasts were established from patient's skin biopsy and were used for determination of α-MPP levels using western blot. Mitochondrial morphology in fibroblasts was assessed with quantification of immunofluorescent confocal microscopy images.

Results: Two novel compound heterozygous variants p.Tyr241-Ser and p.Met251Val were identified in the patient. Compared to other reported patients with *PMPCA* pathogenic variants, the patient's clinical picture corresponds to slowly progressing intermediate to severe form. He has lost his ability to walk with assistance after illness and vaccination at 16 months of age. At present time, he is able to sit, has spastic quadriparesis, developmental delay and intellectual disability. The brain imaging showed cerebellar atrophy and Leigh-like striatum, but also white matter changes. The patient fibroblasts showed decreased α-MPP level and reduction and fragmentation of mitochondrial network although the lactate level was increased only once during illness.

Conclusion: The described case extends the number of patients with slowly progressing *PMPCA*-related disease of intermediate to severe severity. Moreover, the phenotype of the patients was extended also to Leigh-like white matter changes.

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Conflict of Interest: None declared

P07.040.D Evaluating the association of biallelic OGDHL variants with a clinically heterogeneous syndrome

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Oxoglutrate dehydrogenase-like (OGDHL) is a tricarboxylic acid (TCA) cycle gene that encodes one subunit of the α-ketoglutrate dehydrogenase complex. Recently, it has been suggested that biallelic variants in *ODGHL* can contribute to a spectrum of neurological and neurodevelopmental disorders. However, some questionable findings reported have prompt a further investigation to evaluate this proposed gene-disease association.

A large screening of sequencing datasets was conducted to collect an additional cohort of individuals who carry biallelic *OGDHL* variants and presented with variable clinical phenotypes. Sequencing data was then reanalysed to identify other potential variants. Zebrafish models for *odghl* were generated to explore the pathophysiology of the previously published and newly identified *OGDHL* variants.

A total of 20 variants (8 from our cohort and 12 from published studies) were investigated. All 12 individuals from our cohort had at least one other possible candidate variant that could partially explain the clinical variability. Functional analysis of the variants

tested uncovered a wide spectrum of impact, suggesting possible pathogenicity in humans.

This approach provided robust re-classification of 11 *OGDHL* variants and suggest that the *ogdhl* zebrafish model is a useful tool for aiding more accurate molecular genetic diagnoses. Our study also rises the caution of making diagnoses based on finding rare biallelic *OGDHL* variants without taking into consideration in the ambiguity of "*OGDHL*-related disorders".

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Conflict of Interest: None declared

P07.041.A Coding and non-coding transcriptomic profile in pediatric severe obesity

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Background: The increase in children obesity is becoming a major concern for public health. Elucidating the mechanisms underlying pediatric obesity might be important to avoid detrimental consequences, that could exacerbate in adulthood, as cardiometabolic risk. This work aimed at characterizing the transcriptional profile of children's adipose tissue with overweight (OW), obesity (OB) and severe obesity (SV) with respect to normal weight ones.

Methods: Subcutaneous periumbilical adipose tissue (SAT) biopsies were collected from 20 male subjects (1-12 y.o.), divided into 4 groups: SV ($n = 2$; BMI-z score >3); OB ($n = 8$; 2<BMI-z score ≤3), OW ($n = 3$, -1 ≤ BMI-z score ≤ 2), Normal weight (NW, $n = 7$; -2 ≤ BMI-z score ≤ 1). RNA-Seq analyses, performed by the CORALL total RNA-Seq library PrepKit, were sequenced with the Illumina NextSeq500. Raw data were aligned through STAR against the Gencode GRCh38.p13 release as reference human genome. Differential expression analysis was accomplished using the DESeq2 R package. Pathways analysis was performed on g:Profiler.

Results: RNA-Seq revealed hundreds of significantly deregulated coding genes (DEGs), when comparing the SV group with the other ones. Interestingly, the deregulation broadened to non-coding genes: lncRNAs (i.e. OIP5-AS1), snoRNAs (i.e. SNORA23) and snRNAs (i.e. RNU5B-1). Coding and non-coding DEG pathways suggested their implication in mechanisms related to fatty acids and RNA metabolism.

Conclusions: Our work provides for the first time a comprehensive analysis of the different transcriptional signature in children with severe obesity and the integration of RNA-Seq results with lipidomic data will provide an even more complete overview of pediatric obesity.

Conflict of Interest: None declared

P07.043.C Gut microbiome profiles related to type 2 Diabetes, obesity and depression in the Atlas Biomed Group study

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Aims: The gut microbiome is determined by diet, medication, and host genetics. The microbiome composition is a potential predictor of the host's health status. Using the Atlas Biomed Group (ABG) dataset, we aimed to snap-shot the gut microbiome in self-reported type 2 diabetes (T2D), obesity, and depression.

Methods: 16S rRNA gene sequences from 4,548 individuals in the ABG dataset, recruited in the United Kingdom and Eastern Europe, were processed using QIIME-2 microbiome bioinformatics platform. We identified 65 individuals with T2D, 534 with obesity (BMI≥30kg/m2), and 540 with the depressive disorder from self-reports. Bacterial genera ($N = 179$) with relative abundance > 0.1 were evaluated for their association with three phenotypes using linear models in MaAsLin2 and ANCOM-BC software packages in parallel. Models were adjusted by geographical region, age, and gender and included bacterial relative abundance (RA) as the outcome.

Results: T2D status was inversely associated with RA of Terrisporobacter, Senegalimassilia, and Lachnospira and positively associated with Shigella RA (q-value<0.05) in both ANCOM-BC and Maaslin2 analyses. Terrisporobacter was also directly associated with a medical history of depressive disorder and obesity. The microbiome alpha-diversity was significantly lower (p-value<0.05) in individuals with any of these three phenotypes compared to the individuals without them.

Conclusions: Terrisporobacter and Shigella higher abundance, established for T2D, is replicated in the ABG dataset. The link between Terrisporobacter and depressive disorder should be further investigated in sensitivity analyses. Further analyses, considering physical activity, sleep duration, smoking status, and dietary patterns, will account for the environmental component of these effects.

Conflict of Interest: Maria Kardakova current or former employees or contractors of Atlas Biomed Group Limited, sergey musienko current or former employees or contractors of Atlas Biomed Group Limited., holds stock and/or stock options in Atlas Biomed Group., julien terroire: None declared, kirill danilov current or former employees or contractors of Atlas Biomed Group Limited, Vasiliki Lagou: None declared, anna popova current or former employees or contractors of Atlas Biomed Group Limited, Inga Prokopenko: None declared, Ayse Demirkan: None declared

P08

Immunology and Hematopoietic System

P08.001.A RNAseq analysis in Hymenoptera venom immunotherapy- first step towards multi-level biomarker fingerprint

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Background: Hymenoptera venom allergy [HVA] is one of the most severe allergic diseases. For patients with HVA, the only

disease-modifying treatment is allergen-specific venom immunotherapy [VIT]. Continuous VIT for 3-5 years has been shown effective in the majority of patients. No biomarker for therapy monitoring, evaluation of the efficiency or determination of endpoint has been established.

Methods: Whole blood RNA was isolated and RIN was determined using miRNeasy Mini Kit (Qiagen) and Agilent2100 Bioanalyser (Agilent Technologies, USA), respectively. Samples were sent for library preparation and sequencing to Macrogen (Ilumina TruSeq TotalRNA Ribo-Zero-Globin, NovaSeq2x100). RNAseq based whole transcriptome characterization was performed in 19 patients undergoing VIT (before and after 3-5 years). VIT efficacy was evaluated by sting challenge resulting in 14 patients successfully finishing VIT, while five patients had reoccurring allergic reaction. Characterization of differentially expressed genes [DEGs] (cut-off: p-value<0.01) and functional pathway analysis was made using CLC Genomics Workbench and Ingenuity Pathway Analysis, respectively.

Results: Comparing DEGs expressed in patients with successful VIT and in patients with unsuccessful VIT, 300 DEGs were upregulated and 50 downregulated before the treatment, and 108 DEGS were upregulated and 34 downregulated after the treatment. Pathway analysis suggests strongly activated PPAR and IL-10 signalling pathways in a group of patients with unsuccessful VIT before the treatment. A set of genes is proposed for further analysis.

Conclusion: Patients with successful VIT and patients with unsuccessful VIT have distinct gene expression in both time points of sampling. A set of biomarkers distinguishing between both groups is proposed.

Grant References:ARRS-P3-0360

Conflict of Interest: Ajda Demsar Luzar Full, Matija Rijavec Full, ARRS J3-2532, Peter Korošec Full, ARRS J3-2532 **ARRS J3-6787 ARRS J3-3626 ARRS J3-2234, Mitja Košnik Full, ARRS P3-0360, Mihaela Zidarn Full**

P08.002.B Detection and evolutionary dynamics of somatic FAS variants in autoimmune lymphoproliferative syndrome: diagnostic implications

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Somatic pathogenic variants at the *FAS* gene underly up to 20% of ALPS cases. These variants are restricted to double-negative alpha-beta T cells (DNT) which are elevated in ALPS patients but normalized under immunosuppressive treatment. Therefore, the identification of these somatic variants is a major challenge in patients under treatment and can delay the molecular diagnosis.

Here, we present a patient with early-onset ALPS in whom we identified a somatic pathogenic insertion (*FAS*:c.718_719insGTCG). For that, we used Sanger and deep amplicon sequencing (DAS) in CD3+ cells and peripheral blood. Moreover, we studied samples before and during the treatment with Sirolimus (across five years) to explore the detection limits of the technique and study the evolutionary dynamics of the somatic event.

The variant was first discovered in CD3+ enriched samples (RosetteSep™) by Sanger sequencing but was not detected in peripheral blood (7.4% DNT cells). However, using DAS, it was found in blood and CD3+ cells, even in low DNT counts (0.89%). In that scenario, we demonstrated that the variant allele frequency was doubled in CD3+ enriched samples (1.6% CD3+, 0.68% blood) and that there was an excellent correlation between DNT counts and the frequency of the variant (Pearson's R: 0.98).

Our results evidence that somatic variation is more likely to be detected on CD3+ enriched cells and pre-treatment samples but it can also be discovered in peripheral blood of patients under treatment. This highlights the success of sorting-free sequencing experiments and the importance of somatic studies in previously unsolved ALPS cases.

Conflict of Interest: None declared

P08.003.C Somatic revertant mosaicism correlating with clinical improvement in a patient with TNFRSF9 (CD137) deficiency

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Reversion mosaicism is a naturally occurring event involving a spontaneous correction of a pathogenic mutation in somatic cells. *TNFRSF9* (CD137/4-1BB) deficiency is a recently described IEI characterized by lymphocytic defects with early-onset EBV-associated lymphoma.

We report a patient who at 12yo developed severe EBV-related hemophagocytic lymphohistiocytosis. No genetic defects were found at that time and she underwent HSCT from an HLA identical brother with good engraftment. In the following 8y, she presented recurrent EBV reactivations, lymphoproliferation and EBV-associated smooth muscle tumour despite full chimerism. At 21yo, she experienced a spontaneous decrease in EBV viral load and we started an in-depth genetic study of the case in total blood and specific cell populations.

Using whole exome sequencing we identified the homozygous stop-gain variant p.R244Ter in *TNFRSF9*. Strikingly, the HSCT donor was also homozygous for this variant but without overt clinical symptoms. Sanger sequencing results of the region pointed out a possible somatic reversion event. Using deep-amplicon sequencing we confirmed the presence of two independent somatic reversion events in the patient: a second-site mutation in the same codon (STOP to missense) and a "back mutation" (STOP to wild-type). The revertants were specifically located in CD8-T cells in which single-cell RNAseq experiments are ongoing.

We report the first case of reversion mosaicism in CD137 deficiency. This reversion is probably linked to the recent control of EBV viremia and is proof of principle that sets the ground for future gene therapy strategies in this IEI.

Conflict of Interest: None declared

P08.004.D Rete IDEA: solving the unsolved diagnoses of primary immunodeficiency

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Background: Inborn errors of immunity (IEI) are genetic diseases leading to increased susceptibility to infections, autoimmunity, autoinflammation and malignancy, with complex clinical phenotypes. Some unsolved cases are selected by a multidisciplinary network of hospitals (IDEA network) to perform sequencing in trio.

Methods: Twelve IEI patients performed Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS). Our pipeline included alignment, variant calling with GATK4 and annotation with SnpEff. For WGS, copy number variants and structural variants were also analysed.

Results: Three patients (25%) carry variants in known disease-genes, three (25%) in novel putative genes and six (50%) are negative. One patient presented with combined immunodeficiency and an inherited pathogenic variant in *RAG1*, inconclusive for the diagnosis of RAG1 deficiency. Through WGS, an intergenic deletion of 145kb including *RAG1* promoter was detected, reaching the diagnosis. A second patient, with agammaglobulinemia, was diagnosed thanks to WGS reanalysis with an updated pipeline that identified a newly discovered pathogenic variant in *SPI1*, whose functional validation was recently published and for which we demonstrated incomplete penetrance. Lastly, a patient with severe autoinflammatory manifestations, was diagnosed with a frameshift variant in *TREX1*. Given his atypical phenotype, another variant in *TNFAIP3* was considered to potentially contribute to the clinical picture.

Conclusions: The diagnosis of previously unsolved cases was delayed mainly due to: presence of intergenic deletions detected by WGS, need for updated annotations following novel scientific knowledge, and unusual non-mendelian inheritance. To increase the diagnostic yield, these aspects should be considered and applied to complex cases.

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Conflict of Interest: None declared

P08.005.A Genetic diagnosis of inborn errors of immunity: a single-institution study from Korea

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Background/Objectives: Inborn errors of immunity (IEI) are rare disorders characterized by defects in the immune system. Advances in genetic technologies have enabled accurate and rapid diagnosis. This study reports the clinical experience of diagnosing patients with suspected IEI using various genetic methods.

Methods: This study included 291 patients suspected IEI who were referred for genetic analysis at Seoul St. Mary's Hospital from February 2008 to September 2020. Fluorescence in situ hybridization was used to confirm a 22q11.2 microdeletion. Sanger sequencing was conducted to analyze a single clear causative gene. Exome sequencing was performed using the TruSight™ One panel.

Results: A genetic diagnosis of IEI was made in 64 (22%) patients, as shown in Table 1. The most common causative gene identified was *TBX1*, followed by *BTK*, *WAS*, *CYBA*, *CYBB*, *ELANE*, and *NLRP3*. Four patients had novel pathogenic mutations.

Conclusion: This study demonstrates the clinical experience of genetic diagnosis for IEI patients. The results can inform genetic counseling and guide treatment decisions for affected patients.

Table 1. IEI patients and their causative genes

Categories	Genes	No. of Patients
Combined immunodeficiencies	*CD40LG, DCLRE1C, IL7R, JAK3, RAG1*	6
Combined immunodeficiencies with syndromic features	*ATM, STAT3, TBX1, WAS*	18
Predominantly antibody deficiencies	*BTK, NFKB1, PIK3CD, PIK3R1*	10
Diseases of immune dysregulation	*PRF1, SH2D1A, XIAP*	4
Congenital defects of phagocytes	*CYBA, CYBB, ELANE, FERMT3, G6PC3, HAX1*	12
Defects in intrinsic and innate immunity	*IRF7, IRF8*	2
Autoinflammatory diseases	*MEFV, NLRP3*	5
Complement deficiencies	*CFH*	1
Phenocopies of inborn errors of immunit	*KRAS*	1

Conflict of Interest: None declared

P08.006.B The anti-leukemic activity of Shikonin in Core Binding Factor (CBF) AML: in vitro and in vivo biological studies

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Background/Objectives: Acute myeloid leukemia (AML) with t(8;21) or inv(16) is commonly referred to as core-binding factor AML (CBF-AML), that are the most frequent type of de novo AML.

The mutations in CBF genes result in leukemogenic proliferation and impaired differentiation of the hematopoietic progenitors. Kasumi-1 AML cells carring t(8;21) and KIT mutation is an ideal cell model for CBF-AML. We treated Kasumi-1 cells with various phytochemicals and found that shikonin, a natural product from a Chinese herb Zicao, notably inhibited cell growth. We further examined the anti-leukemic activity of shikonin in vitro and in vivo.

Methods: Cell viability, RT-q-PCR, western blot, and microarray were performed to detect the effects of shikonin in vitro. A xenograft model using microinjection of leukemia cells into an optically clear *Absolute* zebrafish was generated to explore anti-proliferative activity of shikonin.

Results: Shikonin significantly inhibited Kasumi-1 cell viability in a time and dose-dependent manner. The expressions of both oncogene KIT and RUNX1-ETO were down-regulated by shikonin. Shikonin caused cell cycle arrest at S-phase, induced apoptosis, and promoted TNFα signaling. Moreover, the zebrafish transplantation of Kasumi-1 cells found that shikonin did not affect zebrafish development, while inhibiting cell proliferation of the CM-DiI-labeled Kasumi-1 cells that were measured using live fluorescent image and RT-q-PCR analysis.

Conclusion: We use Kasumi-1 cells and zebrafish xenograft model to demonstrate the obvious anti-leukemic effects of shikonin on CBF-AML in vitro and in vivo. Our data provide a novel finding of shikonin as a potential agent targeting CBF-AML cells.

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Conflict of Interest: None declared

P08.007.C Neonatal presentation of TNFAIP3-related autoinflammatory syndrome

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Background: *TNFAIP3* encodes a ubiquitin-editing enzyme with a critical role in controlling immune responses, mediated by NF-kappa B and TNF. Loss of function variants in *TNFAIP3* cause autosomal dominant familial Behcet-like autoinflammatory syndrome (haploinsufficiency of A20). Common manifestations are variable and can include oral, genital and gastrointestinal ulcers, skin manifestations, arthritis and uveitis in the first months-years of life. Data on neonatal presentations is scarce.

Case presentation: A two-week-old infant was admitted with fever, diarrhea, vomiting and rashes leading to hypovolemic shock. Sepsis workup was negative. His symptoms improved with conservative treatment but recurred over the following weeks. Family history included Behcet's disease and/or inflammatory bowel disease in multiple individuals, paternal psoriasis and a brother with autoimmune hepatitis and atrophic gastritis. A multigene panel identified a heterozygous deletion encompassing exons 3-9/9 in *TNFAIP3*. Endoscopic assessment demonstrated severe gastritis and duodenitis with multiple colonic ulcerations, supporting the diagnosis.

Review of the literature identified four other cases of neonatal presentations of *TNFAIP3*-related disorders. Features included febrile episodes, gastrointestinal symptoms and rashes. One case was severely affected with CNS vasculitis and pulmonary embolism, and eventually died of complications of the disorder.

Conclusion: *TNFAIP3*-related disorders can present in neonates with signs of a hyperinflammatory state and should be considered in the differential diagnosis of neonatal autoinflammatory syndrome and very early onset inflammatory bowel disease. Intestinal inflammation can occur as early as the first weeks of life. Further studies are required to better characterize the disease in this age group and explore treatment strategies.

Conflict of Interest: None declared

P08.008.D ABO allelic frequency detected by next generation sequencing in Korean patients with hematologic malignancies

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Background: Accurate determination of a patient's ABO blood group is essential for blood transfusion and transplantation. In some patients with hematologic malignancies, blood antigens are altered and serologic ABO typing might be hampered. In this study, we performed ABO genotyping using Next-Generation Sequencing (NGS) in patients with hematologic malignancies and in normal patients.

Method: Targeted sequencing including seven exons of the ABO gene was performed in 548 patients who diagnosed with various hematologic malignancies. In addition, whole exome sequencing (WES) data from 100 healthy Korean adults was analyzed for ABO gene.

Results: The distribution of ABO blood phenotypes among hematologic patients determined by genotyping was A (32.7%), B (27.7%), O (27.4%), and AB (12.2%), and all of the results matched the results of serologic blood typing. Frequencies of ABO alleles were A102 (26.0%), A101 (1.7%), B101 (24.2%), O01 (26.0%), and O02 (22.1%). In addition, rare alleles BW.14, O19, O25, O39, O04, O07, and O61 were found. Phenotypes were determined as A (34%), B (28%), O (30%), and AB (8%). The alleles were distributed as follows: A102 (25%), A101 (2%), B101 (22%), O01 (28%), and O02 (20%), which was not significantly different from the allele distribution in patients with hematologic malignancies.

Conclusions: Using NGS data, ABO genotypes could be accurately determined in patients with hematologic malignancies. Optimized NGS-based testing process may be developed for ABO genotyping, genotyping of other genes, and mutation analysis in hematology patients.

Conflict of Interest: None declared

P08.009.A Prevalence of clonal hematopoiesis in people living with HIV

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Clonal hematopoiesis (CH), the clonal outgrowth of hematopoietic stem and progenitor cells, is associated with age and health-related conditions like cardiovascular diseases and hematologic malignancies. CH is caused by somatic mutations in leukemogenic

genes (clonal hematopoiesis of indeterminate potential, CHIP) or mosaic chromosomal alterations (mCAs), including autosomal mCAs, loss of X and Y chromosomes. We and others recently reported a higher incidence of CHIP amongst people living with HIV (PLWH), suggesting a potential molecular mechanism behind the accelerated aging associated with HIV infection. We here examine the prevalence of CHIP and autosomal mCAs in PLWH in the UK biobank (UKB).

A total of 481 PLWH and 2405 controls, matched on age and sex, were included in our analyses. We identified CHIP mutations from exome sequencing data using mutect2; mCAs were obtained from UKB return #3094. We performed a case-control analysis of CHIP and mCAs frequency using logistic regression.

We did not observe any significant difference in CH prevalence between PLWH and controls ($p > 0.05$ for CHIP and mCAs). Of note, the absolute number of individuals with CH was low due to the limited age range of PLWH in UKB (<70 years old), limiting our ability to detect potential differences.

Our analyses don't replicate previous findings on increased CHIP prevalence among PLWH. However, they are likely underpowered due to the small number of study participants and the age structure of UKB. Additional analyses in older cohorts are needed.

This research was conducted using the UK Biobank Resource under Application #84415.

Conflict of Interest: None declared

P08.010.B Novel DNA variants in IL2RG gene identified in Slovak population of patients with X-linked combined immunodeficiency

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DNA alterations in the interleukin 2 receptor subunit gamma (IL2RG) gene are associated with X-linked severe combined immunodeficiency and cause defects in T and B cells immunity. The IL2RG gene is located on the X-chromosome and associated with a X-linked recessive inheritance. Actually, more than 320 unique pathogenic DNA variants in the IL2RG have been described worldwide. In our study we closely annotated potentially causal DNA variants in IL2RG gene from the biological and clinical point of view, in two X-SCID patients. First patient has diagnosed severe immunodeficiency phenotype, the second one has a milder manifestation. Whole exome sequencing revealed novel variants c.251A>C (p.Asn84Thr) and c.638T>C (p.Val213Ala). Using Varsome and Franklin tools, these variants were evaluated based on theoretical prediction and extremely low frequency in gnomAD databases as variants with uncertain significance. Furthermore, using AGVGD tool variants were predicted as likely pathogenic. Variant c.251A>C was confirmed by direct sequencing and segregation analysis in the family did not confirm the presence of variant in relatives, thus represents de novo substitution. Second variant c.638T>C was also confirmed by direct sequencing and segregation analysis detected its presence in mother, who represents a asymptomatic carrier. Based on the segregation analysis data together with additional biological and phenotype data, we suggest, that both variants should be characterized as potential pathogenic. In summary, we have identified two DNA variants in the IL2RG gene, which represent novel, not yet reported, potentially pathogenic variants in patients with X-SCID in Slovak population.

Conflict of Interest: None declared

P08.011.C Defining gene expression signatures for fatal and non-fatal melioidosis from Thailand

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Background/Objectives: Melioidosis is a neglected tropical infectious disease with an estimated 165,000 infected cases and 89,000 deaths annually. It is caused by a facultative intracellular bacterium *Burkholderia pseudomallei*. The reported mortality is 10-50%. The ability to stratify disease severity by gene expression could enable better understanding of disease mechanisms and inform appropriate choices of treatment, thus improving the patients' outcomes.

Methods: Whole blood for RNA and DNA and comprehensive clinical information were obtained from 370 patients in an endemic region of Ubon Ratchathani, Thailand. Following bulk RNA-seq, mapped reads were filtered, normalised and log-transformed. Differential gene expression (fold-change >1.5, FDR <0.01) analysis was conducted with the limma R package and pathway analysis with pantherdb.org.

Results: We reported a high rate of 28-day mortality among our Thai melioidosis patients (non-survivors n = 94/370, 25%). We identified 1019 differentially expressed genes, with 603 upregulated and 416 downregulated genes in fatal cases. Enriched biological pathways included antigen processing and presentation via MHC-II, T-lymphocyte activities, leucocyte-mediated cytotoxicity, and regulation of IFN-gamma production. This recapitulates the important roles of these immune components in host defence against melioidosis.

Conclusion: We conducted the largest melioidosis bulk RNA-seq research to date to stratify disease severity by gene expression, and increase representation of Thai transcriptomic information. The reported differential gene expression provides biological insights to the heterogeneity of host responses to the infection. This lays foundations for further transcriptomic and genomic studies including incorporation of clinical information, alternative splicing and eQTL analyses.

Grant References: Wellcome 216457/Z/19/Z to CC

Conflict of Interest: None declared

P08.012.D Copy number variations from exome sequencing of Finnish inborn errors of immunity cohort

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Background: Inborn errors of immunity (IEI) are a heterogeneous group of genetic disorders caused by numerous defects in immune functions. To date, 485 known genetic defects are identified as causing IEI. Whole exome sequencing (WES) has become the primary diagnostic approach for rare diseases such as IEI, and it can be used to identify single nucleotide variants as well as copy number variants (CNVs). However, only a fraction of the known IEI-causing variants is CNVs.

Methods: To examine the role of CNVs in Finnish IEI patients using existing WES data, we developed copyCat, an R-based method to discover CNVs from both targeted and whole genome sequencing data, and combined it with two other algorithms, DECoN and CODEX, to produce high-confidence CNV calls. For studying the identified CNVs, we developed an R pipeline for annotation and analysis of structural variants (AnAnaS). AnAnaS combines multiple databases to annotate CNVs, uses ClassifyCNV to calculate a score for CNV interpretation according to ACMG technical standards, and calculates a set of in-house-built metrics.

Results and conclusion: In a sample set of 134 IEI patients and 18 unaffected family members we identified 9690 high-confidence CNVs. We narrowed these down to four CNVs overlapping known IEI genes, which we consider likely causative for IEI in these patients. These CNVs were validated using either SNP microarray technology or quantitative PCR. Our study demonstrates that for the diagnostic assessment of IEIs, it is necessary to screen for both SNV and CNV variants.

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P08.013.A Novel identification of tissue-specific splicing of a PPHLN1-like ZNF638 isoform implicated in determining the severity of multiple sclerosis

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Background: Through international collaboration, we recently completed a genome-wide association study of multiple sclerosis (MS) severity involving over 20,000 people with MS. This study identified genome-wide significant association with rs10191329, carriage of which advances the time to reach EDSS 6 by about 3.7 years. This variant maps close to ZNF638, which is a key recruiter of the HUSH system, a mechanism for silencing viral DNA. *ZNF638* has multiple isoforms, raising the possibility that rs10191329 might exert its effects via regulation of splicing.

Method: We designed a tiled PCR panel to detect known and novel *ZNF638* transcripts. We tested this panel in a range of cell types including monocytes, B-cells, T-cells, LCLs, neutrophils, and oligodendrocytes. Novel unexpected PCR products were characterised by Sanger sequencing. Participants were genotyped for rs10191329 using TaqMan methodology on a Quantstudio 7K Flex.

Results: We identified a novel homology between only one of the ZNF638 isoforms and PPHLN1, which is a major component of the HUSH complex. The expression of this PPHLN1-like ZNF638 isoform was detected in all cell types, apart from B-cells. Also, we identified two novel splice isoforms with cell-specific expression. We are now exploring the impact of rs10191329 genotype on the expression of these three isoforms in monocytes, using RNA collected from 100 individuals.

Conclusion: Identifying the disease-relevant transcript and cell type is of critical value to guiding further experiments to understand the pathogenic function of this MS severity-associated variant in disease progression.

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Conflict of Interest: None declared

P08.014.B Uncovering genetic mechanisms of inflammatory bowel disease through DNA-RNA integration analyses of the inflamed colon tissue

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Background/Objectives: RNA plays a vital role in mirroring the instructions of DNA and delivering the readouts for building the distinct cell's function. We conducted human DNA-RNA integration analysis to uncover genetic mechanisms of inflammatory bowel disease (IBD).

Methods: We integrated genetic variation detected by exome sequencing with the expression of overlapping transcripts quantified in colon tissue 20 cm from the anal verge (*cis*-expression quantitative trait locus analysis) in 523 European adults with IBD (SPARC IBD) from the IBD Plexus program [1]. We further examined the effect of variants associated with IBD in 450K UK Biobank exomes [2] upon gene expression in this dataset.

Results: We identified 33,836 associations between 6,492 protein-coding genes (eGenes) and 21,160 genetic variants (eVariants) in coding regions. Eight of the top ten IBD down-regulated genes identified previously [3] were associated with at least one functional eVariant with identical effect direction. The variant most strongly associated with IBD in the UK Biobank [2], rs4151651-A (p.Gly252Ser, OR[95% CI] = 1.57[1.46, 1.71], $P = 3.64 \times 10^{-27}$), was potentially having a disease- and tissue-specific association with the expression of *HLA-DRB1* (Beta = 0.55, SE = 0.10, $P = 4.18 \times 10^{-8}$), which was one of the most extensively studied genes in IBD.

Conclusion: Our study demonstrated the importance of characterising multi-omics layers in uncovering genetic susceptibility to IBD. We will expand on this work by testing associations of colonic gene expression with interchromosomal genetic variants.

References:
Raffals et al. Inflamm Bowel Dis. 28 (2022): 192-199
AstraZeneca PheWAS Portal, v2.5, https://azphewas.com
Linggi et al. *Sci Rep*. 11 (2021): 18243

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Conflict of Interest: Xiao Jiang AstraZeneca, Margarida Lopes AstraZeneca, Bram Prins AstraZeneca, Junmei Cairns AstraZeneca, Ulf Gehrmann AstraZeneca, Ben Kostiuk Crohn's and Colitis Foundation, Coralie Viollet AstraZeneca, William Rae AstraZeneca, Katherine Smith AstraZeneca

P08.015.C Dissecting the relationship between mosaic loss of chromosome Y and sex hormones

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Background: Mosaic loss of the Y-chromosome (LOY) is the most common somatic alteration in men and linked to a wide range of malignant and non-malignant conditions. LOY is associated with age, smoking and constitutional genetics. Here, we aimed to assess the relationships between LOY, serum biomarkers and clonal hematopoiesis (CH).

Methods: We analysed data from UK Biobank (n = 222,835 men; 44,558 with LOY; 19,296 with CH). Biomarkers included total testosterone (TT), sex hormone binding globulin (SHBG), and calculated free testosterone (FT) and bioavailable testosterone (BAT). Analysis included multivariable linear regression, and Mendelian randomisation (MR).

Results: LOY was strongly associated with SHBG ($\beta = 0.08$, $P = 4.61 \times 10^{-21}$), a key regulator of testosterone bioavailability, and TT ($\beta = 0.05$, $P = 4.13 \times 10^{-9}$), but not BAT or FT. MR suggested a causal effect of SHBG on LOY in Biobank Japan using an inverse variance weighted model ($P = 6.58 \times 10^{-4}$), but reverse causality was absent. In contrast, CH was not associated with sex hormones but myeloid CH (OR = 1.42, $P = 4.52 \times 10^{-3}$), specifically *TET2*, *TP53*, and *CBL* mutations, were significantly associated with LOY in ≥30% of cells, but not *DNMT3A* and *ASXL1*. Interestingly, *JAK2* V617F was negatively associated with LOY (OR = 0.39, $P = 6.78 \times 10^{-3}$). CH without somatic driver mutations was more strongly associated with LOY, and clonal size analysis confirmed that most of LOY is clonal.

Conclusion: We conclude that SHBG is causally associated with LOY but this relationship cannot be explained by the free-hormone hypothesis. CH does not explain the relationship between LOY and SHBG but confirms that LOY is clonal.

Conflict of Interest: Ahmed Dawoud Laday Tata Memorial Trust, international scholarship, william tapper: None declared, nick cross: None declared

P08.016.D Familial aggregation of autoimmune diseases and type 1 diabetes in offspring

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Patients with type 1 diabetes (T1D) and their relatives are often diagnosed with one or more other types of autoimmune diseases (AIDs). However, it remains unsolved to which degree the shared genetic architecture contributes to the observed familial aggregation. We comprehensively investigated the relationship between T1D and 24 common AIDs by leveraging multi-generational health registers (N = 7.2M) and genomic information (N = 400K) from the Finnish population which has the highest T1D prevalence globally. Epidemiological analyses on 58,284 trios shows an increased risk of T1D among individuals whose parents were ever diagnosed with AIDs, six of the parental AID associations were significant after multi-test correction. The results are supported by genetic correlations and novel polygenic scores constructed from human leukocyte antigens (HLA) genotypes. Whereas HLA genotypes, especially the haplotype DRB1*03:01-DQA1*05:01-DQB1*02:01, are more associated with parental coeliac disease and T1D in children (OR = 1.97 [1.75-2.21], $Rg_{non\text{-}HLA} = 0.27$ [0.13-0.42], $Coef_{HLA} = 0.61$ [0.58-0.63]), both non-HLA and HLA genes play a role in parental vitamin B12 deficiency anemia and T1D in children (OR = 1.62 [1.42-1.85], $Rg_{non\text{-}HLA} = 0.53$ [0.35-0.70], $Coef_{HLA} = 0.48$ [0.43-0.53]). Parental inflammatory bowel disease (OR = 1.03 [0.93-1.13], $Rg_{non\text{-}HLA} = 0.00$ [-0.02-0.03], $Coef_{HLA} = -0.37$ [-0.42--0.32]) and multiple sclerosis (OR = 0.99 [0.79-1.22], $Rg_{non\text{-}HLA} = 0.17$ [-0.12-0.46], $Coef_{HLA} = -0.24$ [-0.28--0.21]) are not associated with T1D in children in the registry-based analysis, but they have significantly negative associations with T1D in HLA region. In conclusion, our results implicate that familial aggregation of AIDs can be caused by shared genetic mechanisms in both HLA region and non-HLA region. This design can be extended to other diseases with shared genetic architecture.

Conflict of Interest: None declared

P08.017.A Cross-disorder genetic analysis of immune diseases reveals distinct gene associations that converge on common pathways

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Genome-wide association studies (GWAS) have mapped thousands of susceptibility loci associated with immune-mediated diseases. To assess the extent of the genetic sharing across nine immune-mediated diseases we applied genomic structural equation modelling to GWAS data from European populations. We identified three disease groups: gastrointestinal tract diseases, rheumatic and systemic diseases, and allergic diseases. We identified 92, 95 and 87 genetic loci that predispose to each of these disease groups, with only 12 of them being shared across groups. Although loci associated with the disease groups were highly specific, they converged on perturbing the same pathways. Finally, we tested for colocalization between loci and single-cell eQTLs derived from peripheral blood mononuclear cells. We identified the causal route by which 46 loci predispose to three disease groups. In addition, given that the assessed variants are pleiotropic, we found evidence for eight of these genes being strong candidates for drug repurposing. Taken together, here we show that different constellations of diseases have distinct patterns of genetic associations, but that associated loci converge on perturbing different nodes in T cell activation and signalling pathways.

Conflict of Interest: None declared

P08.019.C Genetic evaluation of Inborn Errors of Immunity in Indian patients

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Background: Inborn Errors of Immunity (IEI) are a group of approximately 485 rare monogenic disorders of the immune system affecting about one in 10,000 people worldwide. We study these disorders in Indian patients by clinical and genomic testing.

Methods: 89 unrelated individuals with phenotypes suggestive of IEIs were recruited. We performed targeted gene testing (1 family), exome sequencing (singleton in 63 families, trio in 22 families), and whole genome sequencing (3 families).

Results: Disease-causing variants were identified in 30 (34%) families. Thirteen variants were pathogenic, 10 likely-pathogenic and 10 were variants of uncertain significance. Homozygous variants were most common (10, 33%), followed by hemizygous (9, 30%), de novo (8, 27%) and compound heterozygous (3, 10%). Six patients had congenital defects of phagocyte number or function. Autoinflammatory disorders were seen in five patients. Combined immunodeficiencies with associated or syndromic features, and predominantly antibody deficiencies were seen in four patients each. Immunodeficiencies affecting cellular and humoral immunity, and defects in intrinsic and innate immunity were seen in three and two patients respectively. Diseases of immune dysregulation, bone marrow failure, and phenocopies of IEI were noted in a patient each. We observed 17 new disease-causing variants in our population in DOCK8, RAG1, SPINK5, WAS, ATM, PEPD, G6PC3, WDR1, NCF4, CYBB, IL12RB1, STAT1, ATP6AP2, CNKSR2, and SFTPC.

Conclusions: Our study provides clinical and genomic insights into IEI in Indian patients and expands the mutation spectrum of IEIs.

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Conflict of Interest: None declared

P08.020.D The Human Leukocyte Antigen-G: a prognostic factor in SARS-CoV-2 infection

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The COVID-19 pandemic has sparked interest in understanding the various factors that may impact the course of the disease. Some populations, such as Sardinia, provide valuable insights into the genetic factors influencing the outcome of the disease. Previous studies have revealed that the β-thalassemia trait, *KIR2DS2/HLA*-C1 and *HLA-B*58:01,C*07:01,DRB1*03:01* haplotype exerted a protective effect, while the Neanderthal-variant (*LZTFL1*:*rs35044562*A>G) had a detrimental consequence in Sardinia. Recently, there has been a growing interest in exploring the role of HLA-G molecules and their impact on the disease. This study aims to shed light on the impact of *HLA-G* polymorphisms on the outcome of SARS-CoV-2 infection.

We compared the immunogenetic and phenotypic characteristics between COVID-19 patients ($n = 381$) and 420 healthy controls from Sardinia (Italy). Moreover, a multivariate analysis was performed to evaluate the *HLA-G* in relation to the other susceptibility genetic factors previously studied in this population.

Our findings indicate that the HLA-G 3'UTR Del/Del genotype frequency decreases as the severity of the patient's symptoms increases. The frequency was highest in paucisymptomatic patients at 27.6%, and decreased to 15.9% in patients with severe symptoms ($X^2 = 7.095$, $P = 0.029$). The frequency reaches its lowest frequency (7.0%) among ICU patients ($X^2 = 11.257$, $P = 0.004$). Finally, the logistic regression model showed that this *3'UTR HLA-G* genotype was independent from the other significant variables [$OR_M = 0.4$ (95%CI 0.2-0.7), $P_M = 6.5 \times 10^{-4}$].

Our results suggest that the outcome of COVID-19 is determined by a complex interaction of many variables, including the *HLA-G* gene. Further research will need to fully comprehend HLA-G's role in SARS-CoV-2 infection.

Conflict of Interest: None declared

P08.021.A Exome sequencing of individuals and families with suspected monogenic primary immunodeficiencies – experiences of a single center

Ulrike Hüffmeier[1], *Cornelia Kraus*[1], *Melissa Rieger*[1], *Katalin Blum*[1], *Annika Willms*[1], *Axel Hueber*[2;3], *Nora Naumann-Bartsch*[4], *Axel Karow*[4], *Gisela Fecker*[4], *Andre Hörning*[5], *Matthias Galiano*[6], *Jürgen Rech*[3;7], *Tobias Krickau*[6], *André Reis*[1;8]

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Background: Primary immunodeficiencies comprise a group of immunologic disease categories caused by single gene defects. We aimed to assess the contribution of coding variants in known genes in 34 affected individuals with autoimmunity ($n = 24$), bone-marrow failure ($n = 5$), increased susceptibility to infections ($n = 4$), suspicion of a specific immunologic diagnosis ($n = 4$) and/or early-onset inflammatory bowel disease (IBD) ($n = 2$).

Methods: We performed exome sequencing and assessed variants in a gene panel for human inborn errors of immunity (Tangye et al. J Clin Immunol 2022), supplemented by single genes based on new findings/ authors' experience [n = 449], and/ or gene panels based on human phenotype ontology.

Results: In total, the diagnostic yield was 29%. It was highest in increased susceptibility to infections/ suspicion of a specific immunologic diagnosis (50%), lower in bone-marrow failure (20%) and autoimmunity (17%) and 0% in IBD. The most common molecularly confirmed diagnosis was VEXAS syndrome, caused by *UBA1* missense variants affecting p.Met41 in four single males with tentative clinical diagnosis/ unspecific autoimmune symptoms. We detected variants of unknown significance in eight individuals (24%). Systematic assessments of all rare variants suggested variants in new candidate genes in five further individuals (15%). Molecularly confirmed diagnoses allowed specific recommendations for therapy, prevention and an individual prognosis. In addition, in two children, they led to informed decision pro/ contra bone-marrow-transplantation.

Conclusions: Exome sequencing in primary immunodeficiencies shows increasing diagnostic yield and is of clinical utility, although genetic defects in many individuals remain to be identified.

Conflict of Interest: Ulrike Hüffmeier part-time, German Research Foundation (DFG), Cornelia Kraus full, Melissa Rieger full, Katalin Blum part-time, Annika Willms full, Axel Hueber full, Nora Naumann-Bartsch full, Axel Karow full, Gisela Fecker part-time, Andre Hörning full, Matthias Galiano full, Jürgen Rech full, Tobias Krickau full, André Reis full

P08.022.B Phenotype-genotype corellation in two families with hereditary spherocytosis

Zuzana Vrzalova[1;2], *Lenka Radova*[1], *Katerina Stano Kozubik*[1;2], *Jiri Stika*[1;3], *Ivona Blaháková*[1;2], *Jakub Trizuljak*[1;2;3], *Sarka Pospisilova*[1;2;3], *Hana Halámová*[2], *Michael Doubek*[1;2;3]

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Background: Hereditary anemias are a heterogenous group of disorders, caused by genetic variants in 70 genes controlling red blood cell production, enzymatic function, membrane structure, as well as production and formation of hemoglobin. The most common hemolytic anemia is hereditary spherocytosis (HS) characterized by spherical-shaped erythrocytes in the peripheral blood smear. HS is clinically manifested by anemia, jaundice and splenomegaly, with variable severity. Here we present two families with HS diagnosis confirmed by molecular genetic analyses.

Methods: Whole exomes were sequenced (WES) in a cohort of patients suspected of HS disorder. WES results were confirmed by Sanger sequencing.

Results: We performed a detailed study of two families with HS and described the clinical phenotype-genotype corellation. WES data were processed using genes coding genetic alterations in α-spectrin, β-spectrin, ankyrin and band 3 protein. In the first family, we identified a known splice variant c.2057+1G>A in *SLC4A1* gene in a proband suffering from macrocytic corpuscular hemolytic anemia, HS, hepatopathy, chronic pankreatitis, and suspected Gilbert syndrome. In addition, a homozygous insertion c.-54insTA of *UGT1A1* gene leading to Gilbert's syndrome was detected. In the second family, we detected a rare multi-locus inheritance presented by a dual molecular diagnosis of hereditary spherocytosis (c.40C>T; *SPTB* gene) and dehydrated hereditary stomatocytosis (c.3012_3025del; *SPTA1* gene).

Conclusion: In this study, we focus on the clinical characteristics of affected patients together with detected genotypes associated with hereditary spherocytosis.

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Conflict of Interest: Zuzana Vrzalova part-time, collaborator, Lenka Radova part-time, collaborator, Katerina Stano Kozubik part-time, collaborator, Jiri Stika part-time, collaborator, Ivona Blaháková part-time, collaborator, Jakub Trizuljak part-time, collaborator, Sarka Pospisilova: None declared, Hana Halámová: None declared, Michael Doubek part-time, principal investigator

P08.023.C Curation and expansion of Human Phenotype Ontology for Systemic Auto inflammatory Disease SAID improves phenotype-driven disease matching

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To diagnose rare disease patients and to discover new diseases, accurate and standardized phenotypic descriptions are essential. Human Phenotype Ontology (HPO) is extensively used for this purpose. The use of HPO has not been widely implemented in the field of Systemic Auto inflammatory Disease (SAID) because the lack of proper HPO terms and SAID annotation. Therefore, in a consortium endeavour HPO for SAID has been improved and curated. However, it has not been investigated if the curation process improved diagnosing SAID patients. Here, we aimed to study if curation enhanced SAID identification. In addition we aimed to demonstrate the potential of phenotype-driven genome diagnostics using curated HPO terms for SAIDs. We collected HPO terms from 93 patients with 27 different genetically confirmed SAIDs from 8 European SAID expertise centres. LIRICAL, a computational algorithm was used to estimate the effect of curation on prioritizing the correct SAID. Prioritizing the correct SAID improved from 62% to 80% after curation. The added value of the curated HPO terms was most prominent when used in WES analysis. In patients with available WES data the correct SAID was prioritized in 6/12 patients using only HPO terms whereas in 9/12 patients HPO-filtered WES analysis prioritized the correct SAID. After HPO-filtering an average of 3 variants per WES analysis remained to be interpreted. This study demonstrates that curation

of HPO terms for SAIDs improved the ability to computationally match SAID patients to their known genetic diagnosis making HPO a promising tool for SAID research and diagnostics.

Conflict of Interest: None declared

P08.024.D Identification of a novel variant in MECOM gene as cause of isolated bone marrow failure

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Background/Objectives: MECOM-associated syndrome (MECOM-AS) is a rare disease characterized by amegakaryocytic thrombocytopenia and caused by alterations of the *MECOM* locus, which encodes MDS1, EVI1 and the respective fusion protein. EVI1 is a transcription factor which plays a fundamental role in hematopoietic stem-cell renewal and is characterized by an N-terminal and a C-terminal zinc-finger domains (ZFD), a repression domain (RD) and a C-terminal acidic domain. Here we reported the functional characterization of a novel de novo variant of *MECOM* gene, identified in a paediatric patient affected by severe thrombocytopenia through NGS analysis

Methods: Mutational screening was performed through targeted NGS analysis. Luciferase reporter assays on the promoters of different target genes were conducted in order to determine the pathogenic role of the variant identified.

Results: In a patient affected by severe, non-syndromic, thrombocytopenia from the first day of life who underwent a hematopoietic stem cell transplantation at the age of three, we identified a novel, de novo variant on *MECOM* gene, which results in an amino acid substitution in the RD of EVI1.The functional effect of this variant was evaluated through gene reporter assays on pAP-1 enhancer element or two direct targets of EVI1 demonstrating that the mutation impairs its activity.

Conclusion: Whereas all missense mutation described in MECOM-AS are localized in the C-term ZFD, we identified the first missense causative mutation in the RD and demonstrated its pathogenic effect. This allowed us to provide a correct molecular diagnosis to the patient.

Conflict of Interest: None declared

P08.025.A Atypical Hemolytic Uremic Syndrome Caused by X-linked C1GALT1C1 Mutation

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Hemolytic-uremic syndrome (HUS) is mostly secondary to infectious diseases. It is a common cause of acute kidney injury in children, characterized by progressive acute kidney failure due to severe thrombotic microangiopathy, associated with nonimmune, Coombs-negative hemolytic anemia and thrombocytopenia. HUS is caused mostly by Shiga toxin-producing E. Coli, and to a lesser extent by Streptococcus pneumonia. In Streptococcus pneumonia HUS (pHUS), bacterial neuraminidase A exposes masked O-glycan sugar residues on erythrocytes, known as the T antigen, triggering a complement cascade causing thrombotic microangiopathy. Atypical HUS (aHUS) is a life-threatening genetic form of the disease, whose molecular mechanism is only partly understood. Through genetic studies, we demonstrate a novel X-linked form of aHUS that is caused by a de-novo missense mutation in C1GALT1C1:c.266 C > T,p.(T89I), encoding a T-synthase chaperone essential for the proper formation and incorporation of the T antigen on erythrocytes. We demonstrate the presence of exposed T antigen on the surface of mutant erythrocytes, causing aHUS in a mechanism similar to that suggested in pHUS. Our findings suggest that both aHUS caused by mutated C1GALT1C1 and pHUS are mediated by the lectin-complement-pathway, not comprehensively studied in aHUS. We thus delineate a shared molecular basis of aHUS and pHUS, highlighting possible therapeutic opportunities.

Conflict of Interest: Noam Hadar Ben Gurion University of the Negev, Ruth Schreiber Soroka Medical Center, Ben Gurion University of the Negev, Marina Eskin-Shwartz Soroka Medical Center, Ben Gurion University of the Negev, Eyal Kristal Ben Gurion University and Soroka Medical Center, George Shubinsky Soroka Medical Center, Ben Gurion University of the Negev, Galina Ling Soroka Medical Center, Ben Gurion University of the Negev, Idan Cohen Ben Gurion University of the Negev, Michael Geylis Ben Gurion University and Soroka Medical Center, Amit Nahum Soroka Medical Center, Ben Gurion University of the Negev, Yuval Yogev Soroka Medical Center, Ben Gurion University of the Negev, Ohad Shmuel Birk Soroka Medical Center, Ben Gurion University of the Negev, ISF, Israeli Ministry of Science and Technology – National Knowledge Center for Rare / Orphan Diseases

P08.026.B First family with recessive ACTN1-related thrombocytopenia: platelet size matters

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ACTN1-congenital macrothrombocytopenia (CMTP) is a rare autosomal dominant disorder characterized by thrombocytopenia, platelet macrocytosis and mild bleeding tendency, caused by monoallelic mutation in the *ACTN1* gene (OMIM: 615193). *ACTN1* encodes a conserved F-actin binding and crosslinking protein. To date, the spectrum of variants includes approximately 45 heterozygous mutations spanning the entire gene. Overexpression of mutant ACTN1 causes disorganization of the actin cytoskeleton in cells.

Mutational screening was performed on genomic DNA from proband's peripheral blood to identify the *ACTN1* variant, while segregation analysis was conducted on other family members Evaluation of F-actin staining was performed by immunofluorescence in U2OS cells ectopically expressing WT or mutant ACTN1.

We identified a pathogenetic *ACTN1* variant transmitted as recessive trait in a Moroccan family. The proband and her sister, homozygous for the variant, present thrombocytopenia, platelet macrocytosis and cardiac valve insufficiency. Proband's father is reported as thrombocytopenic, while the mother's platelet count was borderline to normal. Analysis of blood smear from both parents and proband's brother (all heterozygous for the variant) revealed increased platelet size. Immunofluorescence analysis of U20S overexpressing mutant ACTN1 display aberrant actin cytoskeleton organization with respect to control cells.

We describe for the first time two individuals affected by macrothrombocytopenia caused by a *ACTN1* homozygous mutation. In homozygous individuals, thrombocytopenia varies from mild to moderate with increased platelet macrocytosis compared to heterozygous individuals. Although the involvement of the mutation in the pathogenesis of the heart defect remains to be proven, we recommend echocardiography to broaden the clinical spectrum of *ACTN1*-CMTP patients.

Conflict of Interest: None declared

P08.027.C Profiling of disrupted regulatory networks in COVID19 associated multisystem inflammatory syndrome in children with multiomic single cell technology

Barbara Jenko Bizjan[1], *Robert Sket*[1], *Tine Tesovnik*[1], *Jernej Kovač*[1], *Marusa Debeljak*[1], *Mojca Zajc Avramovič*[2], *Nina Emeršič*[2], *Barbara Slapnik*[1], *Blaž Vrhovšek*[1], *Tadej Avčin*[2]

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Background: Children infected with SARS-CoV-2 usually present minimal symptoms or are asymptomatic. Nevertheless, a subset of children 2-6 weeks after the initial SARS-CoV-2 infection develops a postinfectious SARS-CoV-2-related multisystem inflammatory syndrome (MIS-C). The purpose of our project is to characterize the complexity of cell populations and capture cellular heterogeneity to uncover the regulatory networks that are disrupted during MIS-C flare with simultaneous profiling of gene expression and open chromatin regions.

Methods: Samples of peripheral blood mononuclear cells from patients with MIS-C diagnosed at the University Children's Hospital, University Medical Centre Ljubljana, were collected during the initial presentation before any treatment and at 6-12 months in remission. To enable simultaneous profiling of epigenomic landscape and gene expression from the same nuclei, we are using Chromium Next GEM Single Cell Multiome ATAC + Gene Expression kit from 10X Genomics.

Results: We included 32 MIS-C patients in total. Into single cell multiomic experiment we included 10 patients, with the most viable cell count prior cryopreservation. All included samples had 75% - 99% viability of cells prior cryopreservation. In the protocol the key is to remove remaining granulocytes causing high mitochondrial RNA burden and extensively optimise the dilution factor of lysis buffer and the length of cell lysis step in order to get intact nuclei with no significant blebbing.

Conclusions: The results of this project are expected to enlighten the underlying pathophysiology of MIS-C flare and thus support clinical decision on more targeted treatment.

Grant References: UMC: TP20220090; Interreg CATTEDRA; SRA: J3-3061

Conflict of Interest: None declared

P08.028.D Arrayed screening of immune disease associated genes identifies modulators of CD4+ regulatory T cell suppression

Olivier Bakker[1;2], *Gareth Griffiths*[1;2], *Blagoje Soskic*[3], *Klio Maratou*[4], *Michelle Bartholomew*[5], *Janet Smith*[5], *Carla Jones*[1;2], *Gosia Trynka*[1;2]

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Regulatory T cells (Tregs) have been implicated as one of the key cell types regulated by the common genetic variation associated with immune diseases. Tregs modulate immunity by suppressing autoreactive responses and have a wide ranging impact on immunity. Given this impact, they are of key interest in the development of immune based therapies in the areas of autoimmunity and oncology.

In this study, we evaluate the effect of immune disease associated genes on the suppressive capability of Tregs in an in vitro assay, allowing us to go beyond eQTL prioritisation and assay the effects of selected genes on cell function directly. Due to their rarity, we first expand natural Tregs (eTregs) and profile them during the expansion process to ensure their cellular phenotype remains consistent. Next, we knock-out 15 target genes in eTregs and assess their ability to suppress autologous naive T cell proliferation in a coculture system with macrophages. Our data shows that perturbations in *CTLA4*, *IL10* and *IL2RA* lead to a marked reduction in Treg suppression and an increase in naive T cell proliferation. Effects of these knockouts were further confirmed by analysis of secreted cytokines and single cell RNA sequencing of the cocultures, allowing for the assessment of cellular heterogeneity in the cocultures. In closing, we established a new arrayed CRISPR screen protocol that allows us to assess hundreds of target genes to further our understanding of the regulation of human primary Treg function by immune disease associated genetic factors.

Conflict of Interest: Olivier Bakker Member of Open Targets consortium which actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech, Gareth Griffiths Member of Open Targets consortium which actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech, Blagoje Soskic: None declared, Klio Maratou GSK - Stevenage, Member of Open Targets consortium which actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech, Michelle Bartholomew GSK - Stevenage, Member of Open Targets consortium which actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech, Janet Smith GSK - Stevenage, Member of Open Targets consortium which actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech, Carla Jones Member of Open Targets consortium which actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech, Gosia Trynka Genome Research Limited, Grant reference: 220540/Z/20/A, 'Wellcome Sanger Institute Quinquennial Review 2021-2026', In the past three years Gosia Received honoraria from Japanese Society of Rheumatology, In the past three years Gosia consulted for Relation Therapeutics, is an active member of scientific advisory board for Variant Bio, As a member of Open Targets consortium Gosia is cofounded and actively interacts with: GSK, Sanofi, Pfizer, BMS and Genentech

P08.029.A Mosaic IL6ST variant inducing constitutive GP130 cytokine receptor signaling as a cause of neonatal onset immunodeficiency with autoinflammation and dysmorphy

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Interleukin 6 signal transducer (*IL6ST*) encodes a GP130 protein that transduces pro-inflammatory signaling of the IL6 family of cytokines through Janus kinase signal transducers and activators of transcription pathway (JAK/STAT) activation. Biallelic loss-of-function *IL6ST* variants cause autosomal recessive hyper-IgE syndrome or a type of the Stüve-Wiedemann syndrome. Somatic gain-of-function *IL6ST* variants, particularly small in-frame mono-allelic deletions, of which *IL6ST* Ser187_Tyr190del is the most prevalent, are an established cause of inflammatory hepatocellular neoplasms, but no disease caused by such variants present constitutively has been identified to date. Here, we report a pediatric patient with a novel neonatal-onset immunodeficiency syndrome in which autoinflammation and malformations were associated with a constitutively present *IL6ST* Tyr186_Tyr190del variant. Tyr186_Tyr190del was discovered by exome sequencing and was shown to be de novo (absent in the subject's parents and siblings) and mosaic (present in 15-40% of cells). Functional studies were performed in the Epstein-Barr virus-immortalized patient's B cell lymphoblastoid cell line, which carried the variant in approximately 95% of the cells. Western blotting revealed that the patient's cells had constitutively hyperphosphorylated Tyr705 in STAT3, suggesting IL6-independent activation of GP130. STAT3 phosphorylation could be inhibited by ruxolitinib and tofacitinib, clinically approved inhibitors of JAK1 and JAK3 (and to a lesser extent JAK2 and JAK1), respectively. Given our results and recent reports of the use of ruxolitinib and tofacitinib in diseases caused by direct activation of STAT3 or STAT1, these drugs might be effective in treating our patient's condition.

Conflict of Interest: None declared

P08.030.B Inborn errors of type I interferon immunity in patients with severe acute hepatitis E

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Background/Objectives: The clinical spectrum of human infection by hepatitis E virus (HEV) is highly variable, ranging from asymptomatic to severe acute hepatitis. Furthermore, HEV can cause diverse neurological manifestations, especially neuralgic amyotrophy, also referred to as Parsonage-Turner syndrome (PTS). We here use a large-scale human genomic approach to search for human genetic determinants of severe clinical presentations HEV infection.

Methods: We performed whole genome sequencing on blood extracted DNA from three groups of study participants: A) 24 patients with HEV-induced severe acute hepatitis; B) 12 patients with HEV-associated PTS; and C) 16 asymptomatic blood donors with PCR-proven HEV infection (controls). For variant calling and annotation, we used GATK4 best practices and VEP. For variant classification, we implemented the ACMG/AMP Bayesian classification framework in R. Variants with a probability of pathogenicity >0.9 were considered damaging. We used all genes with at least one damaging variant as input for pathway enrichment analysis.

Results: We observed a significant enrichment of type I interferon (IFN) response pathways in the severe hepatitis group: 10 out of 24 patients carried a damaging variant in one of 9 genes encoding either pattern recognition receptors (*IFIH1*, *DDX58*, *TLR3*, *POLR3B*, *POLR3C*) or other molecules involved in type I IFN response (*IRF7*, *MYD88*, *OAS3*, *GAPDH*). We did not find any enriched pathway in the PTS group nor in the controls.

Conclusion: Our study highlights the essential role of type I IFN to prevent HEV-induced severe acute hepatitis. They also suggest that neurological lesions might be due to different pathogenic mechanisms.

Conflict of Interest: None declared

P08.031.C Analysis of the first Hungarian primary immunodeficency cohort

Máté Bíró[1], ***Eszter Kovács***[1], *Anna Deák*[1], *Beáta Bessenyei*[1], *Katalin Koczok*[1], *László Madar*[1], *Zsuzsanna Szűcs*[1], *Orsolya Nagy*[1], *Szilvia Andó*[1], *Gergely Kriván*[2], *Vera Goda*[2], *Rita Káposzta*[3], *Istvan Balogh*[1]

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Objective: Genetic analysis of the heterogenous diseases group of primary immunodeficiency (PID) has been greatly improved since the introduction of whole exome sequencing (WES). We aimed to collect and analyze the PID results of our laboratory in samples from 2019 to 2022 tested by WES in order to measure diagnostic efficiency. In addition, we performed a bioinformatic reanalysis according to the most recent gene list of International Union of Immunological Societies (IUIS).

Methods: The cohort contained data from 162 PID patients. In case of negative result, a bioinformatic reanalysis was done using the 2022 gene list of IUIS. Pathogenicity analysis was performed by the ACMG guidelines. Pathogenic/likely pathogenic (P/LP) gene variants with were grouped into the disease groups of IUIS.

Results: Of the tested 162 samples, in 31 cases we did find variations associated with the phenotype. Among positive cases, 7 novel mutations were detected. Significant secondary findings were detected in 16 samples. Diagnostic efficiency was shown to be 19.1%. No novel diagnosis could be achieved by the bioinformatic reanalysis.

Conclusion: Diagnostic efficiency was shown to be in line with the international data. Seven novel disease-causing mutations were found. The detected P/LP variants could be grouped into all but two IUIS disease categories. Bioinformatic reanalysis led no novel diagnosis.

Conflict of Interest: None declared

P08.032.D The interplay between gene expression in blood and spontaneous HIV control

Sergey Oreshkov[1;2], *Jenny Meylan-Merlini*[2], *Maude Muriset*[2], *Christian Thorball*[1;2], *Jacques Fellay*[1;2], *Federico Santoni*[1;2]

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Interactions between HIV-1 and the human innate and adaptive immune system play a crucial role in the individual control of infection. Specific host restriction factors such as APOBEC3G/F, Tetherin, SAMHD1 and SERINC5 can block or hamper the viral life cycle. Conversely, other human proteins can be coopted to facilitate viral replication. Interindividual variability in the expression of these host factors might have an impact on spontaneous control of HIV-1 infection. We here examine the role of innate and adaptive immunity related genes in controlling HIV-1 infection, using gene expression levels in blood and their relationship with plasma viral load.

We measured gene expression data in blood samples from 144 untreated and previously genotyped patients using BRB-seq technology. We used a lognormal generalized linear model to determine the correlation strength between gene expression and plasma viral load, including age, sex, mRNA sequencing batch, principal components of the genome-wide genotyping matrix, and genetic variants known to associate with HIV-1 control.

A significant positive correlation between expression levels of interferon related genes and viral load was observed ($p < 2e-7$), as expected. In addition, we observed a negative correlation with RNA translation processes ($p < 1e-53$) and a positive correlation with proteasome activity ($p < 1e-10$). Among negatively correlated genes, we found the recently discovered in vitro restriction factor *TRABD2A* ($p < 1e-7$) and *IL7R*, which encodes the interleukin 7 receptor. Causality was assessed through two-sample Mendelian randomization. This study highlights pathways that are differentially regulated by HIV infection in vivo, with relevance for mechanistic understanding of host-pathogen interactions.

Conflict of Interest: None declared

P08.033.A GINS4 deficiency – an immunodeficiency syndrome with complex developmental aberrations

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About 500 genetically defined inborn errors of immunity (IEI) have been recognized. Affected patients may present with infections, inflammatory disorders, allergies or cancer. Many IEI affect genes and pathways relevant beyond the blood and immune system.

We present clinical, genetic and immunological features of three patients from two pedigrees with biallelic mutations in the gene encoding Go-Ichi-Ni-San complex subunit 4 (GINS4). GINS4-deficiency has recently been described in two patients with human natural killer cell deficiency and congenital neutropenia (Conte et al. 2022).

All five patients share different missense variants affecting the same Valine (position 171) in exon 7 of GINS4 (p.Val171Leu (Western Europe) and p.Val171Met (Turkey).

Our findings suggest variable clinical and immunological phenotypes in patients with GINS4 deficiency. GINS4 deficiency should be considered in patients with developmental aberrations associated with defects in natural killer cells and neutrophil granulocytes.

Conflict of Interest: None declared

Clinical features	II.1 (Conte et al.)	II.2 (Conte et al.)	A-I.1 (NL)	A-II.2 (NL)	B-II. (Turkey)
Clinical history	Male IUGR Growth delay Cryptorchidism Hyperacusis Tonsillar hypertrophy Reccurent fever	Female Normal development	Male Full term SGA Neonatal hypoglycemia Growth delay Micropenis Vanishing testes low-set ears	Female Growth failure	Male Atopic dermatitis Cryptorchism
Infections	CMV Sepsis Pneumonia Varicella Recurrent otitis Gingivitis Recurrent HSV labialis	Varicella Recurrent HSV labialis	FUO Pneumonia Sepsis	Pneumonia Reccuring infections	Pneumonia Recurrent aphthous lesions
Neutropenia	Severe	Moderate	Severe	Moderate	Moderate
NKD	Severe reduction of NK cell number and function	Reduction of NK cell number and function	Severe reduction of NK cell number	Not tested	Not tested

P09

Intellectual Disability

P09.001.D Extending the phenotypes associated with TRIO gene variants in a cohort of 25 patients and review of the literature

Gaby Gazdagh[1], *David Hunt*[1], *Giles Atton*[1], *Anna Maria Cueto-González*[2], *Monserrat Pons*[3], *Ayeshah Chaudhry*[4,5], *Marcus Madruga*[6], *Fleur Vansenne*[7], *Elena Sukarova-Angelovska*[8], *Deborah Shears*[9], *Curie Aurore*[10], *Eva-Lena Stattin*[11], *Britt M. Anderlid*[12], *Slavica Trajkova*[13], *Catherine McWilliam*[14], *Mary O'Driscoll*[15], *Anke Katharina Bergmann*[16], *Pia Zacher*[17], *Leena Mewasingh*[18], *Antonio González-Meneses López*[19], *Olga Alonso-Luengo*[19], *Anne Debant*[20], *Susanne Schmidt*[20], *Diana Baralle*[21], *Pauline Boiroux*[20]

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Health Partners, Mississauga, Canada; [5]University of Toronto, Toronto, Canada; [6]Hospital Viamed Santa Ángela De la Cruz, Sevilla, Spain; [7]University Medical Center, Groningen, Netherlands; [8], University Sv. Kiril i Metodij, Skopje, Macedonia; [9]Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom; [10]Lyon Neuroscience Research Centre, Lyon, France; [11]Uppsala University, Uppsala, Sweden; [12]Karolinska University Hospital, Stockholm, Sweden; [13]University of Turin, Turin, Italy; [14]Ninewells Hospital, Dundee, United Kingdom; [15]West Midlands Regional Genetics Service, Birmingham, United Kingdom; [16]Hannover Medical School, Hannover, Germany; [17]Epilepsy Center Kleinwachau, Radeberg, Germany; [18]Imperial College Healthcare NHS Trust, London, United Kingdom; [19]University of Seville, Seville, Spain; [20]University of Montpellier, Montpellier, France; [21]University of Southampton, Southampton, United Kingdom

The TRIO (Trio Rho Guanine Nucleotide Exchange Factor) gene encodes a guanin exchange factor, the function of which is to exchange GDP to GTP, and has been described to impact neurodevelopment. Specific genotype to phenotype correlations have been established previously describing striking differentiating features seen in variants located in specific domains of the TRIO gene that are associated with opposite effects on RAC1 activity. Currently, 32 cases with a TRIO gene alteration have been published in the medical literature. Here we report an additional 25, previously unreported individuals who possess heterozygous likely pathogenic or pathogenic TRIO variants and review the literature. Variants reported by this study include missense variants, truncating nonsense variants and a deletion. Clinical features were previously described and included developmental delay, learning difficulties, microcephaly, macrocephaly, seizures, behavioural issues, skeletal problems, dental problems (overcrowding/delayed eruption) and variable facial features. We report clinical features that have not been described previously, including specific structural brain malformations such as abnormalities of the corpus callosum and ventriculomegaly, additional psychological and dental issues along with a recognizable facial gestalt linked to the specific domains of the TRIO gene and the effect of the variant on the function of the encoded protein. In addition, functional studies were performed on the c.4394A>G and c.6244-2A>G TRIO variants to provide evidence for their pathogenicity. This study further strengthens the genotype to phenotype correlation that was previously established and extends the range of phenotypes, to include structural brain abnormalities, additional skeletal, dental and psychiatric issues among other features.

Conflict of Interest: Gaby Gazdagh consultant clinical geneticist, David Hunt: None declared, Giles Atton: None declared, Anna Maria Cueto-González: None declared, Monserrat Pons: None declared, Ayeshah Chaudhry: None declared, Marcus Madruga: None declared, Fleur Vansenne: None declared, Elena Sukarova-Angelovska: None declared, Deborah Shears: None declared, Curie Aurore: None declared, Eva-Lena Stattin: None declared, Britt M. Anderlid: None declared, Slavica Trajkova: None declared, Catherine McWilliam: None declared, Mary O'Driscoll: None declared, Anke Katharina Bergmann: None declared, Pia Zacher: None declared, Leena Mewasingh: None declared, Antonio González-Meneses López: None declared, Olga Alonso-Luengo: None declared, Anne Debant This work was supported by grants from the Agence Nationale de la Recherche to A.D (ANR-2019 TRIOTISM), Susanne Schmidt: None declared, Diana Baralle DB is supported by National Institute for Health Research (NIHR) (RP-2016-07-011 research professorship, Pauline Boiroux: None declared

P09.002.B Speech and language abilities in individuals with Kleefstra Syndrome

Lottie Morison[1;2], *Milou Kennis*[3], *Elizabeth Palmer*[4;5], *Adam Vogel*[6;7], *Frederique Liégeois*[8], *Amanda Brignell*[9], *Siddarth Srivastava*[10], *Zoe Frazier*[10], *Di Milnes*[11], *Himanshu Goel*[12], *David Amor*[1;13;14], *Tjitske Kleefstra*[3;15], *Ingrid Scheffer*[13;16;17], *Angela Morgan*[1;2;14]

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Background/Objectives: Kleefstra Syndrome (KS) is a rare, neurogenetic condition caused by haploinsuffiency of *EHMT1*. The KS phenotype is multisystemic and heterogenous, yet small published cohorts (largest $n = 29$) limit our knowledge of KS. This is particularly the case for speech and language abilities, which are currently poorly understood in KS.

Methods: We phenotyped 102 individuals with KS (34 <1Mb, 7 >1Mb and 11 unspecified deletions, 49 point variants, 1 balanced translocation; 40 males; 1-43 years). Health, development and communication were assessed via standardised surveys and direct clinician assessment.

Results: Common health and developmental conditions included: mild to severe hearing loss (35/102), sleep disturbance (64/102), cardiac malformations (32/102), seizures (12/102), and autism (39/102). Regression was reported in 15% (12/79). Mild to severe intellectual disability was common, yet 12 had average cognitive ability. Language and adaptive behaviour were average to severely impaired. There was no significant difference between receptive and expressive language skills. Over one-third of verbal individuals able to be assessed had speech apraxia and dysarthria. Minimally verbal individuals used augmentative and alternative communication (AAC), including sign language, to communicate. Some verbal individuals also used AAC, either to support unclear speech or during periods of regression.

Conclusion: Our large cohort demonstrates average to severely affected language, largely in line with cognitive and adaptive behaviour profiles. Dysarthria and apraxia profiles were pervasive and striking. Regression is a core feature, with onset typically in adolescence or adulthood.

Grant References: LDM and ATM are funded by The National Health and Medical Research Council.

Conflict of Interest: Lottie Morison Murdoch Children's Research Institute, Post-graduate scholarship National Health and Medical Research Council, PhD student at Murdoch Children's Research Institute and The University of Melbourne, Milou Kennis Radboud University Medical Center, Elizabeth Palmer University of New South Wales and Sydney Children's Hospitals Network, NHMRC GeneEqual Project, •Rare Voices Australia Medical and Scientific Advisory Committee (MSAC), Undiagnosed Disease Network International-Diagnostic Working Group Co-Chair, Medical / Scientific advisory to CureCLCN4, SATB2GeneAssociation

Australia, SCN2A Australia, Adam Vogel: None declared, Frederique Liégeois: None declared, Amanda Brignell: None declared, Siddarth Srivastava: None declared, Zoe Frazier: None declared, Di Milnes: None declared, Himanshu Goel: None declared, David Amor Murdoch Children's Research Institute, The University of Melbourne and The Royal Children's Hospital, •Treasurer of the Human Genetics Society of Australia, Preimplantation Genetic Diagnostic Committee and Donor Committee of the Royal College of Pathologists of Australasia, Scientific Advisory of the Royal Australasian College of Physicians, Tjitske Kleefstra Radboud University Medical Center and Vincent van Gogh Institute for Psychiatry, Editoral Board of European Journal of Medical Genetics IDefine and Idefine Europe Scientific Advisory Board, Intellectual disability, Telehealth, Autism and Congenital Anomalies (ITHACA) Neuodevelopmental Disorders Work Group Chair, member of BRAINMODEL consortium, Ingrid Scheffer •Melbourne Laureate Professor, The University of Melbourne, Honorary Senior Research Fellow, The Florey Institute of Neuroscience and Mental Health and Murdoch Children's Research Institute and Director of Paediatrics, Austin Health, Investigator for Anavex Life Sciences, Cerecin Inc, Cerevel Therapeutics, Eisai, Encoded Therapeutics, EpiMinder Inc, Epygenyx, ES-Therapeutics, GW Pharma, Marinus, Neurocrine BioSciences, Ovid Therapeutics, Takeda Pharmaceuticals, UCB, Ultragenyx, Xenon Pharmaceuticals, Zogenix and Zynerba; and has consulted for Atheneum Partners, Biohaven Pharmaceuticals, BioMarin, Care Beyond Diagnosis, Encoded Therapeutics, Epilepsy Consortium, Ovid Therapeutics, UCB and Zynerba Pharmaceuticals, Received funding for travel from Biocodex, Biomarin, Eisai, Encoded Therapeutics, GlaxoSmithKline and UCB, IES may accrue future revenue on pending patent WO61/010176 (filed: 2008): Therapeutic Compound; has a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies; has a patent molecular diagnostic/theranostic target for benign familial infantile epilepsy (BFIE) [PRRT2] 2011904493 & 2012900190 and PCT/AU2012/001321 (TECH ID:2012-009), Scientific advisory boards for BioMarin, Chiesi, Eisai, Encoded Therapeutics, Garvan Institute of Medical Research 2023, GlaxoSmithKline, Knopp Biosciences, Nutricia, Rogcon, Takeda Pharmaceuticals, UCB, Xenon Pharmaceuticals; received speaker honoraria from BioMarin, Biocodex, Chiesi, Eisai, GlaxoSmithKline, Liva Nova, Nutricia, UCB and Zuellig Pharma, Non-Executive Director of Bellberry Ltd and a Director of the Australian Academy of Health and Medical Sciences and the Australian Council of Learned Academies Limited, Angela Morgan Murdoch Children's Research Institute and The University of Melbourne, National Health and Medical Research Council (NHMRC) Centre of Research Excellence in Speech and Language Neurobiology (CRE-SLANG) number 1116976 and NHMRC Project grant number APP1127144; NHMRC Practitioner Fellowship number 1105008 and Investigator grant number 1195955, Batten Disease Support and Research Association Advisory Board, Koolen de Vries Foundation, Redenlab Board, Apraxia Kids Advisory Board, International Association of Communication Sciences and Disorders Committee Chair

P09.003.C GUIDELINES4RARE: an ERN ITHACA project to improve care for individuals with rare genetic disorders and intellectual disability

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Background/objectives: Rare genetic disorders associated with intellectual disability (rare ID) have great impact on physical and psychosocial functioning, requiring lifelong and multidisciplinary care. To optimize care for individuals with rare ID, effective sharing and application of knowledge internationally is indispensable. Clinical practice guidelines bridge the gap between scientific evidence and clinical practice; yet, developing guidelines for this population remains challenging.

Methods: With our partners of European Reference Network ITHACA, the European network on rare malformation syndromes and rare intellectual disability, we develop clinical practice guidelines for various genetic syndromes as well as shared comorbidities. Meanwhile, we conduct research to improve guideline methodology for rare disorders, with special attention to experiential knowledge of clinical experts and individuals and families living with rare ID.

Results: Guidelines for the Phelan-McDermid and Rubinstein-Taybi syndromes are to be published in 2023; work is ongoing on a range of syndrome-specific guideline projects, as well as guidelines for transition of care, polyhandicap, and challenging behaviour. Through critical appraisal of existing guidelines and methodological guidance, as well as qualitative research among clinical experts and individuals and families living with rare ID, we aim to optimize our methodological approach for future guideline projects in rare disorders.

Conclusion: Methodologically sound guideline development is needed to provide evidence-based medicine and improve health outcomes for individuals with rare ID. By providing a blueprint of good care, guidelines may contribute to health equity throughout Europe.

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P09.004.D DISSEQ – Double-blind exome and large gene panel sequencing analyses in the first-line diagnosis of 330 patients with intellectual disability (ID): ES superiority for the identification of CNV, variants in new disease-causing genes, and new candidate genes, as well as the advantage of possible prospective reanalysis

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Next Generation Sequencing (NGS) technologies has revolutionized the field of human genetics, making it possible to have the unprecedented ability to establish molecular diagnoses in a time- and cost-effective manner. DISSEQ study aims to assess the efficiency of solo NGS strategies in the first-line diagnosis of intellectual disability from a cohort of 330 patients, using double-blind strategy 1 (array-CGH+FRAXA+large panel sequencing (459 genes)) and strategy 2 (FRAXA+exome (ES)). Strategies 1 and 2 display a positive diagnosis in 109/330 and 104/330 patients, respectively, including 75 SNV, 44 CNV/SV, 4 FRAXA, and 6 double-hits. Results were concordant in 88% between large panel and ES. Large panel did not miss any SNV while ES missed 5/75 SNV because of atypical phenotype, double-hits, bioinformatics mis-alignments and difficulties to interpret missense variants within a solo strategy. For CNV, large panel appeared limited and required to be coupled with array-CGH, whereas ES appeared totally efficient. Moreover, ES identified variants in new disease-causing genes, recently described and new candidate genes. Reanalysis of data at 12 months and 36 months of negative or non-conclusive ES conferred a significant benefit unavailable in large panel: +5,7% (19 positive of 213 reanalysis) and +2,4% (8 positive of 194 reanalysis) respectively, increasing ES diagnostic yield from 33% to 40% after 36 months of follow-up. In conclusion, solo ES obtained slightly higher results than gene panel, in particular for the identification of CNV, variants in new disease-causing genes, and new candidate genes, as well as the advantage of possible prospective reanalysis.

Conflict of Interest: None declared

P09.006.B Clinical features and developmental trajectories in school-aged children with 16p11.2 deletion

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Background: 16p11.2 deletion (16p11.2DS, BP4-BP5) is a recurrent CNV that occurs de novo in approximately 70% of cases and confers risk for NDD, including ID and ASD. This study focusses on presenting symptoms, developmental milestones and cognitive trajectories.

Methods: Digital medical records and parental interviews on medical and developmental history of 23 children (5-16 years, 12/16 de novo) with a confirmed BP4-BP5 16p11.2DS were reviewed and analyzed. Standardized intelligence tests (Wechsler Scales) were administered in all, and longitudinal IQ-data were available in a subgroup (83%,19/23).

Results: Most prominent clinical issues were nutritional problems (68%,15/22), transient/permanent hearing impairment (52%,12/23), overweight (50%,10/20) and epilepsy/seizures (43%,10/23). Developmental milestones were delayed from infancy on across several developmental domains (motor, language). At least one NDD was diagnosed in 74% (17/23), most commonly ASD and DCD (48%,11/23). Average IQ was in the mild ID range (IQ69.2) with 39% having borderline IQ (IQ70-84). Longitudinal IQ-data with first assessment at a median age of 5y10m and second timepoint at a median age of 10y10m, indicate that children perform statistically significantly lower on the second timepoint ($p < 0.001$) with 53% (10/19) showing a growing into deficit trajectory.

Conclusion: Delayed developmental milestones are frequent in 16p11.2DS carriers (BP4-BP5), as well as medical issues such as feeding problems, overweight and epilepsy. School-aged children with 16p11.2DS show increasing cognitive impairments over time and high rates of NDD (ASD, DCD). Future studies in larger cohorts including carrier relatives are needed to gain more insight into the penetrance and phenotypic variability of 16p11.2DS.

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Conflict of Interest: None declared

P09.007.C Whole genome analysis for 81 families with undiagnosed rare diseases

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Rare diseases are often difficult to diagnose. However,

comprehensive genome analysis is considered useful for such undiagnosed cases, for the reason that about 80% are estimated to be genetic or genomic diseases. In Japan, a national project, the Initiative on Rare and Undiagnosed Diseases (IRUD), has conducted whole-exome sequencing analysis (WES analysis) using short-read next-generation sequencers on such cases. The current diagnostic yield of IRUD is approximately 40%. However, WES targets exons in the gene coding regions and is limited in detecting significant pathogenic variants. Whole genome sequencing analysis (WGS analysis) is performed in cases where the diagnosis was not made after WES analysis in IRUD, or where WGS analysis was 1st tier at the time of analysis. In the present study, we report the results of whole-genome analysis of 81 undiagnosed families with considering to be rare or intractable diseases. WGS was carried out using a short-read next-generation sequencer at an average depth of 30x, and variant calling was performed including CNVs (copy number variants) in addition to the SNVs and indelsl. In almost all cases, a trio-based analysis was performed (243 samples). As a result, causative pathogenic variants (including chromosomal structural abnormalities) were found in 31 families, leading to a final diagnosis. Of these, WGS analysis could have identified the cause in approximately 6% of cases, in which a small deletion involving a 19 bp exon was detected and confirmed. Whole genome analysis appeared to be effective for detailed genomic analysis, except for the cost.

Conflict of Interest: None declared

P09.009.A Glycosylphosphatidylinositol deficiency due to compound heterozygous frameshift GPAA1 variants revealed using whole exome sequencing

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Glycosylphosphatidylinositol biosynthesis defect 15 (GPIBD15, MIM617810) is a rare autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the *GPAA1* (glycosylphosphatidylinositol anchor attachment 1) gene. GPIBD15 is characterized by delayed psychomotor development (DD), variable degree of intellectual disability (ID), hypotonia, early-onset seizures and cerebellar atrophy. *GPAA1* was associated with GPIBD15 in 2017, and since then only 17 patients from 12 families have been reported.

We describe a 9-year-old boy with delayed psychomotor development, moderate intellectual disability, initially normal speech development but currently non-verbal after regression, seizures, ataxia, microcephaly, short stature, scoliosis and quadriparesis spastica.

Exome sequencing revealed compound heterozygous frameshift *GPAA1* variants, paternal c.809 del, p.(Gly270Alafs*48) and maternal c.1477_1478del, p.(Arg493Glyfs*152) (NM_003801.4). The paternal variant was absent from all databases. The maternal variant has already been described in one patient.

Our patient confirms the typical spectrum of features of GPIBD15 such as DD/ID, speech delay, hypotonia, seizures or cerebellar symptoms. The patient is exceptional as homozygous or compound heterozygous loss-of-function variants have not been identified in any of the previous patients. Surprisingly, his phenotype is not the most severe. He started walking even earlier (at 24 months of age) than most of the other patients, and his MRI is normal. Of interest might be his initially normal speech development followed by regression and current absence of speech. Decrease in the CD16 level on granulocytes by flow cytometry is considered an insufficient diagnostic marker, and exome sequencing is thus indispensable in the diagnosis of this rare disorder.

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Conflict of Interest: Miroslava Hančárová Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, Markéta Vlčková Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, Šárka Bendová Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, Darina Prchalová Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, Viktor Stranecky Department of Pediatrics and Adolescent Medicine, Diagnostic and Research Unit for Rare Diseases, Charles University 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic, Zdeněk Sedláček Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic

P09.010.B GenIDA, an international participatory registry to better characterise comorbidities of genetic forms of intellectual disability: insights on POGZ, SETD5 and KBG syndromes

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Background/Objectives: GenIDA is an international participatory registry initiated to better characterise the clinical manifestations and natural history of genetic forms of intellectual disability with or without autism or epilepsy.

Methods: Clinical information reported and updated by the patient's family using a structured questionnaire is analysed to identify new medically relevant information for families and professionals concerned with a given condition. The current questionnaire consists of 41 multiple-choice questions exploring physical parameters, cognitive and behavioural aspects, the presence or absence of neurological disorders or problems affecting major physiological functions (cardiac, renal, etc.). Five

open-ended questions explore families' perception of the events that most affect their relative's health and quality of life, the secondary effects of treatments, etc.

Results: Currently, the questionnaire is available in 8 languages and has been completed for over 1730 patients, the main cohorts being Koolen-de Vries (n = 246) and Kleefstra syndromes (193). Other cohorts have grown significantly over the past year (ANKRD11-KBG syndrome: 48, SETD5 syndrome: 32, POGZ-White Sutton syndrome: 29. Comparing several aspects of these conditions reveals that behavioural problems are major concerns for families affected by these conditions. Vision problems are frequently reported for both POGZ and KBG patients, while sleep disorders appear to be more frequent in POGZ patients, and feeding difficulties are more frequently reported in SETD5 patients.

Conclusion: This validates the interest of our participatory approach: through their direct involvement, families can reveal aspects of the pathology that were previously underestimated.

Conflict of Interest: None declared

P09.011.C Case report demonstrating certain pitfalls and challenges in NGS data interpretation

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Menke-Hennekam syndrome-1 (MKHK1) is a congenital disorder characterized by developmental delay, intellectual disability, variable facial characteristics, as well as, feeding difficulties, autistic behaviour, recurrent upper airway infections, hearing impairment, short stature, and microcephaly. It is caused by heterozygous mutations in exon 30 or 31 of the CREB Binding Protein (*CREBBP*) gene, whereas mutations elsewhere in the *CREBBP* gene result in Rubinstein-Taybi syndrome-1 (RSTS1), which is phenotypically distinct.

The patient, along with his parents, was referred for trio-based clinical exome sequencing (CES). The patient is a 21-month-old boy with severe global developmental delay, failure to thrive, arched eyebrows, long lashes and prominent forehead.

CES was performed on Illumina NextSeq 2000 platform using TruSight One sequencing panel. Bioinformatic analysis, annotation and interpretation were performed with the VarSome Clinical platform (version 11.3, hg19).

Sanger sequencing confirmed the CES findings.

A de novo, missense variant was identified and confirmed by Sanger Sequencing, at exon 31 of NM_004380.3 transcript in *CREBBP* gene (NC_000016.9:3779680A>G), resulting in a cys-to-arg substitution at codon 1790 (Cys1790Arg).

Based on the clinical data, the detected variant was linked to the patient's phenotype. Even though the same gene is responsible for another phenotypically distinct syndrome (RSTS1), the specific exon where the variant is located has differentiated the final diagnosis to MKHK1. This finding highlights the pitfalls and challenges in NGS data interpretation; the importance of a detailed phenotypic description in combination with an in-depth review of all gene-related data available through literature and databases, is determinant to reach an accurate genetic diagnosis.

Conflict of Interest: None declared

P09.012.D Terminal triplications of 1p36.3, including GABRD and SKI, are causing a remarkable overlapping facial and developmental phenotype

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Background: Although rearrangements involving chromosomal region 1p36.3, both gains and losses, are a common cause of developmental disorders, only one patient with isolated triplication of 1p36.3 has been described.

Methods: We describe phenotypic and molecular data (molecular karyotyping and FISH) from four unrelated patients with variably sized 1p36.3 triplications. Their phenotype was compared to that of patients with 1p36.3 duplications, described in literature and patient databases.

Results: Patients presented with severe feeding problems, seizures, global DD, moderate ID and behavioral problems. Their facial gestalt includes ptosis, arched eyebrows and hypertelorism. Patients with isolated duplications present with a similar, but less penetrant and less severe phenotype. The minimal overlapping region of these de novo triplications spans 508kb((hg38) chr1:1892482-2400650) and comprises four disease-related genes of which *GABRD* and *SKI* are most likely to contribute to the phenotype. SKI is a negative regulator of the TGF-β pathway. In patients with Shprintzen-Goldberg syndrome, de novo heterozygous missense variants prevent SKI degradation, resulting in attenuation of TGF-β signaling. Mild-to-moderate ID and hypertelorism are shared symptoms between patients with SGS and patients with 1p36.3 triplications. Heterozygous gain-of-function missense variants in *GABRD* were recently associated with neurodevelopmental disorders with behavioral issues, mild-to-severe ID, and seizures. Further studies are ongoing to link the phenotype of 1p36 triplications to altered expression of these genes.

Conclusion: We introduce a novel chromosomal syndrome with a distinct phenotype, caused by isolated de novo triplications on 1p36.3. The shared triplicated region encompasses *GABRD* and *SKI*, most likely contributing to the phenotype.

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Conflict of Interest: None declared

P09.013.A Development of diagnostic solutions for neurodevelopmental disorders caused by ubiquitin-proteasome system dysfunction

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Background/objectives: Neurodevelopmental disorders (NDDs) affect >3% of individuals worldwide and have a monogenic etiology in ~50% of cases. Strikingly, 10-15% of monogenic NDDs are caused by pathogenic variants in genes coding components of the ubiquitin-proteasome system (UPS), which is essential for selective protein degradation in eukaryotic cells. However, the complexity of the system creates major challenges in assessing the pathogenicity of genetic variants, and good biomarkers indicative of UPS dysfunction are largely lacking, hampering the diagnosis of UPS-related NDDs (UPS-NDDs). Our objective is to provide reliable biomarkers and functional assays to classify UPS-related variants.

Methods: We created the European UPS-NDDiag consortium, structured around six partners from five countries, and enriched by 26 international collaborators. Based on >250 pathogenic variants identified by the consortium across >30 UPS genes associated with NDD, we will implement analysis tools using complementary skills and expertise in genetics, functional genomics; facial recognition; functional studies in hIPSCs/ brain organoids; animal models; bioinformatics; integrative analysis of multi-omics, and pharmaceutical nanotechnology.

Results: We will present a project overview, figuring the complementary approaches and interconnections between the work packages.

Conclusions: Our project is one of the 12 winning projects of the EJP RD JTC 2022 call. In addition to implementing diagnostic tools, UPS-NDDiag aims to yield therapeutic targets that may support drug development for personalized medicine and shed light on our current understanding of the overall pathogenesis of disorders related to the UPS.

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Conflict of Interest: None declared

P09.014.B Linear diagnostic procedure elicited by clinical genetics and validated by mRNA analysis in Neuronal Ceroid Lipofuscinosis 7 associated with a novel non-canonical splice site variant in MFSD8

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Neuronal ceroid lipofuscinoses (CNL) are lysosomal storage diseases that represent the most common cause of dementia in children. To date, 13 autosomal recessive (AR) and 1 autosomal dominant (AD) gene have been characterized. Biallelic variants in *MFSD8* cause CLN7 type, with nearly 50 pathogenic variants, mainly truncating and missense, reported so far. Splice site variants require functional validation. We detected a novel homozygous non-canonical splice-site variant in *MFSD8* in a 5-year-old girl who presented with progressive neurocognitive impairment and microcephaly. The diagnostic procedure was elicited by clinical genetics first, and then confirmed by cDNA sequencing and brain imaging. Inferred by the common geographic origin of the parents, an autosomal recessive inheritance was hypothesized, and SNP-array was performed as the first-line genetic test. Only three AR genes lying within the observed 24 Mb regions of homozygosity were consistent with the clinical phenotype, including *EXOSC9*, *SPATA5* and *MFSD8*. The cerebral and cerebellar atrophy detected in the meantime by MRI, along with the suspicion of accumulation of ceroid lipopigment in neurons, prompted us to perform targeted *MFSD8* sequencing. Following the detection of a splice site variant of uncertain significance, skipping of exon 8 was demonstrated by cDNA sequencing, and the variant was redefined as pathogenic.

Conflict of Interest: None declared

P09.015.C de novo PHF5A variants cause craniofacial abnormalities and developmental delay

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The SF3B complex is composed of SF3B1-6 and PHF5A and recognizes the branch point adenosine during splicing. PHF5A is a ubiquitously expressed and evolutionarily highly conserved nuclear protein. We report on a developmental disorder caused by de novo variants in *PHF5A*. We studied seven individuals with variable congenital malformations, including preauricular tags, growth abnormalities, and developmental delay who had a de novo heterozygous *PHF5A* variant, including two missense, one start loss, and four loss-of-function (LOF) variants. In fibroblasts from four subjects with *PHF5A* LOF variants, we identified wild-type and mutant *PHF5A* mRNAs at a ratio of ~1:1, suggesting escape of nonsense-mediated mRNA decay that was confirmed by similar *PHF5A* mRNA levels in subject and control cells. On protein level, we detected similar amounts of PHF5A with the predicted wild-type

molecular weight as well as of SF3B1-3, and SF3B6 in almost all subject and control fibroblasts. SF3B complex formation was unaffected in two subject cell lines. Our data suggest the existence of feedback mechanisms in fibroblasts with *PHF5A* LOF variants to maintain normal levels of SF3B components. Several genes encoding splicing factors, like SF3B2 and SF3B4, the causative genes for craniofacial macrosomia and Nager syndrome, respectively, negatively autoregulate their own expression to control and maintain homeostatic levels of spliceosomal proteins required for effective pre-mRNA splicing. The observed compensatory mechanisms in certain types of subject cells questions haploinsufficiency as a potential pathomechanism and suggest disturbed autoregulation of mutated splicing factor genes in specific cell types (i.e. neural crest cells) during embryonic development.

Conflict of Interest: None declared

P09.016.D Rare loss-of-function variants in DOCK4 lead to neurodevelopmental delay

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Objectives: Despite increasing improvements in sequencing techniques, 27-42% of patients with developmental disabilities remain undiagnosed. In the present study we evaluate the dedicator of cytokinesis 4 (DOCK) gene as a novel cause for a monogenetic neurodevelopmental disorder.

Methods: Through Trio-Exome analysis, matchmaking platforms and international collaboration we gathered a cohort of 8 individuals (7 males and one female) and one fetus with *de* novo and/or inherited variants in DOCK4 and a neurodevelopmental disorder. Variants were functionally analyzed by an in silico molecular modelling and in a neurite outgrowth assay by transient overexpression or after CRISPR-Cas9-mediated *Dock4*-knock-out Neuro-2A cells.

Results: All individuals show mild to severe developmental delay. Other common symptoms comprise movement disorders such as ataxia or dystonia, hypotonia, autism spectrum disorder, seizures, MRI abnormalities and microcephaly. Molecular modelling of the heterozygous missense variants suggest that the majority of them affect the globular structure of *DOCK4*. After transfection of corresponding expression plasmids, Neuo-2A cells exhibited significantly reduced neurite length compared to overexpression of the wild type *DOCK4*. Furthermore, knock-out of *Dock4* in Neuro-2A cells, resulted also in a reduced neurite length compared to wild type cells.

Conclusion: Our results including clinical, molecular and functional data suggest that rare loss-of-function-variants in *DOCK4* cause a variable spectrum of a novel neurodevelopmental disorder. As predominantly men were affected in our cohort, a gender-specific effect is possible.

Grant References: none

Conflict of Interest: None declared

P09.017.A Investigating mutation-specific mechanisms of SATB2 missense variants to reveal genotype-phenotype correlations in SATB2-associated syndrome

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Introduction: SATB2 is a transcription factor that has been implicated in a neurodevelopmental disorder. The associated phenotype is variable, ranging from mild to more severe symptoms. We recently reported that missense variants in the close gene family member, *SATB1*, result in increased protein activity and more severe phenotypes compared to loss-of-function variants. Therefore, we hypothesized that different *SATB2* variants may have distinct functional consequences as well, important for establishing genotype-phenotype correlations.

Methods and results: Here, we functionally tested 28 etiological *SATB2* missense variants located in the DNA-binding domains, including twelve recurrent variants, as well as three rare missense variants identified in healthy individuals. We characterized their effects on nuclear localization, transcriptional activity and global chromatin binding in cell-based assays. Consistent with a gain-of-function effect, we found 7/28 (25%) of the etiological missense variants to have an altered nuclear localization and increased transcriptional repression on two SATB2 targets (Ctip2-MAR, Nr4a2-MAR), while the other variants showed decreased transcriptional activity indicative of a loss-of-function. Using fluorescent recovery after photobleaching (FRAP) experiments, we demonstrate that the variants associated with increased activity show a decrease in protein mobility, suggesting stabilization of DNA binding. An integrative analysis of the functional data with in-depth phenotypic information deposited in the SATB2 Registry, including disease severity, is currently ongoing, to establish genotype-phenotype correlations for *SATB2* missense variants.

Conclusions: In summary, using a combination of cell-based functional assays, we show that etiological *SATB2* missense variants have distinct functional consequences, which may be linked to severity of the associated neurodevelopmental disorder.

Conflict of Interest: None declared

P09.018.B Epigenotype-genotype-phenotype correlations in SETD1A and SETD2 chromatin disorders

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Background/Objectives: Gene products of *SETD1A* and *SETD2* are key components of the chromatin-mediated regulation of gene expression. Germline pathogenic variants in two genes encoding the lysine-specific histone methyltransferase genes *SETD1A* and *SETD2* are associated with neurodevelopmental disorders (NDDs) characterised by developmental delay and congenital anomalies. There have been specific methylation episignatures identified for a range of chromatin gene-related NDDs that have improved interpretation of variant pathogenicity in clinical practice.

Methods: We performed genome-wide methylation profiling using next generation sequencing based assay of >2M CpGs to determine whether *SETD1A* and/or *SETD2*-related NDDs exhibit a particular episignature.

Results/Conclusion: Comparison of methylation profiles in patients with *SETD1A* variants (n = 7; 6 pathogenic/likely pathogenic and one VUS) did not reveal any strong methylation episignature. In contrast, both *SETD2* subgroups (*SETD2* Luscan-Lumish syndrome (n = 4) and *SETD2* codon 1740 missense variants (p.Arg1740Trp (n = 4) and p.Arg1740Gln (n = 2)) demonstrated a methylation episignature which was characterised by hypomethylation and hypermethylation events respectively. Within the codon 1740 subgroup, both the methylation changes and clinical phenotype were more severe in those with p.Arg1740Trp variants. We also noted that two of 10 cases with a *SETD2*-NDD had developed a neoplasm. These findings reveal novel epigenotype-genotype-phenotype correlations in *SETD2*-NDDs and predict a gain-of-function mechanism for *SETD2* codon 1740 pathogenic variants.

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Conflict of Interest: Sunwoo Lee Rosetrees Trust, Lara Menzies: None declared, Eleanor Hay: None declared, Eguzkine Ochoa NIHR Cambridge Biomedical Research Centre, France Docquier: None declared, Fay Rodger: None declared, Charulata Deshpande: None declared, Nicola Foulds: None declared, Sébastien Jacquemont: None declared, Khadije Jizi: None declared, Alison Kraus: None declared, Katharina Wemmenhove-Lohner: None declared, Patrick J Morrison: None declared, Bernt Popp: None declared, Ruth Richardson: None declared, Arie van Haeringen: None declared, Jose-Ezequiel Martin Rodriguez: None declared, Ana Toribio: None declared, Fudong Li: None declared, Wendy Jones: None declared, Francis H Sansbury: None declared, Eamonn Maher Principal investigator, NIHR Cambridge Biomedical Research Centre

P09.019.C CNOTs-related disorders: a recognized group of neurodevelopmental conditions caused by defective CCR4-NOT complex

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Background: Individuals with variants in genes that encode different subunits of the CCR4-NOT protein complex have been sporadically reported. The aim of this study was to investigate whether they have overlapping phenotypes.

Methods: We included 91 published and unpublished individuals with heterozygous pathogenic variants in *CNOT1*, *CNOT2*, *CNOT3* and *CNOT9*, and reviewed their clinical manifestations.

Results: Affected individuals carrying pathogenic variants in genes codifying subunits of the CCR4-NOT complex were found to share common clinical features. The majority of patients had developmental delay/intellectual disability (83/86, 97%). Hypotonia (53/76, 70%) and behavioral abnormalities (42/69, 61%) were common, whereas seizures were seen in one quarter of individuals. Subtle skeletal anomalies were detected in 28/46 (61%). No recurrent birth defects were seen in this cohort.

Evaluation of available photographs identified common facial features, with a high forehead and abnormal shape of the nose consistently found across the four genes.

Cleft and/or high palate were seen in half of the patients. Interestingly, holoprosencephaly and pancreatic problems were present in only 10 previously reported individuals with the recurrent p.Arg535Cys variant in *CNOT1*, but were not found in any other patient with different variants in either *CNOT1* or other genes of the complex, suggesting that midline defects may be at the most severe end of the spectrum or confined to variants affecting specific domains.

Conclusion: Our study delineates a novel genetically and clinically defined group of neurodevelopmental disorders with a shared phenotype across different genes of the CCR4-NOT complex, which we propose to name "*CNOT*s-related disorders".

Conflict of Interest: None declared

P09.020.D Publicly funded exome sequencing for outpatients with neurodevelopmental disorders demonstrates a high rate of unexpected findings with an impact on medical management

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Background: Exome sequencing (ES) is a powerful tool that facilitates the diagnosis of patients with rare Mendelian syndromes. In 2018 the Israeli Ministry of Health (IMOH) initiated a national pilot program that funds ES for out-patients with global developmental delay (GDD). Here, we describe the 3-year impact of this program on patient care in a single tertiary hospital.

Methods: Between 2018-2020 trio ES was performed on 170 patients fulfilling IMOH criteria: 1) Moderate to severe GDD (DQ or IQ <55) 2) Mild GDD (DQ or IQ <70) with epilepsy or a major congenital anomaly. We retrospectively analyzed this cohort.

Results: A diagnosis was achieved in 74 individuals (43%). There were 82 clinically significant variants, the majority being novel. Consanguinity was reported in 22% and was not associated with a higher diagnostic rate. The presence of autism spectrum was associated with a lower diagnostic rate of 8/33 (24%). Autosomal dominant inheritance was identified in 14% of participants and the parental phenotype ranged between fully affected and asymptomatic. Among the diagnosed patients, 16% had an unexpected diagnosis that did not fit the typical clinical presentation. In 9% the diagnosis changed the medical treatment and in 19% the surveillance recommendations.

Conclusions: The introduction of a national program that funds ES for GDD has transformed patient care, leading to a major effect on patient management and treatment. The high rate of an unexpected inheritance mode and variable phenotypes emphasizes the diagnostic complexity of neurodevelopmental disorders and the strength of a non-targeted approach.

Conflict of Interest: None declared

P09.021.A Diagnostic yield of Intellectual Disability using whole exome singleton analysis, real world data

Nelmar Valentina Ortiz Cabrera[1], *Barbara Fernández Garoz*[2], *Beatriz Bernardino Cuesta*[3], *Verónica Cantarin Extremera*[3], *ELENA GONZÁLEZ*[3], *Laura López Marín*[3], *Victor Soto insuga*[3], *Sara Vila Bedmar*[3], *Luis Gonzalez Gutierrez Solana*[3], *Juan José García Peñas*[3], *Ana Isabel Quinteiro García*[2], *Anna Duat Rodríguez*[3]

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Background: Intellectual disability affects up to 3% of children. Once perinatal affections are rule out, standard etiological studies are complex and with a low diagnostic yield. Our aim is to calculate the diagnostic yield of whole exome analysis in these patients.

Methods: Here we present data from an 3º level paediatric hospital from June 2018 to July 2022.

We studied patients derived to the Clinical Genetics Clinic for study, being Intellectual Disability (HP:0001249) or Global developmental delay (HP:0001263) the phenotypic drivers. We just included those patients with a normal chromosomal study. All patients were studied using singleton Whole Exome Sequencing (WES), variants were filtered using virtual panel approach and/or Human Phenotype Ontology terms (HPO) approach and classified using ACMG recommended criteria different kits were used as all the analyses were done in different commercial labs. All candidate variants were segregated when possible. Some negative cases were re-analysed one to three years after, as the virtual panel and/or HPO were updated.

Results: A total of 292 patients were studied in this period. In 32% of cases diagnostic was reach on primary analysis. 59 studies were re-analysed leading to a 12 (4%) additional diagnosis. In 4 patients a gross deletion/duplication was detected.

Conclusion: The study of patients with intellectual disability or global developmental delay using WES allows the diagnosis in one third of patients, with one non-invasive test. Also, WES allows to re-analyses data without any additional sample or cost, adding up diagnostic yield as new genes are added as disease causing.

Conflict of Interest: Nelmar Valentina Ortiz Cabrera NIMGenetics, Barbara Fernández Garoz: None declared, Beatriz Bernardino Cuesta: None declared, Verónica Cantarin Extremera: None declared, ELENA GONZÁLEZ: None declared, Laura López Marín: None declared, Victor Soto insuga: None declared, Sara Vila Bedmar: None declared, Luis Gonzalez Gutierrez Solana: None declared, Juan José García Peñas: None declared, Ana Isabel Quinteiro García: None declared, Anna Duat Rodríguez: None declared

P09.022.B Delineating the neurobiological mechanisms involved in SETBP1-haploinsufficiency disorder using human brain organoids and transcriptomics

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Introduction: Haploinsufficiency of the *SETBP1* gene causes a highly heterogeneous neurodevelopmental syndrome (*SETBP1*-haploinsufficiency disorder) with the main phenotypic features including moderate-to-severe speech and language impairments, and wide variability in intellectual functioning. The precise functions of *SETBP1*, encoding the SET-binding protein, are yet to be discovered. Therefore, the neurobiological pathways by which rare loss-of function *SETBP1* variants cause a neurodevelopmental disorder remain largely unknown.

Materials and Methods: By employing induced pluripotent stem cell (iPSC)-derived brain organoids and transcriptomic approaches, we aim to dissect the underlying aetiological pathways. We have generated iPSCs from three patients carrying heterozygous de novo truncating variants and sex-matched parents as controls, and *SETBP1* knockout iPSCs with CRISPR/Cas9 gene-editing. *SETBP1* knockout and isogenic control iPSC lines were differentiated into cerebral organoids. Their transcriptomic profiles were analysed at both whole organoid and single-cell levels. Morphological examination, cell-type specific differential gene expression analysis and cell lineage tracing were performed at two selected developmental timepoints.

Results: We found that when differentiated into cerebral organoids, homozygous *SETBP1* knockouts showed striking gross morphological differences, while heterozygous knockout organoids displayed subtle changes in ventricles that resembled neuroepithelium. Preliminary transcriptomic analyses of these organoids suggested alterations in cell fate commitment since early stages of organoid differentiation.

Conclusions: *SETBP1* knockout organoids showed gross structural, ventricular and transcriptomic anomalies during early organoid development, suggesting aberrations in cell fate commitment during embryonic neurodevelopment. Together, this work promises to offer valuable insights into fundamental understanding of neurodevelopmental roles of SETBP1 and aetiological mechanisms that go awry in *SETBP1*-haploinsufficiency disorder.

Conflict of Interest: None declared

P09.024.D OMIXCARE: OMICS technologiessolved about 33% of the patientswith heterogeneous rareneuro-developmental disordersand negative exome sequencingresults and identified 13%additional candidate variants

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Patients with rare genetic diseases, which affect 350 million people worldwide, may experience a diagnostic odyssey. High-throughput sequencing leads to an etiological diagnosis in up to 50% of individuals with heterogeneous neurodevelopmental or malformation disorders. There is a growing interest in additional omics technologies in translational research settings to examine the remaining unsolved cases. We gathered 30 individuals with malformation syndromes and/or severe neurodevelopmental disorders with negative trio exome sequencing and array comparative genomic hybridization results through a multicenter project. We applied short-read genome sequencing, total RNA sequencing, and DNA methylation analysis, in that order, as complementary translational research tools for a molecular diagnosis. The cohort was mainly composed of pediatric individuals with a median age of 13.7 years. Genome sequencing alone identified at least one variant with a high level of evidence of pathogenicity in 8/30 individuals (26.7%) and at least a candidate disease-causing variant in 7/30 other individuals (23.3%). RNA-seq data in 23 individuals allowed two additional individuals (8.7%) to be diagnosed, confirming the implication of two pathogenic variants (8.7%), and excluding one candidate variant (4.3%). Finally, DNA methylation analysis confirmed one diagnosis identified by genome sequencing (Kabuki syndrome) and identified an episignature compatible with a BAFopathy in a patient with a clinical diagnosis of Coffin-Siris with negative genome and RNA-seq results in blood. Overall, our integrated genome, transcriptome, and DNA candidate gene in 4/30 (13.3%) of the patients with rare neurodevelopmental disorders and negative exome sequencing results.

Conflict of Interest: None declared

P09.025.A RBMX2, a novel candidate gene for an X-linked neurodevelopmental disorder

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Background/Objectives: A growing list of genes involved in post-transcriptional regulation of gene expression such as regulation of mRNA splicing, stability or translation have been identified as causing monogenic forms of Neurodevelopmental disorder (NDD) with Intellectual disability (ID).

Methods & Results: We identified de novo missense variants in two girls with NDD in RBMX2, an X-linked gene encoding a component of the retention and splicing (RES) complex. RBMX2 is a strong candidate gene for NDD as it is highly expressed in brain, intolerant to loss of function and strong brain defects have been observed in mutant zebrafish (Fernandez et al., 2008). We collected five additional missense and truncating variants in males and females and found that the recurrent features included moderate to severe ID, seizures and microcephaly. To confirm the involvement of RBMX2 in NDD, we studied the consequences of its inactivation in human neural stem cells (hNSC) on gene expression and splicing (intron retentions), and analysed the effects of missense variants on RBMX2 expression and cellular localisation.

Conclusion: RBMX2 is a strong candidate gene for a novel X-linked form of NDD with ID, microcephaly and epilepsy, affecting both males and females.

Conflict of Interest: None declared

P09.027.C Duplication of Xp21.3-p22.1 region involving ARX gene in a male patient with intellectual developmental disorder

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The Aristaless-related homeobox gene (*ARX*, MIM#300382) encodes a transcriptional protein involved in molecular pathways of neuronal patterning and cerebral development.

Haploinsufficiency of *ARX* causes X-linked neurodevelopmental disorders. Duplications of region including *ARX* were described in only several patients with X- linked intellectual disability, although asymptomatic patients have been described. Such variable phenotype in small number of patients makes clinical diagnosis very challenging.

We present an 8-year old boy with a motor and speech developmental delay, hyperactivity, attention deficit, emotional immaturity and moderate intellectual disability.

His fine motor skills are immature for his age and the muscle tone is hypotonic. He has unspecific slightly dysmorphic features. He has asthenic constitution with locomotor deformations: narrow chest, pectus carinatum type, inverted nipples, thoracolumbar scoliosis, winging scapula, hypoplastic nails and hypermobile joints. Eye examination showed strabismus with hypermetrophia and reduced retino-cortical conduction on the right eye.

Cytogenetic analysis showed a normal male karyotype. Molecular analysis of CTG trinucleotides in DMPK revealed a normal range of CTG triplet 11/11. Array-CGH analysis showed one copy gain of region Xp21.3-p22.11 size of 828 kb, containing three morbid genes: *ARX, PDK3*, and *POLA1*. Functional enhancers of *ARX*: hs118, hs119, hs121, hs122 and hs145 are also present in duplication. He was adopted at the age of 15 months. Biological mother has only mild intellectual disability.

Data from our patient and previously reported cases strongly indicate that duplication of Xp21.3 region including *ARX* and its enhancers is a copy number variant associated with increased risk for X-linked intellectual disability.

Conflict of Interest: None declared

P09.028.D The importance of analyzing genes with weak phenotypic association: PHF12 as a potential cause of developmental disorder phenotype

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Introduction: The analysis of Genes of Uncertain Significance (GUS) is one of the challenges of genetic diagnosis. To that aim, routine molecular diagnostic laboratories are a very powerful source of genetic information; however, the limitation in performing functional studies slows down the novel description of gene phenotypic association. *PHF12* is an example for which an association between its loss-of-function variants and neurodevelopmental disorders has been suggested but for which a molecular confirmation is still pending.

M&Ms: Whole Exome sequencing was performed for two patients. A 11-year-old male, with language disorder, autism, borderline intellectual disability, fine psychomotor disorder and dysmorphisms; and a 6-year-old male, with developmental delay, poor language proficiency and motor clumsiness. After a first-tier gene-targeted inconclusive analysis, it was extended to potentially pathogenic variants in GUS.

Results: Two de novo truncating variants in *PHF12* were detected: c.2059C>T;p.(Gln687*) and c.2011_2014del;p.(Thr671-Profs*36), respectively. Both variants are predicted to undergo Nonsense-mediated decay and they are not described in general population databases. Moreover, *PHF12* is haploinsufficiency intolerant (pLI = 1 gnomAD constraint).

Conclusions: According to the molecular findings identified in these two patients, we propose *PHF12* as the main suspect for causing the pathology; however, further patients and functional studies are needed to stablish this association. These results highlight the need to perform a further analysis including those genes for which a definitive phenotypic association remains unknown, in order to contribute to research by providing with phenotypic data with the aim of better characterizing novel phenotype-genotype associations and, above all, improving genetic diagnosis performance.

Conflict of Interest: None declared

P09.031.C A severe neurocognitive phenotype caused by biallelic CHD3 variants in two siblings

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Background: *CHD3* heterozygous variants are associated with Snijders Blok-Campeau syndrome (SBCS) which consists of Intellectual disability (ID), macrocephaly and dysmorphic facies. Most reported variants are missense or loss of function clustered within the ATPase/helicase domain of the protein. We report a severe neurocognitive phenotype caused by biallelic *CHD3* variants in two siblings each inherited from a mildly affected parent.

Methods: Male and female siblings were referred to the genetic clinic due to severe ID and profound dysmorphism. The parents are first cousins with borderline intellectual abilities. Exome sequencing was performed for the affected female and her parents.

Results: A single homozygous candidate variant in the *CHD3* gene was detected in the proband; c.5384_5389dup. p.Arg1796_-Phe1797insTrpArg resulting in an in frame insertion of 2 amino acids located outside the ATPase/helicase domain at the C-terminal region of *CHD3*-encoding residues. Both affected siblings were homozygous while their unaffected brother did not carry the variant.

Conclusion: Biallelic *CHD3* variants cause a severe neurodevelopmental syndrome that is distinguishable from SBCS. We assume that variant type (in frame insertion) and affected domain may enable CHD3 biallelic variants.

	Variant	ID	Clinical findings	Dysmorphism
Affected female	homozygous	Severe	pes cavus clubfoot ventriculomegaly hypothyroidism epilepsy friendly personality	dolichocephaly large nose synophrys epicanthal folds dental & gingival abnormalities retrognatia
Affected Male	homozygous	Severe	scoliosis pectus carinatum pes cavus strabismus hypothyroidism friendly personality	dolichocephaly, large nose, dental & gingival abnormalities low set protruding ears synophrys retrognatia

Table d. continued

	Variant	ID	Clinical findings	Dysmorphism
Father	heterozygous	Borderline	hernia	-
Mother	heterozygous	Borderline	unilateral microphthalmia	sloping forehead ptosis prognathism
Brother	WT			

Conflict of Interest: None declared

P09.032.D Co-existence of two variants in ANKRD11 and PTEN genes in a dysmorphic patient with intellectual disability

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Background: Atypical patients' phenotype often leads to a diagnostic odyssey. However, since the introduction of exome and genome sequencing, the diagnostic yield in dysmorphic patients with intellectual disability has greatly increased.

Case presentation: 15-year-old boy was referred to the clinical genetics unit due to neurodevelopmental problems. He was born at term, to young, unrelated parents without any birth defects. He had a developmental delay. At the age of 9 years, clinical signs of puberty appeared. On admission, physical examination showed a length of 175 cm (75c), weight of 68 kg (75c), and OFC of 58 cm (90c). Facial dysmorphisms consisted of a triangular face, high forehead, protruding ears, long philtrum, prominent mandible, macrodontia of upper incisors. Additionaly macroorchidism and brachydactyly of the hands and feet were noticed. Behavioral problems such as autism-like features, impulsivity, anxiety, and moderate intellectual disability were observed. Genetic tests were performed, including karyotype, subtelomeric MLPA, *FMR1* gene, and metabolic screening, which were all normal.

Results: NGS panel (including genes associated with intellectual disability) revealed a novel de novo missense heterozygous variant c.4715_4728del in *ANKRD11* (NM_013275.6) gene, which results in the KBG (OMIM #148050) syndrome phenotype (distinctive facial dysmorphism, brachydactyly, precocious puberty, neurodevelopmental delay). Moreover, a likely pathogenic mutation c.63dup in *PTEN* (NM_001304717.5) gene of maternal origin was shown, which may manifests as macrocephaly/autism syndrome (OMIM #605309).

Conclusion: This is the first report of overlapping KBG and Macrocephaly/autism Syndromes. Identification of pathogenic variants has a big prognostic and therapeutic value for presented patient and his family.

Conflict of Interest: None declared

P09.033.A Molecular profiles of MECP2 duplication syndrome and Rett syndrome patients

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Background/Objectives: *MECP2* is a multifunctional gene involved in transcription activation, repression, splicing and microRNA processing. Loss of function mutations in *MECP2* trigger Rett syndrome (RTT) while duplication of the entire gene leads to *MECP2* duplication syndrome (MDS). MDS is usually inherited from healthy asymptomatic carrier mothers. Multi-omics has revealed altered biological processes in RTT samples, but that characterization has not been done in MDS patients.

Methods: RNA and protein from skin fibroblasts of 21 RTT patients, 17 MDS patients, 10 *MECP2* duplication carriers and 13 healthy controls were obtained. RNAseq and proteomics analysis were performed with DESeq2 and LIMMA in a case-control approach. Enrichment of the differentially expressed genes (DEGs) and proteins was evaluated.

Results: MDS male patients have cytoskeleton, vesicular transport and neuronal system related genes dysregulated. MDS female patients have translation and cytoskeleton related genes altered. Interestingly, *MECP2* duplication carrier mothers' molecular profile differs from the controls' profile despite being all healthy. The dysregulation in genes related to transcription, translation and protein degradation could be explaining their asymptomatic phenotype despite carrying the duplication. Cell adhesion and mRNA splicing genes were dysregulated in an opposed way between RTT and MDS, indicating direct MeCP2 effects.

Conclusion: Multi-omics approach has shown gene candidates for biomarkers. Besides, molecular profiles have broadened the understanding of the dysregulated mechanisms in these cohorts and could become a technique for diagnosis and prognosis.

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P09.034.B Evaluation of the 16p11.2 proximal copy number variants in patients with neurodevelopmental disorders

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Background: Deletions and duplications at the chromosomal region of 16p11.2 have a broad range of phenotypic effects including intellectual disability, autism, epilepsy, language and motor delays. Recurrent proximal copy number variants (CNVs) at the 16p11.2 (BP4-BP5 region) are among the most frequent genetic causes of neurodevelopmental disorders (NDDs).

Methods: We have analysed the findings of CNV studies from a cohort of 2430 patients with NDDs referred for clinical geneticist evaluation. Clinical geneticists evaluated all patients before genetic testing, including complete clinical workup. The analysis was conducted using Agilent 60K oligonucleotide array-based

comparative genomic hybridization. World databases of patients were used for case clarification.

Results: Chromosome microarray analysis was performed in 2430 patients with NDDs and the 16p11.2 distal rearrangements were identified in 20 patients (0,8%). We identified one triplication, five duplications and 14 deletions involving chromosomal region 16p11.2 BP4-BP5. Inherited 16p11.2 CNV were identified in four cases; two duplication and two deletion. In three patients second copy number alteration were present. Additionally, duplication was found in two healthy family members.

Conclusion: A prevalence of 0,8% indicate that chromosome 16p11.2 BP4-BP5 CNV is emerging as one of the most common cytogenomic abnormalities seen in patients with NDDs and our findings are in alignment with those reported previously. Counselling for 16p11.2 CNVs remains challenging because of variable expressivity.

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P09.035.C Genotype-phenotype correlations in RHOBTB2-associated neurodevelopmental disorders

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Background/Objectives: De novo missense variants clustering in the BTB domain region of *RHOBTB2* cause a developmental and epileptic encephalopathy (DEE) with early-onset seizures, severe intellectual disability, movement disorders and microcephaly.

Methods: By international collaboration, we assembled individuals with neurodevelopmental phenotypes carrying various *RHOBTB2* variants. Protein levels of RHOBTB2 with different missense variants were determined by western blot.

Results: While de novo missense variants clustering in the BTB domain region result in a severe DEE, de novo missense variants in the GTPase domain are associated with a milder and more variable neurodevelopmental disorder (NDD) with or without epilepsy and without microcephaly. In contrast to variants in the BTB domains, variants in the GTPase domain do not impair proteasomal degradation of RHOBTB2 in vitro, indicating different functional consequences.

Bi-allelic splice site and truncating variants were identified in nine families with variable neurodevelopmental phenotypes including intellectual disability and epilepsy, indicating that not only specific, heterozygous missense variants but also complete loss of *RHOBTB2* is pathogenic.

Conclusion: By identifying a phenotype-genotype correlation regarding location and consequences of de novo missense variants in *RHOBTB2* and by identifying bi-allelic truncating variants, we further delineate and expand the molecular and clinical spectrum of *RHOBTB2* related disorders including both autosomal dominant and recessive NDDs.

Conflict of Interest: None declared

P09.036.D De novo KCNA6 variants with attenuated KV1.6 channel deactivation in patients with epilepsy

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Mutations in the genes encoding neuronal ion channels are a common cause of Mendelian neurological diseases. We sought to identify novel de novo sequence variants in cases with early infantile epileptic phenotypes and neurodevelopmental anomalies.

Following clinical diagnosis, we performed whole exome sequencing of the index cases and their parents. Identified channel variants were expressed in *Xenopus* oocytes and their functional properties assessed using two-electrode voltage clamp.

We identified novel de novo variants in *KCNA6* in four unrelated individuals variably affected with neurodevelopmental disorders and seizures with onset in the first year of life. Three of the four identified mutations affect the pore-lining S6 α-helix of $K_V1.6$. A prominent finding of functional characterization in *Xenopus* oocytes was that the channel variants showed only minor effects on channel activation but slowed channel closure and shifted the voltage dependence of deactivation in a hyperpolarizing direction. Channels with a mutation affecting the S6 helix display dominant effects on channel deactivation when co-expressed with wild-type $K_V1.6$ or $K_V1.1$ subunits.

This is the first report of de novo nonsynonymous variants in *KCNA6* associated with neurological or any clinical features. Channel variants showed a consistent effect on channel deactivation, slowing the rate of channel closure following normal activation. This specific gain-of-function feature is likely to underlie the neurological phenotype in our patients. Our data highlight *KCNA6* as a novel channelopathy gene associated with early infantile epileptic phenotypes and neurodevelopmental anomalies.

Wellcome Trust (WT093205MA, WT104033AIA), Medical Research Council, National Institute for Health Research, University College London Hospitals

Conflict of Interest: Vincenzo Salpietro Full time, Valentina Galassi Deforie Full time, Stephanie Efthymiou Full time, Emer O'Connor Full time, Anna Marce-Grau Full time, Reza Maroofian Full time, Pasquale Striano Full time, Federico Zara Full time, Michelle Morrow Full time, Adi Reich Full time, Amy Blevins Full time, Julia Sala-Coromina Full time, Andrea Accogli Full time, Sara Fortuna Full time, Marie Alesandrini Full time, PY Billie Au Full time, Nilika Shah Singhal Full time, Benjamin Cogne Full time, Bertrand Isidor Full time, Michael Hanna Full time, PI, ALFONSO MACAYA RUIZ Full time, Dimitri Kullmann Full time, PI, Henry Houlden Full time, PI, Roope Mannikko Full time, PI

P09.037.A Whole exome sequencing in patients with developmental delay in routine diagnostics

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Approximately 3% of children worldwide are affected by neurodevelopmental disorders such as autism spectrum disorders, intellectual disability, and language disorders. Many cases have a genetic cause, and the associated genetic variants may be located in many different genes. Several hundred genes have been reported in association with neurodevelopmental disorders. As part of our routine diagnostic procedures, most patients with sporadic developmental delays and other comorbidities undergo initial genetic testing by chromosomal analyses, array-CGH, and *FMR1* repeat expansion analysis. In the case of negative preliminary genetic tests, we performed whole-exome sequencing (WES) as extended diagnostics, preferably as a trio analysis if both parents are available. In 2022, we tested 488 patients with unresolved developmental delay by WES analysis as part of our routine diagnostics. In 163 cases (33.4%), we found a (likely) pathogenic variant (93 cases, 19.1%) or a variant of uncertain significance (70 cases, 14.3%) in genes associated with a neurodevelopmental disorder. In 123 cases (75.4% of the positive cases), the mutated gene was associated with an autosomal dominant (AD) disorder. The variant occurred de novo in 63 cases (51.2% of AD cases); in 38 cases (30.9%), the variant was inherited

from a most often unaffected parent and in 22 cases (17.9%), at least one parent was unavailable for genetic testing. Interestingly, the *MECP2* gene, which is associated with Rett syndrome in females and X-linked intellectual disability syndrome in males, was reported in four cases. Additionally, four genes (*CHD2*, *CREBBP*, *MED13*, and *MED13L*) were repeatedly reported in three cases each.

Conflict of Interest: Christian Albig Medicover Genetics, MVZ Martinsried GmbH, Anna Munzig-Schmidt Medicover Genetics, MVZ Martinsried GmbH, Constanze Kurschat Medicover Genetics, MVZ Martinsried GmbH, Julia Philippou-Massier Medicover Genetics, MVZ Martinsried GmbH, Oliver Wachter Medicover Genetics, MVZ Martinsried GmbH, Steffen Lott Medicover Genetics, MVZ Martinsried GmbH, Konstanze Hörtnagel Medicover Genetics, MVZ Martinsried GmbH

P09.038.B De novo variants in PPFIA2 in individuals with neurodevelopmental disorders – evidence for the first α-liprinopathy

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Liprin-α2, encoded by *PPFIA2*, belongs to the family of four Liprin-α proteins which constitute major synaptic scaffolds with an important role in assembly and maturation of synapses. Furthermore, Liprin-α proteins guide pre- and postsynaptic processes by their ability to undergo liquid-liquid phase separation. Liprin-α2 itself is highly expressed solely in brain and serves as a key component for the assembly of the presynaptic active zone. No member of the Liprin-α family has been associated with monogenic disorders.

Exome/genome sequencing data of individuals with neurodevelopmental disorders were screened for rare de novo variants in *PPFIA2*. Individuals underwent in-depth phenotyping using HPO terms.

Exome/genome sequencing identified heterozygous de novo variants in *PPFIA2* (NM_003625.5) in two unrelated patients: an in-frame deletion of Exon 20-24 (NC_000012.11:g.81685127_81735798del) and a nonsense variant in Exon 29 (c.3367C>T, p.Arg1123*) both affecting the highly conserved SAM3 domain. While one individual presented with mild intellectual disability, speech delay and behavioral abnormalities, the phenotype of the other individual was characterized by learning disabilities and dystonia.

We present two individuals with heterozygous de novo variants in *PPFIA2*, which encodes a key regulator of the presynaptic active zone. The hypothesis of *PPFIA2* as a novel candidate gene for a neurodevelopmental disorder is underscored by the gnomAD gene constraint metrics showing a depletion of loss-of-function variants as well as the description of one individual with a de novo in-frame deletion (Exon 5-7) in *PPFIA2* with overlapping phenotype in the literature. In summary, we provide evidence for the first gene-disease association of a Liprin-α protein.

Conflict of Interest: None declared

P09.039.C Refining pathogenic RAC1 switch II variants

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Background/Objectives: Heterozygous dominant negative or activating *RAC1* missense variants cause a group of microcephalic, normocephalic or macrocephalic neurodevelopmental disorders (RAC1-NDD). The basis of this extreme phenotypic heterogeneity is poorly understood. We recently showed that *RAC1* variants in the switch II region are activating, tend to result in normocephalic RAC1-NDD, and could be therapeutically targetable. However, it is not clear if all switch II *RAC1* variants are activating.

Methods: The cohort of individuals with *RAC1* switch II variants was expanded using collaborations and variants databases. The effects of these variants on RAC1 signalling, lamellipodia formation and cell spreading were evaluated in NIH3T3 cells.

Results: In total, we identified 11 variants in 22 patients spread throughout the RAC1 switch II region, including three novel variants: R66G, P69S, and L70S. Expression of Q61E, Y64D, Y64C, R68S, R68G and R66G variants resulted in increased RAC1 activity, promotion of lamellipodia formation and a significant increase in the cell circularity index. In contrast, expression P69S and L70V, did not significantly alter the cell circularity index compared to the WT.

Conclusion: *RAC1* variants in only Q61-R68 region (but not in P69S-P73) of switch II region are activating. These results potentially also inform the interpretation of variants in switch II region of RAC2, RAC3 and other small GTPases. Importantly, these results have implications for developing variant and consequence-specific precision treatments for RAC1-NDD.

Conflict of Interest: None declared

P09.040.D Resolving pathogenicity of non-truncating ARID1B variants in Coffin-Siris syndrome

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Background/Objectives: Germline variants in different BAF complex subunits lead to recognisable neurodevelopmental syndromes, including Coffin-Siris syndrome (CSS). The most frequently affected CSS gene is *ARID1B*. To date, only heterozygous truncating *ARID1B* variants resulting in nonsense-mediated mRNA decay (NMD) are considered causative. In an individual with CSS clinical presentation we identified a de novo in-frame deletion (p.(Glu2129_Met2132del)) located in the EHD2 domain of ARID1B. Literature search revealed two additional missense alterations, p.(Cys1945Arg) and p.(Ile2031Thr), and one NMD-escaping truncation (p.(Ser2155Leufs*33)) in the same domain.

Methods: The *ARID1B* variant was detected by trio exome sequencing. RNAseq analysis was performed to compare the expression pattern of this individual with six CSS cases harbouring causative truncating *ARID1B* variants and nine controls. We examined the functional consequences of non-truncating variants by using immunofluorescence in HEK293T cells overexpressing wild type and mutant ARID1B.

Results: The transcription pattern of the in-frame deletion clustered together with that of the truncating variants and separately from controls. Mutant proteins formed cytoplasmic aggregates. Co-localisation with vimentin and γ-tubulin identified aggresomes, juxtanuclear inclusion bodies containing poly-ubiquitinylated proteins, surrounded by a vimentin cage and attached to the microtubule organisation centre. Such structures occur when the proteasome machinery is overwhelmed due to overload of misfolded proteins.

Conclusion: Here we present data suggesting that non-truncating variants in the EHD2 domain of ARID1B can be disease causing by impairing protein folding. The clinical presentation did not differ from that of the CSS individuals with *ARID1B* truncating variants, indicating a common pathomechanism.

Conflict of Interest: None declared

P09.041.A Expanding the clinical spectrum of PPP3CA variants: alternative isoforms matter

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Background: *PPP3CA* variants cause developmental and epileptic encephalopathy-91 (DEE91). This gene encodes for a protein with two main tissue-specific isoforms (1 and 2) that differ for the presence of exon13. The cellular function is essential in intracellular signals transduction. It is ubiquitously expressed, mostly in the brain where it dephosphorylates DNM-1 protein. By Whole Exome Sequencing analysis, we identified two *PPP3CA* de novo variants: a 4-nucleotide duplication predicting a premature truncated protein in Pt.1 presenting the classical clinical phenotype, and a splicing mutation altering the exon13 inclusion in Pt.2 showing a mild phenotype, undescribed for this condition.

Methods: we evaluated the isoforms1 and 2 expression in several healthy donor tissues. Then, immortalised lymphoblastoid cell lines (LCLs) derived from the two patients were used to study the effects of the variants, mainly by assessing cell growth, DNM-1 phosphorylation levels and the two isoforms expression pattern.

Results: transcript analyses confirmed the isoform1 prevalence in the brain conversely to all other tissues investigated. Pt.1 LCLs showed a reduced cellular growth and an increase in phosphorylated DNM-1 compared to controls. Interestingly, Pt.2 LCLs showed a growth rate comparable to controls and an absence of phosphorylated DNM-1.

Conclusion: in this study we characterized two *PPP3CA* variants studying the effects on the two main isoforms. The predicted effect of variant altering the phosphatase function is in accordance with the classical Pt.1 phenotype. We hypothesize that the variant identified in Pt.2 with an unusual phenotype could perturb the isoform1 formation with deleterious effects mainly in brain function.

Conflict of Interest: None declared

P09.042.B De novo variants underlying syndromes with intellectual disability in a neurodevelopmental cohort from India

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Background: De novo variants significantly contribute to the burden of intellectual developmental disorders and have been studied in diverse populations worldwide. The growing availability of genomic testing options in low-and middle-income group countries like India is contributing to rapid increase in the genetic diagnosis and gene discovery of neurodevelopmental disorders.

Methods: We evaluated 190 families from October 2019 to September 2022, predominantly presenting with syndromic intellectual disability (ID). Genome wide evaluation was performed using chromosomal microarray and/or exome sequencing.

Results: Eighty-nine families (47%) received a molecular diagnosis of monogenic disorders. Of these, de novo variants were identified in 54 families (60%), 48 of which were autosomal dominant disorders and six of X-linked disorders. Of these 54 families, consanguinity was noted in six families. Genomic alterations were identified in nine clinically recognizable conditions and 45 clinically unrecognizable conditions. The disease-causing de novo variants were observed in genes encoding transcriptional regulators (*FOXG1, BCL11B, CREBBP, QRICH1, ASXL1, TCF12, NSD1, ZBTB18, MEIS2, BCL11A, ANKRD11, SOX10*), chromatin remodelers (*BRWD3, KDM6B, KDM5C, MECP2, SETD5, ATRX, KMT2D, SMARCD1, SMARCA4, KMT2A, GATAD2B, SETBP1, NIPBL, NSD1, SETD2, SMARCA2, SETD1B, ARIDB1, SRCAP*), serine threonine kinases (*AKT3, WASF1, BRAF, RIT1, DYRK1A*), signaling molecules (*DCX, DLG4, CTNNB1, FBN2*) and others (*TSC2, IDS, TUBG1, DYNCH11A, NEXMIF*).

Conclusion: We herein highlight the genetic heterogeneity of syndromic ID due to de novo disease-causing variants in the

Indian population that exhibits variable degree of consanguinity and endogamy and creates a complex genetic background.

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Conflict of Interest: None declared

P09.043.C The ultrarare ASXL-related disorders: two novel cases of Sashi-Peña and Bohring-Opitz syndrome

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Background: Variants in *ASXL1*, *ASXL2* and *ASXL3* are the molecular cause of Bohring-Opitz (BOPS), Shashi-Peña (SPS) and Bainbridge-Ropers syndrome, respectively. All of these conditions share common phenotypic features such neurodevelopmental delay, hypotonia, and some facial features; moreover, each clinical condition is distinguished by peculiar characteristics.

Methods: The two patients underwent an extensive instrumental and clinical evaluation in order to determine their neurodevelopmental delay and phenotypic abnormalities. Molecular analysis was performed via trio-based WES.

Results: Patient 1 has a clinical phenotype characterized by glabellar nevus flammeus, elbow flexion, axial hypotonia, psychomotor delay, epilepsy and growth at lower limits. WES analysis evidenced a de novo, p.Gly1264Glufs*16 in the *ASXL1* gene which allowed one to confirm the diagnosis of Bohring-Opitz syndrome. Patient 2 showed facial dismorphisms (such as thick eyebrows, hypertelorism, large and fleshy lobes), epilepsy, sensory polyneuropathy, global neurodevelopmental delay, skeletal anomalies (scoliosis, irregular profile of 2nd-3rd finger), diffuse hypertrichosis; MRI revealed atrophy of right hippocampal and para-hippocampal structures, dilated ventricles and thinned white matter. Molecular analysis highlighted the presence of a de novo p.Gln437* variant in the *ASXL2* gene, associated with Shashi-Peña syndrome.

Conclusion: To date, the cases described of ASXL-related disorder are fewer than 100 and further studies appear necessary to further define their clinical characteristics. In particular, the overlapping features does not always make it easy to immediately distinguish the various forms of this group of diseases. Our report strengthens the hypothesis that some skeletal and neuroradiological features may be essential in separating cases of BOPS syndrome from SPS.

Conflict of Interest: None declared

P09.044.D Diagnosis of Shukla-Vernon syndrome by reverse phenotyping

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Background/Objectives: Pathogenic variants in the X-chromosomal located gene *BCORL1* cause Shukla-Vernon syndrome (SHUVER), a neurodevelopmental disorder (NDD) characterized by intellectual disability (ID), behavioral abnormalities and dysmorphic features. To date, only 14 affected males from nine unrelated families have been reported.

Methods: Trio exome sequencing (ES) and array CGH were performed using DNA from patient's lymphocytes. Total RNA from the patient was prepared from a PAXgene tube and reverse-transcribed. RT-PCR amplicons were sequenced using exonic primer pairs. Segregation analysis was performed using conventional PCR followed by Sanger sequencing.

Results: We describe a 17-year-old male individual with severe intellectual disability, atypical autism, muscular hypotonia with progressive scoliosis, short stature and epilepsy. Facial dysmorphism comprised large mouth with full lips, uplifted ear lobes and diastema. In addition, cranial MRI revealed cerebral hypomyelination and corpus callosum hypoplasia. Trio-ES identified a maternally inherited intronic variant in *BCORL1* (NM_001379451.1):c.3608-13C>G. RNA analysis confirmed aberrant splicing at the novel splice site, resulting in the inclusion of 12 bp of intronic sequence: r.3607_3608ins[acatcactccag;3608-12_3608-1]. This expansion is predicted to result in the insertion of four additional amino acids: NP_001366380.1:p.(Glu1202_Gly1203insAspIleThrPro).

Conclusion: We present the first individual with Shukla-Vernon syndrome likely caused by an intronic *BCORL1* variant located outside the canonical splice site. Our data expand the molecular and phenotypic spectrum of SHUVER. Furthermore, we show that RNA analysis from peripherally collected blood is feasible for intronic *BCORL1* variants, which may help to validate intronic *BCORL1* variants in patients with NDD/ID.

Conflict of Interest: Angela Abad Perez: None declared, Ronja Adam Charité – Universitätsmedizin Berlin, Gabriele Hildebrand: None declared, Denise Horn: None declared, Nadja Ehmke: None declared, Felix Boschann: None declared

P09.045.A Cluster based approach for deciphering complexity in individuals with neurodevelopmental differences

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Objective: This study aims to assess the use of two different clustering approaches, agglomerative and divisive, in order to group individuals with global developmental delay (GDD) for future basket trials and identify disease related genes. Basket trials have shown success in oncology but have not yet been used in GDD due to the heterogeneity of individuals with neurodevelopmental disorders.

Methods: The study used the largest cohort of individuals with GDD, the Deciphering Developmental Disorders (DDD), and extracted genotypic and phenotypic information from 6,588 individuals. K-means clustering (divisive) and hierarchical agglomerative clustering (HAC) were used to identify subgroups of individuals. Gene network and molecular function information about the clusters were then extracted.

Results: The HAC based on phenotypes identified 16 clusters in individuals with GDD, each presenting one dominant phenotype and other minor phenotypes. The most common phenotypes were delayed speech, absent speech, and seizure. Each phenotypic cluster had several sub-clusters of more closely related genes with diverse molecular functions. K-means clustering also segregated individuals with these phenotypes, but the genetic

pathways identified were different from the ones identified by HAC.

Conclusion: This study highlights the potential of using in a complementary manner both divisive and agglomerative clustering to group individuals with GDD for basket trials. The results suggest that phenotypic clusters should be subdivided into molecular clusters for increased likelihood of successful treatment and that a combination of both clustering approaches may be necessary for comprehensive treatment development.

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Conflict of Interest: None declared

P09.046.B A novel variant in the HX repeat motif of AUTS2 causing a severe phenotype

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Introduction: Pathogenic variants in *AUTS2* cause the autosomal dominant intellectual developmental disorder-26 (OMIM#615834). Eighty-six patients with pathogenic variants in *AUTS2* have been reported. Patients with missense variants in the HX repeat motif (three) and patients with small in-frame deletions in the same motif (five) show a particularly severe phenotype that has begun to be recognized.

Materials and Methods: We present a 19-month-old female who was born after an uneventful pregnancy. She is the only child of non-consanguineous parents. Her weight is 7.7 kg (-2.8SD), length is 71 cm (-3.8SD) and OFC is 46 cm (-1.0SD). Physical examination shows a nevus simplex on the midline of the forehead, trigonocephaly, synophrys, long eyelashes, downslanted palpebral fissures, convex nasal ridge, low-set columella, microstomia, thin upper lip, retrognathia, low-set ears, endotropia, and generalized hypotonia. She has severe global developmental delay. Brain MRI revealed small frontal lobes, small brainstem, and small vermis with abnormal folding of the superior vermis. Our first diagnostic hypothesis was Rubinstein-Taybi syndrome.

Results: Trio-based WES identified a novel *de novo* heterozygous missense variant in the *AUTS2* gene (NM_015570.3, c.1603C>A, p.His535Asn), classified as pathogenic (ACMG classification).

Conclusions: Patients harboring mutations in the HX repeat motif of *AUTS2* exhibit defects in *AUTS2* and *P300* interaction, and a severe developmental disorder reflective of Rubinstein-Taybi syndrome. The reported variant affects the transcription start site of short isoform of *AUTS2* (mRNA quantitative expression of both *AUTS2* full-length and short transcripts using standard protocols is ongoing), which might also be associated with a severe phenotype.

Conflict of Interest: None declared

P09.047.C Rare inherited copy number variants as genetic modifiers of developmental disorders

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Background/Objectives: Neurodevelopmental disorders (NDDs) are a group of cognitive and/or behavioural conditions that have onset in childhood. Rare inherited Copy-Number Variants (CNVs), could play a role in the sensitization of the genome and modulation of NDD phenotypic traits.

Methods: We combined transcriptomics, chromatin conformation analyses, whole-genome sequencing and deep familial phenotyping to analyse 6 family trios, each carrying a unique rare CNV transmitted to the affected child by a seemingly healthy parent.

Results: So far, 3 families are completed. RNA-seq data indicate that the expression of genes within and/or flanking the inherited CNVs could be affected. Capture Hi-C data of our families 2 and 5 (F2, F5), show an altered TAD profile in the carriers at the CNV locus. Interestingly, carriers of a 4q31 deletion in F2 show a depletion of interactions between a microglia-specific enhancer cluster mapping within the CNV and the promoter of *TBC1D9*, located upstream of it. This is accompanied by the down-regulation of *TBC1D9* (pHaplo = 0.89) in F2 carriers, making this gene a potential new candidate for NDDs. WGS data for F2 indicates that the rare inherited CNV is not associated with additional de novo, recessive, X-linked or paternally inherited pathogenic SNV, indels or CNVs. Deep phenotyping analysis, instead, indicates that the segregation of the CNV correlates with progressive aggravation of cognitive and behavioural abilities, with milder symptoms in the carrier parent compared to the child. Overall, our data suggest a contributory effect of rare inherited CNV to NDD.

Grants: KU Leuven C2 internal fund.

Conflict of Interest: None declared

P09.048.D Novel splice TRIO variant results in an in-frame exon deletion affecting the PH domain

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Background: Pathogenic variants in *TRIO*, encoding the guanine nucleotide exchange factor, are associated with two distinct phenotypes: Missense mutations in the spectrin-repeats are responsible for a more severe developmental delay with macrocephaly (MIM: 618825), whereas missense variants in the GEF1 domain and truncating variants throughout the gene lead to a milder developmental delay and microcephaly (MIM: 617061).

Objective: To expand the mutational spectrum and to validate a distinct facial phenotype associated with pathogenic *TRIO* variants.

Patients and Methods: We phenotyped, and sequenced (WGS) three members of a family with variable ID/NDD and microcephaly and reviewed the literature on *TRIO*-associated ID. The facial characteristics of the family members and published cases were evaluated via GestaltMatcher. Splicing analysis of variant *TRIO* mRNA was performed using RT-PCR.

Results: The family carried a novel heterozygous variant at the last coding base of exon 31 of *TRIO* (NM_007118.4:c.4716G>A). RT-PCR confirmed aberrant splicing resulting in the skipping of exon 31 (r.4615_4716del), which is predicted to lead to an in-frame deletion in the PH domain: p.(Thr1539_Lys1572del). We identified 23 facial photographs of patients with *TRIO* associated neurodevelopmental disorder and microcephaly in the literature. Computational analysis of the facial gestalt suggests a distinct facial phenotype not outlined in the existing literature.

Conclusion: The splice variant identified here expands the mutational spectrum of *TRIO* and shows the importance of further

functional analysis of rare variants at the exon-intron boundary. Furthermore, our data indicate that there is a characteristic facial appearance of *TRIO*-associated NDD and microcephaly (MIM: 618825).

Conflict of Interest: None declared

P09.049.A Intersection of obesity and macrocephaly in individuals with neurodevelopmental phenotypes

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Background/Objectives: Individuals with neurodevelopmental phenotypes are at higher risk of medical comorbidities, including obesity. We explored the overlap of macrocephaly and obesity as co-occurring phenotypes and their association with rare genetic variants.

Methods: Retrospective evaluation of genomic clinical testing of patients with neurodevelopmental phenotypes (ASD/ID/DD) referred for a medical genetics evaluation.

Results: Our cohort consisted of 910 cases (evaluated 1/94-3/17), of which 163 had macrocephaly (HC> + 2SD). Genetic testing was completed in 92 of those patients and 43% underwent multiple tests. The vast majority of cases did not complete genetic testing due to loss of follow up or lack of insurance coverage. Within the total cohort, 4% presented with a BMI > 99th percentile in addition to macrocephaly. Analysis of testing results identified a case of 7q11.23 duplication, known to be associated with macrocephaly and obesity, validating our approach. We also identified a number of genetic conditions not previously associated with obesity and/or macrocephaly. These include single gene variants within *TNIK*, *LMTK2*, *GNB1*, *DYNC1H1*, *PTEN*, *GRIN2A*, and *IDH2*, as well as copy number variants 20p12.3 deletion (*PLCB1*), 3q26.31 duplication (*NLGN1*), 15q13.1q13.2 deletion (*APBA2* and *TJP1*). Mutations in *DYNC1H1*, *GNB1*, and *PTEN* highlighted the importance of mTOR signaling, as these genes interact within mTOR-mediated pathways through direct and indirect protein interactions.

Conclusion: Our findings advance our knowledge of rare genomic variants associated with macrocephaly and obesity, demonstrating the clinical utility of these combined phenotypes in identifying individuals with neurodevelopmental phenotypes that would benefit from genetic evaluation.

Conflict of Interest: None declared

P09.050.B Exploring the role of SLC35F1 in the Rett syndrome landscape

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Background: Rett syndrome (RTT) is a neurodevelopmental disorder (incidence of 1:10,000) most frequently affecting girls during infancy, after an early normal development. The genetic cause is known for about 90% of patients (*MECP2* mutations in the classic form, *CDKL5* and *FOXG1* mutations for the two main variants), while the 10% remain without molecular diagnosis.

Methods: We applied trio whole exome sequencing (WES) analysis to a Rett-like proband negative for mutations in RTT known genes. We further characterized in vitro the role of a newly identified variant: we assessed protein product localization by fractionation and immunofluorescence, and protein interactome by mass spectrometry (LC-MS/MS).

Results: In our patient we identified a novel heterozygous missense variant in *SLC35F1*, encoding for a putative solute carrier whose role is unknown. Our in vitro analysis showed that SLC35F1 localization is mainly cytoplasmatic. In addition, immunoprecipitation for endogenous SLC35F1 and LC/LC-MS analysis on 293T cells and *SLC35F1* KO line generated by CRISPR/Cas9 editing led to a preliminary delineation of the interactome of this protein. Further analyses on the effects of the identified variant are currently under investigation, rescuing *SLC35F1* KO cells with FLAG/HA tagged wild type or mutated SLC35F1.

Conclusion: The identification of new RTT genes could expand the genotype-phenotype correlation for RTT-like syndrome, and the characterization of new candidate RTT genes could give insights into the pathogenesis of this neurodevelopmental disorder.

Grants: Grant Aldo Ravelli Center for Neurotechnology and Experimental Brain Therapeutics and Intramural funding of Università degli Studi di Milano.

Conflict of Interest: None declared

P09.051.C Deciphering Developmental Disorders in Africa (DDD-Africa) –the first ~100 cases from South Africa

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Developmental disorders (DD) are individually rare, but cumulatively affect ~6% of individuals globally, with at least 50% having a genetic cause. Clinical and genetic heterogeneity makes diagnosis challenging. The Deciphering Developmental Disorders in Africa (DDD-Africa) study is using a genotype-driven approach to define the genetic epidemiology of DD in Africa, while providing individual level results. The study has recruited 500 probands and family members from South Africa and the DRC for whole exome sequencing (WES) following clinical phenotyping. Preliminary results from the first 119 South African probands are presented. Clinical and variant information were integrated using DECIPHER (www.deciphergenomics.org) and the data interpreted by a multidisciplinary team. Pathogenic single nucleotide variants (SNVs) have been identified in 29 genes in 33/119 (28%) families after analysis for variants in DDG2P genes and/or those reported in Clinvar. Two pathogenic copy number variants (CNV) were

identified. Variants were identified in genes associated with well described phenotypes, including *NSD1, OCRL, CHD7, EHMT1, EFTUD2* and *PTEN*. Atypical presentations or limited to supportive special investigations delayed diagnosis. Patients with African ancestry and variants in *ARID1B, SON, mTOR, STXBP1 and DNMT3A* have now been recognised. Homozygous variants in *MFSD8* and *XYLT2* in 2 patients from non-consanguineous families raise the possibility of local founder effects. WES is valuable, even in limited resource environments, where diagnoses can improve the practice of medical genetics, direct management, limit expensive investigations, assist reproductive decisions. and end families' diagnostic odysseys The project has enhanced research capacity and skills in variant interpretation, while training post-graduate students.

Conflict of Interest: Amanda Krause Krause Full, Barry Shingwenyana Full, Nadia Carstens Full, Phelelani Mpangase Full, Mhlekazi Molatoli: None declared, Patracia Nevondwe: None declared, Nadja Louw Part, Aimé Lumaka Full, Prosper Lukusa Full, Koenraad Devriendt Full, Zané Lombard Full

P09.053.A Five new patients with TAOK related disorders: relevance of TAOK2 gene in neurodevelopment?

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Background/Objectives: Pathogenic variants in the *TAOK1* gene have been associated with neurodevelopmental disorders. To date, approximately 35 patients with *TAOK1* disorders have been reported. Until now only 3 patients with *TAOK2* pathogenic variants have been reported. Patients with *TAOK1* and *TAOK2* variants are mainly characterized by intellectual impairment, behavioural abnormalities, dysmorphia and other characteristics such as hyperlaxity and visual anomalies among others.

Methods: Here we report five patients from four unrelated families with heterozygous variants in *TAOK1 and TAOK2* gene. In patients 1 and 2 the diagnosis was uncovered by whole-exome *sequencing* (WES), in patients 3 and 4 by exome data reanalysis and patient 5 with direct sanger sequencing.

Results: We identified four previously unreported pathogenic variants. Four were de novo and one inherited. All identified variants were novel; c.57_60del/p.(F19Lfs*64) and c.1414C>T/ p.(Arg472Ter) variants in TAOK1 and c.148A>T/ p.N50Y and c.1411A>G/ p.T471A in TAOK2. Common characteristics of the five patients were mainly based on neurodevelopmental involvement, including neurodevelopmental delay, delayed speech and language development and behavioural problems with autístic features. Dysmorphia were nonspecific.

Conclusions: Our cases highlight that *TAOK2* gene should be considered as a gene with clinical relevance related to neurodevelopmental disorders as *TAOK1* gene has been considered previously. The description of more cases should help to better define the clinical spectrum of patients with alterations in both genes.

Conflict of Interest: None declared

P09.054.B Human iPSC-derived brain organoids to model the neurological disease of a patient with a PPP2R2C mutation

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Background/Objectives: Brain organoids are three-dimensional in vitro culture systems generated from pluripotent stem cells that recapitulate the organization of the developing human brain. These mini-brains have emerged as new models for the study of neurological disorders. Our objective was to establish brain organoids from a patient included in the Spanish Undiagnosed Rare Diseases Program (SpainUDP) of the Institute of Health Carlos III in order to investigate the molecular mechanisms of his neurological disease.

Methods: We have performed exome sequencing in the blood of a patient with developmental delay and intellectual disability to identify pathogenic variants associated with the disease phenotype. Induced pluripotent stem cells (iPSCs) were generated from the fibroblasts of the patient and were used to establish cortical brain organoids. Morphological parameters of the organoids were evaluated by microscopy techniques and organoids were further characterized by RNA-Seq, quantitative PCR and immunofluorescence.

Results: We have identified a de novo likely pathogenic missense variant in the gene PPP2R2C, not previously associated with disease, as possibly associated to the patient disorder. Our results show that the iPSC-derived human brain organoids recapitulate aspects of human brain organogenesis. In addition, differences in organoid size and markers of differentiation were found in patient-derived organoids when compared with control-derived organoids.

Conclusion: We have identified alterations in the development of the patient-derived brain organoids. Our results demonstrate that human brain organoids constitute a resourceful tool to investigate neurological diseases.

Grant References: Spanish Undiagnosed Rare Diseases Program (SpainUDP) and Grant ISCIII PT20CIII/00009.

Conflict of Interest: None declared

P09.056.D Multiomic profiling unravels disease mechanisms in complex chromosomal rearrangement carriers with autism spectrum disorder

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Autism spectrum disorder (ASD) affects ~1 in 100 children, and is a highly variable neurodevelopmental disorder typically characterized by difficulties with social interaction, repetitive or restricted behaviour and often delayed language skills. Most cases have an underlying genetic cause, with de novo and/or rare-inherited single nucleotide variants (SNV) to large structural variations (SV), notably copy number variations (CNV), found in ~20% of families.

To date, there are over 700 genes associated with ASD (Genomics England PanelApp), with ~100 'penetrant' genes and CNVs meeting American College of Medical Genetics criteria. For ~80% of families, the possible genetic contributors remain to be resolved.

Here we use Oxford Nanopore Technologies PromethION genome sequencing (ONT-GS) to characterize complex chromosomal rearrangements (CCRs) involving multiple duplications (DUP) that segregate with ASD in 5 families in the Autism Speaks MSSNG consortium study. In total we evaluated 13 CCR carriers and were able to resolve all breakpoint junctions at nucleotide resolution. In addition, we utilize Nanopolish to detect methylation status directly from the ONT-GS data, allowing us the assess the activity of rearranged genes and regulatory regions.

Notable findings include a DUP-NML-DUP rearrangement forming an in-frame fusion-gene involving *L1RAPL1-DMD*. Furthermore, two identical DUP-TRIP-DUP involving *ANK2*, was present in two unrelated families originating from Newfoundland, indicating the presence of a founder variant. In aggregate we demonstrate the utility of ONT-GS data to pinpoint CCRs associated with ASD in five unrelated families, and we highlight the importance of a transcript-centric description of disease associated complex chromosomal rearrangements.

Conflict of Interest: None declared

P09.057.A Three new cases of SRRM2-related neurodevelopmental disorder: a potential expansion of the phenotype

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Background: Heterozygous loss of function (LoF) variants in the *SRRM2* gene, which encodes for the SRm300 splicing factor protein of the SR-related protein family, have been recently associated with a neurodevelopmental disorder also characterized by speech delay, autistic or attention-deficit/hyperactivity, hypotonia, and dysmorphic facial features. To date, only 22 patients have been reported.

Methods: Here we describe three cases of <5 years old patients who presented clinical phenotypes consistent with SRRM2-related syndrome. Singleton whole exome sequencing (WES) and segregation analysis by Sanger sequencing were performed in two patients. Trio WES was performed in one patient.

Results: three previously undescribed truncating heterozygous variants NM_016333.4:c.6170_6176del:(p.R2057Hfs*10), NM_016333.4:c.328G>T:(p.Glu110*) and NM_016333.4:c.5737delT:-p.(Arg1912Glnfs*7), were identified, expecting to undergo nonsense-mediated decay. After segregation, variants were not identified in healthy relatives and were considered de novo. The three patients showed developmental and language delay, with late walking acquisition and dysmorphic facial features. Two of them showed autistic and attention-deficit and hyperactivity features. Moreover, failure to thrive was noted in one patient. Interestingly, one of the three patients showed head tremor/dystonic vertical episodes at the age of 9 months, a phenotype previously unreported in patients with *SRRM2* pathogenic variants.

Conclusions: Our findings expand the clinical and molecular knowledge of SRRM2-related disorder, adding three new cases, and highlighting the relationship between LoF variants in this gene and the neurodevelopmental disorder phenotype. Moreover, two of the patients present clinical manifestations which have not been previously described in SRRM2-reported cases, further expanding the phenotype related to this gene.

Grant: La Marató TV3-Foundation (590/C/2020).

Conflict of Interest: None declared

P09.058.B Biallelic variants in GET4 cause developmental disabilities with a variable degree of phenotypic spectrum

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GET4 is one of the six components of the transmembrane domain recognition complex (TRC) pathway. TRC directs the tail-anchored proteins to the appropriate cellular membranes. A single patient with compound heterozygous variant in the gene *GET4* has been reported with global developmental delay, intellectual disability (ID) and seizures. We studied a five-generation Pakistani consanguineous family having four affected members presenting with variable degree of developmental disability including ID, seizures and developmental delay. Through whole exome sequencing, we identified a rare homozygous missense variant c.803G>A; p.R268Q (NM_015949.3) in *GET4* gene which segregated with the phenotype. By GeneMatcher, we found another homozygous missense variant c.326C>G; p.A109G in *GET4* in a patient of a consanguineous family. The patient presented with global developmental delay, and aspecific dysmorphia. In silico molecular modelling of the identified variants revealed that R268 residue's side chain is forming a hydrogen bond with the Q1035 of *BAG6* gene and p.R268Q variant in *GET4* possibly influences this interaction. It was predicted that this variant could lead to destabilization of the local protein structure. The p.A109G variant was predicted to decrease the stability of GET4 protein by destabilizing the alpha-helix structure, as Gly is known as a helix breaker. Moreover, we have initiated some functional experiments to study possible defects in TRC pathway and to prove the pathogenicity of these variants.

In conclusion, we found two homozygous missense variants, p.R268Q and p.A109G in *GET4* causing variable degree of developmental disability by plausibly influencing the stability and the structure of the GET4 protein.

Conflict of Interest: None declared

P09.059.C Dandelion sign, a highly specific neuroradiological feature for the diagnosis of EBF3-related neurodevelopmental disorder?

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Background/Objectives: Heterozygous pathogenic variants in *EBF3*, encoding for a DNA binding transcription factor, are associated with a rare neurodevelopmental disorder described as HADD syndrome (hypotonia, ataxia, delayed developmental syndrome). Reported individuals present intellectual disability or autistic features. Cerebellar anomalies have also been described. We aim to precise the clinical and radiological phenotype associated to *EBF3* pathogenic variants.

Methods: We report six patients with de novo pathogenic variants in *EBF3* gene detected through various methods (NGS gene panel, trio-WES, trio-WGS), and one patient with 10q26 deletion encompassing the gene detected by array-CGH. We obtain for all of these individuals clinical data and high quality brain MRI (3T-MR750).

Results: All individuals have developmental delay and three have intellectual disability. They all show a typical cerebellar syndrome with gait disturbance and hypermetry. Five individuals have behavior issues and five have autistic features. Language development is highly variable but all have speech delay with an average age of the first word at 36,6 months. Hypotonia is frequent in our cohort (5/7). Six patients present cerebellar anomalies on MRI with vermis dysplasia and a peculiar foliation anomaly (dandelion sign). Extra-neurologic symptoms are observed: orthopedics (6/7), vesico-renal (3/7), ORL (6/7), ophthalmologic (7/7), and dysmorphic features (6/7).

Conclusion: This study present the clinical and radiological phenotype of patients with pathogenic variants in *EBF3*. The cerebellar anomaly is frequent and seems to be recognizable. This feature should be a good handle for diagnosis of HADD syndrome.

Conflict of Interest: None declared

P09.060.D Homozygous 22q11.2 distal microdeletion is associated with syndromic neurodevelopmental delay

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Background/Objectives: Copy number variants (CNVs) in the 22q11.21 region are mediated by non-allelic homologous recombination between pairs of low copy repeats (LCRs), from amongst eight LCRs designated A-H. Heterozygous distal type II deletions (LCR-E to LCR-F) have incomplete penetrance and variable expressivity, and can lead to neurodevelopmental issues, dysmorphic features, and congenital abnormalities. We present two siblings with syndromic neurodevelopmental delay and a homozygous 22q11.2 distal type II microdeletion.

Methods: Following informed consent, both affected siblings and the parents had a clinical CMA (Affymetrix CytoScan 750K array). Exome sequencing was pursued for one sibling, in order to exclude additional monogenic variants.

Results: We report siblings with global developmental delay, hypotonia, dysmorphic features, ocular abnormalities and minor skeletal issues, in whom chromosomal microarray identified a homozygous 22q11.2 distal type II deletion. The deletion was brought to homozygosity as a result of a consanguineous marriage between two heterozygous carriers of the deletion. The phenotype of the children was strikingly more severe and complex than that of the parents and previously reported heterozygous cases, providing further confirmation of a dosage effect for this region.

Conclusion: Homozygous deletions spanning numerous genes are rare, despite the potential contribution of consanguinity to such instances. Our data suggest that the 22q11.2 distal type II deletion harbors a dosage-sensitive gene or regulatory element, which leads to a more severe, syndromic neurodevelopmental phenotype when deleted on both alleles.

Conflict of Interest: None declared

P09.061.A Two in trans pathogenic variants in a patient with neurofibromatosis type I: a case report

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Background: NF1 is an AD genetic disorder with full penetrance. The disease is generalized but, in some patients, the clinical findings appear only in one body segment (mosaic NF1). These cases are due to postzygotic mutations.

We report the case of a young woman affected by NF1 who carries in trans two pathogenic variants, one of them in mosaic.

Methods: High-depth NGS was performed for the analysis of small indel and point mutations in the coding region and splicing sites of *NF1* (*NF1* NextGeneDx®). The capture was performed by PCR amplification of the coding exons and the intronic flanking regions of *NF1*. Subsequently, we prepared the libraries using Nextera XT kit (Illumina), sequenced (2 × 150) in the MiSeq (Illumina), aligned against the hg19 genome and annotated variants with our own pipeline.

Results: A 28-year-old woman with a clinical diagnosis of NF1 (multiple café-au-lait spots, cutaneous neurofibromas and a right latero-cervical plexiform neurofibroma) was referred for *NF1* genetic testing. The following variants were detected in the patient's blood sample in trans:

Gene * 613113	Variants			Classification
	Zygosity	Refseq NM_000267.3	Protein NP_000258.1	
NF1	Mosaic (VAF 24%)	c.2327delC	p.Ala776Valfs*15	pathogenic
	heterozygosis	c.2329delT	p.Trp777Glyfs*14	pathogenic

The study on patient's oral mucosa and the family segregation is ongoing.

Conclusion: >7,000 patients have been genetically diagnosed with NF1 to date but, as far as we know, this patient would be the first with two pathogenic variants in trans in a germline blood sample in the absence of a hematological neoplasms. Her phenotype, however, is not particularly severe.

Conflict of Interest: None declared

P09.062.B Diagnostic yield of NGS data reanalysis on a cohort of patients with intellectual disability

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Background/Objectives: Intellectual disability (ID) can appear alone or occur together with other symptoms that make up a syndrome. Both types can be caused by genetic factors. On average, it takes 5 years for rare disease patients to reach a genetic diagnosis, especially in the case of ID, a very heterogeneous condition.

Methods: In our laboratory, more than 400 WES-based ID panels have been performed. In a first phase, family segregation has been done for the reported variants, allowing its reclassification. Afterwards, in order to increase our diagnostic yield, we will perform reanalysis of NGS data of unsolved cases.

Results: In our cohort, pathogenic or likely pathogenic variants have been reported in 99 cases (frequency of 23,8%), being the *ANKRD11* gene the most frequently reported, followed by *FOXP1*, *GRIN2B*, *NSD1* and *WAC*. Out of these 99 positive cases, 12 involve pathogenic or likely pathogenic CNVs. After segregation studies and reclassification, this frequency has increased up to 25,2%. Several variants of unknown significance, confirmed to be de novo, could be upgraded to likely pathogenic if paternity was confirmed. By performing reanalysis, we expect to increase the overall diagnostic yield by at least 7% (Ewans et al. 2022).

Conclusion: The obtained frequency of pathogenic or likely pathogenic variants is consistent with what is published in literature. As it is widely known in our community, both variant reclassification and reanalysis of NGS studies after a certain time is essential to diminish the diagnostic odyssey and increase diagnostic yield.

Conflict of Interest: Marta Carreño Full, Maria Segura-Puimedon Full, Raquel Garcia Full, Olaya Villa Marcos Full, Mònica Vall Full, Lidia Carreño Full, Hector San Nicolás Full, César Arjona Full, Raquel Muñoz Siles Full, Emma Lorente Ruiz Full, LLuís Armengol Full

P09.063.C Sex difference contributes to phenotypic diversity in individidual neurodevelopmental differences

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Objective: This study aimed to compare males and females with global developmental delay (GDD) in terms of their clinical presentation and genetic profiles.

Methods: Using the Deciphering Developmental Disorders (DDD) dataset, we extracted phenotypic information from 6,588 individuals with GDD and identified the statistically significant differences in phenotypes based on sex. Additionally, we analyzed the genomic data to determine the presence of mutation in GDD genes and then compared males or females. We then analyzed molecular function and gene expression profiles in the function of sex.

Results: The study found significant differences between males and females in terms of phenotypic and genomic features. For instance, microcephaly was found to be significantly more common in females, while macrocephaly was overrepresented in males. The study also found that autism spectrum disorder (ASD) was more prevalent in males with GDD, a pattern already established in individuals with ASD. Interestingly, while we found that some GDD gene mutations were specific to each sex, their overall molecular functions overlapped.

Conclusion: This study highlights the importance of considering sex as a variable in the study of GDD. The findings suggest that there are differences in phenotypic and genomic features between males and females with GDD, which could have implications for the diagnosis and treatment of the condition. Further studies are needed to fully understand the underlying mechanisms and develop targeted interventions that consider these differences.

Conflict of Interest: None declared

P09.064.D Molecular basis of developmental language disorder: a specific condition of language disorder ?

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Background: Developmental Language Disorder (DLD) refers to children who present with language difficulties that are not resulting from a known biomedical condition, or associated with autism spectrum disorder, intellectual disability or apraxia of speech. The prevalence of DLD is ~7%-8% including 2% of severe forms. Regarding aetiology, increasing evidences suggest that DLD have a genetic basis. Until recently, terminology has been confusing leading to a great heterogeneity in clinical cohorts. Our objectives are to characterize the genetic basis of DLD from deeply phenotyped patients and to better define the molecular pathways which are involved in language acquisition.

Methods: Seventeen families, including 38 patients diagnosed with DLD, were enrolled. None had apraxia of speech. Most of the cases were included in multiplex families ($n = 14$) and 3 cases were sporadic. This yielded a set of 71 individuals for whom Chromosomal Microarray Analysis and Whole Genome Sequencing were performed.

Results: CNVs of interest were detected in 4 families. Among those, 3 of them correspond to CNV causing neurodevelopmental disorders with variable expressivity and incomplete penetrance (locus 15q13.3, proximal and distal 16p11.2 locus). In a sporadic case, a de novo loss-of-function mutation in the *ZNF292* gene was detected. In 4 multiplex families, variants of interest were identified.

Conclusion: Overall, the genetic basis of DLD seems more likely to involve a complex pattern of inheritance. Interestingly, for a sporadic case, we could identify one of the first pathogenic variants (i.e., truncation variant in *ZNF292*) in a DLD patient.

Grant References: Entrepreneurs Amis d'Imagine. Eric Perrier

Conflict of Interest: Clothilde ORMIERES APHP Institut IMAGINE Hopitaux universitaires de Geneve, Eric PERRIER, Karine Siquier-pernet Imagine, Marlene Rio APHP, Delplancq Geoffroy Hopital André Mignot Versailles, Rodriguez-Fontenla Cristina: None

declared, marion lesieur-sebellin APHP, Bodineau Alison: None declared, Narcy Lucie APHP, Schlumberger Emilie APHP, Vincent Cantagrel IMAGINE, ERIC PERRIER, VALÉRIE MALAN APHP IMAGINE UNIVERSITE PARIS CITé, ERIC PERRIER

P09.066.B 2000 trio exome sequencing on research base: output and outlook

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When routine NGS is inconclusive and consent is available, we perform exome sequencing (ES) on a research basis to identify novel morbid genes for rare disorders.

Between 2017 and 2022, we performed NGS analyses in 9476 cases. 4337 have neurodevelopmental disorders (NDD). 1800 cases consented to be included in research analysis. Sequencing was performed using standard methods, mostly as trio. The software used was Varvis®.

In 689 cases, we have identified one candidate gene, in 405 cases two or more candidate genes. We uploaded 600 of the candidates to GeneMatcher. For 360 genes, we had contact with other groups. Of these, 45 were published with our contribution as novel disease genes, and 66 genes were funnelled into ongoing studies. De novo variants (1/3 of all candidates) were the easiest to be confirmed (3/4 of published genes, reflecting the advantage of trio analyses), followed by homozygous variants (1/4). Almost all published cases have NDD, reflecting the easiness to identify the genetic causes of severe phenotypes. 3/4 of the published genes were identified before 2020, signalling the long time needed to describe a gene, but also a slowdown in gene discovery using exomes.

We encourage all to take advantage of own unsolved diagnostic cases or to share it with research focused centres. We observe gaps in autosomal recessive as well as in mild (i.e. affected persons can reproduce) disorders.

Conflict of Interest: None declared

P09.067.C Is this our CHAMP1on gene? A 13q34 microdeletion Case Report

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Introduction: Terminal 13q34 microdeletions are rare chromosomal anomalies, associated with developmental delay (DD), intellectual disability (ID), autism, epilepsy and dysmorphisms. *CHAMP1* gene (OMIM* 616327), located within 13q34 deleted region, encodes for a zinc finger phosphoprotein, crucial to chromosome segregation via attachment of kinetochore-microtubule. Loss-of-function pathogenic variants in *CHAMP1* result in a neurodevelopmental disorder characterised by DD, severe ID, speech and language impairment, autism and sleep disturbances, hypotonia, microcephaly, seizures, ophthalmologic issues and gastrointestinal problems. It is hypothesised that *CHAMP1* haploinsufficiency can also be associated with the described phenotype but with a milder presentation.

Case presentation: A 15-year-old boy was first observed in our Genetics Clinic due to ID, autism with absent speech, epilepsy, axial hypotonia and sleep disturbance. At observation, our patient presented with dysmorphic facial features, tall stature and scoliosis. Brain MRI, electrocardiogram and FRAXA study were normal. Microarray analysis identified a 45.6 Kb heterozygous deletion involving 13q34 chromosome region, classified as variant of unknown significance. Parental testing revealed that 13q34 microdeletion was de novo.

Conclusions: To the best of our knowledge, this is the smallest 13q34 microdeletion reported to date, encompassing only one morbid OMIM gene (*CHAMP1*), associated with ID, autism, epilepsy and sleep disturbances. We believe that our case provides evidence that *CHAMP1* haploinsufficiency may contribute to the presented neurodevelopment phenotype, providing a possible genotype-phenotype correlation. Given the rarity of this microdeletion, further evidence is needed to enhance our understanding of the etiology of this condition, contributing to improve diagnosis and provide genetic counselling for these patients.

Conflict of Interest: None declared

P09.068.D Expanding the phenotype in Schaaf-Yang syndrome due to MAGEL2 non-truncating variants

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Background/Objectives: *MAGEL2* haploinsufficiency has been associated with Schaaf-Yang syndrome. *MAGEL2* is a single-exon gene, maternally imprinted and paternally expressed, encoding a protein in the Prader-Willi domain (15q11). The so far 97 reported patients carry protein-truncating variants with a severe phenotype, often responsible for an early death and marked at birth by hypotonia, arthrogryposis, respiratory distress, feeding problems and neurodevelopmental delay resulting in intellectual disability and autism spectrum disorder; We report here 2 patients with variants in *MAGEL2* with high variable phenotype.

Methods: WES was performed in patient 1 while Patient 2 was enrolled in SeqOIA project performing *trio-WGS*.

Results: Patient 1 was a female infant. She presented with hemorrhagic occipital stroke, severe neonatal respiratory distress, feeding difficulties, Pierre Robin sequence, clubfeet, arthrogryposis and died at 1 year-old owing to seizure onset in combination with the persistent respiratory issue. She harbors a novel heterozygous truncating variant in *MAGEL2* mutational hotspot c.1996dup;p.(Gln666Profs*47). Patient 2 presented with pre and postnatal growth failure (<-3.5SD), microcephaly (-3.5SD), right iris coloboma, mild dysmorphic features, thin corpus callosum. She was hospitalized at birth for a perinatal anoxia placed in hypothermia and had then feeding difficulties. Trio-WGS analysis revealed paternally inherited heterozygous in-frame deletion (c.2860_2907del;p.(Ile954_Ala969del)) in *MAGEL2* ; the variant resulted in a aminoacid loss in the U7BS-domain essential for interaction with USP7 protein in the MUST complex.

Conclusion: These two cases further expand the clinical spectrum of *MAGEL2* variants and support genotype–phenotype correlation.

Conflict of Interest: None declared

P09.070.B Clinical significance of genomic variants affecting exons 1-3 of KANSL1: diagnostic pitfalls

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KoolenDeVries syndrome (KdVS) can be caused by chromosome deletions encompassing exons 2-15 of *KANSL1*, or intragenic variants scattered on exons 2-15. Extra copies of exons 1-3 of *KANSL1* are recorded in 40% of the european alleles. We present six genomic abnormalities encompassing exons 1-3 of *KANSL1*: one case of deletion, and five cases of variants affecting exon 2 [c.2785_2786delAG, p.(R929Gfs*44); c.540delA, p.(K180Nfs*22) and c.985_986delTT, p.Leu329Glufs*22 in three cases].

Of them, four were detected by array-CGH and gene panel NGS analysis performed as first tier test. We made: 1) deep clinical evaluation of patients; 2) parental analysis; 3) MLPA of *KANSL1*; 4) cDNA sequencing of *KANSL1*.

We observed: a) the exon 1-3 deletion was de novo; the clinical phenotype was not consistent with KdVS; a diagnosis of Mowat-Wilson syndrome was inferred clinically and confirmed by *ZEB2* sequencing; b) of the three c.985_986delTT variant in exon 2, one was de novo and two were inherited from a healthy parent; none was detected at a cDNA level; the duplication polymorphism affecting exon 1-3 was detected in all patients; the clinical phenotype was not consistent with KdVS; we concluded that these variants affected the non functional duplicated copy of the gene. Of note, the c.985_986delTT has been reported both in healthy subjects and KdVS patients; c) the c.2785_2786delAG and c.540delA variants were de novo; the clinical phenotype was fully consistent with KdVS; the duplication polymorphism was not detected.

This report highlights the pivotal role of clinical genetics in precise diagnosis of syndromic neurodevelopmental disorders.

Conflict of Interest: None declared

P09.071.C CHAMP1-related disorders: pathomechanisms triggered by different genomic alterations define distinct nosological categories

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ABSTRACT

Loss-of-function variants in *CHAMP1* were recently described as cause of a neurodevelopmental disorder characterized by intellectual disability (ID), autism, and distinctive facial characteristics. By exome sequencing (ES), we identified a truncating variant in *CHAMP1*, c.1858A>T (p.Lys620*), in a patient who exhibited a similar phenotype of severe ID and dysmorphisms. Whether haploinsufficiency or a dominant negative effect is the underlying pathomechanism in these cases is a question that still needs to be addressed.

By array-CGH, we detected a 194 kb deletion in 13q34 encompassing *CHAMP1*, *CDC16* and *UPF3*, in another patient who presented with borderline neurodevelopmental impairment and with no dysmorphisms. In a further patient suffering from early onset refractory seizures, we detected by ES a missense variant in *CHAMP1*, c.67G>A (p.Gly23Ser). Genomic abnormalities were all de novo in our patients. We reviewed the clinical and the genetic data of patients reported in the literature with: loss-of-function variants in *CHAMP1* (total 40); chromosome 13q34 deletions ranging from 1.1 to 4 Mb (total 7) and of the unique patient with a missense variant. We could infer that loss-of-function variants in *CHAMP1* cause a homogeneous phenotype with severe ID, autism spectrum disorders (ASD) and highly distinctive facial characteristics through a dominant negative effect. *CHAMP1* haploinsufficiency results in borderline ID with negligible consequences on the quality of life. Missense variants give rise to a severe epileptic encephalopathy through gain-of-function mechanism, most likely. We tentatively define for the first time distinct categories among the *CHAMP1*-related disorder on the basis of pathomechanisms.

Conflict of Interest: None declared

P09.072.D Is the Northern Genetics Service adhering to European best practice guidelines for the assessment and management of Fetal Valproate Spectrum Disorder?

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Background/ Objectives: Exposure to sodium valproate in utero can result in Fetal Valproate Spectrum Disorder (FVSD). FVSD is characterised by a pattern of clinical features with life long impact. These include congenital anomalies, physical health problems, developmental and neuropsychological difficulties. Recent 2019 European guidelines on the diagnosis and management of individuals with FVSD require that other genetic diagnoses are excluded. Expert physical phenotyping and co-ordination of care is also required. Referral to genetics services is therefore a key part of the diagnosis of FVSD. We undertook a database audit to compare our assessment and management of FVSD over the past 20years to the recently published guidelines.

Methods: Patients in the Northern Genetics Service database coded as 'anticonvulsants - fetal teratogenesis' or 'valproate embryopathy' were identified. Notes were reviewed and the assessment and management of exposed individuals compared to the 2019 European Consensus guidelines.

Results: 410 families were identified, of which 79 families were referred for a genetics NHS clinic consultation within the northern genetics service. The remaining 331 families were part of a prospective research project undertaken in 2005 through the department. Diagnosis was inconsistent and surveillance and follow up of exposed individuals is not common practice.

Conclusion: Most FVSD assessments predated the 2019 guidance. Comparison of practice to the 2019 guidelines was not possible due to the lack of follow-up within genetics. Formal clinical review of historically assessed families is warranted to address gaps in past assessments and to inform the development of effective pathways going forward.

Conflict of Interest: None declared

P09.073.A Mother and child with a novel splicing variant in SETD1A gene – impact of reverse phenotyping

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Background/Objectives: *SETD1A* gene encodes a protein involved in histone H3 lysine 4 methylation. *SETD1A* haploinsufficiency causes a neurodevelopmental disorder (OMIM#618832), characterized by developmental delay (DD)/ intellectual disability, facial dysmorphisms, behaviour/ psychiatric abnormalities, and epilepsy.

Methods: We report a 4-year-old male, second child of non-consanguineous parents. His mother has mild dysmorphisms, epilepsy and psychiatric disorder. Pregnancy was complicated by foetal growth restriction and acute foetal distress, with an emergency C-section at 34 weeks. He evolved with hypotonia, feeding difficulties, short stature, mild-to-moderate DD, behavioural problems, and high myopia. Physical examination showed relative macrocephaly, dysmorphic facies, joint laxity, and brachydactyly. Previous normal investigation included cardiac and abdominal ultrasounds, EEG, auditory evoked potentials, array-CGH and molecular study for fragile X syndrome. Brain MRI revealed megalencephaly, Chiari malformation type 1, and platybasia.

Results: Whole-exome sequencing identified a novel heterozygous variant in *SETD1A* gene: c.151-2A>G, classified as uncertain significance. Segregation studies confirmed it is maternally inherited. This variant is predicted to disrupt the highly conserved acceptor splice site and co-segregates with the phenotype. We hope to demonstrate its pathogenicity through future DNA methylation episignature analysis.

Additionally, a maternally inherited uncertain heterozygous variant in *SLC12A2* gene was identified, but the phenotype is not suggestive of any of the *SLC12A2*-related disorders.

Conclusion: We report a novel variant in *SETD1A* gene, and the first case of *SETD1A* related-neurodevelopmental disorder inherited from an affected parent, expanding both genotype and phenotype of this condition. This case also illustrates the impact of reverse phenotyping in variant interpretation and diagnosis.

Conflict of Interest: None declared

P09.074.B Family management of sleep problems in children with Down syndrome

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Background/Objectives: Children with Down syndrome (DS) have a high prevalence of sleep problems and these problems can have long-term negative consequences for not only the child with DS, but other family members. The main purpose of this mixed-methods study was to gain a better understanding of how families of children with DS define and manage their child's sleep problems.

Methods: The Child's Sleep Habits Questionnaire (CSHQ) was completed by 53 parents of children with DS between 24 months to six years of age. Results of the CSHQ were used to select a subset of 15 parents to be interviewed; the goal was to interview parents of children with many problematic sleep habits, as well as those with few problematic sleep habits. Interviews were transcribed verbatim and coded using coding categories from the Family Management Style Framework.

Results: Parents used words like difficult, frustrating, and exhausting to define their child's sleep problems. The most reported physical sleep problems were restless sleep and apneic episodes, while the most reported behavioral sleep problems were bedtime resistance, night awakenings, and daytime sleepiness. Parents used a wide variety of medical and behavioral strategies to manage their child's sleep problems and in many cases they had to find the strategies on their own.

Conclusion: Findings from this study indicate that parents of children with DS devote a great deal of time and energy to managing their child's sleep problems. Parents want greater help and support from health care providers.

Grant References: UNC Sleep Innovation Research Grant

Conflict of Interest: None declared

P10 Neurogenetic and Psychiatric Disorders

P10.002.B A novel PRRT2 variant in hemiplegic migraine: identification by exome sequencing and functional characterization through cellular studies

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Background/Objectives: Hemiplegic migraine (HM) is a rare monogenic autosomal-dominant form of migraine with aura and can occur as a sporadic or as a familial form (FHM). Three disease-causing genes have been identified for FHM: *CACNA1A*, *ATP1A2* and *SCN1A*. However, not all FHM families are linked to one of these three known FHM *loci*. Some *PRRT2* variants have been associated with HM symptoms, therefore, the *PRRT2* gene may be considered the fourth gene causing FHM. PRRT2 plays an important role in neuronal migration and synapse formation/ maintenance during development and calcium-dependent neurotransmitter release.

Methods: We performed a whole exome sequencing (WES) in one Portuguese family to unravel the genetic cause of migraine, resulting in the identification of a novel *PRRT2* variant with further functional studies to confirm its pathogenic effect.

Results: Our findings demonstrated that *PRRT2* variant (c.938C>T;p.Ala313Val) reduces protein stability, leads to the premature protein degradation by the proteasome and alters the subcellular localization of PRRT2 from the plasma membrane to the cytoplasm.

Conclusion: In this study, we identified for the first time in the Portuguese population, a novel heterozygous missense variant PRRT2-A313V in a patient with HM-associated symptoms. *PRRT2* is a highly promising candidate gene causing a fourth form of HM.

Our results highlight the importance of including this gene in the diagnosis of FHM.

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Conflict of Interest: None declared

P10.003.C The impact of socioeconomic status in the polygenic risk of psychiatric traits and disorders: evidence of assortative mating and participation bias in UK Biobank

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Background/Objectives The polygenic architecture of psychiatric traits can be affected by assortative mating (AM) and participation bias. However, it is unclear whether AM and participation bias effects on psychiatric traits are influenced by socioeconomic status (SES). Here, we investigated the SES effect on AM and participation bias with respect to psychiatric traits in the UK Biobank (UKB).

Methods We estimated AM genetic signatures across psychiatric traits in the UKB using three approaches: gametic phase disequilibrium (GPD; n = 243,476 unrelated individuals), within-spouses (n = 45,570 spouse pairs), and within-siblings polygenic risk score correlation (n = 17,911 full-sibling pairs). Then, we conducted a conditional analysis to adjust AM estimates by SES effect. To assess SES effect on participation bias, we compared GPD estimates between UKB mental health questionnaire (MHQ)-responders (n = 118,656) and MHQ-non-responders (n = 243,476). Analyses were performed using data from European-descent individuals.

Results We observed an increase in AM estimates after SES conditioning for *Frequency of drinking alcohol* (2.5% to 6%, p-value = 1.27×10^{-6}), and *Maximum habitual alcohol intake* (1.33% to 4.43%, p-value = 3.97×10^{-6}). We found significantly higher GPD estimates in MHQ-responders compared to MHQ-non-responders for major depressive disorder (3.83% vs. 0.23%, p-value = 1.79×10^{-7}) and alcohol use disorder (1.2% vs. 0.45%, p-value = 4.2×10^{-5}).

Conclusion We found suggestive evidence that SES affects the genetic signatures of AM and participation bias in psychiatric traits, particularly in those alcohol related. Thus, SES should be accurately modeled when investigating the polygenic architecture of mental health.

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Conflict of Interest: None declared

P10.004.D Phenotypic expansion of EGP5-related Vici syndrome: 15 Dutch patients carrying a founder variant

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Background: Vici syndrome (OMIM 242840) is a very rare autosomal recessive multisystem disorder first described in 1988. In 2013, bi-allelic loss-of-function mutations in *EPG5* were reported to cause Vici syndrome. Five principal diagnostic features of Vici syndrome have been proposed: agenesis of the corpus callosum, cataracts, cardiomyopathy, hypopigmentation, and combined immunodeficiency.

Methods: We identified 15 patients carrying a homozygous founder missense variant in *EPG5* who all exhibit a less severe clinical phenotype than classic Vici syndrome.

Results: None of the 15 patients had immunodeficiency, cardiomyopathy, hypopigmentation, or cataracts. All patients had a severe developmental delay and all developed epilepsy (age of onset 1 month to 17 years). To date, 13 patients have died, with a median age of death of 13 years (range 20 months to 23 years). All 15 show typical brain abnormalities on MRI, consisting of corpus callosum agenesis, a deep frontal fissure, limited white matter volume leading to indentation of the dorsal insula into the posterior horns of the lateral ventricles, and a smaller mesencephalon and pons. The homozygous founder variant in *EPG5* they carry results in a shorter in-frame transcript and truncated, but likely still residual, EPG5 protein.

Conclusion: We speculate that the residual EPG5 protein explains their attenuated phenotype, which is consistent with two previous observations that low expression of EPG5 can lead to an attenuated Vici syndrome phenotype. We propose renaming this condition *EPG5*-related neurodevelopmental disorder to emphasize the clinical variability of patients with bi-allelic mutations in *EPG5*.

Conflict of Interest: None declared

P10.005.A Cellular model to predict brain pathology of TBCK encephalopathy patients

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Background: One of the most prevalent causes of intellectual disability are genetic disorders, such as TBCK-encephaloneuronopathy (TBCKE). TBCKE is caused by loss of function mutations in the TBCK gene, which encodes a Tre-2, Bub2 and Cdc16 (TBC)-domain-containing kinase. TBCKE patients experience multiple symptoms, one of which is malformations of the parietal white matter of the brain. Therefore, we sought to investigate how TBCK gene knockdown impacts morphology and behavior of neuronal and glial cells in vitro.

Methods: We used ReNcell VM cells, an immortalized neural stem cell line derived from ventral mesencephalon of human brains. We generated stable knockdown of TBCK gene in ReNcells by transducing lentivirus with shRNA specific to TBCK mRNA. We allowed the cells to differentiate into neurons and glia in vitro, and harvested for qPCR and Western blot analysis. Plus, we utilized immunocytochemistry (ICC) to stain for multiple neuronal and glial cell-specific markers.

Results: Knockdown of TBCK resulted in loss of normal neuronal organization and differentiation. ICC staining using neuron- and glial-specific markers demonstrated loss of soma clustering present in wild-type co-cultures. qPCR plus Western blotting of MAP2, a microtubule-associated protein (MAP) that localizes to neuronal dendrites, showed that expression was reduced. Finally,

we observed that the total number of cells that positively stained for NeuN, a neuronal nuclei marker, was reduced after TBCK knockdown.

Conclusions: Based on the results, we hypothesize that TBCK knockdown impacts neuronal cell differentiation and organization by suppressing expression of neuron-required differentiation and morphological factors, such as NeuN and MAP2.

Conflict of Interest: Kristen Navarro: None declared, Rajesh Angireddy: None declared, Elizabeth Bhoj Principal investigator of the Bhoj lab

P10.006.B Whole exome sequencing for dyslexia

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Introduction: Dyslexia is a neurodevelopmental condition characterised by poor reading abilities and affecting up to 10% of children with heritability estimated to be around 70%. Recently, a large GWAS identified 42 associated loci, but rare variants have not been systematically assessed.

Materials and methods: We performed whole exome sequencing on 65 unrelated individuals selected for a severe dyslexia phenotype (reading score < -1 SD & IQ > 85). For 33 individuals we also sequenced family members who presented reading difficulties suggesting the presence of highly penetrant variants in the family ($N_{total} = 166$). We processed reads following the GATK guidelines, called variants with DeepVariant and annotated them with ANNOVAR. Filtering criteria include allele frequency (<1% in gnomAD) and pathogenicity scores. In the case of families, we also filtered variants for segregation with the affected status.

Results: We report the results for the largest rare variant analysis conducted so far for dyslexia. We identified variants in genes previously reported for reading and language disorders (ZGRF1), mathematical abilities (MYO18B), neurodevelopmental conditions such as Usher syndrome (MYO7A) as well as novel associations in genes related to vision (EYS). No variants in the known dyslexia candidates (DCDC2, DNAAF4, KIAA0319, ROBO1) were detected. At a conference we will present a more in-depth downstream analysis.

Conclusion: We identified variants in genes related to reading and language disorders, mathematical abilities, and other neurodevelopmental conditions. Our findings support the role of rare variants in contributing to dyslexia.

Conflict of Interest: None declared

P10.007.C Report of a third family with a novel biallelic loss-of-function variant causing FAM160B1-related neurodevelopmental disorder

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Background: The FHF complex subunit HOOK interacting protein 2A (*FHIP2A*, also known as *FAM160B1*) is involved in vesicle trafficking by linking dynein to endoplasmic reticulum-to-Golgi cargos. Currently, there are two reports with five affected individuals in two unrelated families carrying biallelic variants in *FHIP2A*.

Methods: We ascertained two individuals (P1 and P2) born to a consanguineously married couple from India. Singleton exome sequencing (ES) followed by Sanger validation and segregation was performed in the family.

Results: P1 and P2 presented with global developmental delay, intellectual disability, microcephaly, difficulty in walking and dysmorphic facies. On singleton ES analysis, we identified a homozygous variant, g.114822124G>T in *FHIP2A* (NC_000010.11) (NM_020940.4:c.45+1G>T) in P1. Sanger validation and segregation showed the variant in homozygous state in P2 and heterozygous state in their parents. This variant is absent in population databases like gnomAD, Singapore genome database, GenomeAsia and our inhouse database of 2673 exomes. Previously reported five individuals with biallelic variants in *FHIP2A* had clinical findings of developmental delay, intellectual disability, microcephaly, cerebellar atrophy, mild facial dysmorphism which were similar to the phenotypes observed in the present family.

Conclusion: This report of two additional individuals provides further evidence for a novel autosomal recessive neurodevelopmental disorder caused by pathogenic biallelic variants in *FHIP2A*.

Grant References: 1R01HD093570-01A1(NIH,USA)

Conflict of Interest: None declared

P10.008.D Neurogenetic syndromes with cerebellar abnormalities revealed by whole-exome sequencing

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Background: Cerebellar hypoplasia is a heterogeneous genetic disorder characterized by intellectual disability, delayed motor development, and/or ataxia. Well-known causes of X-linked cerebellar hypoplasia include MICPCH/CASK syndrome. Neurodegeneration and spasticity with or without cerebellar atrophy or cortical visual impairment syndrome is caused by heterozygous variants in the *KIF1A* gene. Hypotonia, ataxia, and delayed development syndrome are caused by heterozygous variants in the *EBF3* gene. We examined neurogenetic syndromes with cerebellar abnormalities using whole exome sequencing (WES).

Methods: Blood samples were collected from patients and their parents after obtaining written informed consent. WES was used to identify causative variants. Some patients were diagnosed by chromosomal microarrays or Sanger sequencing. Joubert syndrome and triplet repeat associated spinocerebellar ataxias were excluded from the present study. Many patients were included in the Initiative on Rare and Undiagnosed Diseases project.

Results: Causative variants in 16 different genes were identified in 27 (67.5%) out of 40 probands: *CASK* (n = 5), *KIF1A* (n = 5), *EBF3* (n = 3), *CACNA1A* (n = 2), *AARS2*, *ABCB7*, *AHI1*, *ATP2B3*, *EBF3*, *HNRNPH2*, *ITPR1*, *NRROS*, *PTPN23*, *SCN8A*, *SPTBN2*, and *TBCD* (n = 1). Compound heterozygosity was noted in *AARS2, NRROS*, *PTPN23*, and *TBCD*.

Conclusion: Cerebellar hypoplasia may have unique characteristics in the form of a constellation of clinical symptoms, which may facilitate their recognition and shorten the diagnostic process. The present results will broaden the known clinical

manifestations of these syndromes. A definitive genetic diagnosis is beneficial for genetic counseling and the clinical management of individuals.

Conflict of Interest: None declared

P10.009.A The role of WDFY3 and the Wnt signalling pathway in neurodevelopmental disorders and brain size

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Mild to moderate neurodevelopmental disorders (NDD) are poorly understood and late diagnosis and interventions result in a high burden for the society and health system.

Our project investigates the gene *WDFY3*, a monogenic cause for moderate NDD. We performed deep phenotypization on the largest cohort of patients to date. Variants affecting *WDFY3* may result in either increased or decreased brain size. We hypothesize that these different outcomes depend on WDFY3 gain- or loss-of-function. We are using the neuroblastoma cell line SH-SY5Y to assess the effects of *WDFY3* downregulation and overexpression of wildtype and variants identified in our cohort. Our results suggest that *WDFY3* knockdown leads to increased cell proliferation. This is supported by a higher amount of Ki-67 positive cells in *WDFY3* knockdown. Our results also suggests that this increased proliferation might be caused by dysregulated Wnt/β-Catenin signalling supported by decreased levels of endogenous β-Catenin protein and altered *Axin-2* and *Glycogen synthase kinase-3β (GSK-3β)* mRNA expression in WDFY3 knockdown cells. *WDFY3* knockdown also leads to impaired clearance of aggregated proteins as shown by overexpression of an Exon1-Huntingtin-103Q protein. Since pharmacologic stimulation of autophagy could improve the outcome, we are currently running experiments to assess the potential benefit for such patients.

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Conflict of Interest: Moritz Jonathan Paha Student assistant, Neuroimaging Laboratory, Department of Neurology, University of Leipzig Medical Center, Leipzig, Germany Student assistant, Intermediate Care Unit Cardiology and Emergency Department, University Hospital Leipzig, Germany, Antje Garten Pediatric Research Center, University Hospital for Children and Adolescents, Leipzig, Germany, German Research Foundation - project number 209933838 - SFB 1052 - project B10, Diana Le Duc University Clinics Leipzig, Else Kröner-Fresenius-Stiftung 2020_EKEA.42 German Research Foundation SFB 1052 project B10, University of Leipzig

P10.011.C Cryptic or not? Pathogenic intronic variants detected from WES data significantly increase the diagnostic yield in Joubert syndrome

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Background/Objectives: Despite the NGS revolution, the diagnostic yield of many inherited disorders remains unsatisfactory. One example is Joubert syndrome (JS), a recessive ciliopathy. After WES, up to 30-35% patients fail to receive a definite diagnosis. We hypothesized that a proportion of these cases are caused by cryptic variants within known genes, and that at least a subset can be diagnosed by reanalyzing available NGS data, as sequences partly cover introns.

Methods: To prove this, we focused on 39 JS patients carrying a single coding pathogenic variant in a known JS gene. We reanalyzed NGS data searching for CNVs, then confirmed by RT-PCR, in trans with the exonic variant. Next, we searched for rare intronic variants bioinformatically predicted to impact splicing, whose effect was validated through PCR on patients' cDNA or in vitro minigene assays. We are now performing RNAseq in patients lacking candidate intronic variants detectable by WES.

Results: We identified 5 distinct intragenic heterozygous deletions in 7 cases and 7 candidate splicing variants (one recurring in 3 cases), placed up to 200bp from exon boundaries. Notably, all variants were shown to induce overt splicing defects, resulting either in frameshift, inframe deletions or insertions.

Conclusions: In conclusion, WES reanalysis focused on CNVs and intronic variants allowed detecting the "second hit" in patients carrying heterozygous coding variants. We provide proof of principle that cryptic variants are common mutational events in JS, and their search results in a significant increase of the diagnostic yield.

Grant References: Telethon-GGP20070

Conflict of Interest: None declared

P10.012.D Genetic testing in sporadic & familial Amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive, incurable fatal disease. Most cases are sporadic but 5%–10% are familial. The most common genetic etiology is a *C9ORF72* repeat expansion accounting for 3%-10% of sporadic cases and 39%-45% of familial cases. Dozens of other genes are implicated

Methods: We reviewed medical records of 73 ALS patients attending the neurogenetics clinic during 2015-2023. *C9ORF72*

testing was offered to all patients. Negative cases were also an ALS-panel including 60 genes.

Results: 40 patients were Ashkenazi Jews and 32 non-Ashkenazi. All but 3 cases were sporadic. Of the **40** Ashkenazi patients, 8 declined testing. *C9ORF72* testing was positive in **25%** (8/32). Of the 24 C9orf72 negative cases, ALS-Panel yielded a positive result only in 1/18 cases: a known pathogenic *LRRK2* mutation (p.G2019S) as well as variants of unclear significance in *VCP* and *KIF5A*.

Of the 32 non-Ashkenazi patients, *C9ORF72* was tested in 27 cases. Only 2 cases (7%) were positive, both in patients of Moroccan descent. However, ALS Gene-Panel was performed in 12 non-Ashkenazi patients yielded a positive result in 7 cases (42%) including pathogenic variants in *VCP*, *OPTN*, *TARDP*, *TNN* and suspected variants in *GRN* and *SPGM*.

Conclusions: Our results suggest that *C9ORF72* should be a first-tier test in Ashkenazi ALS patients. In non-Ashkenazi ALS patients, a multi-gene ALS panel should be considered as the first step, followed by the *C9ORF72* in negative cases. Such testing will have important implications for prognostication, treatment and prevention

Conflict of Interest: None declared

P10.013.A PSMC genes, variants and their role in the molecular pathogenesis of rare neurodevelopmental proteasomopathies

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Background/Objectives: The ubiquitin-proteasome system (UPS) is a highly conserved pathway across eukaryotes which preserves proteome stability in the cell by targeting ubiquitin-modified proteins to the 26S proteasome for degradation. An important step in this process is the unfolding of substrates, which is ensured by six AAA+ ATPase proteasome subunits encoded by the *PSMC1-6* genes. Over the last couple of years, we have identified an increasing number of de novo heterozygous loss-of-function variants in all six *PSMC* genes in >80 unrelated individuals with neurodevelopmental disorders (NDD). The cellular consequences of such dysfunction remain, however, to be characterized.

Methods: Cells isolated from NDD patients were subjected to transcriptomic and proteomic analysis using NanoString technology, qPCR, flow cytometry and SDS-PAGE/western-blotting.

Results: Our data show that PSMC loss-of-function was consistently associated with protein aggregation and sustained activation of the autophagy-lysosomal pathway resulting in a net loss of mitochondria. Transcriptomic analysis further reveals that *PSMC* variants generated a persistent type I interferon (IFN) gene signature with increased expression of typical IFN-stimulated genes (ISG). Herein, our investigations show that UPS dysfunction is accompanied by the release of immunostimulant danger signals which are subsequently sensed by receptors of the unfolded protein response (UPR) and integrated stress response (ISR) including protein kinase R (PKR) and general control nonderepressible 2 (GCN2), respectively.

Conclusion: Altogether, our work suggests a role for type I IFN in the molecular pathogenesis of NDD caused by *PSMC* variants and identifies molecular targets for potential therapeutic applications.

Grant References: Project UPS-NDDecipher ANR-21-CE17-0005 Project NDD-UBIPRO NExT

Conflict of Interest: None declared

P10.014.B An integrative strategy for the identification of gene-environment interactions in a case series of patients with Autism Spectrum Disorder

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Background/Objectives: Previous studies support the hypothesis that early life exposure to xenobiotics increases the risk of developing Autism Spectrum Disorder (ASD). However, the mechanism underlying this remains unclear. We propose that exposure to xenobiotics increases ASD risk in genetically susceptible subjects, carrying variants in genes involved in regulation of detoxification or selective permeability of physiological barriers (XenoReg genes).

Methods: In 70 ASD patients we sought for evidence of gene-environment interactions by 1) quantifying selected xenobiotics (bisphenol A, glyphosate and persistent organic pollutants) in neonatal blood spots, through mass spectrometry, 2) identifying predicted-damaging variants in a panel of 77 XenoReg genes and 3) defining a gene-environment interaction burden rank.

Results: A total of 35 (50%) ASD subjects had high (one standard deviation above the mean of the dataset) or very high (two standard deviations above the mean of the dataset) neonatal concentrations of at least one xenobiotic. Individuals were ranked according to xenobiotic concentration and presence of a XenoReg gene variant. The top ranking individuals (N = 13) carried predicted-damaging variants in *CYP2D6*, *CYP4X1*, *GSTM1*, *STS*, *UGT2B10*, *ABCB1*, *KIF17* or *TJP3*, suggesting an impaired metabolism of xenobiotics or the dysregulation of physiological barriers permeability as mechanisms contributing to disease risk.

Conclusion: These results suggest that ASD risk is increased in genetically susceptible subjects that are more vulnerable to early life exposure to xenobiotics, when the brain is still immature. This supports a role for gene-environment interactions in the etiology of ASD.

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Conflict of Interest: None declared

P10.015.C The potential impact of TTBK2 missense variants in SCA11: in silico analysis and a phosphoproteome study

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Spinocerebellar ataxia type 11 (SCA11) is a rare form of autosomal dominant cerebellar ataxia. The small number of families with SCA11 described to date harbour small deletions or insertions in *TTBK2*, resulting in truncated proteins. But importantly, *TTBK2* missense variants remain to be functionally validated. *TTBK2* encodes tau tubulin kinase 2 (TTBK2), a ubiquitous protein kinase involved in several molecular processes, e.g., microtubule dynamics, TDP-43 phosphorylation, and ciliogenesis. However, the mechanism underlying cerebellar neurodegeneration in SCA11 is still not established.

In this study, we reviewed and conducted an in silico analysis of the previously reported *TTBK2* missense variants. Also, we performed a phosphoproteomic analysis of a CRISPR/Cas9-cell model expressing a novel *TTBK2* variant (c.625C>T; p.Leu209Phe) identified by our group.

Our results predicted all variants to be benign/likely benign, except three variants, including TTBK2-Leu209Phe, located within the kinase domain, which were predicted as variants of unknown significance (VUS). TTBK2-Leu209Phe cells showed reduced TTBK2 protein levels and phosphorylation changes in several proteins, having 110 differentially expressed phosphoproteins (DEP). The interactome of the DEP revealed eight major clusters, which have as central nodes SMAD2, p62, AMOT and NARS (upregulated DEP), and EEF2, ILK, MAP1B, and KIF18A (downregulated DEP). Functional enrichment analysis of the phosphoproteins uncovered molecular processes likely impaired in TTBK2-Leu209Phe cells, namely cytoskeleton dynamics, gene regulation, protein degradation and TGF-β signalling.

In conclusion, our study confirmed the deleterious impact of a novel *TTBK2* missense variant affecting the kinase domain and opens new perspectives on SCA11 pathogenesis.

Funding: Ataxia UK (small grant ZGRACA).

Conflict of Interest: None declared

P10.016.D Biallelic variants in RCC1 result in fever associated axonal neuropathy with encephalopathy

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Background/Objectives: Acute encephalopathy (AE) is a rare condition characterised by neurological features, including seizures and cognitive impairment following febrile illness. Variants in *RANBP2* and *NUP214*, encoding nuclear pore complex proteins, have been identified in familial necrotising AE.

Methods: Families with AE underwent autozygosity mapping combined with WES/WGS. Thermal shift and enzymatic assays were conducted using recombinantly expressed proteins. Protein localisation was determined by immunofluorescent staining of patient fibroblasts.

Results: We identified two British Pakistani consanguineous families with multiple children affected by a rapid onset axonal neuropathy with AE following infection resulting in death or severe neurological impairment. A novel c.127G>A, p.(Gly43Ser) homozygous missense variant in *RCC1* segregated with the phenotype. In a British Cypriot family, we identified an individual compound heterozygous for *RCC1* c.238G>A;c.1195C>T, p.(Val80-Met);p.(Arg399Cys) with demyelinating, relapsing neuropathy secondary to recurrent infections. A maternal cousin homozygous for p.(Val80Met) had fatal AE. An Iranian homozygous for c.280A>G (p.Asn94Asp) had axonal neuropathy with sudden onset at 11 years.

Rcc1 protein binds Ran nuclear GTPase which controls nucleocytoplasmic transport and binds to RanBP2. $Rcc1^{G43S}$ had reduced thermostability compared to $Rcc1^{WT}$, with no effect on GTPase activity when assessed over a physiological temperature gradient. Immunofluorescence staining of patient fibroblasts cultured at 42°C for 4 hours indicated delocalisation of Rcc1 from chromatin.

Conclusion: We describe a novel form of acute encephalopathy with peripheral neuropathy due to biallelic *RCC1* variants, providing further insight into the role nucleocytoplasmic transport in acute neuropathies.

Grant References: Wellcome Trust PhD Studentship; LifeArc Pathfinder Award PATHFINDER004

Conflict of Interest: None declared

P10.018.B Three-tiered strategy for variant selection in patients with epilepsy

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Background: Epilepsy is one of the most common neurological conditions often characterized by recurrent episodes of seizure. The etiology is heterogeneous, however genetic factors are thought to play a major role. In this study, we sought to discover novel variants associated with epilepsy with the aim to identify candidate genes as therapeutic targets.

Methods: Whole genome sequencing (WGS) was performed on 58 DNA samples from patients with confirmed epilepsy. A three-tiered strategy was used: first to analyze variants in known epilepsy genes, then to identify mutations within epilepsy-associated genetic loci known in literature, and finally expand to unblinding prospective bioinformatic assessment of all sequence variants. Novel associations were predicted using different metrics and database annotations, such as: MAX_AF, SIFT, PolyPhen,

CLIN_SIG, IMPACT. Biological pathways and genes associated with the hits were identify using the g:GOSt module of g:Profiler.

Results: We identified novel disease-gene relationships by prioritizing genes with high number of variants associated with significant high impact rating (*CASKIN2, POMT1*), classified as pathogenic (*FAM161A, SCN1A, TTN*), and probably damaging (*ZBP1, CBWD5, CYP2C19, DNAH10, TRAV19, MDP1, TK2, ENKD1, CTC1, SOGA1*). Enrichment analysis on 200 genes with the highest number of hits was used to pinpoint candidates involved with neuronal development (*NRG1*), neuronal cell adhesion (*NRXN1, PTPRD, DCC,LRRC4C, CNTNAP2, CTNND2, CTNNA2*) and synapse-related functions (*GRID2, NLGN4X*).

Conclusions: This study identifies novel genetic variants in patients with confirmed epileptic conditions, extending scopes of clinical diagnosis and providing the groundwork for future functional studies as well as clues for the development of new treatments.

Conflict of Interest: None declared

P10.019.C Analysis of exome sequencing data for small structural variants impacting cognition in schizophrenia

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Introduction: Cognitive impairment is an important feature of schizophrenia that is associated with poor outcomes. Microarray studies have shown large (>100KB), rare copy number variants affecting loss-of-function intolerant (LoFi) genes are associated with lower cognition in schizophrenia. However, the contribution from smaller structural variants (SVs) to cognition in schizophrenia is unknown.

Methods: We analysed whole exome-sequencing (WES) data in 795 schizophrenia cases for SVs using the 'Indelible' algorithm (Gardner et al., 2021), which can detect SVs as small as 10bp. Rare SVs (<1% allele frequency) impacting LoFi genes (pLI <0.9) were tested for association with quantitative measures of current cognitive function and estimated premorbid IQ, using linear regression.

Results: 28 (3.5%) schizophrenia cases carried a rare SV overlapping at least one LoFi gene: 18 deletions, 9 duplications and 1 small tandem repeat. All SVs were <100bp (mean size = 37bp). No statistically significant association was found between SV carrier status and either current cognitive function ($\beta = 0.142$, $p = 0.59$) or estimated premorbid IQ ($\beta = 0.340$, $p = 0.070$).

Discussion: We found that 3.5% of probands carried a rare, highly constrained SV < 100bp, demonstrating that Indelible can potentially identify small SVs that cannot be identified using microarray data. In our sample we found no evidence that rare, small SVs impact cognition in schizophrenia, even if occurring in LoFi genes. However, our sample is small and accordingly has low power.

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P10.020.D Genetic variability of incretin receptors is associated with Alzheimer's disease biomarkers

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Background: Chronic inflammation can affect insulin signalling in the brain of AD patients and patients with mild cognitive impairment (MCI). Incretins GLP-1 and GIP, peptide hormones that stimulate insulin secretion, have shown neuroprotective effects. Incretin receptors are expressed in the central nervous system, opening up the window of opportunity for application of incretin analogues in AD patients without diabetes. Since genetic variability of *GLP1R* and *GIPR* receptors can modulate the neuroprotective effect of incretins, we evaluated the association of common polymorphisms with cerebrospinal fluid (CSF) biomarkers and cognitive test results in patients with AD and MCI.

Methods: Our study included 62 AD patients, 24 MCI patients with pathological CSF biomarker levels and 31 MCI patients with normal CSF biomarker levels. Patients were genotyped for *GLP1R* rs10305420, rs6923761 and *GIPR* rs1800437 polymorphisms using competitive allele-specific PCR. Association of these polymorphisms with CSF biomarker levels was evaluated using nonparametric tests.

Results: In AD patients, carriers of at least one polymorphic *GLP1R* rs10305420 T allele or *GLP1R* rs6923761 A allele had higher CSF $A\beta_{42/40}$ ($p = 0.041$ and 0.050, respectively). Carriers of at least one polymorphic *GLP1R* rs6923761 A allele had lower CSF p-tau_{181} levels as compared to non-carriers ($p = 0.022$).

Conclusion: *GLP1R* polymorphisms may be associated with CSF biomarkers in AD patients. Our observations could foster further research in the area of impaired insulin signaling. They also suggest the potential of the pharmacogenetic approach in implementation of incretin analogues in AD patients.

Grant References: ARRS P1-0170.

Conflict of Interest: None declared

P10.021.A Parallel in-depth analysis of repeat expansions in ataxia patients by long-read sequencing

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Background: Instability of simple DNA repeats has been known as a common cause of hereditary ataxias for over 20 years. Routine genetic diagnostics of these phenotypically similar diseases still rely on an iterative workflow for quantification of repeat units by PCR-based methods of limited precision. It only partly covers complex repeat disorders such as *RFC1* spectrum disorder in which both, repeat motive and repeat length, determines pathogenicity.

Methods: We established and validated clinical nanopore Cas9-targeted sequencing (Clin-CATS), an amplification-free method for simultaneous analysis of 15 repeat loci associated with hereditary ataxias including the complex ataxias SCA31, SCA37 and *RFC1* spectrum disorder. The method combines target enrichment by CRISPR/Cas9, Oxford Nanopore long-read sequencing, and a bioinformatics pipeline for parallel detection of length, methylation, and sequence of the repeat loci.

Results: Clin-CATS allowed the precise and parallel analysis of 15 repeat loci and revealed additional parameter such as *FMR1* promotor methylation and repeat sequence. We analyzed 125 clinical samples of undiagnosed ataxia patients and identified causative repeat expansions in 32 patients including rare conditions such as a very-late onset Friedreich's ataxia and or a Fragile-X-associated-tremor-and-ataxia syndrome (FXTAS) caused by a full *FMR1* repeat expansions with non-methylated *FMR1* promotor. Biallelic expansions within *RFC1* were identified as the most frequent cause of ataxia.

Conclusion: Our results highlight the power of Clin-CATS as a readily expandable workflow for the in-depth analysis and diagnosis of phenotypically overlapping repeat expansion disorders that enables a molecular diagnosis of ataxias independent of preconceptions based on clinical presentation.

Conflict of Interest: None declared

P10.022.B Phenotype and genotype spectrum expansion of West Syndrome of genetic aetiology in Egyptian children

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Background/Objectives: West syndrome (WS) is characterised with epileptic spasms, developmental delay or regression and hypsarrhythmia with genetic and non-genetic aetiologies. The genetic landscape of WS is heterogenous including genes implicated in various cellular pathways. Herein, we describe the phenotype and genotype spectrum of WS of genetic aetiology in Egyptian children.

Methods: In this study, we retrospectively studied the clinical, neuroimaging, EEG, and genetic data of patients with WS with a genetic aetiology identified by whole exome sequencing and who presented at Cairo University Children Hospitals and National Research Centre in Egypt.

Results: Fifteen patients were identified, and consanguinity was observed in 6 families (40%). The age at onset of seizures ranged between 1-9 months. All patients presented with epileptic spasms, with concomitant generalised tonic clonic seizures and focal seizures in two and one patients respectively. All patients had variable degrees of developmental delay. Hypotonia was common in 11 (73%) patients and hypertonia was seen in 4 (27%) patients. Cerebral cortical atrophy was the most common neuroimaging abnormality in 11 (73%) patients. Other abnormalities included cerebellar hypoplasia, white matter, and corpus callosum abnormalities. Variants in 12 genes were identified with 7 novel variants (in *UBA5*, *WWOX*, *CDKL5*, *SLC25A12*, *STXBP1* and *RNF13* genes) and 8 previously reported variants (in *SCN2A, SCN1B, WWOX, ATRX, RHOBTB2, GRIN2B* and *STXBP1* genes). Obtaining a genetic diagnosis led to a change in epilepsy treatment in five patients.

Conclusion: In this study, we expand the phenotypic and genotypic spectrum of WS and highlight the genetic heterogeneity of WS.

Conflict of Interest: None declared

P10.023.C The genetic and phenotypic correlates of neonatal Complement Component 4 protein concentration with a focus on psychiatric and autoimmune disorders

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Background/Objectives: The complement system, including complement component 4 (C4), traditionally has been linked to innate immunity. More recently, complement components have also been implicated in the risk of schizophrenia and selected autoimmune disorders (e.g. systemic lupus erythematosus; SLE).

Methods: Based on a large, population-based case-cohort study, we examined the genetic architecture of circulating C4 protein concentration in 68,768 neonates. We examined the association between C4 concentration versus the subsequent risk of mental disorders in the case-cohort study. We also explored Mendelian randomisation (MR) and phenome-wide association study (PheWAS) in UK Biobank for selected mental and autoimmune disorders.

Results: A genome-wide association study (GWAS) identified 36 independent loci, 30 of which were in or near *C4* gene. We found no association between measured neonatal C4 concentration versus schizophrenia (SCZ), bipolar disorder (BIP), depression (DEP), autism spectrum disorder, attention deficit hyperactivity disorder or anorexia nervosa diagnosed in later life. MR found a small positive association between higher C4 concentration and an increased risk of SCZ, BIP, and DEP. PheWAS found no association with mental disorders. MR found a decreased risk of SLE, type-1 diabetes, multiple sclerosis, rheumatoid arthritis but an increased risk of Crohn's disease. PheWAS confirmed that the genetic correlates of C4 concentration were associated with a range of autoimmune disorders (including SLE).

Conclusions: Our study provides new insight into the genetic architecture of C4 protein and adds to our understanding of the associations between neonatal C4 concentration versus subsequent mental and autoimmune disorders.

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P10.024.B Application of long read sequencing for diagnosis of spinocerebellar ataxia 36

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Background: Spinocerebellar ataxia (SCA) 36 is an autosomal dominant disease caused by an expansion of hexanucleotide in the NOP56 gene. Traditional genetic diagnosis of SCA36 requires Southern blot due to the large-sized repeats of pathogenic alleles. While SCA36 is becoming more widely recognized worldwide, it has not been reported in Korea. In the present study, we aimed to investigate the prevalence of SCA36 in Korean patients using long-read sequencing (LRS) with Cas9 target enrichment.

Methods: Using repeat-primed PCR (RP-PCR), we screened 47 cerebellar ataxia patients who tested negative for SCA1/2/3/6/7/8/17 and dentatorubral-pallidoluysian atrophy. The target region was enriched with the CRISPR-Cas9 system and prepared libraries were sequenced on SpotON flow cells (Oxford Nanopore technology). Repeat number estimation and data analysis were performed with RepeatLab pipelines based on RepeatHMM.

Results: Six patients whose RP-PCR showed a saw-tooth pattern were subjected to and confirmed by LRS. The depth of coverage of the target region ranged from 17-573X, which accounted for 1~2% of the total obtained reads. The estimated repeat number ranged from 477-1400. The prevalence of SCA36 in our study population was found to be higher compared to those observed in other regions.

Conclusion: LRS using Oxford Nanopore technology with Cas9 target enrichment can be an alternative to Southern blot for the diagnosis of SCA36. Considering the prevalence observed in this study, testing for SCA36 would increase the diagnostic yield of SCA, especially in Korean patients.

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Conflict of Interest: None declared

P10.025.A A novel case provides new evidence of the occurrence of hereditary pathogenic variants in the RERE gene

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Background: The arginine-glutamic acid dipeptide repeats gene (*RERE*) encodes a nuclear receptor coregulator acting within a complex that plays a critical role in controlling retinoic acid signalling during brain, eye, ear, and heart development. Variants in *RERE* have been associated with different phenotypes in the 23 previously reported cases, including developmental delay, intellectual disability, seizures, and an autism spectrum disorder (ASD). *RERE*-related disorders are collectively termed Neurodevelopmental Disorders with or without Anomalies of the Brain, Eye, or Heart (NEDBEH). Inherited pathogenic *RERE* variants are rare, with only one case previously described. This report presents a new case of a family with an inherited pathogenic variant in the *RERE* gene.

Case description: The proband was a 7-year-old female with a diagnosis of behaviour disorder, strabismus, achromic macules, ASD, and intellectual disability. A heterozygous frameshift variant was identified in *RERE* (c.3612_3613insAGGCAGAGCG, NM_001042681.2), resulting in a premature termination signal in the protein sequence (p.Ala1205ArgfsTer6). The mutation was also present in the proband's dizygotic twin, who had been diagnosed with behaviour disorder. Both individuals had inherited the variant from their father, who had a psychiatric disorder.

Discussion: This report provides new evidence of the occurrence of inherited pathogenic variants in the *RERE* gene, underscoring its significance in terms of its implications for genetic counselling and disease management. These findings expand the phenotypic spectrum regarding *RERE*-associated diseases and highlight the necessity of continued research to enhance our understanding of their role in disease.

Grant References: FI-AGAUR (2022FI_B_00262)

Conflict of Interest: None declared

P10.026.B Identification of candidate gene variants for autism spectrum disorders in Slovak population using whole exome sequencing

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Background: Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by persistent impairments in reciprocal social communication and the use of restrictive and repetitive routines, typically manifesting before age of 3 years. ASD are often accompanied by delayed or impaired language development and cognitive deficits. ASD are multifactorial disorders, genetically very heterogeneous and the genetic background of ASD in the Slovak population is rarely investigated.

Methods: Children diagnosed by standard diagnostic tools (ADOS-2, ADI-R) in Academic Research Centre for Autism were involved in the study based on their age, communication and cognitive skills ($N = 24$). The whole exome sequencing and subsequent bioinformatic analysis were performed using the Sophia GENETICS Platfom.

Results: The pathogenic or likely pathogenic variants were identified in 75% of analyzed samples. These were represented by variants in genes *ITPR1, ASAP2, PHIP, VPS13B, TTN, BCAS1, SYNE1, SYNGAP1, DHCR7, PAH, ANKRD11, EFR3A, PCDH19, FBN1, TM4SF19, FBRSL1, ABCA7, DPYD, FGF14, KCNJ10, EP300, KMT2C, INTS1, MMP23B*. In addition, the potential involvement of the variants with uncertain significance needs to be evaluated in more detail.

Conclusion: Identified gene variants will be associated with other biological and psychologic-behavioral parameters to clarify the role of genetic background in ASD etiopathogenesis. We will focus on clarification of the relationships between the core symptoms of ASD, cognitive abilities, problem and adaptive behavior in children with ASD.

Grant References: The study was supported by grants APVV-20-0070, APVV-20-0139 a VEGA 1/0068/21.

Conflict of Interest: None declared

P10.027.C The impact of rare coding variants in schizophrenia associated genes on cognition within the UK Biobank

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Cognitive impairment is a key feature of schizophrenia and is associated with poor functional outcomes, however, the causes of cognitive impairment in schizophrenia are poorly understood. We analysed exome sequencing data from 200,625 UK Biobank participants to investigate the effects of damaging rare coding

variants (DRCVs) in schizophrenia-associated loci on generalised cognition (*g*) in unaffected individuals.

DRCVs, defined as loss-of-function (LoF) or pathogenic missense (REVEL>0.75) variants occurring in only one sample in the dataset, were tested for association with *g* using a linear regression model covarying for demographic and ancestral factors.

DRCVs in 3,063 loss-of-function intolerant (LoFi) genes were associated with lower *g* ($\beta = -0.08$, $p = 4.6 \times 10\text{-}14$). Similar effects on *g* were observed for DRCVs affecting 1,186 genes in developmental disorder (DD) CNV loci ($\beta = -0.05$, $p = 1.15 \times 10\text{-}2$); with considerably stronger effects from DRCVs in LoFi genes ($\beta = -0.23$, $p = 7.0 \times 10\text{-}6$) than in LoF tolerant genes within CNV loci ($\beta = -0.01$, $p = 6.22 \times 10\text{-}1$). There was no association of *g* with DRCV burden in 2,213 genes located within schizophrenia GWAS loci, however when we focused on 106 genes prioritised as most likely causal from these loci, we observed a strong association of pathogenic missense burden and *g* ($\beta = -0.25$, $p = 3.6 \times 10\text{-}4$).

Our results suggest that DRCVs in genes implicated in schizophrenia have pleiotropic effects on cognition in unaffected individuals. The strongest effects on cognition were observed for LoFi genes in DD CNV loci and for prioritised genes in schizophrenia GWAS loci, thus supporting the use of approaches to prioritise genes underling complex genetic associations.

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Conflict of Interest: Eilidh Fenner: None declared, James Walters Receiving grants from Akrivia Health outside the submitted work. Receiving grants from Takeda Pharmaceutical Company Ltd outside the submitted work. Takeda and Akrivia played no part in the conception, design, implementation, or interpretation of this study., Elliott Rees Receiving grants from Akrivia Health outside the submitted work. Akrivia played no part in the conception, design, implementation, or interpretation of this study.

P10.028.D Analysis of Short Tandem Repeat expansions in a cohort of 15,015 exomes from patients with neurological diseases

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Many patients with suspected neurogenetic conditions remain undiagnosed after standard genetic testing, including whole exome sequencing. Repeat expansion disorders (REDs) are a large group of neurogenetic disorders caused by expansions of DNA repeats. It has been recently shown that REDs can be detected by short-read whole genome sequencing. Here, we tested repeat expansion detection by whole exome sequencing in a cohort of patients with genetically undiagnosed neurological disorders.

We run ExpansionHunter on 15,015 exomes on RED loci previously associated with diseases. We tested possible aberrant alleles through PCR based methods following visual inspection (VI). The performance of ExpansionHunter was evaluated based on the sequencing read length, exome capture kit and coverage on target repeat.

ExpansionHunter called 534 cases (3.6%) with likely expanded alleles. Visual inspection of all expanded alleles allowed us to discard 48% total called alleles ($n = 259$). 16% ($n = 84$) were considered Pass after VI, 36% ($n = 191$) Borderline with sort or low-quality reads. We confirmed the presence of an expansion in 98% of the VI Pass cases, while only 14% of the Borderline short-read cases were validated. A clinical diagnosis was confirmed for 12 cases with available clinical data.

The findings presented here suggest that exome sequencing can be used to detected REDs. We find that the performance to distinguish between normal and expanded alleles is affected by the sequencing read length. In cases where the presence of an interruption determines the pathogenicity of the expansion, we observed that visual inspection is essential to differentiate positive and negative cases.

Conflict of Interest: Clarissa Rocca Brain Research UK PhD Fellowship, David Murphy: None declared, Delia Gagliardi: None declared, James Polke: None declared, Robyn Labrum: None declared, Queen Square Genomics: None declared, Henry Houlden: None declared, Arianna Tucci: None declared

P10.029.A Targeted CRISPR-Cas9 and long-read sequencing of the SCA37 (ATTTC)n expanded insertion within the DAB1 gene proves a common origin 859 years ago and differential cerebellar allele-specific hypomethylation

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Background/Objectives: Spinocerebellar ataxia subtype 37 (SCA37) is characterised by a pure cerebellar syndrome with altered ocular movements. SCA37 has been described in ataxic patients from the Iberian Peninsula and one German family. SCA37 patients carry a heterozygous pathogenic (ATTTC)n repeat insertion within the *DAB1* gene leading to toxic DAB1 overexpression. We aimed at determining the possible common origin and the date of the (ATTTC)n SCA37 mutation, and characterising the configuration and the epigenomic signature of SCA37 alleles by targeted CRISPR-Cas9 enrichment and long-read nanopore sequencing.

Methods: Haplotypes of 14 SCA37 Spanish and 16 Portuguese patients from 14 unrelated kindreds were analysed by PHASE and DMLE+. SCA37 pathogenic allele configurations and methylation profiles were established in 14 DNAs from lymphocytes, fibroblasts, and cerebellar tissues, by CRISPR-Cas9 enrichment and long-read sequencing.

Results: All 30 SCA37 patients share a 964-kb region spanning the (ATTTC)n insertion revealing a common origin of the SCA37 mutation in the Iberian Peninsula which originated 859 years ago (95% CI: 647-1,378). The (ATTTT)n(ATTTC)n(ATTTT)n pathogenic allele configuration was accurately determined revealing cerebellar repeat instability. In the cerebellum, SCA37 alleles revealed significant hypomethylation upstream of the repeat sequence possibly affecting binding of 179 transcription factors.

Conclusions: This study provides evidence of a common origin of the SCA37 mutation in the Iberian Peninsula in 1,164 CE. Significant differential hypomethylation was identified in SCA37 alleles that may account for DAB1 pathogenic overexpression in SCA37 cerebellum. CRISPR-Cas9 enrichment and long-read sequencing provides accurate determination of the size and configuration of the complex repeated tracts.

Conflict of Interest: None declared

P10.030.B Yield of a genetic diagnosis in complex and essential autism

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Background/Objectives: Autism spectrum disorder (ASD) is characterized by impaired social communication and restricted behaviors. We compared the diagnostic yield of genetic evaluation in essential vs. complex ASD.

Methods: A retrospective cohort study. Data were collected from medical records of autistic patients referred between January 2011-December 2020 to the Tel Aviv Sourasky Medical Center Genetics Institute. ASD was classified as essential; complex syndromic; or complex non-syndromic. Fisher's Exact Test of Independence was performed to evaluate the yield of genetic tests among these phenotypic subgroups.

Results: Data of 138 patients (81 essential; 34 syndromic; 23 non syndromic ASD) were analyzed. Mean age was 14.2±12.6 years (108 males). All had Fragile X testing, 137 chromosomal microarray and 42 had exome sequencing. Copy number abnormalities (CNA) were detected in 18% (15/81 essential; 10/56 complex ASD). All pathogenic CNA were in the complex syndromic group (5/25, 15%), ($p < 0.05$). Among the essential ASD group high frequency low penetrance 15q11.2 or 15q13.3 CNA were the most common finding (6/15 or 40%). Pathogenic and likely pathogenic single nucleotide variants were detected by exome sequencing in 3/19 (15.7%) of children with essential ASD; 6/9 (33.3%) with complex non-syndromic ASD, and in 6/14 (42.9%) with complex syndromic ASD ($p = 0.039$). One patient (0.7%) had Fragile X syndrome.

Conclusion: The diagnostic yield of genetic testing is lowest in essential; higher in complex; and highest in complex-syndromic ASD. These results may aid in allocation and development of genetic services to ASD patients who might benefit most from clinical genetic testing.

Conflict of Interest: Uri Hamiel: None declared, Chen Patt: None declared, Yam Amir: None declared, Hagit Baris Feldman HBF has received honoraria for scientific talks, grants, and consults from: Sanofi-Genzyme, Protalix, Pfizer, and Takeda-Shire. HBF serves on scientific advisory boards of Sanofi-Genzyme and Igentify and in the past also in Shire and Regeneron., Daphna Marom: None declared

P10.031.C Snijders-Blok-Campeau syndrome (CHD3 gene): Description of seven clinical cases with novel pathogenic variants

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Background/Objectives: Snijders-Blok-Campeau syndrome (SNIBCPS, OMIM#618205) is an extremely infrequent disease with only 60 cases reported. Clinical features of patients with SNIBCPS included global developmental delay, intellectual disability (ID), speech and language difficulties, behavioral disorders like autism spectrum disorder (ASD) and typical dysmorphic features. SNIBCPS is caused by pathogenic variants in *CHD3 (Chromodomain Helicase DNA Binding Protein 3)* which is seems to be involve in remodeling of chromatin by deacetylating histones.

Methods: Here, we report seven additional cases of SNIBCPS from unrelated families with confirmed novel pathogenic variants in *CHD3*. Patients were analyzed by either Sanger sequencing or by whole exome sequencing (WES).

Results: Patients in this study showed different pathogenic variants affecting different functional domains of the protein. In addition, none of the variants described were reported in control population databases and most computational predictors suggest that they are deleterious. Regarding the clinical features of the cohort of patients, the most common are global developmental delay, motor delay and macrocephaly. Other frequent features are intellectual disability, gait delay and different dysmorphic features.

Conclusion: This study expands the number of individuals with confirmed SNIBCPS due to pathogenic or likely pathogenic variants in *CHD3*. Furthermore, we add evidence of the importance of the application of massive paralleled sequencing for neurodevelopmental disorders in which the clinical diagnosis might be a challenge.

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Conflict of Interest: None declared

P10.032.D Interactions of rare copy number variations with polygenic risk scores in psychiatric disorders and quantitative traits

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Background: Copy number variants (CNVs) are major risk factors in neuropsychiatric disorders and are contributing to their shared genetic etiology. We determine associations of CNVs across psychiatric disorders and investigate the modifying role of polygenic risk scores (PRS) on clinical and dimensional traits in CNV carriers.

Methods: Harmonized CNV calling and quality control was performed for 636,737 individuals, including patients with ADHD ($N = 5,276$), ASD ($N = 16,131$), bipolar disorder ($N = 26,863$), PTSD ($N = 18,428$), SCZ ($N = 36,873$), control samples ($N = 107,355$), and UKBiobank ($N = 308,713$). We analyzed 42 multi-genic pathogenic CNVs for association with psychiatric disorders and assessed combined effects of rare CNVs and polygenic risk scores (PRS).

Results: We found evidence for traditional additive liability threshold models of disease risk, as CNV carriers have lower PRS. If effects of multiple CNVs are collapsed, we mainly observe additive effects of CNV and PRS. We also observed non-additive effects, in which effect of PRS differs significantly for specific rare CNVs. We find significant evidence of genetic interactions of CNV genotype and polygenic load on case status and dimensional traits. After meta-analysis of three independent cohorts, we find significant interactions between two CNVs and PRS-Body Mass Index (BMI) with BMI.

Conclusion: Rare variants of large effect can have a significant influence on the effect of other common risk alleles. Common

polygenic factors contributed to variable phenotypic expressivity in CNV carriers. PRS effects can differ by CNV genotype, suggesting that modifying effects of polygenic risk may differ between clinical subtypes.

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Conflict of Interest: Marieke Klein 1 FTE Radboudumc, Nijmegen, NL, Dutch Research Council (grant nos. 45219212 and 09150162010073), Omar Shanta 1 FTE UCSD, La Jolla, USA, Oanh Hong 1FTE UCSD, La Jolla, USA, Jeffrey MacDonald 1FTE The Centre for Applied Genomics and Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada, Bhooma Thiruvahindrapuram The Centre for Applied Genomics and Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada, Agathe de Pins 1 FTE The Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, USA, Alexander Charney 1 FTE The Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, USA, Molly Sacks 1 FTE Department of Psychiatry, University of California San Diego, La Jolla, CA, USA, Guillaume Huguet 1 FTE CHU Sainte-Justine Research Centre, University of Montreal, Montreal, Quebec, Canada, Sebastien Jacquemont 1 FTE CHU Sainte-Justine Research Centre, University of Montreal, Montreal, Quebec, Canada, Data were generated as part of the G2MH Network, supported by NIH grants awarded to: U01MH119739-01 Jacquemont, Sebastien Sainte-Justine University Hospital Center, Stephen Scherer The Centre for Applied Genomics and Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada, Jonathan Sebat 1 FTE University of California, San Diego, La Jolla, USA, Data were generated as part of the G2MH Network, supported by NIH grants awarded to: U01MH119746-01 Sebat, Jonathan University of California, San Diego

P10.033.A Characterization of copy number variants identified by genetic testing of epilepsy

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Background: Copy number variants (CNVs) are the underlying cause of an important proportion of cases of unexplained epilepsy. The sensitivity at which small CNVs are detected varies across genomic testing methods. This study describes intragenic CNVs identified in patients with epilepsy undergoing NGS multigene panel testing to further define their prevalence and clinical implications.

Methods: Retrospective review of 1626 deidentified patients who underwent comprehensive epilepsy panel testing at Blueprint Genetics included sequence and CNV analysis of 194-474 genes by NGS from a validated whole exome or clinical exome assay. An informative case was defined as the identification of pathogenic (P) or likely pathogenic (LP) variant(s) consistent with the patient's reported phenotype and with known associated disease inheritance.

Results: 21.1% of patients received an informative genetic test result. 3.1% of patients had P/LP CNVs. 26.0% of LP/P CNVs were intragenic and 53.8% of these were <1000 bp in size. 4 had CNVs in genes associated with a gene-specific treatment (*SCN1A* n = 3, *SCN2A* n = 1), and 4 had CNVs in genes associated with a gene-specific interventional trial as listed in clinicaltrials.gov (*SCN1A* n = 2, *CLN3* n = 1, *CDKL5* n = 1).

Conclusion: 4% of patients receiving an informative genetic test result had an intragenic P/LP CNV; and greater than 50% of these were <1000 bp in size. Of these patients, 60% had a P/LP CNV in a gene associated with a gene specific treatment or clinical trial. This work highlights the clinical importance of high resolution CNV analysis combined with sequence variant detection in patients with epilepsy.

Conflict of Interest: Lotta Koskinen Blueprint Genetics, Julie Hathaway Blueprint Genetics, Kirsi Alakurtti Blueprint Genetics, Monica Segura Castell Blueprint Genetics, Åsa Hagström Blueprint Genetics, Heli Kuisma Blueprint Genetics, Kimberly Gall Blueprint Genetics, Inka Saarinen Blueprint Genetics, Mikko Muona Blueprint Genetics, Tuuli Pietilä Blueprint Genetics, Janica Djupsjöbacka Blueprint Genetics, Massimiliano Gentile Blueprint Genetics, Pertteli Salmenperä Blueprint Genetics, Sari Tuupanen Blueprint Genetics, Tiia Kangas-Kontio Blueprint Genetics, Kati Kämpjärvi Blueprint Genetics, Eija H. Seppala Blueprint Genetics, Jussi Paananen Blueprint Genetics, Samuel Myllykangas Blueprint Genetics, Juha Koskenvuo Blueprint Genetics

P10.034.B Autosomal dominant MPAN: mosaicism expands the phenotypic spectrum to frontotemporal dementia

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Purpose: Mitochondrial membrane protein-associated neurodegeneration (MPAN) is caused by pathogenic variants in the *C19Orf12* gene which may be transmitted either with recessive (AR-MPAN) or dominant transmission (AD-MPAN). Our aim was to better characterize the clinical, molecular and functional spectra associated with the recently described heterozygous *C19Orf12* dominant pathogenic variants.

Methods: We collected clinical, imaging and molecular information of eight individuals from four AD-MPAN families and obtained brain neuropathology results for one. Functional studies,

focused on energy and iron metabolism, were conducted on fibroblasts from AD-, AR-MPAN patients and controls.

Results: We identified four heterozygous *C19Orf12* variants in eight AD-MPAN patients. Two of them carrying the familial variant in mosaic displayed a very atypical late-onset fronto-temporal dementia. Fibroblasts from AD-MPAN showed more severe alterations of iron storage metabolism and autophagy compared to AR-MPAN cells.

Conclusion: Our data add strong evidence of the realness of AD-MPAN with identification of novel monoallelic *C19Orf12* variants, including at the mosaic state. This has implications in diagnosis procedures. We also expand the phenotypic spectrum of MPAN to late onset atypical presentations. Finally, we demonstrate for the first time more drastic abnormalities of iron metabolism and autophagy in AD-MPAN than in AR-MPAN.

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P10.035.C The importance of genetics in the diagnostic workup of cerebral palsy; results of a monocenter study

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Objectives: Cerebral palsy (CP) is the most frequent cause of motor impairment in children, with perinatal asphyxia long thought to be the leading cause. However, studies have illustrated its causation in only 10% of patients. Hence, the role of genetic factors in CP has gained interest. We systematically performed genetic investigations in a CP cohort. We hereby aimed to improve insight the contribution of genetic variants in the pathomechanism of this disorder.

Methods: Medical files of 647 CP patients were analysed for exclusion criteria: (1) prematurity (<30 weeks postmentrual age); (2) perinatal asphyxia; (3) other aetiology (e.g. perinatal infection, trauma, etc.); (4) refusal of genetic test; (5) trio analysis not possible. This led to the exclusion of 310 patients. In the 337 remaining patients both single nucleotide polymorphism array and exome sequencing were performed.

Results: A genetic disorder was diagnosed in 129/337 patients, resulting in a genetic diagnostic yield of 38.3%. A large proportion of these patients had ≥ 1 comorbidities (intellectual disability/ developmental delay, epilepsy, autism spectrum disorder). In this subgroup the diagnostic yield was even higher, namely 49.6%. The most frequently affected genes were *KIF1A* (8/129) and *COL4A1* (4/129). Other genes with variants in >1 patient were *FRRS1L*, *MECP2*, *BRAF*, *TSEN54*, *DYRK1A*, *RNASEH2B* and *RNU7-1*.

Conclusion: Genetic testing in our CP cohort led to a diagnostic yield of 38.3%, highlighting the substantial contribution of genetic causes underlying CP. Diagnosing these disorders is essential for prognosis, clinical follow-up and genetic counselling.

Grant references: Fund Marguerite Marie Delacroix (RVC/B-458)

Conflict of Interest: None declared

P10.036.D Variants in CEP192 potentially associated to microcephaly

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Background: Microcephaly is defined as having a reduction in head circumference less than 2 standard deviations compared with controls matched for age and sex. Causes may be environmental or genetic. Multiple genes have been identified among which the CEP family. Pathogenic variants in the genes *CEP138*, *CEP152*, *CEP215*, f.i., are established causes of microcephaly. The centrosome-associated CEP family proteins are the active component in centrosome maturation, centriole biogenesis and cell cycle progression control.

Methods: Whole exome sequencing was performed in a 3-year-old boy with a postnatally established profound microcephaly. He was diagnosed with global developmental delay, failure to thrive, hypotonia, severe hearing impairment and vision problems. Brain imaging shows, cerebellar hypoplasia with a thin corpus callosum.

Results: Two variants of unknown significance were identified in *CEP192:* NM_032142.4: c.1935A>G, p. (Ser645) and NM_032142.4: c.3253C>T, p. (Arg1085Trp). Since *CEP192* is a gene with unknown clinical consequences, seven extra individuals were collected through GeneMatcher. These additional individuals with biallelic variants in the *CEP192* gene present hypotonia, neurodevelopmental delay, epileptic encephalopathy, retinitis pigmentosa, oesophageal dysmotility and failure to thrive as main features.

Conclusions: Clinical information of eight patients with biallelic variants in *CEP192* was collected. CEP192 is a pericentriolar protein required for centriole duplication and nucleation of centrosomal microtubules during mitosis and, consequently, plays an essential role during the centrosome maturation process. Pathogenic variants in *CEP192* are likely related to a neurodevelopmental syndrome involving microcephaly and failure to thrive. Functional studies are ongoing.

Grant references: Fellowship Marguerite-Marie Delacroix

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P10.037.A Intermediate and expanded HTT alleles and risk for tauopathies

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Background/Objectives: Previous studies have suggested that there is a link between the CAG repeat number in the *HTT* gene and non-Huntington neurodegenerative diseases. This study aims to analyze whether the size of expanded *HTT* CAG alleles is associated with the risk for developing tauopathies and their behavior as modulators of the phenotype.

Methods: The study involved genotyping the *HTT* gene CAG repeat number and APOE-Ɛ isoforms in a case-control series that included patients with neuropathological diagnoses of tauopathies, including CBD, PSP, and AD/EOAD.

Results: The study identified low-penetrance *HTT* repeat expansions in a small percentage of patients with CBD, PSP, and AD, representing 2.7%, 3.2%, and 0.3% of the respective cohorts, compared to 0.2% in the control cohort. Compared to controls (3.9%), the AD/EOAD group had a higher frequency (6.2%) of HTT Intermediate alleles (IAs) distribution. A clear increase of the number of *HTT* CAG repeats was found among AD and Control groups influenced by the presence of APOE-Ɛ4 isoform. Furthermore, the size of the larger allele of the *HTT* gene, intermediate alleles, and APOE-Ɛ2 isoform influenced the age of onset of patients with EOAD when using multiple linear regression analysis.

Conclusions: The study found that intermediate/expanded *HTT* CAG alleles and their size may be associated with the risk of developing certain tauopathies and may act as modulators of the phenotype. The findings contribute to our understanding of the genetic basis of tauopathies and may aid in the development of more targeted treatments in the future.

Grant: PI21/0467 to VA.

Conflict of Interest: None declared

P10.038.B De novo missense variants in PHLPP1 cause a specific neurodevelopmental disorder (NDD) with epilepsy

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Background: PH Domain And Leucine Rich Repeat Protein Phosphatase 1 (*PHLPP1*) is a ubiquitous protein that is evolutionarily conserved from yeast to mammals and has an important role in the regulation of protein translation, cell growth and proliferation. Originally identified as a tumor suppressor, *PHLPP1* is highly expressed in the brain and plays a key role in memory processes and regulation of circadian rhythm.

Methods: We obtained dental pulp stem cells (DPSC) from twins and fibroblasts from one case. We evaluated *PHLPP1* and its main targets levels by western blot and performed cell viability or proliferation assays. We assessed the effect of *PHLPP1* mutations by in vitro cellular expression.

Results: We identified six syndromic cases with a similar phenotype characterized by mild intellectual disability (6/6), seizures (6/6), delayed speech (4/6), skeletal abnormalities (3/6) with missense changes in *PHLPP1* (monozygotic twins, p.R1047L de novo; p.L177V de novo, p.I1553M, p.A1623D, and p.L1129R). Using DPSC from the two twins, we showed a reduction of PHLPP1 level, and an increased phosphorylation of its downstream targets: Akt-S473, S6K1-T389 and Erk1-2. These change were in agreement with a resulting enhanced cell proliferation. The expression of *PHLPP1*-p.R1047L construct in HEK-293 cells showed lower protein level, suggesting decreased protein stability. In *PHLPP1*-p.L177V fibroblasts, we observed PHLPP1 protein levels similar to controls with a significant increase in Akt (S473) phosphorylation suggesting impaired PHLPP1 function.

Conclusion: Our preliminary data suggest that PHLPP1 is a potential novel gene associated with a new NDD through a likely loss-of-function mechanism.

Conflict of Interest: None declared

P10.039.C Complex phenotype in patients affected by autism spectrum disorder (ASD) explained by oligogenic inheritance of rare and common variants in functional gene networks

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Background: Genetic bases of autism spectrum disorder (ASD) are heterogeneous. The detection of disease-causing genomic variants has focused mostly on the identification of mendelian, highly penetrant variants, however, genetic causes remain unknown for most cases. Increasing evidence suggests that the combination of multiple variants with high/moderate penetrance in more than one gene leads to ASD. To identify genetic bases in complex ASD cases, we used whole exome sequencing (WES) and downstream bioinformatic tools set up for oligogenic analyzes.

Methods: We developed a method for detecting networks of interacting genes, in ASD-relevant pathways, affected by denovo or inherited highly/moderatly penetrant variants identified by WES. Briefly, we selected potentially gene-damaging variants

affecting ASD-related genes; detected networks of physically interacting proteins; performed gene ontology functional enrichment on each network; identified networks significantly enriched for ASD-relevant pathways.

We applied our method to WES data from 54 ASD probands and 100 healthy individuals from the 1000G Project in an unbiased fashion.

Results: In three individuals, undiagnosed after first-level analyzes, we detected a network of genes, with potentially damaging variants, enriched for ASD-relevant pathways/biological processes: gluatamatergic synapse, neuronal action potential, sodium channel activity. In each patient, novel, rare, and common variants inherited from either of the unaffected parents occurred in genes of the same pathway, affecting proteic regions relevant for folding and/or protein-protein interactions.

Conclusion: Using a method to detect gene networks, we identified multiple inherited variants that could contribute to the complex phenotype in 3 of 54 ASD patients according to oligenic inheritance.

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P10.040.D Single-molecule Molecular Inversion Probes provide an accurate diagnostic approach for the detection of low VAF somatic variants in focal cortical dysplasias and brain tumors

Ester Cifaldi[1], *Paola Dimartino*[2], *Lorenzo Ferri*[3], *raffaella minardi*[4], *Laura Licchetta*[3;4], *Marco seri*[1;2], *Marco de Curtis*[5], *Laura Rossini*[5], *Rita Garbelli*[5], *Laura Tassi*[6], *michele rizzi*[7], *matteo martinoni*[8], *Elena Pasinis*[4], *Roberto Michelucci*[4], *Francesca BISULLI*[3;4], *Leonardo Caporali*[4], *Tommaso Pippucci*[1]

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Background: Drug resistant focal epilepsy (DR-FE) accounts for a significant proportion of epilepsy burden worldwide. Surgery is often the best option to remove the area of the brain where seizures occur. Germline or brain-only somatic variants are a recognized genetic determinant of FE. Recurrent genes and hotspot mutations are found in patients carrying circumscribed lesions - e.g. Focal Cortical Dysplasia (FCD) type II and glioneuronal tumors. A genetic origin is suspected in other histological subtypes, but it is still largely unexplored.

Materials and methods: DNA was successfully extracted from matched Fresh Frozen (FF) brain–blood and in some cases from Formalin-Fixed, Paraffin-Embedded (FFPE) samples of 130 surgically treated FE patients. We designed separate panels covering FCD and brain tumor-associated genes (24 total genes) using single molecule Molecular Inversion Probes (smMIPs) and performed the set-up of these gene panels on matched blood-brain samples of two FCD and one tumor patients.

Results: smMIPs sequencing obtained an adequate read depth required for the detection of low VAF variants. We obtained 3000X mean coverage for uncollapsed reads, and target regions were fully covered with >200 unique reads. In the tested samples, we identified a probably pathogenic variant in SLC35A2 with variant allele frequency (VAF) 2.49% in FF lesional tissue of a patient with FCD type I and not present in the blood.

Conclusion: smMIPs showed to be an accurate diagnostic approach for the detection of low VAF variants in FCD and tumor samples.

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P10.041.A FGF14 : a frequent new causal expansion in cerebellar ataxias

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Background/Objectives: The causal variant remains undetermined for nearly half of hereditary cerebellar ataxias despite high-throughput sequencing (Coutelier et al., 2018).

A new intronic GAA expansion of the *FGF14* gene was recently discovered (Pellerin et al., 2023; Rafehi et al., 2023). It would represent more than 10% of the forms hitherto unsolved.

We looked for the presence of this expansion in our French research cohort.

Methods: The search for an *FGF14* expansion was done in individuals with spinocerebellar degeneration who had benefited from an exome for research purposes, after exclusion of the main expansions involved. The protocol of Pellerin et al. was used with a pathogen threshold size of 250 GAA without interruption.

Results: Among the 521 individuals studied, 59 pathogenic FGF14 expansions were confirmed, i.e. 11.3%. The presence of paroxysmal ataxia or downbeat nystagmus increased the diagnostic yield.

The typical clinical picture associated with this expansion was a late cerebellar ataxia beginning after 40 years, slowly evolving, often by attacks. It was associated with diplopia, downbeat nystagmus and tremor. Cerebellar atrophy is often isolated and discreet even after many years of disease progression.

Conclusion: *FGF14* expansion causes a new form of pure autosomal dominant cerebellar ataxia with an oculovestibular phenotype. This work confirmed the importance of the prevalence of *FGF14* expansion in unsolved cerebellar ataxias. The expansions of repeats are therefore responsible for more than half of the ataxias (*ATXN1*, *2*, *3*, *7*, *CACNA1A*, *TBP*).

Conflict of Interest: None declared

P10.042.B Metabolomic investigation of major depressive disorder identifies a potentially causal association with polyunsaturated fatty acids

Eleanor Davyson[1], *xueyi shen*[1], *danni gadd*[1], *elena bernabeu*[1], *robert hillary*[1], *daniel mccartney*[1], *mark adams*[1], *riccardo marioni*[1], *andrew mcintosh*[1]

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Background: Metabolic differences have been reported between individuals with and without Major Depressive Disorder (MDD), but their consistency and causal relevance has been unclear.

Methods: We conducted a metabolome-wide association study of MDD with 249 metabolomic measures available in UK Biobank (N = 29, 757). We then applied 2-sample bidirectional Mendelian Randomization (MR) and colocalization analysis to identify potentially causal relationships between each metabolite and MDD.

Results: One hundred and ninety-one metabolites tested were significantly associated with MDD ($P_{FDR} < 0.05$), which reduced to 129 after adjustment for likely confounders. Lower abundance of Omega-3 fatty acid measures and a higher Omega-6: Omega-3 ratio showed potentially causal effects on liability to MDD. There was no evidence of a causal effect of MDD on metabolite levels. Furthermore, genetic signals associated with Docosahexaenoic Acid colocalized with loci associated with MDD within the FADS gene cluster. Post-hoc MR of gene-transcript abundance within the FADS cluster demonstrated a causal association with MDD. In contrast, colocalization analysis did not suggest a single causal variant for both transcript abundance and MDD liability, but the likely existence of two variants in LD with one another.

Conclusion: Our findings suggest that decreased Docosahexaenoic Acid and increased Omega-6: Omega-3 fatty acids ratio may be causally related to MDD. These findings provide further support for the causal involvement of fatty acids in MDD.

Grant References:

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P10.043.C Systematic reanalysis of genomic data from the Australian Cerebral Palsy Biobank cohort

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Background: Cerebral palsy (CP) is a clinical descriptor, not a distinct disorder. It encompass a spectrum of non-degenerative movement disorders, frequently accompanied by additional neurodevelopmental features e.g. intellectual disability (~50%), epilepsy (~30%), speech impairments (~60%), vision impairments (~40%) and autism spectrum disorders (~9%). The contribution of genetics to CP etiology is being increasingly recognised. The Australian Cerebral Palsy Biobank (ACPB) is an internationally unique resource, containing DNA, patient cell lines and clinical data for >500 Australians with CP, mostly recruited from CP Registers in Australia between 2010-2018 and clinically unselected.

Methods: Exome, genome or gene panel was performed between 2012-2023, with ~350 cases also undergoing RNA sequencing and ~200 profiled for genome-wide epigenetic signatures. We performed systematic reanalysis of our combined genomic data with updated bioinformatic pipelines, and incorporationg our curated CP gene-list (of n>500 CP implicated genes) with updated annotations and gene-disease associations.

Results: With the addition of omics technologies, at least ¼ of the ACPB cohort have a genetic cause for their CP, including known and novel genetic disorders. Our investigations and review of the literature identified variants in *SPAST*, *ATL1*, *CACNA1A*, *CTNNB1*, *KCNQ2*, *ATM*, *PAFAH1B1/LIS1*, *COL4A1* and *SPR* as the most frequent genetic causes of CP.

Conclusions: These results highlight the genetic overlap between CP and other neurodevelopmental and movement disorders. Importantly, genetic findings are not limited to children without other CP-associated risk factors, suggesting that broad genomic testing is warranted in this frequently overlooked group of rare disorders.

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Conflict of Interest: None declared

P10.044.D Elucidating the function of H3-3B alternative transcripts in Bryant-Li-Bhoj syndrome

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Background/objectives: Bryant-Li-Bhoj syndrome is an ultra-rare neurodevelopmental syndrome caused by de novo missense germline mutations in *H3-3A* and *H3-3B*, the genes that encode the replication-independent histone H3.3. Unlike replication-coupled histones, which are encoded by clusters of 10-20 intronless genes, H3.3 is encoded by just two unique genes with alternatively spliced transcript isoforms. These individual genes and their isoforms are understudied even though they encode a highly conserved protein with critical gene regulatory activity. The potential functional role of these transcript isoforms was illuminated by a patient who has a truncating variant on an alternative *H3-3B* transcript, but a synonymous mutation on the canonical transcript. In this work, we characterized the expression of *H3-3A* and *H3-3B* transcript isoforms in both unperturbed and perturbed cell lines to gain insight into the mechanism of patient mutations.

Methods: Using fibroblasts from patients harboring various mutations and isogenic iPSCs pushed to different lineages, we measured transcript (canonical, non-canonical, non-coding) and protein expression to understand the consequences of patient mutations.

Results: Across all cell lines, patient mutations differentially upregulate non-canonical or non-coding *H3-3B* transcripts. We are working to elucidate the roles of these transcripts with currently unknown functions in the context of development and disease.

Conclusion: Profiling the transcriptional regulation of *H3-3A* and *H3-3B* is an unexplored but crucial approach to understand the pathology of Bryant-Li-Bhoj syndrome. This work broadly contributes to the field's understanding of replication-independent histone RNA.

Grant References: Hartwell Foundation, Burroughs-Wellcome Fund

Conflict of Interest: None declared

P10.045.A Contribution of Mendelian neurodegeneration disorders in a population-based pediatric biobank with >100,000 participants

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Background/Objectives: To evaluate Mendelian causes of neurodegenerative disorders in a cohort of pediatric patients as pediatric neurodegenerative disorders are a rare, diverse group of diseases. As molecular testing has advanced, many children can be diagnosed, but the relative contribution of various disorders is unclear.

Methods: Patients enrolled in the Center for Applied Genomics (CAG) Biobank at the Children's Hospital of Philadelphia with neurodegenerative symptoms were identified using an algorithm that consisted of including and excluding selected ICD9 and ICD10 codes. A manual chart review was then performed to abstract detailed clinical information.

Results: Out of approximately 100,000 patients enrolled in the CAG Biobank, 76 had a neurodegenerative phenotype. Following chart review, 7 patients were excluded. Of the remaining 69 patients, 42 had a genetic diagnosis (60.9%) and 27 were undiagnosed (39.1%). There were 32 unique disorders. Common diagnoses included Rett syndrome, mitochondrial disorders and neuronal ceroid lipofuscinoses.

Conclusion: The disorders encountered in our cohort demonstrate the diverse diseases and pathophysiology that contribute to pediatric neurodegeneration. Establishing a diagnosis often informed clinical management, although curative treatment options are lacking. Many patients who underwent genetic evaluation remained undiagnosed, highlighting the importance of continued research efforts in this field.

Conflict of Interest: None declared

P10.046.B Evaluating clinically relevant genes associated with cerebral palsy

Yana Wilson[1;2], *Hayley Smithers-Sheedy*[1;2], *Shona Goldsmith*[1;2], *Tasneem Karim*[1;2], *Georgina Henry*[1;2], *Nadia Badawi*[1;2;3], *Gareth Baynam*[4;5], *Jozef Gecz*[6], *Sarah McIntyre*[1;2]

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Objective: To examine the evidence supporting gene-disease associations in cerebral palsy (CP), through a review of the CP genetic literature.

Methods: We extracted genetic variant and clinical data for pathogenic/likely pathogenic genes reported in CP cohorts of ≥10 individuals, recurrent in ≥ three independent studies. Confidence in CP phenotype for each cohort was scored: high (fulfilled ≥5 criteria), moderate (3-4), or low (≤2). This was based on: CP defined using a well acknowledged definition; CP clinical diagnosis confirmed at recruitment; inclusion/exclusion criteria of the study met the clinical description of CP; CP clinically confirmed at age ≥ four; motor disorders of CP well described; and individual phenotypic data available.

Results: 51 genes were reported in ≥ three studies. Data were extracted from 19 cohorts ($n = 374$). Confidence in CP phenotype was rated high in 8 cohorts ($n = 84$), moderate in 4 ($n = 79$), and low in 7 ($n = 211$). Individual phenotypic data were absent for 59% of cases. The most reported genes were: SPAST ($n = 27$), CTNNB1 ($n = 24$), ATL1 (n = 20) and COL4A1 ($n = 19$). Only TAF1 ($n = 1$) and SPAST ($n = 2$) were associated with a CP phenotype in the absence of any neurodevelopmental comorbidities. Of the 51 genes, 34 were associated with progressive disorders or phenotypes, suggesting the diagnosis of CP required review. When restricting analyses to the high confidence cohort, 35% of genes were not represented.

Conclusion: More than half the cases identified in CP genomic studies had incomplete or ambiguous phenotypes, thus limiting the evidence of gene-disease associations for CP.

Conflict of Interest: None declared

P10.047.C Biallelic variant in the histone acetyltransferase KAT2A identified in individuals with craniofacial anomalies, congenital malformations and developmental delay

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Background: Lysine Acetyltransferase 2A (*KAT2A*) encodes the histone acetyltransferase GCN5, which mainly functions as a transcriptional activator for several genes and pathways. We identified a homozygous variant in *KAT2A* (c.2032_2055del) in two apparently unrelated individuals with a core phenotype of developmental delay, microcephaly, dysmorphism, cleft palate, low-set ears, hypoplasia of the corpus callosum, ventricular septal defect, unilateral renal agenesis and joint contractures.

Methods: Following informed consent, trio ES was carried out on the proband and parents in each family. The effect of the variant was assessed in patient-derived EBV-transformed lymphoblastoid cell lines (LCLs) at the cDNA and protein levels, by reverse transcriptase (RT)-PCR and Western blot.

Results: A homozygous in-frame variant (*KAT2A:* c.2032_2055del; p.Leu678_Gln685del) removing 8 amino acids was identified in both affected individuals and segregated with the phenotype. mRNA analysis indicated expression levels similar to control. However, western blot analysis revealed a decrease of the KAT2A/GCN5 protein in patient cells as compared to healthy controls, suggesting that the protein is less stable.

Conclusion: KAT2A/GCN5 is preferentially involved in the controlling the growth rate by acetylating histones and the genes which are cell cycle-related factors. Previous studies in animal models have demonstrated its importance in embryonic

development, including craniofacial, heart and limb development. Overall, our results suggest that the in-frame deletion leads to a de-stabilized protein and a probable loss-of-function mechanism. Further studies are underway to assess the function of the aberrant protein, and to gain a mechanistic understanding into the disease pathogenesis.

Conflict of Interest: None declared

P10.048.D Role of CACNG2 variants in human pathology

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Background/objectives: *CACNG2* encodes a synaptic trafficking protein (TARPγ2/Stargazin) that binds to AMPA-receptors (AMPARs), which are transmembrane ion channels gated by neurotransmitter glutamate binding. *CACNG2* variants were previously reported only twice in humans : in a patient with non-syndromic intellectual disability, carrying a de novo missense variant p.(Val143Leu), and in a patient with seizures, carrying a c.295+1G>C variant, predicted to affect splicing. By co-immunoprecipitation, the authors demonstrated that the missense variant decreases the ability for CACNG2 to bind to AMPAR subunits, and subsequently reduces glutamatergic transmission. After diagnosing a new patient with a de novo *CACNG2* variant, we launched an international case collection and, report the clinical and genetic data of the series.

Methods: We launched an international collaboration through the GeneMatcher platform to identify additional patients with *CACNG2* variants. Clinical, genetic, imaging and EEG data were collected.

Results: We identified 18 patients with a *CACNG2* variation, ranging from 5 to 42 years old. 80% of the patients showed intellectual disability, mostly mild. 70% presented with autism spectrum disorders, 40% with attention deficit hyperactivity disorders, 70% with seizures (1 atypical rolandic, 1 myoclonic astatic, 1 absence-epilepsy, 2 drug-resistant), and 10% with mild dysmorphic features. Brain MRI did not show any brain malformation. All variants were missense except three, 6 de novo, and 1 inherited among available data.

Conclusion: We report the clinical and genetic features of an international cohort of patients with *CACNG2* variants. The next step will consist in the evaluation of functional consequences of the variants and electrophysiological-clinical correlation.

Conflict of Interest: None declared

P10.049.A HTT and ATXN2 repeat expansions increase risk of ALS in a Norwegian ALS cohort: The GAIN study

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Background: Amyotrophic lateral sclerosis (ALS) is a motor neuron disease caused by varied genetic components as well as metabolic and environmental risk factors. Genetic repeat expansions are frequent cause of neurodegenerative diseases, such as ataxias, frontotemporal dementia, Huntington's disease and Kennedy's disease. Here, we investigate repeat expansions in *AR, ATXN1, ATXN2* and *HTT* in a Norwegian ALS cohort.

Methods: Norwegian ALS patients ($n = 414$) and motor neuron healthy controls ($n = 1092$) was analyzed for repeat expansions in *AR, ATXN1, ATXN2* and *HTT* by next-generation sequencing and ExpansionHunter. Identified repeat expansions were validated by traditional fragment analysis.

Results: Repeat expansions in *HTT* and *ATXN2* were associated with increased risk of ALS ($p < 0.05$). Six ALS patients (1.45%) carried repeat expansions (36-40 repeats) in *HTT*, whereas seven ALS patients (1.69%) carried repeat expansions (29-34 repeats) in *ATXN2*. The clinical information for patients carrying the *HTT*

repeat expansions was re-evaluated by two independent neurologist; all patients had an ALS phenotype. In addition, one ALS patient carried a pathogenic repeat expansion (45 repeats) in *AR*. Repeat expansions in *ATXN1* (≥34 repeats) were not associated with increased ALS risk ($p > 0.05$) in this cohort.

Conclusion: Having more than 36 repeat expansions in *HTT* and more than 29 repeat expansions in *ATXN2*, increases the likelihood of developing ALS.

Grant References: The work was funded by Telemark Hospital Trust to C.N., and the South Eastern Norway Regional Health Authority [2021097] to H.H.

Conflict of Interest: None declared

P10.050.B A time- and cost-effective protocol for GBA sequencing and its application in a large cohort of Italian Parkinson's disease patients with deep brain stimulation

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Background/Objectives: Heterozygous variants in the *GBA* gene are the commonest genetic risk factor for Parkinson's disease (PD). We optimized a short-reads NGS-based method for large-scale *GBA* sequencing; this was applied on a multicentric Italian cohort of PD subjects who underwent deep brain stimulation (DBS)-surgery, to investigate the impact of *GBA* variants on the long-term outcome of DBS.

Methods: *GBA* analysis relied on specific amplification of the whole gene in one PCR fragment (6kb) followed by Nextera sequencing and a customized bioinformatics pipeline masking the pseudogene. Patients were divided in GBA-PD and non-mutated PD (NM-PD); GBA-PDs were further stratified based on the type of variant (severe, mild, risk, complex, unknown). Motor and non-motor features were longitudinally assessed within groups up to five years post-DBS.

Results: We tested 296 DBS-PD patients, of whom 65 (22%) carried *GBA* variants, including 25 severe (38,5%), 12 mild (18,5%), 16 risk (24,6%), 4 complex (6,5%) and 8 unknown variants (12,3%). At pre-DBS evaluation, GBA-PD and NM-PD showed similar clinical features. Five years post-DBS, both groups showed motor and non-motor improvement, with exception of cognitive scores, which worsened faster in GBA-PD. However, only 26 % of GBA-PD were eventually diagnosed with dementia after 5 years from implant.

Conclusion: We propose a time- and cost-effective, highly sensitive strategy for *GBA* sequencing, broadly applicable in clinical diagnostic labs. GBA-PD patients seem to benefit from DBS as NM-PD, although cognitive performance shows a more rapid deterioration in GBA-PD. Correlations between GBA-PD stratified by mutation type and clinical features is ongoing.

Conflict of Interest: None declared

P10.051.C Prevalence and geographic distribution of the C9orf72 expansion among Norwegian ALS patients

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Background/Objectives: The most common genetic cause of the neurodegenerative disease amyotrophic lateral sclerosis (ALS) is the C9orf72 hexanucleotide repeat expansion. The C9orf72 expansion may also result in frontotemporal dementia (FTD) or a combination of ALS and FTD.

Methods: Over the course of 3.5 years, blood samples and clinical data from 449 ALS patients were collected from all 17 neurological departments in Norway. An expansion analysis was performed to identify individuals with the C9orf72 expansion.

Results: The C9orf72 expansion was identified among 9.8% ($n = 44$) of the 449 ALS patients, making the C9orf72 expansion the most common genetic cause of ALS in Norway. This included two intermediate C9orf72 expansions; one in a familial patient with a full C9orf72 expansion, and one in a simplex patient without other genetic findings. Of the 44 C9orf72 carriers, 22 were familial and 22 were simplex cases. Further, 30% ($n = 13$) reported a family history of dementia. The geographic distribution showed a tendency of higher density of C9orf72 expansion carriers in the Northern region of Norway (18%), compared to the Western region (3%).

Conclusion: In Norway, almost 10% of individuals with ALS carry the C9orf72 expansion. Half of them have no family history of ALS. There is a tendency of C9orf72 clustering in the Northern regions.

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Conflict of Interest: None declared

P10.052.D Homozygous variants in SPAST cause severe epileptic encephalopathy

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Spastic paraplegia 4 (SPG4) is the most common form of hereditary spastic paraplegia in western countries. Transmission is autosomal dominant. We can observe huge inter- and intrafamilial variations in age of onset, course of the disease and symptom's severity. SPG4 is caused by heterozygous pathogenic variants in *SPAST*. Four individuals carrier of homozygous pathogenic variants in *SPAST* have been previously described, two siblings with progressive degenerative encephalopathy with tetraparesis since childhood, and two siblings with pure spastic paraparesis, with an onset at 39 years. Here we described two brothers carrier of an homozygous variant in *SPAST* suffering from severe early-onset degenerative encephalopathy.

Both presented developmental delay, progressive microcephaly (-3SD) and progressive spasticity in the 4 limbs. They developed epileptic encephalopathy and died around their twenties from pneumopathy.

Sanger sequencing of *SPAST* identified a homozygous previously described (ClinVar VCV000989091.1) non-sense variant, NM_014946.4: c.447T>A, p.Tyr149*, carried by the two brothers. It is located in the microtubule interacting and trafficking domain of the SPAST protein. Their parents, first degree consanguineous, were carrier of the variant at the heterozygous state. The father suffered from lower limbs spasticity, with paraparesia, with an onset at 45 years. The mother only presented with Babinski sign and increased reflexes in the lower limbs at 53 years. SPG4 was diagnosed first in the parents before identifying homozygosity of the variant in deceased offspring.

SPAST variants could be found at homozygous state responsible for severe degenerative encephalopathy with tetrapyramidal spasticity.

Grants: no funding

Conflict of Interest: None declared

P10.053.A A proteome-wide Mendelian randomization analysis identifies potential causal relationships between circulatory plasma proteins and schizophrenia

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It has been widely reported in observational studies that changes in levels of circulating plasma proteins occur in schizophrenia. However, due to the potential presence of reverse causation and unmeasured confounding it remains unclear whether these changes are causal in nature or artifacts of other disease processes.

We applied 2-sample inverse-variance weighted Mendelian randomization analysis to investigate the evidence for causal associations between circulating blood plasma proteins and schizophrenia. Proteome-wide pQTL data covering 1,247 proteins from 35,559 Icelanders were used as instrumental exposure variables, and outcome summary statistics were taken from the most recent Psychiatric Genetics Consortium schizophrenia GWAS study of 76,755 individuals.

After multiple testing correction, our analysis yielded a panel of 21 circulating plasma proteins with a genetically-predicted causal or protective role in schizophrenia risk. Several of our findings, such as MAPK3 and NPTXR, corroborate previously reported protein markers, whereas many of the proteins that we identified are potentially novel.

Our analysis indicates a causal role for a number of circulating blood proteins in schizophrenia. These results may guide the development of novel therapeutics and pinpoint specific blood-bound biomarkers of psychosis. Further analyses investigating the colocalization of expression of these genes within brain region and cell-type specific datasets are ongoing, with the aim of identifying cellular and biochemical disease mechanisms.

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Conflict of Interest: None declared

P10.054.B Characterization of KDM5A-associated seizures according to the location of genomic variants in functional domains of KDM5A protein

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Introduction: *KDM5A*-related neurodevelopmental disorders manifest mainly by ASD, ID and lack of speech. Concomitant seizures, especially intractable seizures, are observed infrequently.

Methods: We present a 4-year-old boy of Polish origin with unreported variant of the *KDM5A* gene with clinical picture of epileptic encephalopathy. We also reviewed the correlation between epileptic presentation and functional domains of the KDM5A protein in our patient and previously published 10 patients.

Results: Our patient carried unreported heterozygotic non-coding splicing variant *KDM5A* c.2897+1G>A (p.?) de novo (bioinformatics predictions: potentially pathogenic). He experienced his first myoclonic-atonic and atypical absence seizures with focal EEG abnormalities in fronto-central regions at 28 months of age with further rapid progression to intractable seizures and generalized EEG abnormalities. He requires antiepileptic polytherapy (VPA, LEV, RFN, ACZ) and periodic methylprednisolone pulses. We identified epileptic presentation in 4 from 10 previously published patients (M:F = 1:1): 2 patients with early onset focal seizures well controlled in monotherapy (OXC, LTG), 1 patient with unspecified seizures, 1 patient with neonatal onset of polymorphic seizures (Dravet-like clinical presentation). In group with seizures, *KDM5A* variants were indicated de novo and located near protein domains related to structural integrity and stability of protein (JmjN, PHD2, PHD3). In contrast, in group without epilepsy, variants in *KDM5A* had AR mode of inheritance and were located in domains associated with enzymatic activity of protein (JmjC, PHD1).

Conclusion: Inheritance mode and localization of *KDM5A* variants in protein domains may be a good functional marker for predicting the risk of seizures and clinical course of disease.

Conflict of Interest: None declared

P10.055.C Copy number variant analysis of Anorexia Nervosa

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Background: The impact of copy number variants (CNVs) on anorexia nervosa (AN) has not yet been investigated in a large-scale study, even though CNVs may have important functional impacts.

Methods: We investigated the association between CNVs and AN in the Anorexia Nervosa Genetics Initiative (ANGI) study of 5,027 controls and 7,385 cases and the UK Biobank (UKB) 1,246 cases and 381,472 controls. In ANGI, we applied three CNV callers (PennCNV, iPattern, and QuantiSNP) and retained CNVs that were detected by at least two algorithms. For the UKB we accessed CNVs called using PennCNV by Kendal *et al*. We compared the burden of CNVs among cases and controls across a range of frequencies, types, and lengths. We annotated 93 CNVs that have been associated with neurodevelopmental disorders, and tested the association between each locus and AN. We also ran single-breakpoint association analyses to identify novel CNVs.

Results: We found no evidence for differences in overall burden of rare CNVs in cases compared with controls across both ANGI and UKB. For the 93 neurodevelopmental CNVs the control frequencies were not significantly different between controls in ANGI and UKB. We found some nominally significant associations between both the known loci and novel loci with AN.

Conclusion: While large CNVs have been reported to be associated with psychiatric disorders, the role of CNVs in AN is likely of lessor importance. Further increases in sample sizes are needed to fully evaluate if rare neurodevelopmental CNVs are associated with AN.

Conflict of Interest: None declared

P10.056.D Elucidating genetic causes of dystonia by large-scale exome sequencing

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Background/Objectives: Dystonia is a rare movement disorder with high clinical and genetic heterogeneity. Despite its high heritability (>25%), the etiology in most patients remains elusive. This study aimed to further elucidate the genetic causes of dystonia by exome sequencing.

Methods: We performed exome sequencing in ~2000 carefully phenotyped, previously genetically unsolved dystonia patients selected mainly from two large dystonia registries (DysTract [https://www.isms.uni-luebeck.de/en/research/dystract/] and the DystoniaCoalition [https://dystonia-foundation.org/research/dystonia-coalition/]). Patients had undergone two types of previous testing: (1) Hot spot screening for known pathogenic variants in dystonia genes; (2) GenePanel analysis in about a third of the samples. We searched for rare variants in genes previously mentioned in relation to dystonia (n = 412). Variants were Sanger confirmed and tested for segregation when possible.

Results: We identified 132 patients (6.6%) with pathogenic/likely pathogenic variants in 31 genes. We also found 162 patients (8.1%) with variants of uncertain significance (VUS) in 28 genes. Genes with at least 10 mutation carriers included *GCH1, KMT2B, SGCE, THAP1*, and *VPS16*, while the GAG deletion in *TOR1A* was excluded by prescreening. At least four of the detected variants (in *GNB1, IRF2BPL, KCNN2, and KMT2B)* occurred de novo, supporting pathogenicity.

Conclusion: This study demonstrated the value of exome sequencing in establishing diagnoses in a heterogeneous disease like dystonia. Despite prescreening, we found (likely) pathogenic variants in at least 6.6% of patients. We here also confirm the role of several dystonia candidate genes. In the next step, burden analyses will further expand the growing list of dystonia genes.

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P10.057.A Potentially causal associations between placental DNA methylation and schizophrenia and other neuropsychiatric disorders

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Background/Objectives: Increasing evidence supports the role of placenta in neurodevelopment and potentially, in the development of neuropsychiatric disorders. Recently, methylation quantitative trait loci (mQTL) and interaction QTL (iQTL) maps have proven useful to understand SNP-genome wide association study (GWAS) relationships, otherwise missed by conventional expression QTLs. In this context, we propose that part of the genetic predisposition to complex neuropsychiatric disorders acts through placental DNAm.

Methods: We constructed the first public placental *cis*-mQTL database including nearly eight million mQTLs calculated in 368 fetal placenta DNA samples from the INMA cohort, ran cell type- and gestational age-imQTL models and combined those data with the summary statistics of the largest GWAS on 10 neuropsychiatric disorders using Summary-based Mendelian Randomization (SMR) and colocalization. Finally, we evaluated the influence of the DNAm sites identified on gene expression in placenta in the RICHS cohort.

Results: We found that placental *cis*-mQTLs are useful to map the etiology of neuropsychiatric disorders to prenatal stages. Specifically, part of the genetic burden for schizophrenia, bipolar disorder and major depressive disorder could confer risk through placental DNAm. The potential causality of several of the observed associations is reinforced by secondary association signals identified in conditional analyses, regional pleiotropic methylation signals associated to the same disorder, and cell type-imQTLs, additionally associated to the expression levels of relevant immune genes in placenta.

Conclusion: The genetic risk of several neuropsychiatric disorders could operate, at least in part, through DNAm and associated gene expression in placenta.

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Conflict of Interest: None declared

P10.058.B Joint analysis of multiple trio genomic datasets for the discovery of novel dominant epilepsy genes

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Background/Objectives: The epileptic encephalopathies (EEs) and epilepsy with co-morbid intellectual disability (ID) are groups of epilepsy characterized by refractory seizures and developmental regression. Both groups have been shown to have underlying monogenic causes. However, despite state-of-the-art testing, a significant proportion of people with these types of epilepsy do not receive a molecular diagnosis, suggesting yet-to-be-identified genetic causes. Our objective was to identify novel epilepsy with ID and EE genes through joint analysis of multiple trio genomic datasets.

Methods: We assembled WGS/WES datasets with associated EE or epilepsy with ID HPO terms. Datasets were from the FutureNeuro Research Centre (149 trios, 9 quads), the Epilepsy Genetics Initiative (29 trios), Epi4K/EPGP (337 trios), UDN (9 trios,1 quad), CSER (21 trios, 1 quad) and the UK 100,000 Genomes Project (269 trios). A GATK4.2.0 pipeline was used for variant calling. A statistical model using denovolyzeR was utilized to identify genes with a significant excess of DNVs.

Results: A total of 814 trios and 11 quads were included in the final analysis. We identified 13 genes with a significant excess of DNVs, of which 11 were established monogenic causes of epilepsy. Among the potentially novel genes, predicted damaging MAST4 variants were observed in three unrelated patients. All MAST4 patients had epilepsy and a similar developmental phenotype.

Conclusion: Combining genetic and phenotypic data, we report the significant enrichment of DNVs across over 2,000 individuals who underwent WES/WGS. We implicate de novo variants in MAST4 as a cause of epilepsy with ID.

Grant References: 18/CRT/6214 and H2020-MSCA-COFUND-2019-94538

Conflict of Interest: None declared

P10.059.C Cas9 target enrichment and nanopore squencing to replace Southern-blot for the diagnosis of progressive mycolonic epilepsy

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Long-read sequencing has induced a significant boom in the identification of pathogenic repeat expansions. While repeat-primed PCR (RP-PCR) is a valuable first-tier tool for many loci, and considering the costs of long-read genome sequencing, Southern-blot remains necessary in many situations. For Unverricht-Lundborg disease (MIM #254800), the most frequent form of progressive mycoclonic epilepsy, most of the disease-causing variants consist of an expansion in the promoter region of *CSTB* (MIM # 601145). The CCCCGCCCCGCG dodecamer motif is present <4 times on a normal allele and more than 40 times on a pathogenic allele, the transmission being autosomal recessive. While Southern-blotting can be time-consuming and difficult to maintain in a diagnostic setting as other loci benefit from RP-PCR alone, we tested a Cas9 target enrichment combined with nanopore sequencing. This approach allows combined detection of the recurrent expansion and less frequent pathogenic SNVs. After standard DNA extraction, we validated through different iterations the combined use of 2 pairs of CRISPR guides following Oxford Nanopore Technologies (ONT) protocol and downstream sequencing on a R9.4.1 flongle. Data were basecalled with Guppy.

Minimap2 was used for genomic alignment. On average, one percent of all reads were on-target allowing a 10x coverage convenient for a second-tier diagnostic test. We now plan to multiplex samples and sequence libraries on R10.4.1 flowcells. To answer long-standing questions on epigenetic consequences of the expansion we will study methylation on future runs using V14 ONT chemistry in parallel of diagnostic transfer and ISO accreditation of this new economically competitive technique.

Conflict of Interest: None declared

P10.060.D Investigating the molecular genetic basis of hereditary spastic paraplegia due to a large chromosomal insertion in the SOX3 topologically associating domain

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Background: Hereditary spastic paraplegias (HSP) are monogenic neurological disorders characterised by degeneration of the corticospinal motor neurons and progressive limb spasticity. Here, we are investigating the molecular basis of a new X-linked HSP. It is linked to a 149 kilobase duplication from chromosome 4 inserted in a palindromic region of Xq27.1 and 50 kilobases away from the nearest protein-coding gene, the SRY box transcription factor 3 (*SOX3*). Other insertions in the same hotspot cause different phenotypes, such as hypertrichosis or peripheral polyneuropathy.

Methods: The insertion was identified by linkage analysis and whole genome sequencing. We generated an induced pluripotent stem cell (iPSC) line from an affected family member by reprogramming lymphocytes using Sendai virus. Isogenic control iPSC and partial-insertion iPSC lines were generated by two-guide deletions using the CRISPR/Cas9 system. Global gene expression was investigated by whole-RNA-Seq and genome architecture by high-throughput chromosomal conformation capture (Hi-C). iPSCs are being differentiated into neural progenitors and forebrain-type neurons.

Results: The 149 kb insertion causes dysregulation of *SOX3*, resulting in an eight-fold downregulation of mRNA levels in iPSCs. Hi-C analyses indicate reduced interactions between *SOX3* and an upstream regulatory region in patient cells. iPSC-derived neurons and additional CRISPR-edited iPSCs are being analysed to identify the origin and cell-type specificity of the *SOX3* dysregulation.

Conclusion: The results from these experiments demonstrate the complex relationship between insertions, chromatin interactions and gene regulation in the insertional hotspot palindrome at Xq27.1 and suggest a role of *SOX3* in this HSP.

Grant References: Independent Research Fund Denmark (9039-00337B).

Conflict of Interest: None declared

P10.063.C Relationship of genetic polymorphisms of the GABAergic system, BDNF and NPY genes with susceptibility to alcohol use disorders

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Background/Objectives: Alcohol use disorders (AUDs) may be favoured by the presence of certain genetic polymorphisms in neurotransmitter pathways. We thus examined the relationship between AUDs and genetic polymorphisms of brain-derived neurotropic factor (*BDNF*), neuropeptide Y (*NPY*) and gamma-aminobutyric acid type A receptor subunit α (*GABRA*)*1*, *GABRA2* and *GABRA6*.

Methods: Genotyping of the aforementioned polymorphisms was carried out by PCR in peripheral blood mononucleated cells of 300 patients with alcohol use disorders (AUDs) (according to DSM-IV or DSM-5 criteria) with alcohol consumption of at least 100 g of alcohol/day for 10 years and 157 healthy volunteers. Single-marker and haplotype analysis were performed to analyse the influence of genetic variants on AUDs.

Results: The A allele of rs6265 of *BDNF* was significantly more frequent in controls (47.8% vs. 35.7% in alcoholics, $p = 0.018$). The C allele of rs28383487 of *BDNF* was significantly more frequent in alcoholics (91.7% vs. 71.5% in controls, $p = <0.001$). The T allele of rs3219151 of *GABRA6* was more frequent in alcoholics (57.9% vs. 49.3% in controls, $p = 0.02$) and certain haplotypes were also significantly associated with AUDs.

Conclusion: The A allele of r26265 of *BDNF* is associated to lower risk of AUDs while C allele of rs28383487 of *BDNF* and T allele of rs3219151 of *GABRA6* are potentially associated with susceptibility towards AUDs.

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Conflict of Interest: None declared

P10.064.D Exploring the utility of a multi-polygenic risk score to capture the common genetic architecture of autism spectrum disorder

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Background: Compared to psychiatric disorders of similar heritability, few genome-wide significant variants have been implicated in the genetic architecture of autism spectrum disorder (ASD). This limitation may undermine the utility of polygenic risk scores (PRSs) in the context of ASD.

Methods: Using the genetic data of >22K individuals with ASD and >27K of their unaffected family members from the SSC, SPARK, and MSSNG cohorts, we computed the PRSs for ASD and 11 of its genetically-related traits. We applied an unsupervised learning approach to derive a "multi-PRS" variable, which captured the variation of all 11 ASD-related PRSs in a single dimension.

Results: We found that individuals with ASD had a significantly greater burden of PRS-ASD ($OR = 1.16$) and the multi-PRS dimension ($OR = 1.10$), compared to their unaffected family members. The derived multi-PRS variables also captured a

significant difference in clinical outcomes amongst cases with ASD. Notably, the best-performing multi-PRS dimension (PC4) and PRS-ASD similarly increased the cognitive ability and lowered the age of walking amongst individuals with ASD.

Conclusion: Our findings support the use of a multi-PRS variable as a proxy for PRS-ASD in cases where non-overlapping and well-powered GWAS summary statistics are difficult to obtain, or accounting for genetic heterogeneity in a single dimension is preferred. This approach may also capture the overall liability for a mental disorder diagnosis (i.e.: genetic "P-factor"). Altogether, we present a novel approach to study the role of inherited, additive, and non-specific genetic risk factors on the genetic architecture of ASD.

Conflict of Interest: None declared

P10.065.A Transcriptomic analysis of hiPSC-derived neurons from patients with Williams Beuren Syndrome and 7q11.23 microduplication syndrome

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Background/Objectives: Williams-Beuren syndrome (WBS) and 7q11.23 microduplication syndrome (7DUP) are rare multisystemic disorders caused by a deletion or duplication, respectively, in the 7q11.23 region. Both syndromes affect the neurodevelopment, with patients presenting some opposite phenotypical characteristics but also some similarities. The generation of human induced pluripotent stem cells (hiPSC) and derived neuronal (iNeu) cultures has revolutionized the field of in vitro modeling for neurodevelopment disorders. Here, we aim to evaluate the single-cell transcriptomic profiles of hiPSC-derived iNeus generated from WBS and 7DUP patients.

Methods: We differentiated hiPSCs from four WBS, four 7DUP patients and two controls into mature neurons using a *NGN2* based lentiviral transfection protocol. At day 15 post-transfection, after FACS sorting, cells were processed to scRNAseq at CNAG-CRG (10x Genomics single cell 3′ mRNA library preparation, NovaSeq 6000 library sequencing). We performed quality controls, cluster analysis and annotation of cell populations. Following analyses include differential expression and gene enrichment analysis of the under/overexpressed markers for each cluster. Simultaneously, we confirmed neural identity of the obtained iNeus by immunocytochemistry and quantitative-PCR, and their conductivity by calcium current imaging.

Results: iNeus from all hiPSCs expressed mature neuronal markers, specific neuron type markers, formed synapses and presented conductivity. The scRNAseq analysis displayed 24 clusters of cell populations, showing different stages of neuronal differentiation. At least three clusters expressed mature neuronal markers. We are currently completing the annotation adjustments and differential expression analyses.

Conclusion. We present a comprehensive transcriptomic profile of WBS and 7DUP iNeus for the first time at single-cell level.

Conflict of Interest: None declared

P10.066.B Prenatal diagnostic exome sequencing analysis in 207 fetuses with congenital CNS anomalies

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Objective: To determine the genetic contribution of established disease genes to congenital CNS anomalies in fetuses prior to termination of pregnancy (ToP).

Methods: 207 affected fetuses were examined at the Department of Gynecology and Obstetrics at the University Hospital Bonn. ToP was performed on request and blood samples for DNA extraction were obtained. All proceedings were conveyed according to the Declaration of Helsinki and national law. Exome Sequencing (ES) results were evaluated for genetic variations in known CNS disease genes identified by literature review.

Results: We detected 14 disease-causing variants in 16 fetuses with CNS anomalies. These were six missense variants in *L1CAM*, *MKS1*, *POMGNT1*, *PSAT1*, *TUBB3 and TUBA1A*; three nonsense variants in *CSPP1*, *RXYLT1* and *ZEB2;* and three splice variants in *CC2D2A*, *L1CAM* and *OFD1*. These variants were reported previously in the ClinVar database as (likely) pathogenic. Additionally, we identified four CNVs (0.35 Mb – 24.7 Mb) encompassing genomic locations known to contribute to syndromic CNS anomalies: 1q21.1del, 6p25del, 6q21-23del, 6q25.3-27del. We further detected nine novel variants (absent in gnomAD v.3.1.2) of unknown significance in established disease genes.

Conclusion: In this study, we were able to identify a clear genetic diagnosis in 9.6 % and a potential diagnosis (VUS) in 3.8 % of fetuses. Implementing ES in the ToP situation has the power to unravel a prenatal diagnosis and may provide a better basis for counseling of affected families.

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Conflict of Interest: None declared

P10.067.C Single-nucleus RNA-sequencing of the cerebellar cortex reveals oligodendrocytes as underlying disease risk in essential tremor

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Background/Objectives: Essential tremor (ET) is one of the most common movement disorder, affecting close to 1% of the world

population. The disease is characterized by an upper limb action tremor that can severely impact quality of life. The cerebellum has previously been identified as a key brain region in the disease, yet our understanding of the contribution of cerebellar cell types in the pathophysiology of ET is still unknown. To this end, we profiled 50,000 nuclei from ET and control cerebellar cortices to identify affected cell types in the disease.

Methods: 16 ET and 16 control post-mortem cerebellar cortices were processed using split-pool ligation-based transcriptome sequencing. Seurat was used to cluster cells. Differential gene expression was performed using DESeq2. MAGMA was used to assess cell-type enrichment of ET GWAS signals. Coloc was used for colocalization of GWAS signals with cell-type specific eQTL data.

Results: Amongst the 12 cell types identified, the most dysregulated cell types were oligodendrocytes, Bergmann glia and oligodendrocyte progenitor cells (OPCs). Pathway enrichment of dysregulated genes in oligodendrocytes identified myelin sheath constituents ($p = 7.85E-03$) as significant. Furthermore, oligodendrocytes were found to best explain the heritability of ET ($p = 4.20E-04$), in part due to their expression of *BACE2*. Colocalization analysis notably found the lead risk variant for ET, rs9980363, to be an oligodendrocyte-specific eQTL for *BACE2* (posterior probability = 0.978).

Conclusion: Our results indicate that oligodendrocytes might be key drivers of disease in ET and that their initial dysfunction might perturbate neuronal hemostasis in the cerebellum.

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P10.068.D Screening of coding pathogenic variants in Bulgarian patients with unspecified dementia

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Background/Objectives: Unspecified dementia (ICD-10 F03) is underrepresented in genetic research. In order to deepen the knowledge of the genetics of this disease we have used the time and cost effective approach of whole exome sequencing (WES) of a pooled DNA sample and have screened for rare pathogenic variants.

Methods: WES was performed on a pooled DNA sample of 91 Bulgarian patients diagnosed with unspecified dementia. Reads were aligned to the reference genome (GRCh37/hg19) and variants were annotated using wANNOVAR.

Results: We have detected 453631 variants, which were screened for rare variants (gnomAD MAF < 0,001) categorized as pathogenic in ClinVar. The rare pathogenic variants were not found in genes for monogenic forms of dementia, but in genes for ciliopathies (*B9D1*-rs373478202, G>T and *CC2D2A*-rs200904521, C>T) and citrullinemia type 1 (*ASS1*: rs148918985 and rs121908641, G>A). Two of these four variants (rs373478202, G>T and rs148918985, C>T) show statistically significant difference in frequency between the analyzed patients and gnomAD Bulgarian controls and may be involved in the development of unspecified dementia in part of the analyzed patients. Thus, rs373478202, G>T is causative for Joubert syndrome - autosomal recessive ciliopathy characterized by complex midbrain-hindbrain malformation; whereas rs148918985, C>T leads to argininosuccinate synthase 1 inactivation, causing hyperammonemia, which has neurotoxic effect.

Conclusion: The two nominated variants (rs373478202, G>T and rs148918985, C>T) should be further investigated in individual DNA samples and should be correlated with a specific imaging and biochemical phenotype, respectively.

Grant References: KP-06-N33/5 from 13.12.2019 - National Science Fund of Bulgaria

Conflict of Interest: None declared

P10.070.B Comprehensive genomic investigation into the aetiology of Malformations of Cortical Development in an Indian cohort of 47 paediatric cases

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Background: Malformations of cortical development [MCDs] include lissencephaly, pachygyria, polymicrogyria and nodular heterotopia due to disrupted neuronal migration or organisation of cortical neurons. The diagnostic yield even after comprehensive genomic testing is only around 20-30%. Genes implicated in MCDs are either members of the PI3K-AKT-mTOR or glycosylation pathways or those encoding cytoskeletal proteins. Low diagnostic yield is often due to somatic mosaicism or locus heterogeneity with yet unidentified loci.

Methods: We present here a cohort of paediatric MCDs [N = 47] from a single tertiary care centre. After ethical clearance and consenting process Whole Exome Sequencing and Chromosomal Microarray of simplex / Trio / quad was performed along with validation and segregation analysis. Custom panel of genes was developed for analysis based on gene ontology dependant scores, constraint metrics, extended network of genes and foetal brain expression for each subtype. RNAseq to look for specific RNA phenotypes was performed for select variants. In-depth genotype-phenotype correlation was also performed.

Results: MCDs cohort included polymicrogyria (26/47), pachygyria/lissencephaly (13/47), schizencephaly (2/47) and heterotopias (9/47). Phenotypes observed were global developmental delay (44/47), epilepsy (32/47), dysmorphism (29/47) and neurological defects (40/47). We identified 9 previously reported and 9 novel pathogenic single nucleotide variants in the cases segregating in a mendelian pattern, and 2 pathogenic copy number variants achieving a diagnostic yield of 42.5%. Novel phenotypes were observed in some cases.

Conclusion: Comprehensive genomic testing as well as novel gene sets utilised for analysis has improved the diagnostic yield by around 12.5%.

Grants: NIMH/PROJ/GAU/00580/2018-19

Conflict of Interest: None declared

P10.072.D The contributions of mitochondrial and nuclear mitochondrial genetic variation to neuroticism

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Background/Objectives: Neuroticism is a heritable trait composed of separate facets, each conferring different levels of risk, or protection, to health. Large scale genetic analysis has been performed on neuroticism, but so far no investigation has examined the contributions of mitochondrial DNA. Here we examine the association between mitochondrial and nuclear mitochondrial genetic variation to three factors of neuroticism (general neuroticism, anxiety/tension, and worry/vulnerability) in 269,505 unrelated white British samples in UK Biobank.

Methods: Three factors of neuroticism were derived using a bi-factor model. Mitochondrial (MT) markers were imputed using 1000G in IMPUTE2. MT haplogroups were identified by HaploGrep2. MT associations were performed in R and permutation was conducted to examine independent signals. Haplogroup-stratified nuclear-GWAS and nuclear-by-MT interaction GWAS were performed in PLINK2. Genetic correlation was estimated in LDSC. Co-expression analysis was performed using GTEx data.

Results: We identified five MT-haplogroup and 15 MT-marker associations across the neuroticism factors at MT-wise significant level ($P < 1 \times 10\text{-}3$). Autosomal SNP associations with worry/vulnerability differed for the H haplogroup compared to other haplogroups ($rg = 0.87 \pm 0.05$, $P = 3.65 \times 10\text{-}3$). Furthermore, genetic correlations derived using GWAS data showed that pleiotropy between worry/vulnerability with cognitive, physical, and mental health traits differed by mitochondrial haplogroup. An interactions between nuclear DNA on chromosome 9 and MT haplogroups was identified at $P < 5 \times 10\text{-}8$, which revealed associations between general neuroticism and anxiety/tension with brain-specific gene co-expression networks.

Conclusion: Mitochondrial genetic variation is associated with neuroticism traits at the haplogroup and single variant level. Furthermore, autosomal variation associated with neuroticism is influenced by MT haplogroup.

Grant References: MR/T030852/1

Conflict of Interest: Charley Xia Full, Medical Research Council (MRC) [MR/T030852/1], Sarah Pickett Full, Wellcome Trust Career Re-entry Fellowship (204709/Z/16/Z), the Wellcome Centre for Mitochondrial Research (203105/Z/16/Z) and also receives support from a L'Oreal UNESCO FWIS Award, David Liewald Full, Medical Research Council (MRC) [MR/T030852/1], Alexander Weiss Full, Gavin Hudson Full, Parkinson's UK (G-2003), the Michael J Fox Foundation (MJFF-007574 and MJFF-007690) and the Wellcome Centre for Mitochondrial Research (203105/Z/16/Z), David Hill Full, Medical Research Council (MRC) [MR/T030852/1]

P10.074.B Functional analysis of KCNQ2 variants

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Background/Objectives: $K_V7.2$ potassium channels play a key role in the initiation and propagation of action potential essential for controlling excitability of neurons in the central nervous system. Alterations of the *KCNQ2* gene encoding $K_V7.2$ can lead to a spectrum of diseases including benign familial neonatal seizures and developmental and epileptic encephalopathy. Exploring *KCNQ2* mutations in patients with epileptic syndromes is critical for understanding their pathogenesis and possibly for the selection of the most appropriate treatment.

Methods: Accordingly, we aimed to fully characterize the biophysical properties of $K_V7.2$ channels containing novel heterozygous mutations of p.Ser113Phe localized in the extracellular S1-S2 loop and p.Ala306Val located in the pore domain identified in patients with epilepsy. We subsequently compared the main gating parameters of mutant channels to wild-type $K_V7.2$ channels. The channels were transiently expressed in CHO cells and potassium currents were measured using the standard whole cell patch-clamp technique.

Results: We found that the peak current density is substantially reduced by the p.Ser113Phe mutation. We also observed slower activation kinetics of I_K current and a significant rightward shift on the steady-state activation in this mutant. The p.Ala306Val mutation resulted in non-functional channels. All observations confirmed that the mutations alter the function of the channels. Pharmacological studies revealed that Retigabine might restore the normal function of $K_V7.2$ containing p.Ser113Phe mutation.

Conclusion: Our findings may facilitate the understanding the mechanism of epilepsy associated with *KCNQ2* defects. Moreover, understanding the structure-function relationship of $K_V7.2$ will shed new light on exploiting new therapeutic drugs for *KCNQ2* channelopathies.

Conflict of Interest: None declared

P10.075.C Biallelic variants in POLR3A encoding catalytic subunit of human RNA polymerase III cause primary microcephaly through perturbation of the mTOR signaling pathway

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Background/objectives: Primary Microcephaly is the observation of head circumference >2.5 SDs below the mean for a given sex, age, and ethnicity. It is caused by biallelic variants of 30 genes.

Methods: We analyzed the family through exome sequencing and linkage analysis. The consequences of the candidate variant were explored at cellular and molecular levels by immunofluorescence, immunoblotting, transient expression of tagged proteins, pulldown assay coupled with mass spectrometry, RNA-seq, and knockdown of Polr3a in chicken embryos.

Results: We studied a large six-generation Pakistani family with seven affected members manifested primary microcephaly. The genetic analysis identified a biallelic variant (NM_007055.3: c.40A>G; p.(Lys14Glu)) of *POLR3A*. Studying further patients, we were able to find four compound heterozygous variants of *POLR3A* in two unrelated patients manifesting microcephaly and variable features of global developmental delay, seizures, hypotonia, and dental abnormalities, featuring leukodystrophy. Pathogenic variants in *POLR3A* are known to cause hypomyelinating leukodystrophy but have never been implicated in the etiology of microcephaly. Patient-derived primary fibroblasts and ectopically expressed HeLa cells revealed reduced immunoreactivity of POLR3A. The variant plausibly compromises the interaction of POLR3A with crucial proteins involved in mTOR signaling and gene expression pathways and exerts effects on the differential expression of genes involved in translation and cell cycle. Knocking down of Polr3a in chicken embryos shows striking features of reduced brain size.

Conclusion: Our findings suggest that biallelic disruption of *POLR3A* should be considered in molecular diagnosis of primary microcephaly. Notably, mTOR signaling contributes to determining normal brain size.

Conflict of Interest: None declared

P10.076.D Functional study of PRRT2 missense variants in epilepsy patients

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Objective: Mutations in *PRRT2* gene encoding proline-rich transmembrane protein 2 is the cause of a wide spectrum of neurological diseases: benign familial infantile epilepsy, infantile convulsions with choreoathetosis syndrome, and paroxysmal kinesigenic dyskinesia (PKD). Since PRRT2 protein is a key component in synaptic contacts and neurotransmitter release, determining the pathogenicity of missense variants occurring in this gene may help in elucidating the mechanisms leading to derangement of network function in neurological disorders. We detected the c.883C>T (p.Arg295Trp) mutation in two families with epilepsy symptoms. In addition to the c.883C>T variation, we choose previously uncharacterized missense alterations in the *PRRT2* gene and analyzed the possible functional consequence of the mutations.

Methods: Six missense mutations were introduced in the cDNA of *PRRT2* cloned into pcDNA3.1 by site-directed mutagenesis. Protein expression levels of the mutated recombinant proteins were investigated in HEK293 cells by western blot using monoclonal anti-PRRT antibody and ECL as substrate.

Results: Functional study revealed that protein expression of c.883C>T (p.Arg295Trp) was somewhat decreased in comparison with wild type. Three of the reported variants located in the loop domain (p.Ser294Asn, p.Gln299Pro, p.Arg295Pro) showed reduced levels of the recombinant mutant proteins while p.Met293Leu and p.Ser297Asn did not show decreased expression.

Conclusion: Functional analysis of the mutations predicted to be pathogenic/likely pathogenic is of great importance in clinical genetic decision making. In this study we tested *PRRT2* missense variants and found that in some cases, decreased amount due to the mutation might be associated with the pathogenicity.

Grant References: Stipendium Hungaricum scholarship

Conflict of Interest: None declared

P10.078.B Delineating the CANVAS-associated genetic locus in a Greek late-onset ataxia cohort

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Background: CANVAS syndrome is characterized by cerebellar ataxia, neuropathy, and bilateral loss of the vestibular reflex. The syndrome is due to a pentanucleotide biallelic expansion in the *RFC1* gene. The expansion is highly polymorphic, with variability in both the repeat pattern and the number of repeats. Initially, 4 different conformations were observed: $AAAGG_{11}$, $AAAAG_{(n)}$, $AAAGG_{(n)}$ and the pathological $AAGGG_{(n)}$, n>400. We have recently completed a study of 5 genetically confirmed CANVAS cases after screening a cohort of 77 Greek patients with late-onset ataxia. As a continuation of this study, we presently aimed to delineate the genotypic variability of the CANVAS locus in the remaining patients of that cohort.

Methods: Sixty-four patients from the previously described Greek ataxia cohort, presenting at least one band of sizing PCR in agarose gel (negative for homozygous expansions) were further examined through fragment analysis and RP-PCR.

Results: Overall, the pathological expansion $AAGGG_{(exp>400)}$ was found in 3/64 (4.7%) cases in heterozygous state, while it was also found with fewer repeats (<400) in 6/64 (9.4%) cases. The non-pathological expansions $AAAGG_{(exp)}$, $AAAAG_{(exp)}$ were found in 4/64 (6.3%) and 16/64 (25.0%) respectively, in the heterozygous state. Additionally, 12/64 cases (18.8%) carried two non-expanded alleles differing in 1-2 repeats.

Conclusions: This study further delineates the genetic heterogeneity of the CANVAS locus in Greek patients with late-onset ataxia without biallelic pathological expansions. By comparing these findings with future data on Greek healthy controls, and data from other ataxic populations, we hope to better characterize the CANVAS locus in health and disease.

Conflict of Interest: None declared

P10.079.C Case report: A young patient with Brunner syndrome (MAOA deficiency) responding to a combination of pharmacological and non-pharmacological interventions

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In 1993, Brunner syndrome (MIM# 300615), an X-linked recessive disorder caused by pathogenic variants in the Monoamine Oxidase A (MAOA) gene was first described. The phenotype in affected males features explosive aggression, violent behavior, arson, autism spectrum disorder and mild intellectual disability. Only a handful of cases has been published since, all concerning (young) adults.

However, since the introduction of whole exome sequencing (WES) in common clinical genetic diagnostics, multiple young boys were found to have (likely) pathogenic variants in MAOA. Here we describe a 7-year old boy with a severe psychiatric phenotype already at young age and an approach that proved successful in alleviating his symptoms with a follow-up of one year.

The boy was evaluated because of developmental delay at age two years. WES revealed a de novo pathogenic variant in MAOA (p.(Trp116*)). He developed severe sleeping problems, anxieties, anger and explosive obsessions. Symptoms were worse in wintertime. Metabolite evaluation showed high serotonin, norepinephrine and normetanephrine in blood, and low HVA and VMA in urine. Treatment was started with risperidone, a 5HT2 (serotonin) and D2 (dopamine) receptor blocker, 0,2mg/day. Overtime the dose was increased to 0,7mg/day. Combined with a tryptophan low diet and sun-exposure, the boy significantly improved including his sleep, mood and obsessions. Interestingly, metabolite measurements did not normalize.

This case exemplifies that Brunner syndrome can also be symptomatic at young age. The number of young cases will likely increase given the increasing availability of WES. This clinical experience may assist in treatment of these cases.

Conflict of Interest: None declared

P10.080.D Whole-exome and array-CGH analysis of patients with epilepsy in Slovakia

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Introduction: Epilepsy is a heterogeneous neurological disorder caused by variants in several hundred genes. Genetic diagnosis thus requires the use of high throughput methods such as aCGH and NGS.

Methods: Cohort of 47 patients was analyzed by whole exome sequencing (WES) using Nextera Exome Rapid Capture Enrichment kit. Variants were validated by Sanger sequencing. 20 patients underwent aCGH analysis (SurePrint G3 Human CGH + SNP Microarray Kit 2 × 400K protocol).

Results: Seizure onset in cohort was between 1 day to 6 years and 55% of patients experienced first seizure before 1 year of age. All patients were negative for mutation screening in *SCN1A, SCN1B, SCN2A, GABRA1, GABRD, GABRG2* and *CDKL5* genes. WES revealed probable causal variants in 18 of 47 patients in genes *ALG13, AP3B2, CACNA1A, CDKL5* (mosaic), *GNAI1, KCNQ2, KCNT1, PCDH19, PRRT2, SCN8A, SIK1, SMC1A, TBC1D24* and *TPP2*, of which 8 were novel variants. Possible causal variants were identified in another 20/47 patients mostly in non-epilepsy genes based on their function and prediction of the pathogenicity of the variants. We found 11 variants inherited from a parent, 7 de novo variants and a recessive form of inheritance in 6 patients. aCGH showed 16p11.2 deletion (ch16:29652999-30198611) in one patient.

Conclusion: Clinical and genetic heterogeneity of epileptic disorders complicates molecular diagnostics, therefore WES in combination with aCGH is currently one of the most effective ways to identify variants in epileptic genes but also in genes whose impact on epileptic phenotype must be elucidated.

Grant References: Supported by VEGA 1/0731/17.

Conflict of Interest: None declared

P10.081.A A novel nonsense variant of NF1 gene in two related patients with different clinical manifestations of neurofibromatosis

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Background/Objectives: Neurofibromatosis (NF) is an autosomal dominant disease characterized by cafe-au-lait spots, cutaneous neurofibromas, and Lisch nodules of the iris. Pathogenic variants in the Neurofibromin 1 (*NF1*) gene are largely responsible for the disease. In our case, a 19-years-old female patient with intracranial venous anomaly and hamartomatous lesion in brain MRI was seen in our clinic with her father. They both had cafe-au-lait spots. Therefore they were both evaluated for *NF1* gene variants.

Methods: Exons and exon-intron transition regions of *NF1* gene were analyzed by next generation sequencing. Detected variants were assessed with variant information servers (dbSNP, ClinVar, Ensembl, VarSome). "American College of Medical Genetics and Genomics (ACMG)" criteria were used for variant evaluations.

Results: Analysis of the sequencing data revealed a heterozygous variant of c.3800T>A in 28th exon of *NF1* gene (NM_001042492.3) in both patients. This stop codon forming variant (p.Leu1267*) was classified as "likely pathogenic" by the ACMG criteria and was not observed in population databases nor any publication in the literature. Although one missense, one frameshift, two synonymous variants were published in the same aminoacid region.

Conclusion: We present a truncating novel *NF1* gene variant in a patient with cafe-au-lait spots, congenital glaucoma, lumbar arachnoid cyst, scoliosis, and no Lisch nodules, observed also in patient's father showing cafe-au-lait spots in his first current prediagnosis.

Variant's segregation analysis in other family members is requested and functional analysis' are needed to better elucidate

the phenotypic manifestations of the variant and its pleiotropic effects.

Grant References: No grants were received.

Conflict of Interest: None declared

P10.082.B Genomics of neurodegenerative diseases caused by repeat expansions: NGS long-read sequencing reveals an unexpected complexity of repeat architecture in C9orf72/ FTDALS1 and RFC1/CANVAS genes

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Background/Objectives: Expansions of repeated sequences are an important cause of neurodegenerative diseases (ataxias, FTD/ ALS, HD) but are still difficult to define with the commonly adopted laboratory techniques. The aim of this study was the development of sequencing approaches that allow to precisely define the length and architecture of repeat expansions involved in different neurodegenerative diseases.

Methods: To this aim, two complementary NGS sequencing technologies were used: a short-read (amplicon-based SRseq) and a long-read (LRseq) approach with CRISPR/Cas9 enrichment of the target region and nanopore/ONT sequencing of the entire expanded region.

Results: The SRseq approach applied to polyglutamine diseases allowed to precisely define the architecture, stability, and size of the repeats, and to establish the high error rate (up to 75%) of the methods commonly used for repeat sizing. The LRseq approach applied to longer and more complex repeats, as in CANVAS/*RFC1* and FTDALS1/*C9orf72*, allowed to identify the frequent presence of expanded alleles with a complex and hitherto unknown architecture of the repeat. The LRseq approach in the study of these complex genomic regions proved indispensable in a case of suspected neuronal intranuclear inclusion disease (NIID) in which the expansion in the *NOTCH2NLC* gene had escaped the conventional method due to an ~80bp deletion flanking the repeat.

Conclusion: These results demonstrate the essential contribution that sequencing approaches make to the understanding of the variability and structure of repeat regions, as well as their intergenerational and somatic instability, with crucial implications for diagnosis and studies of the pathogenic mechanisms.

Grants: FRRB-CP-20/2018

Conflict of Interest: None declared

P10.083.C Deep intronic FGF14 GAA repeat expansion is a frequent cause of Episodic Ataxia in Italian patients

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Background/Objectives: Recently, a heterozygous GAA repeat expansion in intron 1 of *FGF14* gene (fibroblast growth factor 14) was identified as a common cause of a dominant adult-onset form of spinocerebellar ataxia (SCA27B). Uninterrupted expansion ≥250 GAA may be considered pathogenic, with a potential incomplete penetrance (1,2).

Objectives: 1) to screen for *FGF14* expansion a cohort of Italian patients with ataxia (n = 128) or episodic ataxia (EA) negative for *CACNA1A*/EA2 and *KCNA1*/EA1 pathogenic variants (n = 48); 2) to assess the distribution of *FGF14* alleles in a control population (n = 183).

Methods: The *FGF14* expansion was analysed by long-range PCR encompassing the repeat and by fluorescent repeat-primed PCR (1,2).

Results: *FGF14* expansions >250 GAA were identified in 11 EA cases from 8 families (8/48 = 16.6%, 4 AD, 4 S) but only in two patients with chronic-progressive ataxia (2/128 = 1.5%). In the controls' cohort, we identified only one uninterrupted allele >200 (GAA = 236) but no uninterrupted alleles ≥250 GAA. In both patients and controls, approximately 1% of alleles may have an alternative/interrupted expanded repeat configuration.

Conclusions: *FGF14* expansion accounts for up to 16% of Italian non-EA1/non-EA2 EA families. Patients present with late-onset (mean = 55 yrs) EA characterized by disequilibrium, gait ataxia, and eye movements abnormalities, followed by a slowly progressive chronic ataxia (mean onset = 60 yrs). Cerebellar atrophy was present in 60% of patients. Sequencing of uncertain alleles by long-read approach is undergoing and is expected to be crucial to determine their pathogenic role.

References

1. Rafehi et al., 2023 PMID36493768
2. Pellerin et al., 2023 PMID36516086

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Conflict of Interest: None declared

P10.084.D Integration of multi-omics data for the diagnosis of Rett syndrome spectrum disorders

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Background/Objectives: Rett syndrome (RTT) spectrum disorders are a group of neurodevelopmental disorders with overlapping phenotypes and diverse genetic aetiologies. The current gold-standard for molecular diagnosis is WES, but diagnostic yield is <50%. WGS has potential to uncover new diagnoses, but the interpretation of the amount of data generated poses a challenge to clinical usage of this test.

Methods: We generated WGS data coupled with transcriptomic and proteomic profiles from fibroblast cultures of 8 RTT-spectrum patients without molecular diagnosis. We used DROP-framework, Watershed-framework and Protrider tools to detect outlier behaviours in RNAseq and proteomics data. Aberrant molecular phenotypes were used to prioritize variants in WGS analysis.

Results: WGS detected ~5M SNVs, indels and SVs per patient, of which <1% would be identified via WES. Almost 50% were intronic, in 5′/3′-UTR regions or upstream a gene, potentially causing aberrant expression, aberrant splicing or monoallelic expression events. Analysis of the fibroblasts transcriptome revealed that 85% of RTT-spectrum genes were covered in our

experiment, and that ~99% of detected transcripts (TPM>0.5) correspond to genes with some degree of expression in the nervous system. Limiting WGS analysis to the 110-1380 transcriptomics or proteomics outlier genes per patient reduced the call set to 1% of the original data.

Conclusion: The set of fibroblast-expressed transcripts indicates this is an adequate surrogate tissue to carry out expression studies in RTT-spectrum patients. An integrated multiomics approach contributed to variant prioritization by identifying candidate genes with aberrant molecular phenotypes that streamline variant interpretation in WGS.

Grant References: PI20/0289, FPU18/02152, 2020/FI-B/00888

Conflict of Interest: None declared

P10.085.A Human models for White Sutton syndrome: POGZ mutations change the transcriptome and induce defects in neural progenitor cell biology

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Background: Pogo transposable element derived with ZNF domain (*POGZ*) has been identified as one of the most recurrently mutated genes in patients with neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD), intellectual disability and White-Sutton syndrome; however, the underlying disease-causing cellular and molecular mechanisms are still unclear.

Methods: We generated several induced pluripotent stem cell lines (iPSCs) with heterozygous *POGZ* mutations, either derived from patient fibroblasts or introduced by CRISPR/Cas9 genomic editing, and differentiated them into neural progenitor cells (NPCs).

Results/Conclusion: We demonstrate that frameshift mutations, either in the N-terminus or in the HP1-binding zinc finger-like (HPZ) domain, decrease POGZ protein expression but do not impair its nuclear localization. By using a 3D neurosphere model, we show that *POGZ* deficiency impairs self-renewal activity and enhances NPC differentiation and neuronal migration.

POGZ binds to chromatin as transcriptional regulator. RNA sequencing of NPCs revealed widespread transcriptome changes in the NPCs carrying POGZ mutations. The differentially expressed genes were significantly enriched in the GO terms of chromosome and mitotic chromatid segregation, DNA repair by homologous recombination and alternative splicing, among others. Of note, the transcriptomes of different cell lines all carrying POGZ mutations were more similar to each other than were the transcriptomes of different wildtype cell lines. This indicates that POGZ mutations strongly affect cell expression levels and give rise to a POGZ-specific transcriptome. To identify direct POGZ targets, we are carrying out CUT&RUN sequencing of the NPCs. At the ESGH conference, we will report more on the results of these experiments.

Conflict of Interest: None declared

P10.086.B Diagnostic implementation of WGS for rare neurological disorders: The first Bulgarian experience

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Introduction: According to EURORDIS rare diseases affect 3.5-5.9% of the worldwide population, currently, 80% of them are genetic. Many of them have neurological component and about 90% manifest during childhood. Implementation of WGS in the routine clinical workflow improves diagnostic yield and shortens patients' diagnostic odyssey.

Materials and Methods: Through WGS we examined 40 patients aged 6 months to 16 years consecutively referred for genetic testing due to a neurological disease of unknown etiology. WGS was performed on a BGISEQ-500 platform followed by targeted bioinformatic analysis based on every patient's clinical phenotype.

Results: Seventeen patients received a definite diagnosis based on the identification of pathogenic/ likely pathogenic gene variants relevant to the phenotype. Variants of unknown clinical significance that are potential candidates associated with clinical symptoms were observed in another 6 patients. After extensive bioinformatic analysis, 17 remained without a diagnosis, and re-analysis of the data after one year as also a more detailed evaluation of the phenotype was recommended.

Conclusion: This is the first WGS study of pediatric patients with rare neurologic disorders in Bulgaria. The results had a major impact on clinical management in all 17 patients and added valuable genetic knowledge about pediatric neurologic disorders in our country. The results assessed the perspective of WGS for the diagnosis of Bulgarian patients with rare diseases. There is a lack of medical treatment for many of these disorders, but the diagnosis allows prognosis of the course of the disease and enables prevention in the affected families.

Conflict of Interest: None declared

P10.087.C NKX6.2- related leukodystrophy: natural history study, clinical phenotype, biomarkers, and gene therapy

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Background/ Objectives: Hypomyelinating leukodystrophies are a heterogeneous group of genetic disorders characterized by detectable myelin deficiency in the brain with a wide spectrum of phenotypes. A phenotype associated with bi-allelic mutations in the *NKX6.2* gene that leads to spastic ataxia 8 (SPAX8), autosomal recessive, with hypomyelinating leukodystrophy, has been described. Despite the discovery of various other genetic forms of hypomyelinating leukodystrophies, many remain unsolved.

Methods: The study included affected individuals with spastic ataxia and hypomyelination from unrelated families, and homozygosity mapping and exome sequencing were implemented to identify and characterize the causal variants in *NKX6.2*.

Results: 33 individuals from 21 families carrying compound heterozygous and homozygous pathogenic variants in *NKX6.2* were identified. The key neuroimaging feature in most cases was of hypomyelination.

Conclusion: Phenotypic and neuroimaging expression in *NKX6.2* mutations vary from a complex, neonatal onset at the severe end to a childhood onset at the milder end of the spectrum. We plan to expand our analysis to conduct a natural history study to evaluate disease progression, and to identify serial biomarkers. There is an important need to develop gene transfer methods to replace and rescue *NKX6.2* protein loss.

Grant References: Spastic Paraplegia Foundation; Medical Research Council; Wellcome Trust (Synaptopathies); Ataxia UK;

British Neurological Surveillance Unit; National Institute for Health Research.

Conflict of Interest: Kristina Zhelcheska full-time PhD student on studentship award, Viorica Chelban full-time, Henry Houlden full-time, Ataxia UK Grant awarded for PhD studentship

P10.089.A New exome sequencing data provides preliminary evidence for three novel schizophrenia risk genes

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Schizophrenia is a debilitating psychiatric disorder whose causes remain poorly understood. Rare variant sequencing studies have implicated twelve genes at exome-wide significance in schizophrenia, yielding novel biological insights. However, larger sample sizes are required to implicate genes in more substantial numbers.

Here, we meta-analysed a new exome sequencing sample of 4,661 cases and 5,713 controls with sequencing data from the SCHEMA Consortium (24,248 cases, 97,322 controls and 3,402 trios). We tested individual genes for enrichment of ultra-rare coding variants in cases for four variant classes, incorporating evidence from both loss-of-function and missense variants.

Our meta-analysis identified 11 genes significantly enriched for ultra-rare coding variants in schizophrenia after correction for multiple testing, including six genes previously reported by SCHEMA, two genes reported in a subsequent targeted sequencing meta-analysis, and three novel genes *(STAG1*, *ZNF136*, and *MAGEC1)*. *STAG1* has previously been implicated in schizophrenia by common variants, strongly supporting its pathogenic role in schizophrenia. *STAG1* mutations have additionally been identified as a cause of neurodevelopmental syndromes, and *MAGEC1* has previously been implicated in autism spectrum disorder.

In our study, we combined a new sample with data published by the SCHEMA Consortium to increase the total number of exome sequenced schizophrenia cases by 19%, which identified three novel schizophrenia risk genes. Our findings provide further evidence that rare and common variants in *STAG1* confer risk for schizophrenia, and that the genetics of schizophrenia overlaps with other psychiatric and neurodevelopmental disorders.

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Conflict of Interest: Sophie Chick: None declared, Valentina Escott-Price: None declared, Peter Holmans: None declared, Michael Owen Dr Rees and Professors Walters, O'Donovan, and Owen reported receiving grants from Akrivia Health outside the submitted work. Professors Walters, Owen and O'Donovan reported receiving grants from Takeda Pharmaceutical Company Ltd outside the submitted work. Takeda and Akrivia played no part in the conception, design, implementation, or interpretation of this study., Michael O'Donovan Dr Rees and Professors Walters, O'Donovan, and Owen reported receiving grants from Akrivia Health outside the submitted work. Professors Walters, Owen and O'Donovan reported receiving grants from Takeda Pharmaceutical Company Ltd outside the submitted work. Takeda and Akrivia played no part in the conception, design, implementation, or interpretation of this study., James Walters Dr Rees and Professors Walters, O'Donovan, and Owen reported receiving grants from Akrivia Health outside the submitted work. Professors Walters, Owen and O'Donovan reported receiving grants from Takeda Pharmaceutical Company Ltd outside the submitted work. Takeda and Akrivia played no part in the conception, design, implementation, or interpretation of this study., Elliott Rees Dr Rees and Professors Walters, O'Donovan, and Owen reported receiving grants from Akrivia Health outside the submitted work. Professors Walters, Owen and O'Donovan reported receiving grants from Takeda Pharmaceutical Company Ltd outside the submitted work. Takeda and Akrivia played no part in the conception, design, implementation, or interpretation of this study.

P10.090.B Recessive variants in SLC9A1 cause a syndrome of cerebellar ataxia, amelogenesis imperfecta and variable sensorineural hearing loss

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Background: The SLC9A1 gene encodes the mammalian Na + /H + exchanger isoform 1 (NHE1), a ubiquitously expressed membrane- bound enzyme involved in intracellular pH regulation. Ultra-rare recessive variants in SLC9A1 have previously been described to cause autosomal recessive spinocerebellar ataxia type 19 (SCAR19) in two families.

Design/Methods: Patient phenotyping was performed through serial clinical assessments, dental examinations, audiograms, and brain MRIs. Candidate variants were first identified by whole-exome sequencing and characterized in vitro. The expression and enzymatic activity of mutant NHE1 proteins were respectively examined by immunoblotting and transient induction with ammonium chloride of transfected NHE1-deficient cells.

Results: We identified 12 patients belonging to 8 consanguineous families with homozygous SLC9A1 variants. Eight novel variants were discovered, including two nonsense, four missense, one frameshift and one splicing variant. Patients presented with moderate to severe cerebellar ataxia from infancy associated with cerebellar atrophy (10/11; 91%) and occasional thinning of the corpus callosum (3/11; 27%) on MRI. In addition to developmental delay, all patients exhibited amelogenesis imperfecta, which had not been previously reported with SLC9A1 mutations. Sensorineural hearing loss of variable severity was present in 9 of the 12 subjects (75%). All identified variants caused lower protein expression, reduced NHE1 enzymatic activity and protein mislocalization.

Conclusions: This study expands the mutational and phenotypic spectrum of SCAR19 and provides functional evidence for the pathogenicity of the newly identified variants. Mutations in SLC9A1 should be specifically sought for in the presence of early-onset cerebellar ataxia and amelogenesis imperfecta.

Grant: None

Conflict of Interest: None declared

P10.091.C Evaluation of CNVs in Czech cohort of 400 patients with syndromic and nonsyndromic epilepsy

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Epilepsy is chronic neurological heterogeneous disorder with strong genetic component in more than half of cases. Copy

number variations (CNVs) are responsible for 2 - 11 % of cases. We present cohort of 400 patients with epilepsy, divided in four groups according to their clinical manifestation (e.g. age of onset, comorbidities, brain MRI scan result). Two aCGH platform (SurePrint G3 ISCA 4 × 180, 8 × 60; Agilent Technologies) were used for analysis.

Using the aCGH method, we have revealed the cause of epilepsy in 7,5 % patients, the most in the group with comorbid neurodevelopmental disorders, malformations and dysmorphic features. We detected 86 putative CNVs in 76/400 of patients. 41 deletions, 44 duplications and one triplication. In 8/76 patients, more than one CNV was detected. The size of aberration ranged between 13 kb to 22,8 Mb. 39/86 (45 %) CNVs were evaluated as variant of uncertain clinical significance (VUS), 24/86 (28%) were classified as pathogenic and 9/86 (10%) as likely pathogenic. Ten recurrent variants (10/86) were classified as HFLP (high frequency, low penetrant CNV). Remaining 4/86 CNVs were secondary finding. The origin of CNV was verified 46/76 families (61 %).

Unique CNVs classified as VUS have been detected and should be studied in more details in future. In some cases, subsequent analyses proved additional point mutation which suggest possible "second hit" model of ethiology of epilepsy. Regarding our results, we designed diagnostic algorithm, which can help to clinical geneticist and improve the efficiancy of genetic testing.

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Conflict of Interest: None declared

P10.092.D CGH-array analysis in 466 patients with autism spectrum disorder (ASD): from diagnosis to uncovering new neurodevelopmental disease (NDD) genes

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Background/aim: CGH-array is the first-tier genetic analysis for patients with ASD, with a diagnostic rate between 5-10%. We describe a cohort of ASD patients screened by CGH-array and explore its potential for discovering new ASD-associated genes.

Methods: we enrolled both pediatric and adult patients with ASD and CGH-array data available. We re-classified CNVs using the ACMG criteria and searched for candidate disease-associated genes by evaluating gene's role in NDDs and their scores of haploinsufficiency/triplosensitivity.

Results: among the analysed 466 ASD cases (78% males; 22% females; age 3-59 years, mean 19.2±12.6), 23 (4.9%) had pathogenic/likely pathogenic CNVs, 128 (27.5%) had VUS CNVs, 5 (1.1%) benign variants, and 310 (66.5%) were negative. Eleven patients with ACMG VUS variants, carried indeed known microduplication/microdeletion syndromes (e.g., distal del22q11.22) increasing the total number of cases with causative CNVs to 34 (7.3%). A resolutive CGH-array was more likely in patients with perinatal distress (13.8%; p = 0.01). An in-depth gene-content analysis for VUS-CNVs allowed us to highlight 27 new genes potentially associated with ASD, 11 haploinsufficient (*ACTN2, DAB1, DDX5, HEATR1, LSG1, MAGI2, PTPRT, RAC1, RBFOX1, TJP1, ZNF536*) and 16 triplosensitive (*ADARB1, CACNA1E, EP400, FGFR2, FRMD5, GATAD2, ILF2, INO80, ITCH, KMT2C, MSI2, PRPF8, PTPN1, TNRC6B, UBAP2L, UBR5*).

Discussion: CNVs can explain approximately 7% of ASD cases with an increased rate for patients with perinatal distress. ACMG criteria failed in classifying several known ASD-associated CNVs, indicating their limits especially for variants with incomplete penetrance. Finally, 27 novel ASD-candidate genes, 11 of which are in the SFARI database, deserve further investigation.

Conflict of Interest: None declared

P10.093.A Delineating the role of genetic modifiers in Parkinson's disease-associated LRRK2-G2019S mutation: a large multi-ethnic study

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Background/Objectives: Variants in Leucine-rich repeat kinase 2 (LRRK2) are the most frequently reported monogenic cause of PD. Among them, the glycine to serine substitution (G2019S) is the most common. Despite genetic homogeneity, penetrance variability is observed among carriers suggesting the influence of additional genetic variations.

Methods: We recruited the world-wide largest clinic-based cohort of LRRK2 G2019S carriers including 1486 participants from three different ethnical background (Caucasian, N = 598, North African, N = 578, Ashkenazi, N = 310). SNP array were performed on all samples followed with imputation. We used genome-wide association analysis (GWAS) for related/unrelated G2019S carriers to find out loci of interest.

Results: When using age at onset (AAO) as quantitative trait on our North African cohort, we identified two suggestive SNPs (rs1762792, pvalue = 8.64E-08; rs1360821 pvalue = 1.00E-07) by GWAS within the intergenic region between FABP5P4 and GPC6 (chromosome 13q31.3).These findings where confirmed when AAO was used as a categorical variable (dichotomised by median onset, 53 years, rs1762792 - pvalue = 1.25E-07; rs1360821 - pvalue = 2.07 E-07). Survival analysis showed that homozygotes for risk allele had a median AAO of 45 years while TT homozygotes had a median AAO of 58 years ; this suggests a protective role of the wild type allele.

Conclusion: Our data suggests that genetic variability in 13q31.3 region may influence AAO for LRRK2 G2019S parkinsonism. Further analyses are currently carried out on our other cohorts as replication studies.

Conflict of Interest: None declared

P10.094.B The changing landscape of genetic testing in paediatric neurology – A retrospective review of genetic testing in a large tertiary centre

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Background: Increasing access to investigations has allowed for effective diagnosis and treatment of paediatric neurological

conditions. This allows for positive patient outcomes but an increased workload.

Aims: To understand the landscape and outcomes of genetic testing for paediatric neurology patients in a large tertiary centre over a 5 year period. We present interim data from 2019 until July 2022 and expect to have complete data set.

Methods: The period of study ranges from Jul 2017 to July 2022. A retrospective analysis of all genetic testing from the paediatric neurology department is underway.

Results: Interim result have identified patients of three paediatric neurologists and one covering consultant working at CHI Crumlin over 3.5 years. 588 genetic investigations were ordered, representing 369 patients. 55% of patients were male and 45% female, median age was 79.1 months.

Diagnostic results (likely pathogenic or pathogenic variant) were delivered in 18.4% of patients. Of 68 diagnostic results, 84% were molecular variants and 16% cytogenetic. 81% were autosomal dominant inheritance, 12% autosomal recessive and 7% X-linked. These represented 42 different genes. Exome diagnostic yield was 28% (24/85 orders), panels 12% and microarray 5%. VUS were identified in 49 patients (17 cytogenetic & 32 molecular).

There is an increase in exome testing and decrease in single gene. Exomes accounted for 35% of all diagnostic results during that period despite accounting for 15% of all testing.

Conclusion: The study has shown an apparent decrease in testing during the COVID 19 pandemic but subsequently an increased uptake in overall testing.

Conflict of Interest: None declared

P10.095.C Reverse phenotyping of novel COL4A1 variants in fetuses with brain malformations expands the phenotypic spectrum

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Background: The spectrum of *COL4A1*-related disorders comprises small-vessel brain disease including porencephaly, variable eye defects and systemic abnormalities involving the kidneys, hemolytic anemia, muscle cramps and cerebral aneurysms. Imaging studies may reveal a spectrum of different associated phenotypes such as porencephaly, intracerebral hemorrhage, and lacunar infarctions. Heterozygous variants in *COL4A1* have been identified as the cause, that are predominantly inherited in ¾ of cases. Here we report 4 fetuses with novel *COL4A1* variants and their phenotype.

Methods: Exome sequencing was conducted in 4 unrelated fetuses with prenatally diagnosed CNS anomalies.

Result: We identified four novel heterozygous missense variants in *COL4A1* absent in publicly available databases. Of those, two fetuses were reported with multifocal cerebral ischemia and porencephaly. The other two fetuses were reported both with (semi)lobar holoprosencephaly. To our knowledge holoprosencephaly has not been associated with *COL4A1* variants.

Conclusion: Our patients with novel heterozygous missense variants in *COL4A1* extend the phenotypic spectrum of the *COL4A1*-related disorders and emphasize the power of exome sequencing in patients with brain malformation.

Conflict of Interest: None declared

P10.096.D Loss of function variants in ZEB1 cause dominant agenesis of the corpus callosum with incomplete penetrance

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The neurodevelopmental prognosis of agenesis of the corpus callosum (ACC), one of the most frequent brain malformations, varies extremely from normal development to profound intellectual disability (ID). Numerous genes are known to cause syndromic ACC with ID, whereas the genetics of ACC without ID remain poorly understood.

We describe here ZEB1, a gene previously involved in an ophthalmological condition called type 3 posterior polymorphous corneal dystrophy (PPCD3), as a new dominant gene of ACC. We report a series of nine individuals with ACC (including three fetuses) carrying a *ZEB1* heterozygous LoF variant. In five cases, the variant was inherited from a healthy parent who carried the variant at homogeneous or mosaic state, illustrating the incomplete penetrance of ACC in *ZEB1*. All patients reported normal schooling and none of them had ID. Neuropsychological assessment in six patients showed either normal functioning or heterogeneous cognitive in tested patients. Moreover, two patients had a bicornate uterus, three patients had a cardiovascular anomaly, and four had macrocephaly at birth, suggesting that the spectrum of malformations in *ZEB1*-related disorders is probably extensive. Thus, this study shows that ACC is part of an extra-ocular phenotype related to *ZEB1* and that the neurodevelopmental associated prognosis is favorable.

Conflict of Interest: None declared

P10.097.A Improving benefit-cost ratio of diagnosis amongst patients with rare movement disorders – Update from single center Ataxia Clinic

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Background: Diagnostic yield of rare movement disorders is increasing as NGS is incorporated into routine workup. We present a single center Ataxia Clinic patient cohort and demonstrate the advantages of direct WES trio amongst adult population presenting with complex neurodegenerative phenotypes.

Methods: Retrospective study of patients seen at Ataxia Clinic between the years of 2019-2022.

Results: We report 105 patients (59 female) diagnosed with ataxia at ages 4-84 years. Of the newly referred 69 ataxia patients, diagnosis was reached in 18 patients (26%), 4 (22%) of which were diagnosed using repeat expansion analysis and 14 (78%) of which were diagnosed using NGS. Diagnostic yield was increased by incorporating WES trio, as illustrated by three adult cases of autosomal recessive CA (AOA2, SPG39 and SCAR28) and a pediatric case of autosomal dominant CA (SCA29).

Spastic paraplegia type 39 (SPG39) was diagnosed in a 36 year old male of Ashkenazi Jewish ancestry presenting at 24 years of age with chronic progressive parkinsonism, spastic paraparesis and cerebellar ataxia. WES trio revealed two PNPLA6 variants, a maternally inherited pathogenic missense variant (c.4003C>T; p.Pro1335Ser), and a paternally inherited likely pathogenic missense variant (c.4003C>T;p.Gly980Ala) thus confirming diagnosis of SPG39 and enabling avoiding recurrence in future family planning.

Conclusions: Our Ataxia Clinic diagnostic yield is in accordance with current reported yield (20-30%). WES trio increases diagnostic yield significantly and should therefore be incorporated as initial workup, irrespective of patient age and specifically amongst pedigrees of common ethnic origin and complex phenotypes, improving benefit-cost ratio of diagnostic procedures.

Conflict of Interest: None declared

P10.098.B Deciphering the role of CR1 haploinsufficiency in Alzheimer´s Disease

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Alzheimer's disease (AD) is the most common neurodegenerative dementia in the elderly, with a significant socio-economic burden to the aging population. Previous studies have identified genes that increase the risk of the late onset form of the disease (LOAD), one of which is *CR1*, codes for a receptor in the complement system. A particular CR1 isoform, CR1*2, is expressed at lower levels and it is speculated it provokes lower Aβ clearance, activation of the microglial response, and neuronal death.

Here, we describe two *CR1* variants related to AD, one in familial LOAD, rs764542666, coding for p.R136* and other related to Early Onset AD (EOAD), rs55998388 coding for a truncated variant identified in this work, p.R2418Hfs54. These results would support the notion that *CR1* nonsense variants could be a cause of AD.

To explore the consequences of these changes and the physiological role of CR1 in AD, we first determined the effect in CR1 synthesis and found that mRNA and protein levels were reduced, possibly through nonsense-mediated mRNA decay (NMD). We have also generated a cellular model of neuroinflammation including neurons (SH- SY5Y), astrocytes (1321N1) and microglia (HCM3). In this system, both Aβ and lipopolysaccharide toxin (LPS) are able to activate the glial cells and to increase the expression of proteins of the complement complex. Our results also suggest that silencing of *CR1* in this model decreases neuronal viability in cells treated with Aβ, concluding that CR1 haploinsufficiency may be related to neuronal vulnerability to Aβ, both in LOAD and EOAD.

Conflict of Interest: None declared

P10.099.C Genetic variability of oxidative stress and inflammation pathways in alcohol dependence

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Background/objectives: Oxidative stress and neuroinflammation are involved in alcohol dependence. We performed a pilot study to evaluate the impact of common functional genetic polymorphisms in inflammation and oxidative stress genes on the risk of alcohol dependence and comorbid psychosymptomatology.

Methods: Pychosymptomatology was assessed in 89 hospitalized alcohol-dependent patients and 93 healthy individuals. All were genotyped for *PON1* rs705379, rs705381, rs854560, rs662, *SOD2* rs4880, *GPX1* rs1050450, *IL1β* rs1143623 rs16944, rs1071676, *IL6* rs1800795, *IL6R* rs2228145, and miR146a rs2910164. Statistical analysis was performed using the Kruskal-Wall and Mann-Whitney tests for additive and dominant genetic models.

Results: *IL6* rs1800795 CC genotype frequency was significantly higher among alcohol-dependent patients, both before and after adjustment for age, education, smoking, environment, and partnership ($p = 0.038$ and $p = 0.043$, respectively). In alcohol-dependent patients, *SOD2* rs4880 was associated with obsession and compulsion ($p = 0.016$ and $p = 0.046$, respectively), *PON1* rs705381 with social phobia ($p = 0.041$), *IL1β* rs1071676 with AUDIT scores ($p = 0.045$) and *IL6R* rs2228145 with compulsion ($p = 0.033$), In healthy individuals, *PON1* rs705381 was associated with compulsion ($p = 0.027$), *PON1* rs705379 with social phobia ($p = 0.001$), depression ($p = 0.005$), anxiety ($p = 0.002$), hostility ($p = 0.040$), and aggression, *PON1* rs854560 with obsession ($p = 0.038$), social phobia ($p = 0.018$), and anxiety ($= 0.005$), and *IL6R* rs2228145 was associated with hostility ($p = 0.014$).

Conclusions: Among the investigated polymorphisms, only *IL6* rs1800795 was found to be associated with alcohol dependence, while genetic variability in oxidative stress and inflammation pathways seems to influence the psychosymptomatology of alcohol-dependent patients and controls. Further studies with larger cohorts are needed to confirm these preliminary findings.

Grant reference: ARRS P1-0170

Conflict of Interest: None declared

P10.100.D STUB1 variants in ataxia with and without intermediate TBP repeat expansion

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Background: Pathogenic variants in *STUB1* cause both recessive (SACR16) and dominant (SCA48) forms of spinocerebellar ataxia. Because of this, difficulties may arise in the interpretation of variants, especially when the family history is unclear. Furthermore, a digenic inheritance pattern in SCA48 with intermediate repeat expansion in *TBP* (SCA17) has recently been described. Here we present 5 families with *STUB1* variants, identified during routine clinical testing.

Methods: Exome analysis of genes involved in movement disorders was performed (Agilent Sureselect XT Human All Exon V7 capture, paired-end Illumina sequencing). In addition, repeat expansion testing was performed for *ATN1, ATXN1, ATXN2, ATXN3, ATXN7, FMR1, PPP2R2B, TBP, FXN* and *CACNA1A*.

Results: Three families showed an autosomal dominant inheritance pattern of a progressive cerebellar syndrome with cognitive decline, starting between 45-60 years. In these three families, a heterozygous *STUB1* variant was identified (c.146A>G, c.256G>C and c.453+4_453+6delAGT), without intermediate *TBP* expansions. In another family, compound heterozygosity for c.433A>C and c.196C>T was found in a patient with early onset cerebellar syndrome. The patient also carried a heterozygous pathogenic repeat expansion in *FXN*. In the last family, progressive neurological symptoms (ataxia, pyramidal signs and cognitive decline) started at age 26. A heterozygous start loss (c.1A>G) was identified together with an intermediate TBP repeat expansion (n = 47).

Conclusions: These families illustrate the diverse inheritance patterns in *STUB1* related ataxia. Our data suggest that intermediate *TBP* expansions are not a requisite for developing symptoms in SCA48. Whether other genetic factors (such as *FXN* repeat expansions) are involved, needs further research.

Conflict of Interest: None declared

P11 Neuromuscular Disorders

P11.001.A Spinal muscular atrophy testing: A clinical laboratory perspective

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Spinal Muscular Atrophy (SMA) is a degenerative disease of the lower motor neurons and brain stem nuclei characterized by progressive and symmetrical muscle weakness and atrophy. Targeted treatments for SMA have emerged within the past decade, prompting the United States federal government to include SMA on the Recommended Uniform Screening Panel for Newborn Screening programs (NBS), as early diagnosis and initiation of treatment before motor neuron death increases the likelihood of positive outcomes. Given the benefits of early detection, a historical review of positive cases identified in our laboratory was undertaken to ascertain whether enhanced population screening practices outside of NBS would be beneficial. From 2016-2022, we identified 228 cases with zero copies of *SMN1* through prenatal screening, diagnostic testing, and carrier screening. Indications for prenatal screening (n = 120) included a positive family history or known parental carrier status. Approximately 90% of these pregnancies would be expected to result in more severe and earlier onset of disease, including one pregnancy in which one parent was a SNP negative silent carrier of SMA. Among postnatal cases, the reasons for testing included positive family history, known carrier parents, clinical symptoms of disease, and screening. The age of postnatal cases ranged from newborn to 63 years. Predictably, age at testing was generally correlated with *SMN2* copy number, but a surprising number of cases (13.9%; 15/108) were identified through screening. Taken together, these data suggest that broader population screening may be beneficial to identify pre-symptomatic adults and inform decisions regarding prenatal screening for reproductive partners.

Conflict of Interest: Jennifer Reiner Labcorp, Robert Pyatt Labcorp, Lynne Rosenblum Labcorp, Zena Wolf Labcorp, Winnie Xin Labcorp, Zhaoqing Zhou Labcorp, Hui Zhu Labcorp, Natalia Leach Labcorp

P11.002.B Clarifying the diagnosis: Genomic findings in a cohort with suspected multiple sclerosis

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Multiple sclerosis (MS) is a neuroinflammatory condition characterized by demyelination of the central nervous system, likely resulting from a combination of genetic and environmental factors. Symptoms of this condition are variable but include muscle weakness, ataxia, sensory dysfunction, bladder and bowel neurogenic dysfunction, and optic neuritis. Although there are no known monogenic causes of MS, exome or genome sequencing (ES or GS) may have utility in clarifying diagnosis. There are several genetic disorders that mimic the symptoms of MS, which raises the possibility of misdiagnosis. Therefore, we sought to summarize the genomic findings in a cohort of individuals with MS symptoms to determine the utility of testing in such individuals.

A total of 268 individuals with symptoms of MS underwent ES or GS from 2017-2022.

Seventeen individuals (6.3%) received a report with genetic findings related to their neurological symptoms: 3 had pathogenic (P) findings, 4 had likely pathogenic (LP) findings, and 10 had high-priority variants of uncertain significance. Two individuals (0.75%) had LP/P variants associated Charcot-Marie-Tooth disease (*MFN2, MME*) and one had an LP variant in *CSF1R*, which is associated with hereditary diffuse leukoencephalopathy with spheroids. Eight individuals (3.0%) had a LP/P variant in an ACMG secondary finding gene (*SCN5A, HFE, BRCA2, KCNQ, RYR1, PMS2, TP53*).

Here we summarize the molecular findings in a cohort of individuals with suspected MS. Our findings highlight the utility of incorporating ES/GS into clinical evaluation for patients with MS in terms of clarifying diagnosis and providing accurate recurrence risk counseling and medical management.

Conflict of Interest: None declared

P11.003.C Three heterozygous GAA cases mimicking late-onset Pompe disease

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Background: Late-onset Pompe disease (LOPD) is a recessive disorder caused by acid α-glucosidase (GAA) deficiency. Carriers of one *GAA* pathogenic variant are asymptomatic. There are several cases reported, where only one pathogenic *GAA* variant has been identified. Here, we present three unrelated cases with suspected LOPD carrying one pathogenic *GAA* variant together with other heterozygous variant(s) related to glycogen storage or structural muscle protein.

Methods: All patients were examined by a neurologist and underwent WGS (60x, PCR-free, PE150). The GAA enzyme activity was measured in dried blood spot, leukocytes and/or in fibroblasts (also used for total RNA sequencing, PE75).

Results: The phenotype of all three patients included adolescent to adult disease onset, proximal weakness and myalgia. GAA was decreased, and creatine kinase was normal to mildly elevated. WGS revealed following heterozygous phenotype-related variants: Patient 1 maternal *GAA* c.-32-13T>G (pathogenic), paternal *PHKB* (VUS) and *AMPD1* (VUS); Patient 2 *GAA* c.-32-13T>G and *AMPD1* (VUS; same variant as in Patient 1); Patient 3 *GAA* c.1082C>T p.(Pro361Leu) (pathogenic) and *FHL1* (VUS). In fibroblasts of Patient 1, the activity of GAA was 0.83 (NR 6.04-17.06) nmol/min/mg protein and RNA sequencing confirmed abnormal transcripts due to c.-32-13T>G but no other splicing defects in *GAA*.

Conclusion: There may be a small cohort of LOPD-like cases where a symptomatic heterozygosity or digenic/oligogenic inheritance can be considered.

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Conflict of Interest: None declared

P11.004.D Objective evaluation of clinical actionability for genes involved in myopathies: 63 genes with a medical value for patient care

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Background/Objectives: The generation of large amounts of mutational data though the diagnostic implementation of high-throughput diagnostic sequencing has been associated to complexification of interpretation and related long delays of genetic results. It has been important to prioritize certain analyses, particularly those of "actionable" genes in diagnostic situations, involving specific treatment and/or management. In this French national project, we carried out an objective assessment of the clinical actionability of genes involved in myopathies, for which only few data obtained methodologically exist to date.

Methods: We applied the ClinGen Actionability criteria to evaluate and score the clinical actionability of all 199 genes implicated in myopathies published by FILNEMUS for the "National French consensus on gene Lists for the diagnosis of myopathies using next generation sequencing".

Results: 63 myopathy genes were objectified as actionable based on the ClinGen Actionability criteria, with the currently available data. Among the 36 myopathy genes with the highest actionability scores, only 8 had been scored to date by ClinGen.

Conclusion: Our work presents the first systematic evaluation of clinical actionability for genes involved in myopathies. The data obtained through these methodological tools are an important resource for strategic choices in diagnostic approaches and the management of genetic myopathies. The clinical actionability of genes has to be considered as an evolving concept, in relation to progresses in disease knowledge and therapeutic approaches.

Grant References: This study was supported by the Filière nationale des Maladies Rares Neuromusculaires FILNEMUS.

Conflict of Interest: None declared

P11.005.A DEPISMA: a prefiguring newborn screening project for spinal muscular atrophy in France

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Spinal Muscular Atrophy (SMA) is a genetic disorder of the nervous system, affecting about 1 in 7000 births and mainly due to

homozygous *SMN1* gene deletion. Currently, the three available treatments all have demonstrated a much higher efficacy in presymptomatic patients than in symptomatic patients. This led several countries in Europe and worldwide to implement newborn screening (NBS) program for SMA, based on a molecular genetic analysis.

To date, NBS in France targets 13 disorders using only biochemical assays as first-tier method. In August 2022, the evolution of the national bioethics rules enabled NBS using molecular genetic test as first-line method.

The objective of DEPISMA project is to demonstrate the feasibility of SMA NBS in two large French regions, for two years. The project is intended for all the newborns in the two regions, around 110,000 births and 16 affected children expected per year. The main judgment criteria will be the exhaustiveness of the screening and the time required, for positive cases, to effective treatment initiation. Deletion detection is done by a commercial automated qPCR method.

The project, started in mid-December 2022, is currently in the launch phase with a gradual increase in inclusions. 1,158 newborns have been tested, corresponding to 88% of births. No positive case has been identified yet. An update of the results will be presented in June.

This project is a prerequisite for the generalization of SMA screening to the entire country and opens the way to other genetic screenings in France.

Conflict of Interest: Nadège Calmels Strasbourg University Hospital, French Biomedical Agency, Technical evaluator for the french comity of accreditation, Valerie Biancalana Strasbourg University Hospital, Invitation for a meeting (Perkin Elmer), Didier LACOMBE Bordeaux University Hospital, Invitation to a meeting (Biogen), Virginie Haushalter Strasbourg University Hospital, Virginie Raclet Bordeaux University Hospital, Marie-Pierre Reboul Bordeaux University Hospital, Presentation to a symposia (Novartis), Sarah Romain Strasbourg University Hospital, Caroline Stalens AFM-Téléthon, Amandine Vaidie Strasbourg University Hospital, Vincent Laugel Strasbourg University Hospital, Novartis, Roche, Biogen, AFM-Telethon, Novartis, Roche, Biogen, Pfizer, Sarepta, PTC-Therapeutics, Novartis, Roche, Biogen, Pfizer, Sarepta, PTC-Therapeutics, AFM-Telethon

P11.006.B Rapid and comprehensive diagnostic method for repeat expansion diseases using nanopore sequencing

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We developed a diagnostic method for repeat expansion diseases using a long-read sequencer to improve currently available, low throughput diagnostic methods. We employed the real-time target enrichment system of the nanopore GridION sequencer using the adaptive sampling option, in which software-based target assignment is available without prior sample enrichment, and built an analysis pipeline that prioritized the disease-causing loci. Twenty-two patients with various neurological and neuromuscular diseases, including 12 with genetically diagnosed repeat expansion diseases and 10 manifesting cerebellar ataxia, but without genetic diagnosis, were analyzed. We first sequenced the 12 molecularly diagnosed patients and accurately confirmed expanded repeats in all with uniform depth of coverage across the loci. Next, we applied our method and a conventional method to 10 molecularly undiagnosed patients. Our method corrected inaccurate diagnoses of two patients by the conventional method. Our method is superior to conventional diagnostic methods in terms of speed, accuracy, and comprehensiveness.

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Conflict of Interest: None declared

P11.008.D Exon 53 skipping of the human dystrophin transcript with different chemically modified AONs in a mouse model for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the *DMD* gene encoding for dystrophin. The absence of dystrophin results in continuous contraction-induced damage in skeletal muscle. Antisense oligonucleotide (AON)-mediated exon skipping is a promising therapeutic approach to restore dystrophin expression by reframing the disrupted open reading frame of the transcript. AONs bind the pre-mRNA dystrophin transcript which results in skipping of the target exon and leads to the production of a shorter, semi-functional dystrophin protein. AONs targeting some exons have been conditionally approved, however, treatment efficacy remains low.

To optimize AON efficiency, we assessed exon 53 skipping with 2'OMe, FRNA, LNA-2'OMe and LNA-FRNA modified AONs, all with a phosphorothioate backbone, in hDMDdel52/*mdx* mice. Mice received weekly subcutaneous injections of 50 mg/kg of AON for a duration of six weeks, starting at 4 weeks of age. All AONs were well tolerated based on plasma markers for liver and kidney function. Pronounced exon 53 skipping levels (up to 68%) were observed with the LNA-FRNA and LNA-2'OMe modified AONs in skeletal muscles and heart. No increase in dystrophin protein levels was observed, potentially due to early timepoint of sacrifice. Furthermore, the discrepancy between RNA and protein can be attributed to the strong binding nature of the LNA modification to RNA resulting in underestimation of wildtype RNA product using PCR based analyses. Our study suggests that caution should be taken when assessing AON efficiency and that both the effect on RNA and protein level should be taken into account.

Conflict of Interest: Sarah Engelbeen: None declared, Daniel O'Reilly: None declared, Davy Van De Vijver: None declared, Ingrid Verhaart: None declared, Maaike van Putten: None declared, Vignesh Hariharan: None declared, Anastasia Khvorova: None declared, Annemieke Aartsma-Rus Project funding is received from Sarepta Therapeutics., LUMC also received speaker honoraria from PTC Therapeutics, Alnylam Netherlands, Pfizer and BioMarin Pharmaceuticals and funding for contract research from Italfarmaco, Sapreme, Eisai, Galapagos, Synnaffix and Alpha Anomeric., AAR discloses being employed by LUMC which has patents on exon skipping technology, some of which has been licensed to BioMarin and subsequently sublicensed to Sarepta. As co-inventor of some of these patents AAR is entitled to a share of royalties., AAR further discloses being ad hoc consultant for PTC Therapeutics, Sarepta Therapeutics, Regenxbio, Alpha Anomeric, Lilly BioMarin Pharmaceuticals Inc., Eisai, Entrada, Takeda, Splicesense, Galapagos and Astra Zeneca. Past ad hoc consulting has occurred for: CRISPR Therapeutics, Summit PLC, Audentes Santhera, Bridge Bio, Global Guidepoint and GLG consultancy, Grunenthal, Wave

and BioClinica. AAR also reports having been a member of the Duchenne Network Steering Committee (BioMarin) and being a member of the scientific advisory boards of Eisai, hybridize therapeutics, silence therapeutics, Sarepta therapeutics. Past SAB memberships: ProQR, Philae Pharmaceuticals. Remuneration for these activities is paid to LUMC., Masad Damha: None declared

P11.009.A Detection of 5q-Spinal Muscular Atrophy by short-read next-generation sequencing - unexpected results

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Background: The importance of early diagnosis of 5q-Spinal muscular atrophy (5q-SMA) has heightened as early intervention can significantly improve clinical outcomes. In 96% of cases, 5q-SMA is caused by a homozygous deletion of *SMN1*. Around 4% of patients carry an *SMN1* deletion and a single-nucleotide-variant (SNV) on the other allele. Traditionally, diagnosis is based on MLPA to detect homozygous or heterozygous exon 7 deletions in *SMN1*. Due to high homologies within the *SMN1/SMN2* locus, sequence analysis to identify SNVs of the *SMN1* gene is unreliable by standard Sanger or short-read next-generation-sequencing (srNGS) methods.

Objective: The objective was to overcome limitations in high-throughput srNGS with the aim of providing SMA patients with a fast and reliable diagnosis to enable their timely therapy.

Methods: A bioinformatics workflow to detect homozygous *SMN1* deletions and *SMN1* SNVs on srNGS analysis was applied to a diagnostic patient cohort undergoing whole exome and panel testing for suggested neuromuscular disorders. SNVs were detected by aligning sequencing reads from *SMN1* and *SMN2* to an *SMN1* reference sequence. Homozygous *SMN1* deletions were identified by filtering sequence reads for the „gene-determining variant" (GDV).

Results: 10 patients were diagnosed with 5q-SMA based on (i) *SMN1* deletion and hemizygous SNV (2 patients), (ii) homozygous *SMN1* deletion (6 patients), and (iii) compound heterozygous SNVs in *SMN1* (2 patients).

Conclusions: Applying our workflow in srNGS-based panel and whole exome sequencing (WES) is crucial in a clinical laboratory, as otherwise patients with an atypical clinical presentation initially not suspected to suffer from SMA remain undiagnosed.

Conflict of Interest: None declared

P11.010.B Zebrafish models of complex hereditary spastic paraplegia caused by variants in the Kennedy pathway gene, PCYT2

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Introduction: PCYT2 is the rate-limiting enzyme in the Kennedy pathway, responsible for production of the majority of phosphatidylethanolamine (PE) which accounts for 45% of the phospholipid content in the brain. Bi-allelic hypomorphic *PCYT2* variants cause complex progressive hereditary spastic paraplegia type 82 (SPG82). Patient fibroblasts have reduced levels of phosphatidylcholine (PC) and PE but have increased levels of O-PCs. However, the underlying mechanisms of this disorder are poorly understood.

Methods: CRISPR-Cas9 and gRNAs targeting exons 3 and 13 of *pcyt2* were used to create zebrafish models. Survival analysis, body lengths and head size measurements, and movement tracking using Daniovision were done in F2 animals. Untargeted lipidomics by supercritical fluid chromatography coupled to electrospray ionisation time-of-flight mass spectrometry was performed in F0 embryos.

Results: We created two zebrafish models, one null ($pcyt2^{ex3}$) and one hypomorphic ($pcyt2^{ex13}$). Survival up to 5 days post-fertilisation was not affected. Homozygous larvae had significantly smaller body lengths, head sizes and impaired mobility than the heterozygous and wild-type siblings. $pcyt2^{ex3}$ knockout zebrafish larvae were more severely affected than $pcyt2^{ex13}$. Lipidomic analysis showed significant reduction in PC and PE species and significant increase in ether PC species.

Conclusion: We have created two *pcyt2* zebrafish models that recapitulate the clinical and the lipidomic phenotypes of SPG82. These models can be used to study the pathophysiology and to test treatments for this disease.

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Conflict of Interest: None declared

P11.011.C Whole exome sequencing in Bulgarian patients with late-onset hereditary ataxia expands the genetic diversity of the disease

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Objectives: Hereditary ataxias are a group of neurodegenerative disorders characterized clinically by slowly progressive gait incoordination often associated with poor coordination of hands, speech, and eye movements. Late-onset forms are extensively studied during the last years exhibiting considerable genetic overlap with other neuromuscular disorders. In about 10% of the cases, the genetic defects represent expansions of dynamic repeats in the SCA 1, 2, 3, 6, 7, 8, 10, 12, 17, and FRDA loci.

Methods: We performed whole exome sequencing in 50 patients with a clinical diagnosis of late-onset spinocerebellar ataxia in whom the most common forms due to expansion repeats had been excluded beforehand. Analysis of variants was carried out using a broad ataxia-gene panel including genes for other neuromuscular disorders.

Results: We succeed to identify disease-causing or potentially pathogenic variants in more than 50% of the cases. However, with the well-defined clinical picture of late-onset spinocerebellar

ataxia in some cases we found variants in genes causing early-onset phenotype as well, as variants in genes currently associated with other neuromuscular disorders or in novel candidate genes.

Conclusion: Our results not only confirmed the genetic overlap between ataxias and other neuromuscular disorders but brought to light other genes that might be involved in the molecular pathogenesis of hereditary ataxias. They suggest that we have to reconsider the genotype-phenotype correlations in hereditary ataxias in light of new molecular genetic data.

Grant References: MU-Sofia: D-216/15.12.2021; MES: D01-395/18.12.2020; D01-278/14.12.2022; D01-302/17.12.2021; D01-165/28.07.2022.

Conflict of Interest: None declared

P11.012.D Statins Induce myopathy phenotypes in Drosophila melanogaster reminiscent of human myopathy: Evidence for the role of chloride channel inhibition in myofibrillar damage

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Background: Statins are the first-line therapy for the management of cardiovascular diseases, yet statin-induced myopathy (SIM) hinders patients' adherence to the treatment. The aetiology of SIM remains debated. In the present study, we employ *Drosophila melanogaster* to scrutinize underlying mechanisms for SIM.

Methods: We used UAS-GAL4 to knock down the gene of interest, *Drosophila* locomotion monitoring system (DAMS), transmission electron microscopy (TEM) and as well as qPCR and western blot.

Results: Chronic fluvastatin treatment for 5 days induced significantly lower general locomotion and climbing ability in *Drosophila*. Additionally, TEM of dissected skeletal muscles of fluvastatin-treated flies reveals severe myofibrillar damage, including longer sarcomere and Z-line streaming, reminiscent of human myopathy, along with fragmented mitochondria that were more round-shaped and larger compared with controls. Mechanistically, selective *Hmgcr* knockdown in the skeletal muscle recapitulates fluvastatin-induced lowered locomotion and mitochondria phenotypes, but it didn't affect sarcomere length or cause noticeable myofibrils damage in comparison with the control groups or fluvastatin treatment. Additionally, chronic fluvastatin treatment induced lowered expression of the chloride channel, ClC-a (*Drosophila* homolog of CLCN1). Intriguingly, selective knockdown of *ClC-a* in skeletal muscle recapitulates fluvastatin-induced lowered climbing ability and myofibril damage, including longer sarcomere and Z-line streaming. Surprisingly, exercising fluvastatin-treated flies for 6 hours reinstated ClC-a expression and normalized sarcomere lengths, indicating that fluvastatin-induced sarcomere change is perhaps linked to muscular ClC-a inhibition (Al-Sabri et al.,2022).

Conclusion: These results may underline the role of skeletal muscle chloride channel inhibition in SIM.

Grant Reference: Swedish Research Council:2019-01066 Al-Sabri et al.,'Statins-Induce-Locomotion-and-Muscular-Phenotypes-in-Drosophila-Melanogaster-That-Are-Reminiscent-of-Human-Myopathy' Cells.2022,Nov 8;11(22):3528.

Conflict of Interest: None declared

P11.013.A Functional characterization suggests that ACTN2 frameshift variants cause distal myopathy through protein aggregation

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ACTN2 encodes alpha-actinin-2, an important sarcomere structural protein that links actin and titin to the sarcomere Z-disk. Variants in *ACTN2* have previously been associated with cardiomyopathy, but recently also with skeletal distal myopathy phenotype. In *ACTN2*-related diseases, actininopathies, several variants have been identified as disease-causing, however, new variants are continuously discovered, and the significance of many variants remain unknown. Thus, lack of clear genotype-phenotype correlations in actininopathies persists. Further, the molecular mechanisms underlying actininopathies are largely unknown.

Here, functional characterization in C2C12 cell models of several *ACTN2* variants is conducted, including frameshift and missense variants associated with dominant and recessive distal myopathies. The results show that the variants associated with a recessive form of actininopathy do not cause detectable alpha-actinin-2 aggregates in the cell model. Thus, alternate methods should be explored to investigate the molecular mechanisms of these recessive actininopathies. Conversely, the results show that the frameshift variants causing a protein extension do produce alpha-actinin-2 aggregates in the cell model, indicating that the aggregation is a molecular mechanism contributing to the disease phenotype associated with these variants.

Together, the results suggest that alpha-actinin-2 aggregation is the disease mechanism underlying some actininopathies, however, this disease mechanism is likely associated with only a limited number of variants. Alternative methods of functional characterization should be explored to further investigate other molecular mechanisms underlying actininopathies.

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Conflict of Interest: None declared

P11.014.B Longitudinal analysis of CTG repeat somatic instability in myotonic dystrophy type 1 patients

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Background/Objectives: Myotonic dystrophy type 1 (DM1) is the most common adult muscular dystrophy caused by CTG repeat expansion in *DMPK* gene. DM1 mutation shows tissue-specific instability with continuous change in repeat number, contributing to disease progression. We aimed at increasing the knowledge of change in CTG expansion size over time.

Methods: Twenty-five patients with two blood samples available in an interval >5 years, absence of repeat interruptions and congenital form were selected from the Serbian DM registry. We used single-molecule small-pool PCR with 30–60 pg of DNA and sized >150 alleles per sample per time point. Allele frequency distributions were compared for each patient using Wilcoxon–Mann–Whitney test and described by kurtosis.

Results: In 24 patients significant difference in distribution over time was found (p for individual patient ≤0.026), with modal allele change biased towards further expansion. When comparing 10th percentile of the distribution, 2 patients showed decrease and 14 an increase of >50 repeats over time. Three patients sampled at the age of 44-50 and having expansion size range of 450-1600 repeats showed a wide distribution in both time points (kurtosis <3). In 67-year-old patient with 170-730 repeats, we observed an equal shift in 10th percentile and modal allele size (~250 repeats) with no change in 90th percentile.

Conclusions: Although our longitudinal analysis confirmed that DM1 mutation continues to expand throughout patients' lifetime, individual differences are observed and require further research.

Grant references: Research supported by the Science Fund of the Republic of Serbia, #7754217, READ-DM1.

Conflict of Interest: None declared

P11.015.C IMAGINE Study: Single gene disorder identified in half of children with complex presentations that include cerebral palsy (CP)

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Background/Objectives: A genetic cause of major effect explains the etiology in many children with cerebral palsy (CP), but genetic testing is infrequently done in such patients.

Methods: Whole Genome sequencing (WGS) was performed on 100 trios or quads with one or more children affected by CP that was complicated by features suggesting a genetic etiology, such as congenital anomalies, multisystem disease, or multiple affected family members. We used a custom bioinformatics pipeline to identify SNVs, indels, SVs, and mitochondrial variants. Genotype-phenotype correlation and clinical interpretation of variants were performed by a multi-disciplinary team. Findings were confirmed with targeted clinical testing and returned to participants.

Results: We studied a total of 109 children (101, counting similarly affected sibs only once) in 100 families. Genetic variants that definitely or probably caused the CP were found in 49 cases, and VUS in 10 cases. WGS was uninformative in 40 cases. In two cases, variants found are still under investigation. Importantly, the diagnostic rate was lower but still substantial among 30 cases with previously uninformative exome sequencing. In this group, 8 had definitely or probably causal variants, 8 had VUS, and 14 remained uninformative on WGS.

Conclusions: Given the high yield in this cohort, diagnostic sequencing is indicated for all patients presenting with CP as part of a complex medical picture or in the context of a positive family history. Determining a diagnosis allows for improved clinical care and accurate genetic counselling for affected patients and their families.

Grant References: CHILDBRIGHT Network

Conflict of Interest: Colleen Guimond University of British Columbia, Collaborator, Patricia Birch University of British Columbia, Collaborator, Madeline Couse University of British Columbia, Anna Lehman University of British Columbia, Co PI, Jill Mwenifumbo BC Children's Hospital, Clara van Karnebeek University of Amsterdam, Co-investigator, Jan Friedman University of British Columbia, Principal investigator

P11.016.D Preliminary report of the DMD twins analysis

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Introduction: Natural history of Duchenne muscular dystrophy (DMD) is well known – it progressively leads to muscular weakness, loss of ambulation, and cardiopulmonary failure. But, only single cases of DMD twins have been described in the literature.

Patients and methods: Rare Disease Centre in Gdansk, Poland, takes care of 120 DMD patients, among whom: 3 pairs of twins with mutations (No1,2&3; aged 5,8 and 11yrs), one couple of twins (No4), where only one brother is affected, and couple of twins (No5): boy and a girl, both with DMD. Medical history and physical examination was performed with clinical diagnostics including PFTs, ECHO, AbdoUSS, DXA, ophtalmological, endocrinology and psychology consultation with laboratory tests. Multiple STR marker analysis of the zygosity pattern results of the twins are pending.

Results: Majority of the results didn't show significant differences between siblings – small diversity included weight, BMI, PFTs, and cholesterol level. Interestingly, the girl from the twin couple No5 probably is a manifesting carrier – she had increased activity of LFTs and CK, but due to young age (4 years) it is difficult to fully evaluate and predict the DMD progression in the future. Her karyotype is normal (46,XX), there is skewed X-inactivation pattern present.

Conclusions: Our analysis is a primary report of the DMD twins natural study. It may enrich the knowledge about the effect of the social environment on the course of DMD. It requires further research and evaluation on a larger number of subjects, preferably in the framework of an international project

Conflict of Interest: None declared

P11.017.A Unravelling a familial case of DOK7 congenital myasthenic syndrome by analyzing RNA in patients' cultured cells

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Background/Objectives : Congenital myasthenic syndromes are characterized by impaired neuromuscular transmission inducing muscle weakness. *DOK7* encodes for an adaptor protein that is crucial to form and maintain neuromuscular synapses. Bi-allelic *DOK7* mutations are found in 10-15% of congenital myasthenic syndromes. Here, we present a family with two brothers diagnosed with congenital bilateral vocal cord palsy who experienced stridor and respiratory distress during the neonatal period. The oldest brother presented later with muscle weakness.

Methods : A quatuor-based whole exome sequencing (WES) followed by RNA analysis in EBV-transformed patients' cells were performed.

Results : A pathogenic (class 5) heterozygous variant was found by WES in both brothers and their father in the *DOK7* gene (c.1263dup p.(Ser422Leufs*97), NM_173660.5). No other variant was found in this gene by WES. RNA analysis highlighted a 88 bp-insertion between exons 3 and 4 corresponding to a sequence within intron 3 and predicted to induce a frameshift in both bothers and their mother. A long-range PCR of intron 3 on genomic DNA of these three individuals, followed by Sanger sequencing, revealed the presence of the substitution c.331+347A>T predicted to introduce a cryptic donor splice site which could trigger the retention of a part of intron 3 in the mRNA. This rare variant (0.0007% in gnomAD v3.1.2) was classified as potentially pathogenic (class 4).

Conclusion : The use of patients' cultured cells to study RNA allowed us to solve a familial case of *DOK7* congenital myasthenic syndrome for which only one pathogenic variant had been identified by WES.

Conflict of Interest: None declared

P11.018.B Ehlers-Danlos Syndrome/Rectus Femoris myopathy: a novel phenotype associated with COL12A1 mutations

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A hybrid phenotype, combining connective and muscle tissue abnormalities, has been recently associated with *COL12A1* variants, referred as myopathic-Ehlers-Danlos-Syndrome (mEDS). To date only 17 families have been described, all but one carrying monoallelic mutation in *COL12A1*. Here we enlarge this cohort of patients and focus on a characteristic sign associated with this novel phenotype. Our observation started from three families characterized by soft skin, scoliosis, joint hyperlaxity/hypermobility with recurring dislocations, axial/proximal/distal muscle hypotrophy. All these families were characterized by rectus-femoris atrophy. Whole Exome Sequencing (WES) detected two splicing variants, c.8415+1G>A and c.8319+1G>T (NM_004370.6) in *COL12A1* gene, in two of analysed families. RNA analysis confirmed the involvement of both variants in the skipping of exon 56 and 55, respectively, resulting in an in-frame deletion in the COL2 protein domain and a dysregulation of COL12A1 cellular localization. In 2018 Nanna Witting et al. also reported the association between a splicing variant in *COL12A1*, causing skipping of exon 52, and mEDS with rectus femoris atrophy. In our third family, WES revealed the already published pathogenic variant c.3309G>A, p.Pro1103= (NM_000393.5) in *COL5A2*, reported to potentially cause skipping of the exon 46. Interestingly, String analysis showed strong association between COL12A1 and COL5A2. Ongoing study on patient fibroblasts could reveal if there was a dysregulation in COL12A1 as consequence of *COL5A2* mutation, thus speculating that patient phenotype is due to aberrant expression of COL12A1.These data could improve the characterization of the pathogenic phenotype associates with COL12A1 dysfunction, thus defining a novel syndrome, the Ehlers-Danlos/Rectus Femoris myopathy.

Conflict of Interest: None declared

P11.019.C Biallelic SORD mutations: frequency and associated phenotypes in a cohort of previously genetically unconfirmed Charcot-Marie-Tooth disease

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Biallelic *SORD* mutations were recently identified as a novel cause for autosomal-recessive CMT type 2 (OMIM# 618912). Clinically, its associated disease is mainly characterized by progressive distal limb muscle atrophy and weakness. *SORD* codes for the enzyme sorbitol dehydrogenase, which together with aldose reductase converts glucose to fructose via sorbitol, indicating potential for the study of possible disease specific-treatments such as aldose reductase inhibitors.

We retrospectively screened 166 patients with predominantly axonal neuropathy without identified genetic etiology for possible pathogenic *SORD* mutations by next-generation sequencing and Sanger sequencing to specifically amplify *SORD* and not the pseudogene *SORD2P*. Clinical and electrophysiology exam findings were analysed for genotype-phenotype correlation.

Five patients harbored pathogenic *SORD* mutations (3%) with the most common homozygous pathogenic frameshift variant c.757delG (p.Ala253Glnfs*27) present in four (ClinVar# 929258). One case carried this variant and the pathogenic missense variant c.458C>A (p.Ala153Asp) in a compound heterozygous state (ClinVar# 1012846). Age of onset ranged from early infancy to mid twenties, and clinical and electrophysiological findings comprised signs of slowly progressive distal weakness with predominantly axonal neuropathy in all identified cases.

In conclusion, we confirmed *SORD* mutations as causative for a small subset of prior genetically unconfirmed axonal CMT in our cohort. Our findings thus strengthen the concept that screening of the *SORD* gene should be performed in patients with genetically unconfirmed CMT, especially axonal CMT. We plan to investigate the possibility that *SORD*-associated hereditary neuropathy could be treated with pharmacological inhibition of aldose reductase and are currently expanding our cohort.

Conflict of Interest: None declared

P11.020.D Bi-allelic loss-of-function variants of FILIP1 encoding a filamin A binding protein cause autosomal recessive arthrogryposis multiplex congenita with microcephaly

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Background/Objectives: Arthrogryposis multiplex congenita (AMC) refers to a broad group of clinically and etiologically heterogeneous congenital conditions characterized by joint contractures in at least two body areas. Until now more than 400 genes have been associated with arthrogryposis. Still, the molecular pathogenesis and the underlying genetic cause remains unclear in a large number of cases.

Methods: We performed a detailed clinical characterization of 5 patients from three independent families presenting with an overlapping arthrogryposis multiplex congenita spectrum phenotype characterized by multiple joint contractures, scoliosis, reduced palmar and plantar skin folds, microcephaly, and facial dysmorphisms, and we used whole-exome sequencing to identify causative variants in these individuals.

Results: We were able to identify bi-allelic loss-of-function variants in the filamin-A-interacting-protein 1 (FILIP1) gene in all affected individuals. FILIP1 is a regulator of filamin homeostasis and plays an important role in the initiation of cortical cell migration in the developing neocortex as well as in differentiation processes in cross-striated muscle cells during myogenesis.

Conclusion: In summary, our finding that bi-allelic, loss-of-function variants in FILIP1 cause a novel, autosomal recessive form of arthrogryposis multiplex congenita associated with microcephaly expands the spectrum of genetic factors related to arthrogryposis.

Conflict of Interest: None declared

P11.021.A A recurrent ACTA1 amino acid change – mosaic form causes milder asymmetric myopathy

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Background: Mutations in the skeletal muscle alpha-actin gene (*ACTA1*) are the second most common cause of nemaline myopathy. Previously, a number of families with confirmed somatic or inferred gonadal mosaicism for *ACTA1* variants have been described. Here, we present three new patients with somatic mosaicism for variants leading to the same amino acid change p.(Gly247Arg) and milder phenotypes than that of a fourth patient with the same amino acid change in heterozygous form.

Methods: The variants were identified using neuromuscular gene panels and subsequently verified by Sanger sequencing. Droplet digital PCR mutation detection assays were used to verify the mosaicism.

Results: All three patients with mosaicism (variant allele frequencies ca. 20%, 30%, and 35%) showed milder phenotypes, muscle weakness, course of the disease, and abnormalities on muscle biopsy than the patient carrying the variant in heterozygous form. Our results indicate, that the grade of mosaicism may correlate with the severity of the phenotype. Furthermore, the muscle weakness and body growth in the three mosaic patients were asymmetric, likely due to their mosaicism.

Conclusion: Low-grade mosaic variants may escape monogenic and oligogenic disease panels, as they are optimized for detecting heterozygous and homozygous variants. We suggest that in cases where mosaicism is suspected (e.g. based on an asymmetric phenotype) but no causative variant has been found, data should be reanalyzed using adjusted pipelines.

Grant References: Folkhälsan Research Foundation (101003), Svenska Kulturfonden (157710), Jane and Aatos Erkko Foundation

Conflict of Interest: None declared

P11.022.B Discovering novel repeat expansions in hereditary ataxia patients

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Background: Repeat expansions (REs) underlie over 40 genetic disorders. Finding novel REs in short read sequencing data has proven to be a challenging task due to their size and the lack of a template in the reference genome. Tools such as Expansion Hunter De Novo have been developed to overcome these

difficulties. These softwares are however limited in their ability to eliminate the high degree of false positives due to the abundance of benign polymorphic repeats in the human genome. Here we present the software motif sub-read counter (MFSC) which is designed to take regions nominated by the initial round of de novo discovery and filter false positives out and provide a distilled list of the candidates that are most likely to be pathogenic repeats.

Methods: MFSC counts the occurrence of motifs in the nominated loci across the list of BAM files to perform a Mann-Whitney U-test to compare between those labelled as cases and controls.

Results: Using our own in-house data we perform de novo motif discovery in Ataxia patients. We reproduce known REs and putative novel ones.

Conclusions: This rudimentary approach complements the more elaborate analyses used for the initial process of discovery in that it narrows down the more likely candidates in that it deals directly with the prevalence of a nominated motif as opposed to any other proxy data.

Grants: UKRI grant number MR/S006753/1

Conflict of Interest: None declared

P11.023.C Genetic screening of Hungarian patients with focal dystonia identified several novel putative pathogenic gene variants

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Introduction: Dystonia is a rare movement disorder characterized by sustained or intermittent muscle contractions causing abnormal and often repetitive movements, postures or both. From a genetic point of view, dystonia can be considered a very diverse disease. Although the number of known monogenic forms of dystonia is constantly increasing, genetic cohort studies performed on these patients yield very few relevant results. The purpose of our work was to screen a Hungarian patient subpopulation with the two most common forms of adult-onset focal dystonia, cervical dystonia (CD) and benign essential blepharospasm (BSP).

Methods: 133 unrelated patients diagnosed with dystonia were recruited for this study. For the screening of variants associated with their disease, next generation panel sequencing (24 dystonia related genes, selection based on literature) was used for 71 patients with CD and 44 patients with BSP and whole exome sequencing was used in 18 cases. Copy number variation analysis of the sequencing was also performed by bioinformatic assessment of the NGS data.

Results: We have identified several already known dystonia-related genetic variants and several putative pathogenic novel ones. To assess the pathogenicity of the individual variants, family screening and extensive clinical examinations, including paraclinical measures (e.g., magnetic resonance imaging), were performed in individual cases.

Conclusion: With our study, we provide a genotype-phenotype correlation of the Hungarian dystonia population from a population-specific aspect. The importance of the investigation is emphasized by the fact that to our knowledge no similar study has been carried out in any Central-European dystonia population yet.

Conflict of Interest: None declared

P11.024.D Identifying the biological role of anoctamin 10 protein

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Introduction and Objectives: Anoctamin 10 (ANO10) is an endoplasmic reticulum-resident protein with scrambling and channel activity. ANO10 was found to be associated with acetylated tubulin of spindles in mouse macrophages, while defects in the *ANO10* ortholog in Drosophila, *Axs*, were found to cause abnormal spindle assembly and chromosome segregation. These findings suggest implication of ANO10 in spindle formation and cell cycle progression. Variants in the *ANO10* gene are linked to a rare type of autosomal recessive spinocerebellar ataxia (SCAR10), probably mediated by degeneration of Purkinje cells in the cerebellum due to ANO10 defects. The aim of this study is to further uncover the biological role of human ANO10, and especially, to investigate the effects of ANO10 depletion at the cell division level.

Methodology: Immunofluorescence microscopy was performed to assess ANO10 localization in SH-SY5Y and U2OS cells. *ANO10* silencing using RNAi technology, followed by validation, was employed to resemble and study the effects of a pathogenic deletion identified in three Cypriots with SCAR10 phenotype.

Results: ANO10 was found to localize at the centrosomes of mitotic and non-mitotic cells, and at the ER in agreement with previous studies. Transfection of cells with siRNA targeting *ANO10* mRNA resulted in a significant reduction in the expression of both gene and protein levels.

Discussion: Centrosomic localization of ANO10 indicates a potential role of the protein in cell division. The effects of *ANO10* silencing are currently being investigated to further characterize the protein and delineate its role in the cell cycle, cell growth and ciliogenesis.

Conflict of Interest: None declared

P11.025.A ENTPD1-related syndromic spastic paraplegia – two brothers with strikingly distinct phenotype severity

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Context: Hereditary spastic paraplegia is a genetically heterogeneous entity associated with variants in more than 80 different genes. *ENTPD1*-related phenotype was recently widened by Calame D. et al (2022) to a complex form of an autosomal recessive syndrome that is characterized by early onset and progressive spastic paraplegia, intellectual disability, dysarthria, and white matter abnormalities.

Case Report: We report a 32-year-old man, wheelchair-bound, who presented with early childhood-onset and progressive spastic tetraparesia, ataxia and lower limbs muscle atrophy, severe intellectual disability with severe speech impairment, significative behavioural problems, epilepsy, and mild non-specific dysmorphisms. Molecular studies identified the splicing

variant (NM_001776.6):c.574-6_574-3del, in homozygosity, in *ENTPD1* gene, previously described but initially classified as a VUS. RNA studies demonstrated the absence of exon 6 confirming that it causes exon skipping (Calame et al, 2022). Noteworthy, his younger brother (21 years old), who also carries the same variant in homozygosity, presents a similar but much milder phenotype – non progressive mild spasticity with independent walking, mild-moderate intellectual disability with understandable speech, partially autonomous in daily activities, and behavioural problems.

Conclusion: This family, in here described in detail, contributes to widen the genotype and phenotype in *ENTPD1*-related syndrome. It also points to the possibility of significant intra-familial phenotypic variability, although further studies are needed to comprehensively understand which factors could contribute to this. A second disease hypothesis is also being considered for the older brother. This knowledge would help clinicians to establish a more accurate prognosis in patients with *ENTPD1*-related spastic paraplegia.

Conflict of Interest: None declared

P11.027.C Genetic background of Czech myopathy patients

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Background: According to the Gene Table of Neuromuscular Disorders (www.musclegenetable.fr), 1,173 neuromuscular diseases and 658 associated genes are currently known. Individual diseases are divided into 16 disease groups, three of which represent (1) congenital myopathies, (2) distal myopathies, and (3) other myopathies. In total, 90 genes are included in these three groups. Our study presents an overview of molecular characteristics of a large cohort of Czech neuromuscular patients with clinically and genetically confirmed diagnosis of congenital/distal/other myopathy.

Methods: Custom capture panel sequencing and/or whole exome sequencing; bioinformatic analysis detecting small-scale gene variants and large gene deletions/duplications (by analysing copy number variations).

Results: 92 patients from our cohort have gene defects and phenotypes associated with congenital myopathy (CM), 26 patients with distal or other myopathy. As regards CM, the myopathy related to the *RYR1* gene was the most common form (35 patients), followed by myopathy associated with *ACTA1* (13 patients), *MTM1* (10 patients), *NEB* (7 patients), *SELENON* (7 patients) and other genes (*CFL2*, *DNM2*, *KBTBD13*, *LMOD3*, *MYH7*, *SCN4A*, *TPM2*, *TPM3*, *TTN*).

Conclusion: We present 118 unrelated patients in whom pathogenic/likely pathogenic variants have been identified. In addition to a spectrum of affected genes, we present unique clinical and genetic findings, like recessive CM associated with pathogenic variants in genes previously associated with dominant disease (and vice versa), and cases of CM presenting in utero.

Grant References: This work was supported by funds from AZV - Czech Health Research Council (NU21-06-00363).

Conflict of Interest: None declared

P11.028.D A recurrent missense mutation in TUBA4A causes spastic ataxia

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Background: Several neurodevelopmental and, less frequently, neurodegenerative disorders are caused by mutations in genes encoding for different isotypes of α- and β-tubulins, the structural components of microtubules. However, little is known about the functional requirements of tubulins or how mutations in tubulin genes cause cell-specific pathologies. We performed an in-depth study of a multigenerational family with an autosomal dominant form of spastic ataxia to investigate the underlying genetic cause.

Methods: Blood samples from 11 patients with spastic ataxia and three unaffected individuals from our family were collected. We performed whole exome sequencing (WES) on eight affected patients, and Sanger sequencing on the remaining affected patients and unaffected family members to confirm variant segregation. We also performed TRIO-WES in a second family enrolled from the Fondazione Stella Maris Institute, in which the proband showed the same undescribed phenotype characterized by cerebellar and pyramidal signs.

Results: In all affected individuals from both families, we found a novel missense mutation, p.Glu415Lys, in *TUBA4A* (NM_006000) gene. *TUBA4A* encodes for α-tubulin, a major component of the microtubule network. The variant resulted deleterious from prediction tools, is not currently reported in public databases, and segregates with the disease.

Conclusion: To date, variants in *TUBA4A* have been proposed as a rare genetic cause of amyotrophic lateral sclerosis and reported in patients with frontotemporal dementia. Our findings widen the phenotypical and genetic manifestations of *TUBA4A* variants and add a new gene to investigate in the differential diagnosis of spastic ataxia.

Conflict of Interest: None declared

P11.029.A A synonymous codon change in the DYSF gene alters mRNA splicing and causes autosomal recessive limb girdle muscular dystrophy type 2B

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Background: Clinical genomic testing produces many variants of uncertain significance (VUS). Without appropriate functional studies the status of these variants remains uncertain. This is the case for 60% of variants in ClinVar. Here we report the utilization of RNA-Seq to reclassify such variants.

Methods: Two unrelated cases presented with predominant proximal weakness of lower limbs and upper limbs. Both having difficulty in walking tip toes with bilateral calf atrophy. Clinical, biochemical, and radiological examination suggested Dysferlinopathy. Exome sequencing had revealed a homozygous synonymous variant [NC_000002.12(NM_003494.4):c.2889C>T] in DYSF gene with predicted aberrant splicing in both individuals. RNA was extracted from peripheral blood along with two age matched controls. Stranded mRNA sequencing was performed to ascertain the effect of the rare variant on splicing after appropriate consenting process.

Result: The variant is predicted to introduce a novel donor splice site by splice site algorithms, resulting in an activation of a new cryptic donor site 38 nucleotides upstream of the consensus 3′ donor site. This is predicted to result in shifting of the reading frame and appearance of a premature termination codon (PTC) 9 codons downstream and theoretically many more PTCs further downstream. Stranded mRNA sequencing analysis showed extreme low expression of the DYSF gene as compared to the controls suggesting nonsense mediated decay due to the above-mentioned effects.

Conclusion: Our study reveals that the previously reported synonymous variant of uncertain significance in DYSF, affects RNA splicing. It emphasises the critical role of transcriptome analysis of uncertain variants in improving molecular diagnosis.

Conflict of Interest: None declared

P11.030.B Identification of potential genetic modifiers underlying phenotypic variability in a French family with striated muscle laminopathies

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Background: *LMNA* gene mutations are responsible for a wide spectrum of disorders called laminopathies, the majority of which affecting striated muscles. Among them, Emery-Dreifuss muscular dystrophy (EDMD) and Limb-Girdle muscular type 1B (LGMD1B) show skeletal muscle involvement of different severity but share the same cardiac involvement, i.e., dilated cardiomyopathy with conduction system disease (DCM-CD) that can also be present in an isolated manner. Clinical heterogeneity is well known among the *LMNA* mutation carriers. Modifier genes have been suggested to explain such variability. The *LMNA* mutation p.Gln6*, identified in a large French family (EMD1), is associated with a wide range of age at onset of myopathic symptoms (AOMS). According to this latter, three phenotypic subgroups have been described within the family: before 20 years (early AOMS), after 30 years (late AOMS) and isolated cardiac disease without musculo-skeletal symptoms. Our objective was to identify genetic modifiers underlying the intrafamilial phenotypic variability within EMD1 family.

Method: Whole genome sequencing (WGS) was performed in 16 *LMNA*-mutation carriers exhibiting the 3 phenotypic subgroups in EMD1 family.

Results: We identified 2 splice variants with a potential aggravating effect, under functional validation. Moreover, 4 structural variants have been detected only in early AOMS patients. An identity by descent analysis specific to phenotypic subgroups was performed and identified one region shared on chromosome 1, containing the *LMNA* gene.

Conclusion: Our results suggests that a single genetic modifier may not be solely responsible for phenotypic variability in this family, but that a combination of several factors is more likely.

Conflict of Interest: None declared

P11.031.C TAF8 has a crucial role in CNS development

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Background: *TAF8* is part of the transcription factor TFIID complex that consists of TBP and TBP-associated factors. TFIID is crucial for recruiting the transcription factor complex containing RNA polymerase II. TAF8 deficiency was recently reported for the first time as causing a severe neurodevelopmental disorder in eight patients.

Methods: We have ascertained three family related Arab Muslim couples who came for genetic counseling due to brain malformations discovered on prenatal ultrasound of current pregnancies including massive cerebellar atrophy, microcephaly, cerebellar anomalies, and corpus callosum anomalies. Clinical, pathological, imaging, biochemical and molecular analyses were performed.

Results: Four pregnancies were terminated and the fetuses were found to carry a novel likely pathogenic homozygous variant (c. 45 + 5 G>A) in *TAF8*, that affect splicing. Segregation analysis supported autosomal recessive inheritance with no healthy homozygous family members. WB analysis in fetuses' fibroblasts demonstrated a significant reduction of TAF8 protein level.

Prenatal MRI studies of two of fetuses show microcephaly small vermis abnormal sulcation pattern with malformation and shortening of corpus callosum.

Post-mortem examinations confirmed microcephaly, hypoplastic cerebellum and incomplete corpus callosum in two fetuses and dysmorphic features including hypertelorism, wide nasal bridge, clinodactyly and hirsutism were present.

Conclusions: We report here for the first time the fetal representation of TAF8 deficiency. Our study might contribute to understanding the role of TAF8 in the development of the fetal brain, and how its absence affects the transcriptome and proteome in the early developmental stages in humans.

Conflict of Interest: None declared

P11.032.D Genome sequencing with comprehensive variant calling identifies structural variants and repeat expansions in a large fraction of individuals with neuromuscular disorders

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Introduction: Neuromuscular disorders (NMDs) have a heterogeneous etiology. A genetic diagnosis is key to personalized

healthcare and access to targeted treatment for the affected individuals.

Methods: In this study, 861 patients with NMDs were analyzed with genome sequencing and comprehensive variant calling including single nucleotide variants, small insertions/deletions (SNVs/INDELs) and structural variants (SVs) in a panel of 895 NMD genes, as well as short tandem repeat expansions (STRs) at 28 loci. In addition, for unsolved cases with an unspecific clinical presentation analysis of a panel with OMIM disease genes was added.

Results: In the cohort, 27% (232/861) of the patients harbored pathogenic variants, of which STRs and SVs accounted for one third (71/232). The variants were found in 107 different NMD genes. Furthermore, 18 pediatric patients harbored pathogenic variants in non-NMD genes.

Discussion: Our results highlight that for children with unspecific hypotonia, a genome wide analysis rather than a disease-based gene panel, should be considered as a diagnostic approach. More importantly, our results clearly show that it is crucial to include STR- and SV-analyses in the diagnostics of patients with neuromuscular disorders.

References: None

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Conflict of Interest: Marlene Ek: None declared, Daniel Nilsson: None declared, Martin Engvall: None declared, Helena Malmgren: None declared, Håkan Thonberg: None declared, Maria Pettersson: None declared, Britt-Marie Anderlid: None declared, Anna Hammarsjö: None declared, Hafdís Helgadóttir: None declared, Snjolaug Arnardottir: None declared, Karin Naess: None declared, Inger Nennesemo: None declared, Martin Paucar: None declared, Helgi Thor Hjartarson: None declared, Rayomand Press: None declared, Göran Solders: None declared, Thomas Sejersen: None declared, Anna Lindstrand Honoraria from Illumina, Advisor for Oxford Nanopore and Pacific Biosciences, Malin Kvarnung: None declared

P11.033.A Pathogenic variants in RS1 and ERLIN2 – a case report

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Background: Complex phenotypes in genetic disease may be caused by multiple genetic disorders, which can impede obtaining a diagnosis. Our case is a 16yo boy presented with motor developmental delay, progressive walking problems resulting in wheelchair dependency. He also suffered from visual problems due to an unspecified retinal disorder.

Methods: SNP array was performed, followed by whole exome analysis (480 genes involved in movement disorders, Agilent SureSelectXT Human All Exon V7, paired-end Illumina sequencing). RNA from cultured fibroblasts (+/- cycloheximide (CHX) treated), was analysed by RTPCR and Sanger sequencing.

Results: Physical examination of the patient showed leg muscle weakness with pyramidal signs, dysarthria and dysmorphic facial features. MR imaging of the brain was normal. Selective metabolic screening in plasma and urine was without abnormalities. SNP array analysis showed a small region of homozygosity comprising two potentially relevant disease genes, ERLIN2 and DDHD2. Exome analysis showed hemizygosity for a known pathogenic variant NM_000330.4(RS1):c.214G>A, p.Glu72Lys. Furthermore, homozygosity for NM_001362878.1(ERLIN2):c.557+74_557+78delCCAAA, p.? was detected. In vitro splicing analysis of the ERLIN2 variant showed that, in RNA from fibroblasts treated with CHX, primarily the aberrant transcript r.557 + 1_557+70ins, p.(Met186fs*1) was present, caused by usage of a novel donor splice site at c.557+70.

Conclusions: Targeted exome testing led to a diagnosis where functional tests aided the variant classification. The ERLIN2 variant was classified as likely pathogenic. In conclusion, the patient's eye disorder and motor problems can be explained by two independent genetic disorders, caused by (likely) pathogenic variants in RS1 and ERLIN2, respectively.

Conflict of Interest: None declared

P11.035.C Myoclonic dystonia and a genetic variant in the SGCE gene skipping two generations - the value of wide examination of the family history for genetic counseling

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A 3-year-old girl with upper limbs jerks underwent WES genetic testing to diagnose the cause of the condition. The family history seemed to be negative including an asymptomatic parents, brother, father's parents and father's brother. Only the great-grandmother of the father (mother of the father's mother) showed jerking of the head since childhood, she was diagnosed of extrapyramidal syndrome and treated for Parkinson's disease, which however was spectacularly not effective.

As a result of the WES-TRIO study, a heterozygous previously unknown paternally inherited potentially pathogenic variant p.(Cys248Gly) in the *SGCE* gene (NM_003919.3:c.742T>G) was selected as a possible candidate to explain the girl's symptoms which were consistent with *SGCE*-related myoclonic dystonia with a known mechanism of maternal imprinting. Variant in the same location (c.742T>A) was reported in the ClinVar database as a probable pathogenic and previously described in a single patient with dystonia.

Extensive analysis of variant segregation within family revealed its presence in the subject's father's brother, father's mother, father's maternal grandmother (affected, probably misdiagnosed), and subject's younger brother.

Studies in 4-generations family allowed the patient to be unequivocally diagnosed. Furthermore, it was possible to assess the risk for other carriers - the paternal uncle and the patient's asymptomatic brother (10-month-old at the time of examination). In addition, the subject's undiagnosed great-grandmother was also definitively diagnosed decades later.

Our results show the value of genetic testing in extended families, and that seemingly unrelated symptoms in distant relatives can linked as the same disease skipping two generations due to genomic imprinting.

Conflict of Interest: None declared

P11.036.D Impact of processed pseudogene insertions in genetic testing as cause of monogenic diseases: insertion in CLCN1 gene causing Myotonia Congenita

Kornelia Tripolszki[1], *Javier Martini*[1], *Kapil Kampe*[1], *Vasiliki Karageorgou*[1], *Mohammad Al Muhaizea*[2], *Catarina Pereira*[1], *Stephanie Weissgraeber*[1], *Omid Paknia*[1], ***Jorge Pinto Basto***[1], *Peter Bauer*[1]

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Background: In this work, we report the first case of autosomal recessive myotonia congenita associated with a homozygous

insertion of a processed pseudogene in the *CLCN1* gene. Myotonia congenita is a rare skeletal muscle channelopathy characterized by muscle stiffness and an inability of the muscle to relax after voluntary contraction (myotonia). It is caused by mutations of *CLCN1* gene (7q34).

Methods: We report on a 3-year-old female patient, presenting with myotonia at the age of 14 months. Her parents are consanguineous, and she has a 13-year-old similarly affected brother. To identify the causative variant, genome sequencing (GS) was performed. PCR amplification and Sanger sequencing of the inserted region and the breakpoint were also performed.

Results: Using GS, we identified a deep intronic homozygous insertion of ~750bp located in intron 14 of the *CLCN1*. The group of discordant reads at the breakpoint in intron 14 of *CLCN1* have mates that map to the *UQCRH* gene on chromosome 1. The sequence of the inserted region was obtained by targeted PCR amplification and Sanger sequencing and was determined to map exclusively to the coding region and UTR of the *UQCRH*. Since the inserted region showed the hallmarks of a retrotransposition event (antisense poly(A) tail, target site duplication, coding sequence of *UQCRH*), the retrotransposition of a processed mRNA was suspected.

Conclusion: To our knowledge, this is the third reported case of a processed pseudogene insertion causing a monogenic disorder. This further emphasizes the role of GS as a first-tier approach in rare disorder diagnostics.

Conflict of Interest: Kornelia Tripolszki Employee of Centogene AG, Javier Martini Employee of Centogene AG, Kapil Kampe Employee of Centogene AG, Vasiliki Karageorgou Employee of Centogene AG, Mohammad Al Muhaizea: None declared, Catarina Pereira Employee of Centogene AG, Stephanie Weissgraeber Employee of Centogene AG, Omid Paknia Employee of Centogene AG, Jorge Pinto Basto Employee of Centogene AG, Peter Bauer Employee of Centogene AG

P11.037.A Genotype-phenotype correlation in patients with SMA in Ukraine

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Introduction: Spinal muscular atrophy (SMA) is an autosomal recessive disease, the main cause is a deletion in the *SMN1* gene. The SMN2 is a highly homologous copy of the SMN1 gene and produces a small amount of functional SMN protein. An increase in the number of copies of the SMN2 modifies the disease phenotype.

Aim: The genotype-phenotype correlation in patients with SMA in Ukraine.

Methods: The 7 and 8 exons copy number of SMN1, SMN2 was detected by the MLPA method (MRC Holland) in 31 patients with SMA from Ukraine.

Results: The 31 patients aged from 10 days to 31 years with SMA were examined. The complete deletions of alleles in the SMN1 were detected in 9 patients, the deletion of the region of 7-8 exons in 5 patients, and the deletion of only exon 7 - in 4 patients.

The largest group of patients (21 patients, 68%) had 3 copies of 7-8 exons of the SMN2 with a very wide range of clinical manifestations – from 4 months to 24 months. Whereas 8 patients with the 2 copies had an early onset of the disease (2 to 9 months). In one proband, only 1 copy of the SMN2 gene was identified, which led to an early start and severe clinic with death at the age of 3 months.

Conclusions: The very wide range in the age of disease manifestation in patients with three copies SMN2 is most likely the result of modifying factors both genetic and environmental.

Conflict of Interest: Nataliia Samonenko Sanofi, Takeda, Biomarin, Roche, Sanofi, Takeda, Biomarin, Roche, Nataliia Olhovich Sanofi, Takeda, Biomarin, Roche, Sanofi, Novartis, Nataliia Mytsyk: None declared, Svetlana Kormoz: None declared, Tetiana Shklyarskaya: None declared, Nataliia Gorovenko: None declared

P11.038.B Genetic testing for the diagnosis of inherited peripheral neuropathies in Latvia

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Background: Charcot-Marie-Tooth (CMT) is a common hereditary motor-sensory neuropathy, although peripheral neuropathy may be a part of a complex phenotype of different hereditary neuropathy diseases.

Methods: Genetic testing was performed on 180 adults with hereditary neuropathies. Initially, genetic testing included MLPA to discover *PMP22* gene duplications/deletions and Sanger sequencing for *GJB1* gene point variants followed by exome sequencing (ES) for patients with still unknown genetic causes. On ES 458 neuropathy-associated genes are analysed. Currently, the patients are undergoing ES as the first-line genetic testing covering SNV and CNV analysis.

Results: Among 180 participants, a pathogenic variant was identified in 99 individuals, with overall diagnostic yield 55%. The most frequent pathogenic variant was *PMP22* duplication (51 [28.3%], including 8 [4.4%] identified through ES) and *GJB1* (17 [9.4%]). Next most common affected genes were *HINT1* (6 [3.3%]), *PMP22* other (5 [2.2%]), *MFN2* (4 [2.2%]), *HSPB1* (4 [2.2%]), *BSCL2* (2 [1.1%]), *AARS1* (2 [1.1%]) and only a single individual was identified with a variant in *SPG11, TRPV4, BICD2, TARDBP, KIF5A, OPA1, MPZ, MORC2* genes. Notably, eight [4.4%] individuals had pathogenic variant in a gene not associated with isolated hereditary neuropathy (e.g., *SPG11, TRPV4, BICD2, TARDBP, BSCL2, KIF5A, OPA1)*.

Conclusion: We have solved the molecular diagnosis in 99 of 180 patients. Individuals with hereditary neuropathy have a high genetic diagnostic yield if tested for a broad gene panel/ES with genes where neuropathy can be just a part of the phenotype.

Conflict of Interest: None declared

P11.039.C Bioinformatic analysis of whole-genome sequencing detects a rare pathogenic multi-exon deletion in the CACNA1A gene associated with episodic ataxia, type 2

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Neurological disorders encompass a large number of diseases with heterogeneous genetic etiology and often overlapping clinical manifestation making the search for the possible genetic cause challenging. A 9-month-old male infant with gross motor delay and vertical nystagmus was referred for genetic analysis in order to unambiguously establish the diagnosis. Whole-genome sequencing (>30X) was performed followed by targeted bioinformatic analysis of 2928 genes associated with various neurological, neuromuscular and neurodevelopmental disorders. Analysis of single-nucleotide variations and indels up to 50 bp did not detect any pathogenic or likely pathogenic variants that could explain the clinical symptoms observed in the patient. Copy-number variation analysis by bioinformatic algorithms detecting coverage fluctuations in the genes from the gene panel, however, identified a heterozygous multi-exon 56-Kb deletion in the *CACNA1A* gene associated with episodic ataxia, type 2. Single-read analysis from the WGS data allowed for precise mapping of the breakpoints down to single-nucleotide resolution annotating the variant as *CACNA1A*:c.4250+814_*3032del. The deletion encompasses exons 27-47 or 43% of the gene coding sequence, which was subsequently confirmed through MLPA analysis. The variant is expected to result in loss of function (a common mechanism for the disease) and was classified as pathogenic following ACMG-AMP guidelines. This clinical case clearly demonstrates that WGS is currently the methodology with the highest diagnostic sensitivity and analysis flexibility enabling clinical geneticists to detect the main genomic variation types - SNVs, indels, CNVs and SVs. WGS should therefore be the primary strategy for diseases with many differential diagnoses requiring analysis of many genes.

Conflict of Interest: None declared

P12 Multiple Malformation/Anomalies Syndromes

P12.001.A Detection of monogenic disorders by microarray

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Background/Objectives: Microarray is widely used for detection of CNVs. But it is also a strong tool for detection of monogenic disorders caused by deletions/duplications of whole or part of genes. We set to determine the frequency of monogenic disorders and carrier status revealed by aCGH and SNPaCGH. In patients with an absence of heterozygosity (AOH) above 5Mb and monoallelic variants we hypothesised about a possible association with autosomal recessive (AR) disorder on the basis of genotype-phenotype correlation.

Method: Retrospective analysis of 581 patients - 442 aCGH (8 × 60) and 139 SNPaCGH (4 × 180) (Agilent Technologies). Segregation studies, MLPA and sequencing were used for verification of the detected variants and for exclusion/confirmation of the mutation on the second allele.

Result: In 89 patients pathogenic/likely pathogenic variant was detected. In 14 patients the variants were a cause of monogenic disorders: homozygotic deletion *STRC*, hemizygotic deletions *STS*, heterozygotic deletions *PAX3*, *PBX1*, *KIF1A*, FOXC1(2 patients), duplications *CYP19A1*(3 patients), *FBXW11*, *LMBR1*, *SOX3* (2 patients). In 42 patients there was a heterozygotic loss of the genes associated with AR or X linked diseases (XLD). SNPaCGH revealed AOH in 29 patients, in 11 patients the size of AOH suggested consanguinity.

Conclusion: Frequency of patients with monogenic disorder revealed by microarray in the study was 2.41%, compared with 5.68 % patients with recurrent large pathogenic CNVs. In 7.23% patients carrier status for AR or X linked condition was detected.

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Conflict of Interest: None declared

P12.002.B Disruption of endosomal membrane protein recycling pathway causes a variety of clinical phenotype in Ritscher-Schinzel/3C syndrome

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Background: Ritscher-Schinzel syndrome (RSS) is a rare genetic disorder characterised by multiple congenital anomalies which include craniofacial-cardiac-cerebellar manifestation as triad of RSS, as well as global developmental delay, skeletal, endocrinological, and renal complications. Three responsible genes, WASHC5, CCDC22, and VPS35L encode for proteins associated with SNX17-mediated integral membrane protein recycling through the endosomal network, suggestive that disrupted protein recycling underlies the pathogenecity. However, it is unclear whether the condition of patients with mutations in either WASHC5, CCDC22, or VPS35L is the same or distinct, and furthermore the functional role of this system in human development remains to be uncovered.

Methods: VPS35L, CCDC22, WASHC5, or SNX17 were depleted in cells from key pathogenic tissues, namely neurons, kidney and skeleton, followed by cellular analysis. Generation of knockin or conditional knockout mice for VPS35L provided a route to investigate in vivo tissue pathology.

Results: Cell surface proteomics identified numerous proteins significantly decreased in gene-edited cells. Some of these proteins were changed in all the gene-edited cells, while others were only perturbed upon specific gene ablation. Immunoprecipitation analysis confirmed the interaction between some of those proteins with SNX17. Functional analysis suggested loss of function of those proteins, which included solute transport and signal transduction. The mice models captured patients phenotypes and suggested histopathological findings.

Conclusion: We suggest RSS as a 'recyclinopathy' given that many clinical manifestations can be explained by the reduced recycling and function of a specific cohort of cell surface integral membrane proteins.

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Conflict of Interest: None declared

P12.003.C Del2Phen: Developing a novel patient-centric genotype-to-phenotype prediction tool for chromosome deletions – the Chromosome 6 Project

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Background: Information on the clinical consequences of rare chromosome aberrations is often limited, impacting both patients and their families. The Chromosome 6 Project (C6P) aims to empower parents of children with a structural chromosome 6 aberration by providing them with reliable information on the expected phenotypes of their child. To achieve this, C6P collects phenotype and genotype data directly from parents and combines it with data from literature reports. In the current study, we developed Del2Phen, a software tool that uses the collected data to produce deletion-specific phenotype information for all chromosome 6 deletions.

Methods: Del2Phen was developed to produce a phenotype description for a patient based on clinical features observed in a group of patients with similar deletions. Similar deletions are grouped using pre-set parameters, based on the predicted haploinsufficiency effect of the involved genes. In this study, we assessed which parameter settings result in sufficiently large groups of genotypically homogeneous individuals and accurate phenotype predictions.

Results: Del2Phen produces an accurate phenotype prediction when deletions are grouped using both of the following parameters: (i) concordance in the involvement of genes known to have a highly penetrant phenotypic effect and (ii) an 80% overlap in haploinsufficiency gene content.

Conclusion: We developed a phenotype prediction tool that uses the parent-derived data collected by C6P to provide patient-specific clinical information directly to parents. Although Del2Phen was developed for chromosome 6, it can be adjusted for use in other chromosomes, making it a valuable resource for parents of children with such aberrations.

Conflict of Interest: None declared

P12.004.D Identification of variants underlying biparietal perisylvian polymicrogyria in Finnish families

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Bilateral perisylvian polymicrogyria (BPP) is the most common form of regional polymicrogyria (PMG) that belongs to malformations of cortical development (MCDs). PMG is the most common form of MCDs accounting for 20 % of all malformations of cortical development. BPP is characterized by overfolding of the cerebral cortex and abnormal cortical layering. The major clinical featuring includes oromotor dysfunction, dysarthria, and hemiparesis. Cognitive impairment and epilepsy are common. By now, more than 50 genes are reported to underlie PMG covering only about 20% of cases. The aim of this study was to identify genetic background of BPP in Finland. DNA from peripheral blood from a total of 21 families and one singleton case was studied using exome sequencing (ES) and eight using optical genome mapping (OGM). In all cases brain imaging using MRI showed BPP. To date, pathogenic (P) or likely pathogenic (LP) variants were found in 6/22 (27 %) patients using ES. Of them, four were de novo/likely de novo *(SCN3A, TUBA1A, DDX23* and *TUBB2B)*, one autosomal recessive (*CCDC82), one X-linked (TAF1)* and one VUS (*AFF2*). In addition, two novel candidate genes were found (*RUFY4* and *BOC*). As mosaicism has been reported in BPP we will perform deep ES (>200x) from DNA of buccal epithelial samples from cases of with unknown cause as the next step. Our results confirm the previously established high etiological heterogeneity of BPP.

Grants: NIH.

Conflict of Interest: None declared

P12.005.A Loss of function LRP6 variant causing tooth agenesis and hand malformation

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Background/Objectives: Low-density lipoprotein receptor-related protein 6 (LRP6) gene is associated with selective tooth agenesis-7(OMIM#616724). While limb malformation has been reported in Lrp6-deficient mice, this association has been rarely discovered in humans with LRP6 variants. Here, we present a rare phenotype associated with a loss of function LRP6 variant.

Methods: Clinical, imagistic, interdisciplinary assessments and genetic testing were performed for the patient and parents.

Results: A 14 years old male presents with multiple anomalies and normal cognitive development: right hand finger II and III syndactyly; oligodontia (permanent dentition missing 6 teeth); hypotrichosis; hyperhidrosis; tall stature(+2SD); arm span/height = 1,01; high myopia; inequality of lower limbs (femur and tibia)-causing scoliosis; dysmorphic small ears and mild recurrent neutropenia. The patient and parents had normal cardiology evaluation. Clinical suspicions included hyperhidrotic ectodermal dysplasia and Marfan syndrome. WES trio was perform to show de novo, heterozygous, pathogenic, exon 1 deletion, c.(?_-1)_(55 + 1_56-1)del, in LRP6 gene (confirmed using digital PCR), in the patient's blood. This variant deletes the canonical start codon. Thus, confirming the diagnosis of selective tooth agenesis-7. Syndactyly was considered within the LRP6 spectrum, as hand anomalies have been (rarely) reported (PMID:34759310). Additionally, the FBN1 NM_000138.5:c.4282C>T(p.Arg1428Cys),

heterozygous, maternal, was classified as VUS. Although reported in a person with Marfan syndrome (PMID:27112580), the variant is observed in the general population (gnomAD). The phenotype of patient and his mother do not meet the criteria for Marfan syndrome.

Conclusion: The spectrum of loss of function LRP6 variants should include hand anomalies, supported by Lrp6-deficient mouse models and case reports phenotype.

Conflict of Interest: None declared

P12.006.B BBS1 impairment affects vesicular trafficking and TGFβ receptor signal transduction

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Background/Objectives: Bardet-Biedl syndrome (BBS MIM# 209900) is a ciliopathy characterized by mutations in genes that mainly code for a series of proteins that interact to form a protein complex, the BBSome. The BBSome is a protein complex well-known for its role in the mobilization of molecules along the primary cilium, but little is known about its relationship to extraciliary vesicular trafficking. The purpose of this work is to study the relationship of the BBSome with the endocytosis of membrane receptors and thus to multiple signalling pathways independent of its ciliary function.

Methods: CRISPR/Cas9 BBS1 KO model was made in RPE1 cells. Biotin labelling followed by a pull-down and HPLC-ESI-MS/MS assay was performed to isolate and identify the membrane proteins. An immunofluorescence detection assay of labelled transferrin and its colocalization with EEA1-positive and RAB11-positive vesicles was carried out to determine the recycling rate. WB was performed to assess the level of abundance and phosphorylation of metabolic intermediates of the TGFβ pathway.

Results: An increase in the TGFBR1 receptor and in molecules associated with the TGFB pathway such as ENG is detected, which does not correspond to an increase in the expression of the respective genes. In addition, there is a 5-min delay in the endocytosis process by the KO.

Conclusion: The absence of functional BBS1 is related to deficient endocytosis of receptors, especially TGFBR1 in this cell type. A process as generic as receptor endocytosis would explain the wide range of phenotypes that BBS patients present depending on the cell type.

Conflict of Interest: None declared

P12.007.C Biallelic CC2D2A variants, missense variant and LINE-1 insertion simultaneously identified in siblings using long-read whole-genome sequencing and haplotype phasing analysis

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[Abstract] Joubert syndrome (JBTS) is characterized by a magnetic resonance imaging (MRI) appearance called 'molar tooth sign', neonatal breathing dysregulation and hypotonia, and developmental delay. To date, 38 genes have a known relation to the JBTS phenotype which is mostly caused by recessive inheritance mode. Though whole-exome analysis has often contributed to the identification of causative variants, approximately 10% of them are still undiagnosed even though a single possible pathogenic variant is identified. We present a successful identification of biallelic variants with examining samples from affected siblings and their mother using continuous long read (CLR) data generated from PacBio Sequel II. [Patients] The affected siblings were born to non-consanguineous parents. Both presented neonatal breathing dysregulation and hypotonia. They are bedridden with severe mental retardation. Brain MRI of them reveal an abnormality of their cerebellar vermis. [Results] A novel nonsynonymous variant (*CC2D2A*:NM_001080522.2:c.4454A>G:p.-Tyr1485Cys, PM2 and PP3) and an exonic insertion of Long INterspercsed Element-1 in *CC2D2A* (LINE-1, PVS1 and PM2) were identified in both affected siblings. These variants over 100kb apart were clearly proven biallelicity only in their genomic data by allele-phasing analysis (PM3). In silico survey of in-house genomic data revealed that 2 of 2,843 had the heterozygous LINE-1 insertion, suggesting that there are rare carriers of *CC2D2A*-related disease in Japanese. [Conclusion] These variants were considered compatible to account for the clinical features of the affected siblings. CLR sequencing may be applicable for finding SNV and SV at one time and for confirming the biallelicity of variants even without parental data.

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P12.008.D "Knowing and Treating Kosaki/Penttinen syndromes" international collaborative consortium: a real-life observational study about the natural history of KOGS & PS and the safety & efficacy profile of TKI in these indications

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Background: It has been three years since the creation of the multidisciplinary consortium "Knowing and Treating Kosaki/Penttinen syndromes", two ultra-rare multisystem syndromes, due to heterozygous activating variants in *PDGFRB*. Neurological, orthopedic and vascular deterioration may arise. Biannual remote meetings enable to share the knowledge about these syndromes, and to discuss the best management of patients.

Methods: In March 2022, the consortium validated the implementation of a database in order to enrich our knowledge on the natural history of these syndromes and to evaluate the real-world safety & efficacy profile of Tyrosine Kinase Inhibitors (TKI) by comparing treated & untreated patients. Standardized follow-up guidelines have been proposed. The regulatory framework is in progress.

Results: As of February 2023, 18 teams in 12 countries have joined the consortium. More than 25 patients with KOGS or PS are now identified worldwide, either published or unpublished; 7 of them are treated with a TKI (Imatinib, Dasatinib or Sunitinib). Most of the treated patients reported a clinical improvement, sometimes occurring rapidly after initiation of treatment. However, some reported a plateau, or even loss of efficacy, which led to a substitution of TKI in some patients. TKI were generally well tolerated.

Conclusion: The consortium welcomes new teams, in order to be as comprehensive as possible. Real-life observational study seems an appropriate model for the study of ultra-rare diseases, including the evaluation of treatment efficacy, when the prevalence of the disease does not allow the development of a clinical trial.

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Conflict of Interest: None declared

P12.009.A The novel variants in the LARP7 gene as a cause of Alazami Syndrome in 3 patients

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Background/Objectives: Alazami syndrome (ALAZS, OMIM #615017) is a very rarely seen autosomal recessive disorder characterized by severe growth restriction, severe intellectual disability, and recognizable facial features. It was first reported in 2012.There are less than 50 patients in the literature. Herein, we describe three patients affected with ALAZS.

Methods: Patient 1(P1,13y,male) and Patient 2(P2,20y,female) were two siblings. They had normal prenatal periods. There was parental consanguinity. Birth weight and height were normal. However, there were growth retardation and microcephaly in the last physical examinations. Both of them had severe neuromotor and speech retardation and their seizures had treatment with antiepileptic drugs. P1 had corpus callosum agenesis on MRI. Patient 3 (5y,female) had low birth weight. On physical examination, she had growth retardation, microcephaly, and developmental delay. No seizure was seen. MRI was reported normal. All 3 patients had distinctive dysmorphic features such as malar hypoplasia, low-set ears, deep-set eyes, broad nose, flat and wide nasal bridge, short philtrum, macrostomia, wide mouth, full lips, and they had anxiety during an examination.

Results: Whole-exome sequencing analysis revealed that pathogenic novel homozygous mutation for P1, P2 [NM_015454.2, c.1056_1057del (p.Leu353GlufsTer7)], and novel compound heterozygous mutation for P3 [NM_015454.2, c.377_378del (p.Thr126SerfsTer11), NM_001370982.1, c.1050_1051del (p.Asn350LysfsTer16)] in the *LARP7* gene which was inherited from their father and mother.

Conclusion: ALAZS is caused by biallelic loss-of-function variants in the *LARP7* gene.The description of new patients is significant to increase our knowledge of disorders to precisely define molecular characteristics and clinical phenotype.

References: https://doi.org/10.1002/ajmg.a.62778
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Conflict of Interest: None declared

P12.010.B Xq26.3 duplication with position effect on SOX3 in a family with hypopituitarism

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Background/Objectives: Intragenic single nucleotide variants and copy number variants of *SOX3* (Xq27.1) are associated with growth hormone deficiency and multiple additional anomalies (*OMIM 312000 and 300123). Here we report on a family in which three male patients (two cousins and their maternal uncle) showed short stature, growth hormone deficiency, hypoplasia of the pituitary gland and cryptorchidism. The maternal uncle whose retractile testis on both sides had been treated successfully with HCG-therapy at the age of 7, developed a seminoma at the age of 41 years. The seminoma was resected and he has remained free of recurrence for 12 years. One of the cousins started treatment with growth hormones at the age of 6 and presently (at 12 years) his body height falls in the 47th percentile, while he is still prepubescent.

Methods: Genetic analyses were conducted using array CGH analyses, fluorescence in situ hybridization (FISH) and whole genome sequencing on the Illumina Novaseq 6000 sequencing platform.

Results: Array CGH analyses revealed a 3.3 Mb duplication in Xq26.3-q27.1 that was located 86 kb downstream of *SOX3* in all three male patients showing the symptoms mentioned above. The

duplication was further confirmed by genome sequencing in one of the cousins. Three female carriers of the duplication identified in the family were healthy.

Conclusion: We hypothesize that the duplication in Xq26.3-q27.1 exerts a position effect on *SOX3* transcription.

Conflict of Interest: None declared

P12.012.D Phenotypic spectrum and genetic landscape of syndromic microcephaly

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Microcephaly is defined by a head circumference at least two standard deviations below the mean for sex, age and ethnicity. It can be isolated or syndromic (SMC) when associated with neurological entities and/or multiple congenital anomalies. The confirmation of the underlying genetic etiology is challenging. Our study aimed to describe the clinical and genetic characteristics of Tunisian patients with SMC.

Methods: We performed clinical assessment and used different tools (standard karyotype, Fluorescent in situ hybridization (FISH), array-comparative genomic hybridization (CGH-array), gene panels and exome sequencing) to explore patients referred with SMC over 10 years.

Results: We included 104 infants. The most common disorders associated with microcephaly were facial dysmorphism (86%), intellectual disability (89%), musculoskeletal anomalies (80.7%), structural brain lesions (48%), and epilepsy (38.4%). Microcephaly was classified as primary and secondary in 33.8% and 32.6% of all patients, respectively. A genetic cause of SMC was confirmed in 37.5% of cases. A chromosomal aberration was detected by standard karyotype in 6.7% of the cases. A cryptic chromosomal abnormality was confirmed by FISH and CGH-array in 19% of patients. We established a molecular basis in 36% of patients explored by next-generation sequencing. Appropriate genetic counselling was provided to the families of confirmed cases and prenatal diagnosis was performed in five of them.

Conclusion: Our results confirm the high complexity of exploring SMC and the importance of combining different diagnostic approaches to elucidate the undefined cases. We have also contributed to widen the phenotypic spectrum and the genetic landscapes of microcephaly.

No Grant References

Conflict of Interest: None declared

P12.013.A Syntelencephaly (Middle interhemispheric variant): an holoprosencephaly (HPE) like the others?

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Background: Middle interhemispheric variant (MIH/ Syntelencephaly) is a subtype of HPE in which the posterior frontal and parietal areas lack midline separation. The anatomic and neuroradiologic features have been well detailed, but the clinical and genetic aspects are largely unknown.

Objective: 1) To identify among the 1560 probands of our HPE database (45% live/ 55% fetus), those who presented with MIH; 2) to highlight the differences in imaging, genetics and prognosis between cases with MIH and other forms of HPE.

Methods: Based on review of 39 cases of MIH, including 15 live children and 24 foetuses, neuroimaging/pathological features, clinical data and results of molecular study were collected.

Results: The face was normal, microcephaly was inconstant, myelination was normal, intellectual disability ranged from absent to severe, spasticity and dystonia were frequent, as well as hypotonia in the first months. No choreoatherosis or endocrine deficits were noted. The de novo pathogenic SNVs or CNVs involved mainly the *ZIC2* gene except in one case with a de novo class 5 variant in *SHH*. All other variants were class 3. Clinical outcome was more favourable in cases where MIH was isolated, without microcephaly, and was characterized by Sylvian fissures that did not connect across the midline, with separation of both thalami, with the presence of a residual part of the corpus callosum.

Conclusion: *ZIC2* is the main gene. The prognosis of MIH is better than that of other forms of HPE. Prospective studies are required to improve knowledge of this rare brain malformation.

Conflict of Interest: None declared

P12.014.B Expanding the phenotypic and mutational spectrum of STAG1-related cohesinopathy

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Background/Objectives: Cohesinopathies represent rare syndromic form of neurodevelopmental disorders arising from a dysfunction in the cohesin complex, which plays an important role in chromosome segregation, DNA repair, replication and gene expression. Up to date eight cohesin complex genes have been reported with *STAG1* being the most recently discovered, with only 18 patients reported in medical literature.

Methods: We describe a 2 yo girl with neurodevelopmental delay, born from nonconsanguineous parents. She was born term with birth weight 3300gr and birth length 50cm and presented with congenital clubfoot and feeding difficulties at birth. Dismorphic features included micrognathia, high palate, low-set ears, anverted nares, wide nasal bridge, thick eyebrows,

brachycephaly, high forehead, strabismus, ptosis, unilateral microphtalmia.

Results: Whole exome sequencing (WES) was performed and a novel c.1183C>T, p.(Arg395*) variant was identified in *STAG1* gene. The variant generates a premature stop codon in *STAG1* exon 12 and is predicted to lead to loss of normal protein function. This variant has not been described in the medical literature or reported in disease-related variation databases such. In addition, the pLI value of the *STAG1* gene in the gnomAD is 1, indicating that the gene is intolerant to loss-of function variation. Parental testing confirmed de novo status of the variant.

Conclusion: Our case expands the phenotypic and mutational spectrums of *STAG1* and confirms application of WES as a first-line diagnostic test in individuals with developmental delay and/or multiple congenital anomalies. More studies are needed to define whether genotype-phenotype correlations exist.

Grant References: No funding.

Conflict of Interest: None declared

P12.015.C Father and son with a pathogenic variant c.614dup p.(Gln206Thrfs*20) in the NR5A1 gene

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Background: Steroidogenic factor 1 (SF-1) is a key transcriptional regulator of the adrenal and gonadal development. Pathogenic variants in the *NR5A1* gene are responsible for various degrees of insufficient virilisation, including gonadal and testicular dysgenesis with or without Müllerian remnants, ambiguous genitalia, mild and severe forms of hypospadias, micropenis, cryptorchidism, anorchia and male infertility. We report a paternal pathogenic variant in the *NR5A1* gene in a 46,XY 3-year-old patient with bifid scrotum, palpable testes, micropenis and proximal-perineal hypospadias. He was conceived by in vitro fertilisation (IVF). The father of the patient had a similar phenotype prior to genital surgery.

Methods: Exome sequencing was performed using Illumina's Twist Comprehensive Exome and Twist Mitochondrial DNA panel with a virtual panel of 130 genes associated with disorders of sex development (DSD). Sanger sequencing was used to verify the results.

Results: We identified a heterozygous pathogenic variant c.614dup p.(Gln206Thrfs*20) in the *NR5A1* gene in the patient and his father.

Conclusion: The c.614dup variant has already been reported. However, 3 out of 4 reported patients were 46,XY DSD females. The clinical information on the fourth patient was not included in the publication. We demonstrate that our patient and his father do not exhibit dysgenetic gonads typical for pathogenic variants in the *NR5A1* gene, but rather hypoplastic, palpable testes. We report that the patient's father, who carries a pathogenic variant, was able to conceive children by IVF.

Grant References: MH CZ - DRO, Motol University Hospital, Prague, Czech Republic 00064203.

Conflict of Interest: Júlia Martinková Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic, Michaela Zelinová Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic, Miroslava Balaščaková Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic, Anna Křepelová Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic

P12.016.D Description of eight novel cases with variants in DNAH14 gene and review of the clinical and molecular features

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Background/Objectives: The *DNAH14* gene is an axonemal dynein heavy chain located in cilia and flagella and plays an important role in cell motility and in a variety of physiological processes. Dysregulations in the function of dyneins are poorly understood, although genes have recently been described as the cause of a group of disorders called primary ciliary dyskinesia (PCD, MIM #242650). To date, no human pathologies with *DNAH14* defects have been described.

Methods: In this study we have described 8 patients with variants of uncertain significance in the *DNAH14* gene detected by different massive sequencing techniques, including WGS, WES and different customised virtual panels.

Results: We identified 8 individuals with variants in the *DNAH14* gene. Among these variants we found heterozygous or homozygous biallelic variants and a de novo variant in heterozygosity. As for the clinical characteristics of our cohort of patients, we found that the most frequent were global developmental delay (63%) and generalized hypotonia (50%). Other phenotypic features include seizures, intellectual disability, delayed speech and language development, autistic behaviour and microcephaly.

Conclusion: This study shows that variants in the *DNAH14* gene cause a new neurodevelopmental disorder. According to the clinical characteristics of each of the patients and the location of the variants in the gene, a genotype-phenotype correlation has not been possible. Therefore, identifying new individuals with defects in the *DNAH14* gene may help in the diagnosis of neurodevelopmental disorders.

Grant References: PI20/01053

Conflict of Interest: None declared

P12.017.A A 5'UTR variant in JAG1 results in translational repression and Alagille syndrome

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Alagille syndrome (ALGS) is an autosomal dominant disorder that affects multiple organs including liver and heart. Due to the hepatic ductopenia, affected newborns often become cholestatic

and often require a liver transplantation. Most pathogenic ALGS variants are located in the *JAG1* gene and cause proteintruncations. While the molecular mechanisms of these variants are often easy to grasp, the impact of non-coding variants are difficult to disclose in many instances. Previously, we discovered a de-novo variant (c.-100C>T) in the 5′ UTR of the *JAG1* gene of an ALGS patient with a severe phenotype. This 5′UTR variant was reported in one other patient and thought to affect transcription. However, our initial data delineated a translational phenotype. To clarify the pathological mechanism, we fused the 5′UTR of *JAG1* and the variant to firefly luciferase. The single point mutation decreased luciferase expression to 2-10% of wild type levels, thus mirroring the patient data. The variant shows high expressivity in various cell types using different promotors demonstrating its independency from transcription. The reduction in luciferase activity was neither due to lesser RNA nor accelerated RNA decay leaving translational repression as the most likely explanation. In the future, we aim to dissect the pathological mechanism further by ribosomal profiling and base editing of the natural *JAG1* locus. This study highlights the importance of non-coding variants as they can exert drastic effects on gene expression. In addition, the underlying cellular regulatory mechanism may also operate on other genes and 5′UTR variants.

Conflict of Interest: None declared

P12.018.B Recessive mutations in SCNM1 are a new cause of Orofaciodigital syndrome due to errors in minor intron splicing affecting primary cilia

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Background/Objectives: Orofaciodigital syndrome (OFD) is a genetically heterogeneous ciliopathy characterized by anomalies of the oral cavity, face and digits. This work was aimed to identify the genetic cause and molecular mechanisms underlying OFD in four individuals from three unrelated consanguineous families lacking a molecular diagnosis.

Methods: Genetic analysis included exome sequencing and homozygosity mapping. Functional studies were performed in patient-derived dermal fibroblasts and relevant findings were subsequently validated in CRISPR-Cas9 knockout and siRNA knockdown hTERT-RPE1 cellular models.

Results: We identified bi-allelic loss-of-function variants in *SCNM1* (sodium channel modifier 1) in all patients of this study. *SCNM1* encodes a protein component of the human activated minor spliceosome, a ribonucleoprotein complex that catalyzes the excision of an atypical class of introns termed minor introns. Comparative transcriptome analysis between patient-derived fibroblasts carrying *SCNM1* mutations and control fibroblasts revealed a set of genes with altered minor intron processing, including some known to be related to primary cilia. In agreement with this, SCNM1-deficient fibroblasts showed abnormally elongated cilia. Minor intron splicing alterations and increased cilia length were reproduced both in *SCNM1* knockout and knockdown cellular models. Conversely, these defects were restored in *SCNM1*-rescued patient cells. Hedgehog signaling evaluation in *SCNM1*-transduced fibroblasts also suggested that SCNM1 acts as a positive regulator of this ciliary-dependent pathway.

Conclusion: These results prove, for the first time, that loss-of-function mutations in a protein subunit of the minor spliceosome lead to primary cilia defects that result in a typical ciliopathy.

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Conflict of Interest: None declared

P12.019.C Analyses of genetic variants for Congenital Anomalies through whole genome sequencing in Korean patients

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Background: Congenital Anomalies (CA), also known as birth defects, are structural and functional anomalies occurred with a frequency of 5~6% during fetal development and are present at birth. These anomalies can range from minor physical variations to serious, life-threatening conditions resulting in long-term treatment and economic burdens. Several genetic variations and environmental factors are known as causal, but the underlying cause remains unknown. Recently, whole-genome sequencing (WGS) is emerging as a diagnostic test for rare genetic diseases such as CA to identify disease-causing genetic variants.

Methods: We established WGS analysis pipeline and performed the trio-based WGS analysis of thirty CA patients and their parents. The clinical characteristics of thirty patients were documented and annotated using HPO terms. The WGS data was analyzed through in-house pipeline, incorporating DRAGEN-HAIL for SNV/Indel, GATK for CNV and MANTA for SV.

Results: The highest distribution of phenotypes is in cardiovascular followed by digestive and nervous disorders. From the analysis of SNV/Indel, 2,583 de novo (DNV) variants including 51 coding DNV, 107 compound heterozygous and 271 rare homozygous variants in coding regions were discovered. Additionally, 95 CNV and 76 SV were also identified. All candidate variants were confirmed by visual inspection using Integrative Genomics Viewer for SNV/Indel and Samplot for CNV and SV.

Conclusion: Several SNVs/Indels, CNVs and SVs were identified in known or novel genes through variants interpretation and genotype-phenotype analysis. Further investigation, such as functional validation through cellular or zebrafish model, could help to elucidate the disease mechanisms of CA.

Conflict of Interest: None declared

P12.020.D Novel mechanism of craniosynostosis associated with chromosome 4q21 duplication: modelling in mice and iPSCs

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Background: Craniosynostosis (premature fusion of cranial sutures) is associated in ~3% patients with chromosomal rearrangements such as copy number variants (CNVs), but demonstrating a causative role for specific CNVs in disease can be challenging. The organisation of the 3D genome into regulatory units termed topologically-associating domains (TADs), provides a useful framework to explore pathogenic mechanisms of CNVs.

We aimed to investigate causation in a family in which a de novo 630kb duplication on chromosome 4q21 segregated from a mother to her daughter, both of whom had severe multi-suture craniosynostosis.

Methods: We generated a mouse model by engineering a 590kb duplication of the syntenic region in embryonic stem cells, and derived induced pluripotent stem cells (iPSCs) from a maternal blood sample.

Results: The tandem duplication encompasses *FGF5* and *CFAP299*, with other potential candidates such as *BMP3* nearby. Deep-C was used to predict the effect on TAD structure, this suggested that the second *FGF5* gene copy localises within a neo-TAD, potentially leading to dysregulated expression.

A mouse chimeric for the duplication exhibited a very severe craniofacial phenotype, including multisuture synostosis (confirmed by CT scan) and severe malocclusion with disproportion of the upper and lower jaws.

Patient-derived iPSCs were differentiated towards a cranial neural crest identity and are currently being analysed using RNAseq, ATACseq and Capture-C to explore the functional disturbance around the duplicated region.

Conclusion: The results support a novel mechanism of craniosynostosis caused by the chromosome 4 duplication and illustrate a generic approach to investigation of other CNVs in craniosynostosis.

Conflict of Interest: None declared

P12.021.A DNA episignature for White Sutton syndrome due to POGZ episignature

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Abstract

Purpose: White-Sutton syndrome (WHSUS) is a rare, autosomal dominant neurodevelopmental disorder caused by heterozygous loss-of-function alterations in the *POGZ* gene. WHSUS has variable expressivity that commonly overlaps with other neurodevelopmental disorders. In this study, we characterized a distinct DNA methylation epigenetic signature (episignature) distinguishing WHSUS from unaffected individuals as well as individuals with other known neurodevelopmental disorders with episignatures, from a cohort of published and unpublished individuals with *POGZ* haploinsufficiency.

Methods: Genome-wide DNA methylation profiles from a total of 13 DNA samples from peripheral blood of individuals with a pathogenic or likely pathogenic POGZ mutations were assessed and compared to the EpiSign™ classifier database to identify a diagnostic episignature. The diagnostic model was used to investigate the methylation pattern of 3 individuals with variants of unknown significance in *POGZ*.

Results: A predominantly hypomethylated DNA methylation profile specific to WHSUS was identified, and the classifier model was able to conclude on the pathogenicity of the VUS. The episignature was sensitive enough to detect individuals with varying degrees of phenotypic severity carrying *POGZ* haploinsufficient variants.

Conclusion: We identified a novel episignature in WHSUS due to *POGZ* haploinsufficiency. This episignature has the potential to aid identification and diagnosis of individuals with WHSUS. Additional samples are being tested to increase robustness of these results.

Conflict of Interest: None declared

P12.022.B ATP5PO levels regulate Enteric Nervous System development in zebrafish, linking Hirschsprung disease to Down Syndrome

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Background/Objectives: Hirschsprung disease (HSCR) is a complex genetic disorder characterized by the absence of enteric nervous system (ENS) in the colon. Down syndrome (DS) patients have a >50-fold higher risk of developing HSCR than the general population, suggesting a role of chromosome 21 (Hsa21) genes in ENS development. However, identification of causative genes remains challenging.

Methods: Potential candidate genes located on Hsa21, were screened using the zebrafish. Selected genes were located in the DS-HSCR susceptibility region; expressed in human intestine; biomarkers for DS prenatal diagnosis; and had a zebrafish orthologue. mRNA encoding these genes was overexpressed in zebrafish, and ENS development was evaluated. Immunohistochemistry in intestinal biopsies of controls, HSCR patients and DS-HSCR patients, as well as in vitro assays using a stable overexpressing neuroblastoma cell line, SH-SY5Y, were performed. Moreover, epistasis with *RET*, the major HSCR gene, was evaluated.

Results: Four genes were selected for further analysis: *RCAN1*, *ITSN1*, *ATP5PO* and *SUMO3*. Only overexpression of *ATP5PO*, coding for a component of the mitochondrial ATPase, caused a significant reduction in the number of ENS cells. In vitro studies showed that overexpression of *ATP5PO* led to a reduction of ATP5PO protein levels, as well as impaired neuronal differentiation and reduced ATP production. Finally, epistasis was observed between *ATP5PO* and *ret* in vivo.

Conclusions: Our results identified *ATP5PO* as the gene responsible for the increased risk of HSCR in DS patients in particular, if *RET* common variants are also present, and show that balanced expression of ATP5PO is required for ENS development.

Conflict of Interest: None declared

P12.023.C The functional enrichment of whole exome sequencing (WES) data suggests that the canonical Wnt pathway plays a role in Chiari malformation type I with possible oligogenic mechanisms

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Background/Objectives: Chiari malformation type I (CMI) is a cranio-vertebral junction anomaly characterized by a protrusion of the cerebellar tonsils of at least 5 mm across the foramen magnum. CMI has an estimated prevalence of 1:1.280 and complex genetics largely unknown. CMI is considered to be caused by hypo-development of cranial posterior fossa. While some patients are asymptomatic, a wide spectrum of neurological manifestations may characterize CMI, due to the brainstem compression. Most cases are sporadic, although familial recurrence is observed with different patterns of inheritance, incomplete penetrance, variable expressivity. Recent evidence indicates that chromodomain genes, collagen genes, and molecules of the canonical Wnt pathway may play a role in CMI.

We aimed identifying pathogenetic mechanisms in CMI families using WES data and functional gene enrichment analyzes.

Methods: We selected damaging WES variants from 45 pediatric cases and affected relatives of a total of 30 families, and 100 in-house control individuals using standard pipelines; evaluated functional networks (biological processes, KEGG pathways) through gene set enrichment analysis (GSEA); explored oligogenic combinations of candidate variants using a prediction platform (ORVAL) and performed phenotype-driven analysis of the results.

Results: Overall, we detected a network of genes, with potentially damaging variants, involved in canonical Wnt signaling in 8 families, one with possible oligogenic inheritance with variable expressivity.

Conclusion: By using a bioinformatic multiple approach, we found that the canonical Wnt pathway could account for at least part of CMI families, with possible synergic effects of multiple variants in the same patient.

Grant References: Volpati Trust

Conflict of Interest: Maria Cerminara: None declared, Patrizia De Marco: None declared, Michele Iacomino: None declared, Diego Vozzi: None declared, Valeria Capra: None declared, Marco Fontana: None declared, Marco Pavanello: None declared, gianluca piatelli Volpati Trust, Federico Zara: None declared, Aldamaria Puliti: None declared

P12.024.D Clinical presentation in two unrelated patients with PERCHING syndrome

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Background: PERCHING syndrome (# 617055) is multisystemic autosomal-recessive disorder with only a handful of patients described. It is characterized by profound developmental delay, joint contractures, spasticity, abnormal posturing, and severe feeding difficulties. Biallelic mutations in *KLHL7* gene coding for protein involved in proteasome-mediated degradation are responsible for the clinical presentation. High inter-and intrafamilial phenotypic variability has been reported, as well as overlapping features with other syndromes as Crisponi/CISS1(# 272430) or Bohring-Opitz (# 605039).

Material and methods: We performed trio exome sequencing (ES) in male and female proband from two unrelated non-consanguineous families that originated from a very small Macedonian village.

Results: Both pregnancies were complicated including poor fetal growth, polyhydramnios and diminished fetal movements with a respiratory distress after birth. Common phenotypic features include distal arthrogryposis, multiple pterygia, ulnar deviation of the hands with clenched fingers, microcephaly, and similar facial appearance. The boy had glandular hypospadias and feeding difficulties with frequent vomiting in infancy which led to a placement of a gastric tube. Unexplained hyperpyretic episodes were present in one, none of them had abnormal sweating. Profound developmental delay was present in both. Ophthalmologic evaluation was not performed. ES analysis revealed a homozygous truncating variant in *KLHL7* (NM_001031710.3):

c.1051C>T (p.Arg351Ter) present in both probands. Heterozygous mutation was confirmed in parents of one patient.

Conclusion: Both children originated from an isolated small community. Since their parents were not related up to fourth generations, we could suspect that this is a founder mutation within this small population. Further investigations should be performed.

Conflict of Interest: None declared

P12.025.A Biallelic KDM8 variants cause severe failure to thrive, intellectual disability and peculiar facial dysmorphism

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2-oxoglutarate (2OG) oxygenases that catalyze histone lysine demethylation belong to a subfamily known as 'Jumonji-C' (JmjC) enzymes. *KDM8* is a highly conserved gene and is the only member of the JmjC-only hydroxylase family that has been demonstrated to be essential for early mouse embryonic development. However, no germline variants in *KDM8* have yet been described that are associated with any human pathology. We present a family with two sibs who presented a pre-and postnatal severe failure to thrive, relative macrocephaly, facial dysmorphism, brain atrophy, moderate intellectual disability, and muscular hypotonia. Growth hormone replacement therapy had a positive impact on their growth. A quadruplicate whole genome sequencing discovered biallelic variants in the *KDM8* gene. The paternally inherited variant is a deletion in intron 7-8 (*c.1086+14_1200_21del*) resulting from the inappropriate removal of exon 7, which reside within a highly conserved section of the JmjC domain responsible for binding the co-factor 2OG. The maternally inherited *KDM8* variant is a missense variant (*c.482G>A*, *p.Cys123Tyr*) that causes the substitution of a highly conserved cysteine residue in the N-terminus. Functional analyses demonstrated that biallelic germline *KDM8* pathogenic variants severely compromise normal KDM8 mRNA splicing and protein stability, rendering cells hypomorphic for KDM8 function. We demonstrated that patient-derived cells exhibit increased levels of replication stress and genome instability in a manner that is critically dependent on the hydroxylase activity of KDM8. This work identifies KDM8 as a critical regulator of the replication stress response that is essential for suppressing neurodevelopmental abnormalities in humans. Grant: PRG471.

Conflict of Interest: None declared

P12.026.B Parents as partners: data consistency and data availability of parent-reported phenotypes

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Background: Despite the introduction of techniques that allow accurate genomic characterisation, knowledge about the phenotypic spectrum of rare chromosomal disorders remains limited, both in literature and existing databases. Yet this clinical information is of utmost importance for health professionals and parents of children with these rare diseases. Since existing databases are often hampered by the limited time and willingness of health professionals to enter new data, the Chromosome 6 Project collects phenotype data directly from parents of children with a chromosome 6 disorder.

Methods: Two studies were performed to assess whether parent-reported phenotypes, collected via the online Chromosome 6 Questionnaire, are reliable and usable for research purposes. First, a data consistency study compared parent-reported phenotypes with phenotypes extracted from copies of medical files on the same individuals. Second, a data availability study compared parent-reported data on specific characteristics to data available in existing literature.

Results: This was the first study to compare parent-reported phenotypes with medical files on the same individuals. Data were 85-95% consistent for all main questions ($n = 115$) in the questionnaire, and 77–96% for sub-questions. However, data from parents were generally more complete and detailed. There was also significantly more data available from parents than could be extracted from literature. Only for the topics developmental delay and brain abnormalities, no significant difference in the amount of available data was found.

Conclusion: Our study shows that parent-reported phenotypes are reliable and we encourage active parent and/or patient participation in data collection for all rare disease research.

Conflict of Interest: None declared

P12.027.C Chromosomal microarray analysis identifies a novel SALL1 deletion, supporting the association of haploinsufficiency with a mild phenotype of Townes–Brocks syndrome

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Background/Objectives: *SALL1* heterozygous pathogenic variants cause Townes–Brocks syndrome (TBS), a condition with variable clinical presentation. The three major diagnostic criteria are stenotic/imperforate anus, dysplastic ears, and thumb malformations. The most common pathogenic variant (p.Arg276Ter) was proven to escape nonsense-mediated mRNA decay and to cause disease via a dominant-negative mechanism. Haploinsufficiency may result in milder phenotypes, but only four families with distinct *SALL1* deletions have been reported to date. We report on a family with a novel *SALL1* deletion and review the clinical findings of known individuals with *SALL1* deletions.

Methods: Array-CGH analyses were performed using CytoSure Oligo array ISCA v2 8 60 K OGT, resolution ~150–210 kb. We reviewed the literature for patients with *SALL1* deletions (haploinsufficiency) and patients with the common p.Arg276Ter mutation (dominant-negative) and compared the prevalence of

clinical features in the two groups of patients using Fisher's exact text.

Results: Anal malformations are equally represented in the two groups, whereas the other anomalies appear to be more frequent in individuals with p.Arg276Ter mutation, although only "dysplastic ears" and "congenital heart defects" reach a statistically significant difference (p- value = 0.0230 and 0.0095). Patients with *SALL1* deletions show a milder phenotype, since they are less likely to fulfil the three major diagnostic criteria and have a lower frequency of renal and cardiac disease.

Conclusion: Abnormal truncated *SALL1* protein, acting in a dominant-negative way, is responsible for the full spectrum of developmental defects seen in TBS, whereas *SALL1* haploinsufficiency causes a milder TBS-like phenotype.

Grant References

Conflict of Interest: None declared

P12.029.A Novel biallelic variants expand the phenotypic spectrum of FAR1-related disorder

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Background: Fatty acyl-CoA reductase 1 (FAR1) is a ubiquitously expressed peroxisomal membrane protein that generates fatty alcohol required for the biosynthesis of plasmalogens. To date, biallelic loss of function variants in *FAR1* have only been reported in six individuals from five unrelated families linked with infantile-onset neurological and developmental features.

Methods: We conducted a thorough clinical characterisation followed by whole exome sequencing and Sanger segregation analysis in four patients from two unrelated families. Splicing and metabolic assays were subsequently conducted in genomic DNA and patient-derived cells.

Results: We report the first North African and South Asian patients with novel biallelic splicing and missense *FAR1* variants. All affected individuals are the offspring of consanguineous parents and present with a broad phenotypic spectrum, including developmental delay, intellectual disability, microcephaly, hypotonia, and spastic diplegia. RNA studies using a minigene assay revealed whole exon skipping and cryptic activation of a donor splice site, resulting in a frameshift/premature termination codon and an in-frame deletion of 19 residues, respectively.

Conclusion: In this study, we report four novel cases of *FAR1*-deficiency disorder in two unrelated families and further expand the molecular and phenotypic spectrum of *FAR1* variants. Our findings will enable clinicians to distinguish clinical features of similarly affected patients to further improve our understanding of this ultra-rare autosomal recessive peroxisomal disorder.

Conflict of Interest: Hatice Tasan: None declared, Arisha Rasheed: None declared, Amatul Raqeeb: None declared, Jonas Setzke: None declared, Barbara Vona Principal Investigator, Go Hun Seo: None declared, yamna kriouile: None declared, Javeria Raza Alvi: None declared, Elisa Cali: None declared, Vincenzo Salpietro: None declared, Tipu Sultan: None declared, Reza Maroofian: None declared, Sadaf Naz Principal Investigator, Henry Houlden Principal Investigator, Sacha Ferdinandusse: None declared, Stephanie Efthymiou: None declared

P12.030.B Near miss — Long-read DNA sequencing uniquely enabled diagnosis of oculo-facio-cardio-dental syndrome caused by mosaicism of a complex duplication of BCOR

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Background: A mother and her daughter with overlapping symptoms including congenital cataract, glaucoma, heart defect and dental abnormalities presented at our clinic for genetic diagnosis. No specific disease was clinically suspected.

Methods: DNA samples from both patients were analyzed by SNP array, exome sequencing, and Oxford Nanopore long-read DNA sequencing, followed by confirmatory breakpoint junction PCR to establish the diagnosis.

Results: No causal variants satisfying ACMG criteria were detected by SNP array and exome sequencing of the mother. However, exome sequencing of the daughter revealed a segmental duplication within the BCOR gene. As clinical presentation suggested the same diagnosis in both patients, genetic testing was extended to long-read sequencing and junction PCR.

Long-read sequencing of the daughter identified a complex duplication of 20 kb on Xp11.4 encompassing exons 2 to 6 of the BCOR gene. The same rearrangement was detected in a minority of reads of the mother, suggesting a mosaic of the duplication. The identified tandem duplication is interrupted by an inverted intronic fragment. PCR amplification of the various breakpoint junctions ultimately confirmed the duplication in the mother in a mosaic state. No alteration in BCOR was found in the unaffected grandmother, confirming de novo mosaic status in the mother. Thus, we were able to confirm the diagnosis of oculo-facio-cardio-dental syndrome in both patients.

Conclusion: In patients with strongly suspected genetic diagnosis, complimentary genetic testing by long-read sequencing may be beneficial. Mosaicism of complex rearrangements may remain difficult to identify even by long-read sequencing due to limited sequencing depth.

Conflict of Interest: None declared

P12.032.D Two sisters with Hermansky Pudlak syndrome type 2 presenting with pediatric-onset interstitial lung disease: an underrecognized entity

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Background/objectives: Hermansky-Pudlak syndrome type 2 (HPS2) is a severe disorder caused by bi-allelic variants in *AP3B1*. It is characterized by oculocutaneous albinism, bleeding diathesis, and neutropenia.

Methods: We report two sisters with pediatric-onset interstitial lung disease (ILD). Clinical data were collected from medical records. Whole exome sequencing (WES) was performed using a trio-approach.

Results: The index patient presented with respiratory insufficiency 14 hours after birth. At age one week, a CT-thorax showed ground-glass opacities and bronchiectasis. Lung biopsy showed immature lung tissue with irregularly enlarged, simplified alveoli and vacuolization of alveolar type II cells. She also had ocular albinism and a thrombocyte aggregation defect. WES revealed two compound heterozygous variants in *AP3B1*: an earlier described variant c.177delA p.(Lys59Asnfs*5) and a novel variant c.2816T>C p.(Leu939Pro). Family history revealed that her 2-year old sister also had HPS2-features: oculocutaneous albinism, a thrombocyte aggregation defect, neutropenia, and features of ILD. She had the same variants in *AP3B1*. Due to the typical HPS2-features and bi-allelic *AP3B1* variants in both sisters, the diagnosis of HPS2 was established. HPS2 has now been described in ~40 patients. Pediatric-onset ILD is an underrecognized entity, which has been reported in ~40% (15/40) of HPS2 patients.

Conclusion: we describe a novel likely pathogenic *AP3B1* variant in two sisters with HPS2 and pediatric-onset ILD. This underscores the importance of detailed phenotyping and genetic testing for patients with pediatric-onset ILD. Finding a genetic cause can improve patient care, help to identify relatives at risk and it can be of importance for reproductive choices.

Conflict of Interest: None declared

P12.034.B New CDC42 missense mutations give rise to diverse functional alterations and cause heterogeneous spectrum of neurodevelopmental and immune-hematologic rare conditions

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CDC42 (Cell Division Cycle 42) is a small GTPase of the RAS super family regulating key developmental processes. We have recently characterized different CDC42 missense mutations associated with a broad spectrum of immune-haematological and neurodevelopmental disorders, which include RASopathies-related phenotypes.

Here, we report the identification and molecular characterization of six additional CDC42 variants associated with neurodevelopmental and immunological conditions. In particular, patients present with various degree of cardiac defects, neurodevelopmental delay, facial dysmorphisms, inflammatory features, and recurrent infections. CDC42 mutants variably perturb GTPase activity, effector binding (i.e. IQGAP1, RHOGDI, and N-WASP) and RAS-mitogen-activated protein kinase (MAPK) pathway in transiently transfected cells. The comparative functional analysis of the CDC42 variants so far described indicates that the IQGAP1-binding defective CDC42 mutants are associated with the inflammatory phenotypes, suggesting a common pathogenic mechanism for these variants. Moreover, the majority of CDC42 variants significantly increases MAPK activation, indicating a role of the RAS-MAPK pathway in the pathogenesis of CDC42-associated disorders. A heterogeneous alteration of developmental programs were observed in transgenic C. elegans lines overexpressing the mutant alleles, providing further evidence of the pathogenicity of the variants and explaining, at least in part, phenotypic variability.

Finally, our study expands the spectrum of CDC42 pathogenic variants and confirms the relevance of functional validation of unclassified variants to assess their possible role in disease pathogenicity.

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Conflict of Interest: None declared

P12.035.D Clinical exome and panel sequencing in consanguineous Kuwaiti families yield novel variants in different conditions

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Background: The growing of rare genetic disorders, genes associated with, and novel variants is primarily caused by high consanguineous marriages in the Arab populations. It is estimated that consanguineous rate is >50% of population in the Middle east (1,2). This study show how exome sequencing in consanguineous Kuwaiti population identifies novel variants in rare genetic disorders.

Methods: Exome sequencing were applied to 9 probands with different clinical genetic conditions seen at the Kuwait Medical Genetics Centre, Kuwait City. Furthermore, parental analysis was carried out to assess the variant in each family.

Result: This work present 9 novel variants were found in 8 probands who are results from consanguineous parents (mostly first cousin) with genetic disorders includes the *NEXN* gene in cardiomyopathy, *ATP8A2*, and *AGXT* genes in metabolic disorders, *DDX11*, *KCNJ1*, *EDAR*, *SCARF2* in craniofacial syndromes, and *DZIPL1* in polycystic kidney disease. Furthermore 1 variant was reported once in disease associated database including *GALNT3* gene in metabolic disorder.

Conclusion: High consanguineous rate in Arab population is one of the strong tools to demonstrate the yield of novel variants in different genetic disorders. This in-turn will tremendously help to expand in discovering more variants, as well as novel genes to increase our understanding of the mechanism and function of each gene, and how it effects the human development.

Grant: not applicable

References:

[1] Ben-Omran T et al. *Mol Genet Genomic Med*. 2020;8:e1051
[2] Alkuraya FS. Clin Genet 2013:84: 203-208.

Conflict of Interest: None declared

P12.036.A : Unusual mode of inheritance in various genes and syndromes: Examples of genes with more than one mode of inheritance

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Background / Objectives: Classically, it is known that each gene has one mode of inheritance (MOI). However, since exome and genome testing become widely available in clinical practice; more reports reveal various genetic syndromes with unusual MOI. The aim of this study is to show that some genes can unusually have more than one MOI.

Methods: Patients from three families with various clinical phenotypes were chosen for whole exome sequencing (WES): trio and Quadro testing were applied for two families; while WES solo with parental testing was applied for the third family.

Result: Although *DNM1* and *PPOX* genes are autosomal dominant (AD) genes; c.107T>G variant was detected in *DMN1* gene in homozygous state in all three affected children of the first family; while both parents heterozygous carriers. Similarly, both affected girls in the second family are homozygous for c.164A>C variant in *PPOX* gene; while both parents are heterozygous carriers. This clearly shows autosomal recessive inheritance in both variants detected in *DNM1* and *PPOX* genes respectively. For the 3rd family, a heterozygous non-sense c.1096G>T variant detected in *IRF2BPL* gene; while parental testing showed the father carries it raising great possibility of incomplete penetrance of previously known AD with complete penetrance.

Conclusion: Surprisingly, different MOI in three different genes are shown in this study raising a possibility that many genes can have more than one MOI. Therefore, it is wise to report any variant with unusual MOI in order to have better understanding of nature and function of various genes.

No grant.

Conflict of Interest: None declared

P12.037.B Ritscher-Schinzel syndrome: broadening the phenotype in prenatal and adulthood

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Background/Objectives: Ritscher-Schinzel syndrome (RTSCS), is a rare genetic condition associated with recognizable dysmorphic features, and cerebellar and cardiovascular malformations. Four genes have been associated to this disorder (*WASHC5, VPS36L*, *DPYSL5* and *CCDC22*) and no genotype-phenotype correlations were identified. We collected genetically confirmed patients in order to better characterize the clinical and molecular spectrum of this syndrome.

Methods: Patients were gathered by an international collaboration of genetic and paediatric departments trough Genematcher. Clinical data was collected by the treating physicians.

Results: Six patients from two unrelated families were identified through exome sequencing. A child and three foetuses from family 1 carried compound heterozygous *WASHC5* variants: a known pathogenic nonsense and a novel missense affecting a highly conserved amino acid residue at the WAHS strumpellin domain. Two adult brothers from family 2, carried a missense hemizygous *CCDC22* variant affecting a highly conserved amino acid residue at the DUF812 domain. Besides the typical craniofacial dysmorphisms, developmental delay and intellectual disability, the patients presented some clinical features reported for the first time in RTSCS: the foetuses showed prenatal ultrasound findings (increased nuchal thickness, short nasal bridge, and/or heterogenous choroid plexus), and the adults parkinsonian features (rigidity, stooped posture, reduced arm swinging and neurocognitive regression).

Conclusion: We expand the genotypic and phenotypic spectrum of RTSCS, describing some pre- and postnatal findings hitherto not related to this syndrome, which have direct impact on postnatal outcome, management, and familial counselling.

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Conflict of Interest: None declared

P12.039.D Identification and functional characterization of a -47 intronic variant in KMT2D causing Kabuki syndrome

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Kabuki syndrome type I (OMIM #147920) is a congenital intellectual disability syndrome associated with distinctive dysmorphic features and caused by pathogenic variants in *KMT2D* gene (PMID: 20711175). To date, more than 700 pathogenic or likely pathogenic variants have been reported in Clinvar, with most of them being loss-of-function variants (85%). Despite this, to the best of our knowledge, no intronic variant >20 bp from authentic junctions interfering with canonical splicing has ever been described.

We report a novel de novo heterozygous intronic variant (NM_003482.4:c.10356-47A>G) in *KMT2D* in a five years old patient with complex heart disease, global developmental delay, suspicion of moderate intellectual disability, unilateral multicystic kidney, stagnant weight and height, and phenotype reminiscent of Kabuki syndrome. The in vitro functional characterization of the variant, performed on RNA from the patient's blood, shows that it causes splicing alteration, consisting on the creation of a cryptic acceptor splice site located at position -47 of intron 36. As a result, 46 nucleotides are inserted (r.10355_10356ins10356-46_10356-1), resulting in the alteration of the reading frame and the formation of a premature termination codon, p.(Arg3452SerfsTer31).

In this work we performed an exhaustive review of Kabuki syndrome candidate genes, due to a high clinical suspicion, that resulted, for the first time, in the discovery of a -47 intronic variant in KMT2D that affects its canonical splicing and causes the syndrome. Our findings urge a review of other intronic variants further than 20 bp from the nearest exon-intron junction in similar undiagnosed clinical cases.

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Conflict of Interest: None declared

P12.040.A In trans reciprocal deletion and duplication of the NXN gene causing Robinow syndrome escaping routine diagnostic testing

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NXN-related autosomal recessive Robinow syndrome (MIM#618529) has been reported in four patients from three families. We present a young, adopted female with clinical features suggesting *NXN*-Robinow syndrome, including omphalocele.

A routine genomic investigation was normal, including an exome sequencing (ES) in silico gene panel encompassing 183 genes and genome-wide high-resolution chromosomal microarray (CMA). Because of the high suspicion of *NXN*-related disease, we performed a manual inspection of *NXN* in ES and CMA raw data, which indicated two small deletions in exon 8 and a limited proximal part of the large intron 1, respectively. However, further investigation with short-read genome sequencing (GS) revealed almost completely overlapping gain and loss on opposite alleles, resulting in an apparent largely neutral copy-number state. One allele carried a 93 kb deletion, starting proximal in intron 1 and ending downstream of *NXN*. The other allele showed a tandem duplication, encompassing most of intron 1 to exon 7 (of total 8 exons) of *NXN*, interrupted by a short segment of haploid CN state in the distal part of intron 1.

NXN maps to 17p13.3, a region characterized by high *Alu* density. Two of the three described families with *NXN* pathogenic variants have deletions affecting one or several exons. *Alu*/*Alu*-mediated rearrangement (AAMR) was involved in at least one of the published deletions, and seemed to be the mechanism for both CNVs in our patient.

We believe that the CNVs in *NXN* explain the patient's Robinow phenotype. However, the prevalence of variants in *NXN*, including CNVs, is still unknown.

Conflict of Interest: Siren Berland: None declared, Eirik Bratland: None declared, Lene Hjertnes: None declared, Tuva Barøy: None declared, Anna Lindstrand Honorarium from Illumina, Advisor for ONT and PacBio, Claudia Carvalho: None declared

P12.041.B The emerging role of mitochondrial dysfunction in a cohort of patients with Rasopathy

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Background/Objectives: Recently, a link between the RAS-MAPK pathway, mitochondrial function and bioenergetics has been hypothesized but the underlying mechanisms are still largely unexplored. The aim of the current prospective case-control study was to investigate the role of mitochondrial dysfunction in clinical outcome of patients with RASopathies.

Methods: 11 patients (4 F, 7 M) were enrolled to explore mitochondrial dysfunction. Anthropometric evaluation and fasting biochemical data were investigated. The analysis of the expression of specific genes was performed by RT-PCR. Determination of the main serum amino acids and acylcarnitines was conducted by tandem mass spectrometric analysis.

Results: Although 2/11 showed poor weight gain, mean BMI values were similar in patients and in controls. Noteworthy mean serum fasting glucose levels were significantly lower in patients than in controls ($p = 0.0036$). At RT-PCR the mRNA levels of *CPTA1, NRF1, AKT3* genes were significantly lower in patients than in controls ($p < 0.05$). The analysis of plasma acylcarnitines demonstrated a statistically significant reduction ($p < 0.05$) of C4, C6, C8, C10, C14, C4DC, C5DC, C16:OH, C12:1, C14:2, C16:1. Moreover C0/C16 + C18 was significantly increased in patients ($p = 0.0017$). The analysis of serum amino acids showed a statistically significant reduction ($p < 0.05$) of Alanine, Valine, Methionine, Aspartate, Glycine, Ornitine, and Citrulline in patients compared to controls.

Conclusion: The impairment of RAS-MAPK pathway is associated with mitochondrial dysfunction. The obtained data might explain the alterations of energy metabolism recently reported in patients with RASopathy. These studies need to be further investigated in order to better characterize potential therapeutic targets.

Conflict of Interest: None declared

P12.043.D Buccal cell whole exome sequencing improves the diagnostic yield in a Cornelia de Lange Syndrome Brazilian cohort

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Background/Objectives: Cornelia de Lange Syndrome (CdLS) is a rare multisystemic developmental disorder with physical, cognitive, and behavioral features. Mutations on the *NIPBL* gene are responsible for about 60% of CdLS cases. Mosaicism occurs in 15%-20% of cases, not usually identified in peripheral blood. Thus, alternative tissues, such as buccal cells, should be used to investigate mosaicism. In this study, we aimed to diagnose patients that remained undiagnosed after performing whole exome sequencing (WES) and RNA sequencing from DNA extracted from peripheral blood.

Methods: Sixteen patients that remained undiagnosed after performing WES and RNA sequencing were included. Buccal cells were collected by scrubbing Kolplast cervical brush for two minutes on each side of the patients' cheeks. DNA was extracted with Gentra Puregene buccal cell kit (Qiagen) and submitted to a

cleaning and concentration step using Genomic DNA Clean & Concentrator™ Kit-10 (Zymo Research). WES was performed with paired-end libraries on Illumina NOVASEQ platform. Data were processed using the DRAGEN DNA Pipeline. Variants were classified according to the ACMG guideline.

Results: Preliminary data revealed likely pathogenic variants in *NIPBL* in four patients (25%). Three patients presented indels (NM_133433.4:c.3821del:p.Gln1274ArgfsTer10, NM_133433.4:c.3878_3881del:p.Glu1293GlyfsTer25, and NM_133433.4:c.195dup:p.Val66CysfsTer3), and the other presented a missense variant (NM_133433.4:c.6893G>C:p.Arg2298Pro). These data improved the diagnostic yield of our cohort from 75.8% (50/66) to 81.8% (54/66).

Conclusion: Combined methodologies are required to improve the diagnostic yield of CdLS patients. Buccal cells are great alternative material because of their easy obtaining, storage, and improved diagnostic yield.

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Conflict of Interest: None declared

P12.044.A Clinical phenotype of Noonan syndrome due to RRAS2 mutations: 6 new cases and review of the literature

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Noonan syndrome (NS) is the most common disease among RASopathies. It is characterized by the association of characteristic dysmorphic features, congenital malformations, growth retardation, neurodevelopmental disorders and predisposition to cancer. NS is genetically heterogeneous with activating mutations in more than 12 genes coding for proteins involved in the RAS-MAPK pathway.

RRAS2 gene mutations were reported in a limited number of patients with NS. The role of TC21, encoded by the RRAS2 gene, in the RAS-MAPKinase signalling pathway as well as its level of homology with other RAS proteins left little doubt about its possible involvement in RASopathies. Biochemical, cellular and animal model studies have been able to confirm the responsibility of activating RRAS2 mutations in 13 patients with NS or related disorders.

Fifteen patients have been reported to date and we have gathered information about 6 new cases. The 10 mutations identified (8 published and 2 new mutations) are distributed over 2 regions of the gene, 5 mutations happened to be recurrent in unrelated patients. The phenotypes observed are variable ranging from a mild form, to lethal form with 5 cases of perinatal death, 2 terminations of pregnancy, 1 case with an intermediate phenotype between NS and Cardio-Facio-Cutaneous syndrome and 3 cases are qualified as not recognizable for a RASopathy. Two cases developed a cancer, 2 cases had ophthalmologic coloboma, 2 cases had bifid uterus and 2 case had hearing loss.

Description of new cases is important to deepen our knowledge of the clinical spectrum of this gene in NS.

Conflict of Interest: None declared

P12.045.B Spanish Undiagnosed Rare Diseases Program (SpainUDP): A review after 8 years of experience

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Background/Objectives: The underlying etiologies for many rare diseases remain undiscovered and knowledge gaps continue regarding genotype-phenotype correlation. To address these issues, the Spanish Undiagnosed Rare Diseases Program (SpainUDP) was officially established in Spain by the IIER-ISCIII in 2015.

Methods: The first phase of SpainUDP protocol consists of deep phenotyping and trio-based WES in blood samples. However, more than 50% of patients continued undiagnosed after WES, so additional diagnostic strategies were implemented to improve diagnostic rates of unsolved cases, such as WGS or RNA-Seq.

Results: Since the official launch, 83 patients with ultrarare diseases have been diagnosed and resolution of 2 cases required technologies "beyond the exome". The majority of the solved cases corresponds to pediatric neurological disorders. The overall diagnostic rate is 40%, although diagnostic rate is higher for the pediatric population (44%) compared to the adult group (26%). On the other hand, 124 patients remain undiagnosed. Regarding these unsolved cases, some candidate variants are being evaluated by using cultured cells, organoids and animal models for functional assays which are expected to allow the characterization of the molecular mechanisms of unknown diseases.

Conclusions: Our experience highlighted the need for establishing an integrated approach consisting of the use of combined omics methods, as well as functional assays to support the phenotype-genotype relationship of a putative variant. Also, our results suggest that the discovery of adult genetic diseases remains a challenge for undiagnosed rare diseases programs.

Grant References: Spanish Undiagnosed Rare Diseases Program (SpainUDP). Platform ISCIII Biobanks and Biomodels grant PT20CIII/00009.

Conflict of Interest: None declared

P12.046.C A compound heterozygosity of a large deletion and a missense variant in PNPLA6 causing Boucher-Neuhäuser syndrome in elderly patients presenting with chronic progressive ataxia, chorioretinal dystrophy, and hypogonadotropic hypogonadism

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Background: We report three sibs, a 67-year-old male, a 65-year-old female, and a 62-year-old female, from a Thai family presenting with chronic progressive ataxia and chorioretinal dystrophy in the adult onset. The subsequent biochemical investigation showed remarkable findings for hypogonadotropic hypogonadism in all patients, especially the male case commencing early-onset osteoporosis.

Objectives: To characterize the molecular basis causing a distinctive phenotype in this family.

Methods: Whole-exome sequencing (WES) was performed in these patients. Large gene deletion was confirmed by exon arrays.

Results: The WES revealed heterozygous c.3931C>T (p.Arg1311Trp) in PNPLA6 (NM_006702.4) and was initially classified as a variant of uncertain significance. Meanwhile, a large region of polymorphisms appeared to be monomorphic, suggesting a significant copy number loss. Finally, a PNPLA6 deletion from exons 17 to 31 were confirmed by exon arrays. The results were consistent in all relatives.

Conclusion: The genomic finding was consistent with a molecular diagnosis for Boucher-Neuhäuser syndrome due to compound heterozygosity of a large deletion and a missense variant in PNPLA6. The future work includes the technical development for a deletion carrier screening in the family using either quantitative or gap PCR. We conducted this study to provide better clinical care, make a final diagnosis and help patients deal with emotions raised by a disorder like guilt, fear, and helplessness.

Grant References: Cultivating Medical-Scientific Expertise for Medical Students Program, Faculty of Medicine Ramathibodi Hospital, Mahidol University

Keywords: Boucher-Neuhäuser syndrome, PNPLA6, ataxia

Conflict of Interest: Pannaporn Kalkoljuck Cultivating Medical-Scientific Expertise for Medical Students Program, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Nareenart Iemwimangsa Centre for Medical Genomics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, sommon klumsathian Centre for Medical Genomics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bhakbhoom Panthan Centre for Medical Genomics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Pichet Termsarasab Faculty of Medicine Ramathibodi Hospital, Mahidol University, Chutintorn Sriphrapradang Faculty of Medicine Ramathibodi Hospital, Mahidol University, Tharikarn Sujirakul Faculty of Medicine Ramathibodi Hospital, Mahidol University, Takol Chareonsirisuthigul Faculty of Medicine Ramathibodi Hospital, Mahidol University, Objoon Trachoo Faculty of Medicine Ramathibodi Hospital, Mahidol University

P12.047.D Digenic inheritance of PAX3 and SFRP5 underlies syndromic myelomeningocele

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Neural tube defects (NTDs) are clinically and genetically heterogeneous developmental disorders. We report 2 fetuses with syndromic myelomeningocele due to digenic inheritance.

Trio Exome sequencing was analysed on the basis of an oligogenic inheritance hypothesis. Functional studies using an iPSC-derived human spinal organoid model was performed to explore the phenotypic consequences of combined expression of 2 variants on 2 candidate genes.

We report a family in which the mother, with Waardenburg syndrome (WS) due to a *PAX3* pathogenic variant c.181G>T, p.(Glu61*), had 2 fetuses with clinical signs of WS and myelomeningocele. Exome analysis identified the *PAX3* variant and a second rare and predicted deleterious variant in the *SFRP5* gene, c.307G>A, p.(Asp103Asn) inherited from the asymptomatic father. Loss of *PAX3* alters the structure of epithelia emerging in organoids and results in defects in neuronal specification. The organization of these epithelia is also modulated by the expression of the *SFRP5* variant and a phenotypic synergy was revealed between the loss of *PAX3* and the presence of the SFRP5 variant. Furthermore, the divergence of the phenotypes of organoids expressing the *SFRP5* variant from those subjected to loss of function suggests a neomorphic function of this variant.

The oligogenism inherent to myelomeningocele complicates our understanding of the etiology of NTD. Our study shows that two genes controlling two apparently distinct cellular processes (transcriptional regulation and planar polarity pathways) converge to regulate cell cohesion within the neuroepithelium. Our study also explains how myelomeningocele can be a rare clinical sign associated with WS.

Conflict of Interest: None declared

P12.048.A Mutations in CPLANE2 are responsible of a new form of orofaciodigital syndrome

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Background: Orofaciodigital syndrome (OFD) is a genetically heterogeneous ciliopathy characterized by anomalies of the oral cavity, face, and digits. We describe individuals with OFD from two unrelated families having bi-allelic variants in *CPLANE2*.

Methods: Whole Exome sequencing (WES) of two patients with clinical suspicion of OFD syndrome was performed. No variants in known genes related to phenotype were detected but biallelic variants in *CPLANE2* where found in the two probands. A collaborative effort using the web-based tool GeneMatcher was done and allowed to connect the two probands with OFD and *CPLANE2* variants. Besides collecting and comparing clinical and molecular data, functional analyses consisting in the evaluation of gene expression and protein expression of CPLANE2 and, GLI1 and PTCH1 (two target genes of the hedgehog signalling pathway) were also done.

Results: Main clinical characteristics of our patients include polydactyly of hand and feet (2/2) with high/cleft palate (2/2), lobulated tongue (2/2), hypertelorism and epicanthus (2/2), congenital heart defect (2/2), feeding difficulties (2/2), breathing issues and recurrent respiratory tract infections (2/2) and

developmental delay (2/2). In patient 1, biallelic variants in *CPLANE2* were identified (c.226G>C/c.-25C>G,) whereas in patient 2 (c.353G>A) a homozygous variant was identified. In expression analyses performed in patient 2 we observed that the mutation did not cause significant changes in CPLANE2 expression. However, this mutation causes a significant decrease in GLI1 and PTCH1 expression.

Conclusions: Our results contribute to describe a new subtype of OFD due to *CPLANE2* variants.

Conflict of Interest: None declared

P12.049.B A novel case of parental mosaicism in SMC1A gene causes inherited Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS, OMIM #122470, #300590, #610759, #614701, #300882) is a multisystemic genetic spectrum characterized by growth retardation, facial dysmorphia, intellectual disability and limb reduction defects. The molecular bases of this disorder are associated with pathogenic variants in genes related to the cohesin complex (*NIPBL, SMC1A, SMC3, RAD21, HDAC8, BRD4, ANKRD11 or MAU2*). Originally, CdLS, as most dominant genetic diseases, was thought to be caused by de novo pathogenic variants. Nevertheless, unexpected transmitted cases resulted from parental gonadosomatic mosaicism are arising over the last few years in many genetic diseases, principally due to the enhancements in diagnostic techniques, namely, next-generation sequencing of DNA, that allows the detection of variants with very low allele frequencies.

Regarding CdLS, up to date, only a few cases are known to follow this inheritance pattern. However, the high prevalence of somatic mosaicism recently reported in this syndrome (~13%), together with the disparity observed in tissue distribution of the causal variant, suggests that its prevalence could be underestimated.

Here we describe a new case of parental gonadosomatic mosaicism in *SMC1A* gene that causes inherited CdLS, in which the unaffected mother carries the causative variant in very low allele frequencies in buccal swab and blood.

Overall, DNA deep-sequencing techniques are highly recommended when it comes to molecular diagnosis of patients and co-segregation studies. The identification of parental mosaicism could substantially alter recurrence risk, with a significant impact in reproductive genetic counseling.

Grant References: Spanish Ministry of Health-ISCIII (FIS Project PI19/01860) and Diputación General de Aragón.

Conflict of Interest: None declared

P12.051.D An inherited PUF60 variant expands the genetic and phenotypic spectrum of the Verheij syndrome

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PUF60 encodes the Poly-U Binding Splicing Factor 60, a component of the spliceosome. Heterozygous missense and nonsense variants in *PUF60* are causative for the Verheij syndrome (OMIM # 615583), which is variably associated with mild developmental delay, learning difficulties, behavioral problems, short stature and microcephaly. Additional congenital malformations of the heart, kidney, eyes and skeleton can be present.

We report the case of a nine-year-old boy who presented with developmental delay, behavioral problems, strabismus and a unilateral preaxial toe polydactyly as well as a syndactyly. Learning disabilities, mild intellectual disability, short stature, strabismus and unilateral toe polysyndactyly were also present in the mother.

Duo exome sequencing identified a maternal heterozygous variant in *PUF60* (NM_078480.3: c.109C>T, p.(Gln37*)) that is predicted to undergo nonsense mediated decay and matches the phenotype. A maternal uncle with bilateral toe syndactyly, strabismus and behavioral problems was not yet tested.

To our knowledge, this is the first case report of an inherited likely pathogenic *PUF60* variant in two generations. The similar phenotype, including toe polysyndactyly and strabismus, between affected individuals might indicate a small intrafamilial variability and could be explained by shared modifying genetic factors. This broadens the clinical and genetic spectrum of *PUF60*-associated Verheij syndrome.

Conflict of Interest: None declared

P12.052.A Brothers in arms? - Somatic HRAS and germline RAF1 mutation as potential cooperative events underlying urothelial carcinoma in a RASopathy patient

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Background/Objectives: Noonan syndrome (NS) is associated with increased risk for childhood solid and haematologic malignancies and (often transient) myeloproliferative disorders. However, the specific tumour risk for *RAF1*-positive NS patients is currently unclear. To date, malignant manifestations have been noted in a few individual reports only, and, particularly, a predisposition to urothelial carcinomas is not known.

Case Report: We report on a 39-year-old patient with characteristic features of NS (NS with multiple lentigines) including postnatal biventricular hypertrophy as cardiac manifestation. At the age of 34, urothelial carcinoma (UC) of both pyelons were diagnosed. In the meantime, the patient developed a seminoma of the right hypotrophic testis.

Results: In NGS panel diagnostics for RASopathies (Custom Panel, Illumina) and for tumour disposition syndromes (TruSight Cancer v02, Illumina), heterozygosity for the pathogenic *RAF1* variant p.(Pro261His) in the CR2 mutation hotspot was identified in the blood. Since the etiological role of the *RAF1* germline variant for the UC manifestation remained unclear, we performed additional sequencing (xGen® Exome Research Panel v2.0, IDT) for UC-associated genes of the RTK/RAS/PI(3)K pathway using a DNA sample obtained from FFPE tissue of the right pyelon. In this analysis, the *HRAS* hotspot variant p.(Gly13Arg) was detected (VRF 29%/31x) as an additional potential somatic driver event. The analysis of DNA from blood and from a gastric antrum biopsy gave

no evidence for a constitutional *HRAS* mosaicism at high sequencing depth.

Conclusion: For the UC tumorigenesis in this case, we postulate an unreported potential cooperative effect of constitutional *RAF1* and somatic *HRAS* variant.

Conflict of Interest: None declared

P12.053.B FAM118A a candidate gene for a new syndrome of mild developmental delay, distal arthrogryposis, exostoses of manubrium sterni, missing adult teeth, and amblyopia

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Background/Objectives: A mother-daughter pair presented to our clinic with an unusual phenotype of mild developmental delay, distal arthrogryposis, exostoses of manubrium sterni, missing adult teeth, and amblyopia. Chromosome analysis identified a de novo balanced reciprocal translocation t(7;22)(q36.2;q13.32) in the mother and inherited in daughter suggesting gene rearrangement. We characterized the patients with a novel cytogenetic technique to determine the breakpoints and identify a candidate gene.

Methods: Clinical laboratory genomics workup. Optical genome mapping (OGM) was performed at the Bionano Services Lab in France. For each sample, ultra-high molecular weight DNA was purified, labelled and stained. The Saphyr chip was ran on both samples aiming for at least 100X coverage. The de novo assembly and Variant Annotation pipelines were executed on Bionano Solve v3.7 and reporting and direct visualization of structural variants was done on Bionano Access v1.7.

Results: aCGH and short sequence WGS were negative. OGM was able to better define the breakpoints (ch22: 45,704,175.5 ± 2387.5bp. ch7: 155,867,681.5 ± 8,052.5bp) and revealed the potential involvement of one gene *FAM118A*.

Conclusion: We elucidated the breakpoints of this de novo translocation in patients which led to an identification of a candidate *FM118A* gene for a previously undescribed syndrome. The *FAM118A* gene encodes for an intramembrane protein with unknown biological function. It is not a known disease gene, but several possibly relevant associations with bone-related phenotypes have been reported. Further analysis is needed to determine molecular rearrangements in the *FAM118A* gene affecting pleiotropic effects.

Grant: Departmental service. First two authors equal contribution.

Conflict of Interest: None declared

P13 Cancer Genetics

P13.001.A The miR-590-3p/MDM2 axis regulates FOXO3 expression in Hepatocellular Carcinoma

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Background/ Objective: There is considerable evidence that miRNAs rely on their downstream genes to carry out their biological roles. miRNA-590-3p is a small non-coding RNA that has previously been implicated in cancer progression. Interestingly, our previous work revealed that miR-590-3p exhibits tumor suppressive activity in HCC, and the MDM2 gene had been identified as its downstream target. The present study aimed to silence the expression of MDM2, as a validated target of miR-590-3p, to identify possible targets in the miR-590-3p/MDM2 pathway that may potentially be implicated in HCC carcinogenesis.

Methods: Bioinformatics analysis was employed to identify downstream targets in the "miR-590-3p/MDM2" axis. Potential target genes predicted by bioinformatics tools were subjected to RT-qPCR. miR-590-3p (mimics and NC) transfected HepG2 cells were used to assess the effect of miR-590-3p overexpression on the predicted target gene. RNAi-mediated knockdown was used to inhibit mRNA and protein levels of MDM2.

Results: FOXO3, a transcription factor belonging to the FOXO family, was selected among various genes that were predicted by bioinformatics analysis as a novel possible target. FOXO3 expression was slightly upregulated and activated in HCC tissues, as indicated by the GENT2 database. miR-590-3p overexpression caused a significant reduction in FOXO3 expression. Similarly, the knockdown of MDM2 induced by RNAi led to an obvious inhibition of FOXO3 expression.

Conclusion: These results not only suggest that FOXO3 expression is negatively regulated by miR-590-3p via its action through miR-590-3p/MDM2 axis but also defend the oncogenic function of FOXO3 in HCC.

Grant References: The American University in Cairo

Conflict of Interest: None declared

P13.002.B Clinical evaluation of paediatric patients with solid tumours, for features of cancer predisposition syndromes in the Gauteng province of South Africa

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Background: We define cancer predisposition syndrome (CPS) as a germline genetic alteration that places an individual at a significantly higher risk of developing cancer than individuals in the general population. Early identification of a CPS could help stratify risks for a proband and their close relatives.

Methods: Our aim was to evaluate the role of histology, family history and personal clinical criteria in identifying an individual with a possible CPS and develop clinical guidelines for genetic testing that can be utilized in a limited resource setting.

Results: Sixty-two (62) children with solid tumours were assessed as 'likely' to have a CPS or 'unlikely'. Among the participants, 44/62 (71%) patients were classified as 'likely CPS', whereas 18/62 (29%) were 'unlikely CPS'. Amongst 'likely CPS' cases, 33/44 (75%) were identified based on personal history criteria, whereas 9/44 (20.5%) were based on histology criteria and 2/44 (4.5%) on family history criteria. Seven (7/44) 'likely CPS' participants had a recognizable genetic syndrome (e.g. Beckwith Wiedemann, Proteus and Li Fraumeni syndromes).

Discussion: Determining a likely CPS is important for directing appropriate genetic testing. We propose a guideline in which histology should be considered first, followed by family history

and lastly personal history in determining the likely CPS status of an individual. We suggest genetic testing of patients identified as likely CPS in our proposed algorithm. If molecular profiling studies show this guideline to have a significant positive predictive value, then limiting genetic testing to those identified clinically as likely CPS would be more cost effective.

Conflict of Interest: Njabulo Mabaso Full, Candice Feben: None declared, Lindiwe Lamola Full, Jennifer Geel Full, Gita Naidu Full, Janet Poole: None declared, Amanda Krause Krause Full

P13.004.D Dysregulated gene expression through TP53 promoter swapping in osteosarcoma

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Background: How massive genome rearrangements confer a competitive advantage to a cancer cell has remained an enigma. The malignant bone tumour osteosarcoma harbours an extreme number of structural variants and thereby holds the key to understanding complex cancer genomes. Genome integrity in osteosarcoma is generally lost together with disruption of normal *TP53* gene function, the latter commonly through either missense mutations or structural alterations that separate the promoter region from the coding parts of the gene. The consequences of such a relocated promoter have, however, yet to be unravelled.

Methods: We performed in-depth genetic analyses of osteosarcoma biopsies (n = 148) and cell models. Genome-wide copy number analyses were carried out using SNP-array, structural variants were identified using mate-pair whole genome sequencing, while RNA-sequencing was used for gene expression analyses and detection of fusion genes, single nucleotide variants and indels.

Results: We show that *TP53* structural variants are early events that not only facilitate further chromosomal alterations, but also allow the *TP53* promoter to upregulate genes erroneously placed under its control in a fashion accentuated by ongoing DNA damage. Paradoxically, many of the induced genes are part of the *TP53*-associated transcriptome, suggesting a need to counterbalance loss of *TP53* function through 'separation-of-function' mutations via promoter swapping.

Conclusion: Our findings demonstrate how massive genome errors can functionally turn the promoter region of a tumour suppressor gene into a constitutively active oncogenic driver. *TP53* structural variants in osteosarcoma likely function similarly to separation-of-function hotspot *TP53* missense variants seen in adult carcinomas.

Conflict of Interest: None declared

P13.005.A A phase III study to determine the breast cancer risk-reducing effect of denosumab in women carrying a pathogenic germline BRCA1 variant (The BRCA-P Study)

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Background: Women with *BRCA1* pathogenic variants (PV) have a 50-85% lifetime risk of developing breast cancer. Currently, prophylactic mastectomy is the only strategy shown to significantly reduce this risk. Recent evidence suggests that the RANK/RANKL pathway plays a pivotal role in development of *BRCA1*-mutated tumors. Targeting RANK/RANKL reduces breast epithelial proliferation in vitro and in vivo and attenuates mammary tumor development in mouse-models. The RANKL inhibitor, denosumab, an FDA approved osteoporosis drug, has promise as chemoprevention for women with *BRCA1* PVs as it could reduce breast cancer risk and improve bone density in post-menopausal women.

Methods: A randomized, double-blind, placebo-controlled, multi-center international phase 3 study to determine breast cancer risk-reducing effect of denosumab in women carrying *BRCA1* PV. Women with *BRCA1* PV aged 25-55 years with preserved breasts and no cancer history cancer are randomized to 120 mg of denosumab or placebo q6months for 5 years and followed for 5 additional years. Primary endpoint: Time to breast cancer occurrence. Secondary endpoints: Time to triple negative breast cancer; other *BRCA1*-associated malignancies; fractures. A 35% reduction in breast cancer risk would be detected with 80% power and 5% two-sided significance level if 167 breast cancer cases are observed. We expect to observe number of events needed if 2,918 subjects are randomized. The study is enrolling participants in Austria, Australia, Israel, Spain, and US, and shortly in Germany and UK.

Grant references: BRCA-P is coordinated by the Austrian Breast & Colorectal Cancer Study Group and supported by Amgen and US Department of Defense.

Conflict of Interest: Rachel Michaelson-Cohen 1. Austrian Breast & Colorectal Cancer Study Group 2. US Department of Defense, ABCSG has received research support from Amgen for the conduct of this trial, Joan Brunet AstraZeneca, MSD, Rita Katharina Schmutzler: None declared, D Gareth Evans Consultancy AstraZeneca, Board Everything Genetic Ltd, Michael Gnant personal fees / travel support: AstraZeneca, DaiichiSankyo, EliLilly, Menarini-Stemline, MSD, Novartis, PierreFabre, Veracyte;, Dominik Hlauschek full, ABCSG (affiliated organization) has received research support from Amgen for the conduct of this trial, Aleksandra Mystek full, Yes – Vanguard Health Care Index Fund

ETF Shares (ISIN: US92204A5048), ABCSG (affiliated organization) has received research support from Amgen for the conduct of this trial, Nizar Bhulani: None declared, Geoff Lindeman NHMRC Grant (Australia) APP1140715, Breast Cancer Trials Australia, US department of defense, Pfizer, Christian Singer: None declared, Judy Garber: None declared

P13.006.B RNA sequencing could provide with functional evidence to an intronic variant (APC c.1409-2delA) in splicing site consensus sequences

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Introduction: Next generation sequencing (NGS) is widely used in clinical laboratories and provides large amounts of genetic information. Many intronic variants are interpreted as 'variant of uncertain significance' (VUS) because of their insufficient evidence. RNA sequencing could provide with functional evidence to help elucidate the pathogenicity of variants. We identified a novel splice site variant in the *APC* (*adenomatous polyposis coli*) in a patient with familial adenomatous polyposis (FAP) by NGS and confirmed its impact on splicing by RNA sequencing.

Methods and Results: 19-year-old and 16-year-old brothers visited to our hospital for further evaluation and management of FAP family history. To find genetic variants causing FAP, NGS with a multi-gene panel composed of 171 hereditary tumor-related genes was performed. One variant was detected in the intron of the *APC* gene with the deletion of one of the acceptor splicing site consensus sequence: c.1409-2delA. To confirm the splicing change at RNA level, we performed RNA sequencing. The change was described as r.1494_1633del and predicted to result in entire exon 11 skipping. According to ACMG (American College of Medical Genetics and Genomics) standards and guidelines, PS3 evidence could be applied to this variant and it was classified as 'pathogenic'.

Conclusion: Clinical sequencing is mostly performed at the DNA level only, therefore many intronic variants are classified as VUSs due to lack of evidence. RNA sequencing could elucidate the impact of sequence variants at the messenger RNA level in intronic variants and reduce the number of variants classified as VUS.

Conflict of Interest: JoonSang Yu Full, Sollip Kim Full-time, Sail Chun Full-time, Won-Ki Min Full-time, WooChang Lee Full-time

P13.007.C Genetics of multiple primary cancers

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Background/Objectives: Within the frame of inherited cancer predisposition, single gene carriers of pathogenic variants (PVs) have been extensively represented in the literature, whereas the oligogenic coinheritance of heterozygous PVs in cancer-related genes is a poorly studied event. Currently, due to the increment of cancer survivors, the probability of presenting multiple primary cancers (MPC) is higher.

Methods: This study included MPC patients ≤45 years without known PVs in common cancer predisposition genes. We used WES of germline and tumoral DNA, and CMA on germline DNA to detect variants associated with the disease.

Results: The twelve patients included in the study presented a mean of 3 cancers per patient. CMA was normal in 8 patients. WES of the germline DNA identified 1-3 variants possibly related to the disease in each patient, and most of them were classified as variants of uncertain significance. The mapping of the germline variants into their pathways showed a possible additive effect of these as the cause of the cancer. Fourteen somatic samples from 6 patients were available for sequencing. All the germline variants were also present in the somatic samples, while no second hits were identified in the same genes.

Conclusion: The sequencing of patients with early cancers, family history and multiple tumors is already a standard of care. However, the growing evidence suggests that patient´s assessment should not stop at the identification of one pathogenic variant in a cancer predisposition gene.

Grant references: Televie fellowship 7451419F, CHU Liège, WALGEMED 1710180

Conflict of Interest: None declared

P13.008.D Occurrence of cancer in Marfan syndrome: report of two females with neuroblastoma and review of the literature

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Background: Marfan Syndrome (MFS) is an autosomal dominant connective tissue disorder caused by pathogenic *FBN1* variants. Neonatal MFS is the most severe form of the disease spectrum with a dismal prognosis. The association between MFS and cancer is hitherto unknown. Here, we present two girls with MFS who were diagnosed with the pediatric tumor neuroblastoma and review reported cases of MFS and cancer.

Methods: Literature review. Whole genome sequencing (WGS) and RT-PCR on blood samples. WGS, SNP array and histopathological examinations on tumors.

Results: The girl with neonatal MFS was diagnosed with neuroblastoma at 8 months of age. Tumor analysis revealed hyperdiploidy and structural chromosomal high-risk aberrations (1q-gain/11p-gain/11q-deletion/17q-gain). Germline WGS and RT-PCR analyses revealed a de novo pathogenic *FBN1* variant

(p.D1322N), affecting exon 32 splicing. The girl died shortly after cancer diagnosis due to cardiac insufficiency.

The female with classic MFS, caused by a de novo nonsense mutation in *FBN1* (p.C805X), was diagnosed with metastatic neuroblastoma at the remarkable age of 18 years. Tumor analysis revealed triploidy, structural aberrations, and high-risk alterations *ALK* (p.R1275Q) and 17q-gain.

A total of 40 patients with MFS and cancer have been described. Of 32 with reported age, 12 (37.5%) occurred in childhood, and 20 (62.5%) before age 30.

Conclusion: We present the first two patients with MFS and neuroblastoma. We also report early age at cancer diagnosis in previously reported MFS patients. Further epidemiological and functional studies are needed to clarify possible links between MFS and cancer.

Grant references: The Swedish Childhood Cancer Fund.

Conflict of Interest: None declared

P13.009.A MCM8 and MCM9 as germline predisposing genes for early-onset cancer, polyposis and primary ovarian insufficiency

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Background/Objectives: *MCM8/MCM9* are involved in multiple DNA maintenance/repair mechanisms and recently proposed cancer-predisposition genes. We aimed to establish the phenotype and cancer risks of *MCM8/MCM9* variant carriers.

Methods/Results: Through literature review, we identified 65 and 72 carriers of class three-five *MCM8* and *MCM9* variants, respectively (**Table 1**). In addition to MCM8/MCM9 deficiency-associated infertility, multiple carriers were reported with cancer. Moreover, through international collaboration, we identified new families with germline *MCM8/MCM9* variants and cancer. Follow-up of these families, including whole-genome-sequencing/mutational signature analysis on available tumors, is pending to explore a potential causative link with cancer.

Conclusion: Germline *MCM8/MCM9* variants may predispose carriers to cancer.

Grant References: MLDS(FP16-06); FIS-FEDER(20/00113); Marató(TV3-202008-10); AECC(PRYGN211085CAST); CERCA Program and AGAUR(GRC2021SGR01185).

Conflict of Interest: Noah Helderman PhD candidate at the department of Clinical Genetics at the Leiden University Medical Centre. His research focusses on germline variants of DNA repair genes and the consequences on carcinogenesis., Yael Goldberg Head of the Adult genetic and Oncogenetic Unit in Recanati institute of medical genetics in Rabin Medical Center in Israel. She is a professor of medicine in Tel Aviv University. Her research focusses on germline variants contributing to adult onset diseases and cancer ., Sergi Castellví-Bel Leads the Genetic predisposition to gastrointestinal cancer Group at the Fundació de Recerca Clínic Barcelona-IDIBAPS. His research focuses on finding genetic alterations involved in the germline predisposition to colon cancer, gastric cancer and pancreatic cancer., Fondo de Investigación Sanitaria/FEDER (20/00113); Fundació La Marató de TV3 (2019-202008-10); Fundación Científica de la Asociación Española contra el Cáncer (PRYGN211085CAST); CERCA Program and Agència de Gestió d'Ajuts Universitaris i de Recerca (GRC 2021SGR01185)., Maartje Nielsen PhD and MD at the department of clinical genetics at the Leiden University Medical Center. Dr. Nielsen's research primarily focusses on inheritable forms of bowel cancer and polyposis. Her recent work focuses on cancer risk and carcinogenesis in the Lynch syndrome and polyposis patients by studying the molecular profile of carcinomas and adenomas., MLDS (FP16-06)

Table 1. Previously reported *MCM8/MCM9* carriers.

Gene	***MCM8***				***MCM9***			
Class	**3**		**4-5**		**3**		**4-5**	
Descriptive	**Mono-allelic (n = 30)**	**Bi-allelic (n = 20)**	**Mono-allelic (n = 8)**	**Bi-allelic(n = 7)**	**Mono-allelic (n = 22)**	**Bi-allelic (n = 9)**	**Mono-allelic(n = 26)**	**Bi-allelic (n = 15)**
Sex								
Male	10/30 (33%)	2/20 (10%)	4/8 (50%)	2/7 (29%)	3/22 (14%)	2/9 (22%)	10/26 (38%)	
Infertility				1/2 (50%)		1/2 (50%)	2/10 (20%)	
Female	20/30 (67%)	18/20 (90%)	4/8 (50%)	5/7 (71%)	13/22 (59%)	7/9 (78%)	15/26 (58%)	15/15 (100%)
Infertility	9/20 (45%)	17/18 (94%)	1/4 (25%)	5/5 (100%)	9/13 (69%)	6/7 (86%)	2/15 (13%)	15/15 (100%)
Unknown					6/22 (27%)		1/26 (4%)	
Cancer								
Polyposis	1/30 (3%)				1/22 (5%)	2/9 (22%)	6/26 (23%)	3/15 (20%)
Breast	1/20 (5%)	1/18 (6%)						
Colorectal	2/30 (7%)			1/7 (14%)	5/22 (23%)	4/9 (44%)	3/26 (12%)	3/15 (20%)
Gynecological						1/7 (14%)		2/15 (13%)
Lymphoma	1/30 (3%)							
Melanoma						1/9 (11%)		
Stomach						1/9 (11%)		

P13.010.B Large-scale analysis of chromosomal aberrations in non-cancer tissue in patients with cancer

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Mosaicism, the occurrence of the subpopulation of cells harbouring mutations is known to contribute to ageing and disease. It has been detected in various tissue, including non-cancer tissue adjacent to the tumour. Here, we aimed to sensitively detect and characterize mosaicism in non-cancer tissue adjacent to tumours in patients with bladder (BLC), prostate (PC), and colorectal cancers (CRC). The variants shared between the non-cancer tissue and tumour indicate shared clonal lineage and so shed light on the earliest events that set a normal cell on the path to cancer. Our study is designed to capture those events by studying non-cancer tissue adjacent to the tumour. We applied a unique tissue collection protocol, for each diagnosis up to 12 tissue samples were collected (up to 4 tumours, 8 non-cancer samples) and blood. Non-cancer samples were collected from tissue from 1 to 5 cm away from the tumour. In total 1112, 1152 and 612 samples were collected from PC, BLC and CRC patients, respectively. The Infinium Global Screening Array was utilised for DNA genotyping. The aberrations were sensitively detected with Mosaic Chromosomal Alteration Caller (MoChA). We detected 62 and 92 somatic variants in non-cancer tissue that were present in the corresponding tumour in PC and BLC, respectively. Some of which span known cancer genes (*TP53*, *ERBB2* and *FAT1*). Those aberrations are now being further studied with Whole Genome Sequencing. To conclude, somatic aberrations occur in normal tissue and may play a role in the initiation of tumorigenesis in sporadic cancers. (No. MAB/2018/6)

Conflict of Interest: None declared

P13.011.C Clinical practice guidelines for the diagnosis and surveillance of BAP1- tumour predisposition syndrome

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Background: BRCA1-associated protein-1 (BAP1) is a recognised tumour suppressor gene. Germline *BAP1* (likely)/pathogenic variants are associated with predisposition to multiple tumours, including uveal melanoma, malignant pleural and peritoneal mesothelioma, renal cell carcinoma and specific non-malignant neoplasms of the skin, as part of the autosomal dominant *BAP1*-associated tumour syndrome. The overall lifetime risk for *BAP1* carriers to develop at least one *BAP1*-associated tumour is up to 85%.

As for many rare cancer predisposition syndromes there is limited scientific evidence of reduced morbidity or mortality for surveillance and opinions can vary greatly between clinicians based on experience and the health care systems in which they work.

To date, European recommendations for *BAP1* carriers have not been published, but are necessary due to the emerging phenotype of this recently described syndrome and increased identification of *BAP1* carriers via large gene panels or tumour sequencing.

Methods: To address this, the Clinical Guideline Working Group of the CanGene-CanVar project in the United Kingdom invited European collaborators to join them to develop guidelines for *BAP1* heterozygotes to harmonize surveillance programmes within Europe.

Results: We present the final recommendations with respect to considerations for testing and surveillance achieved following literature review and Delphi survey completed by the core group and an extended expert group of 34 European specialists including Geneticists, Ophthalmologists, Oncologists, Dermatologists and Pathologists. It is recognised that these largely evidence-based but pragmatic recommendations will evolve over time as further data from research collaborations adds to data on phenotypic spectrum and surveillance outcomes.

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Conflict of Interest: Fiona Lalloo: None declared, Anjana Kulkarni: None declared, cindy chau: None declared, Maartje Nielsen: None declared, michael sheaff: None declared, jeremy steele: None declared, Remco Van Doorn: None declared, Karin Wadt: None declared, monica hamill: None declared, beth torr: None declared, marc tischkowitz: None declared, Helen Hanson Honoraria for AstraZeneca Advisory Board on results from Olympia trial

P13.012.D Genetic counseling and testing in invasive ovarian cancer - a nationwide study

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Background/objectives: There has been increasing emphasis on genetic risk predisposition in ovarian cancer, a leading cause of malignant gynecological deaths. The aim of this study was to obtain an overview of genetic counseling and testing in all women diagnosed with ovarian cancer in Iceland.

Methods: Cohort: All women diagnosed with invasive ovarian, tubal, and peritoneal cancer in Iceland 2005-2018. Information was gathered from the Icelandic Cancer Register, a nationwide population-based prospective database, and patients' medical records. Follow-up extended to January 1st, 2023.

Results: In total, 319 patients were identified. The majority had serous epithelial carcinoma (62%) and stage III disease (51%). Of all, 80 (25%) had genetic counseling of which 79 had clinical genetic testing. Patients tested had lower median age at diagnosis (59 vs. 69 years, $p < 0.001$). A pathogenic variant was found in 42%, most of which appeared in *BRCA2* (18, 5.6%) or *BRCA1* (12, 3.8%). A pathogenic variant was found in seven other disease-related genes. In women with stage-III serous carcinoma, median time until relapse was longer for *BRCA*-positive women compared to

others (15 vs. 11 months, $p = 0.03$). Overall five-year survival for *BRCA*-positive women was higher (79%, 95%CI: 66-96% vs. 40%, 95%CI: 35-46%). However, ten-year survival did not differ significantly.

Conclusion: The high diagnostic yield among our cohort suggests that more should be offered testing and counseling. The results might imply that women with pathogenic variants in *BRCA1* or *BRCA*2 genes have better prognosis, although the small proportion of patients tested limits the study.

Grant references: Not applicable.

Conflict of Interest: Arna Rut Emilsdóttir: None declared, Vigdis Stefansdottir: None declared, Anna Margrét Jónsdóttir: None declared, Hans Bjornsson Dr. Bjornsson is a consultant for Mahzi Therapeutics., Elísabet Arna Helgadóttir: None declared

P13.013.A Pathogenic germline variants in SMARCA4 and further cancer predisposition genes in early onset ovarian cancer

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Background/Objectives: *SMARCA4*, a gene associated with the small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), appears to be the sole ovarian cancer (OC) predisposition gene associated with an early age at diagnosis.

Methods: To assess the role of germline pathogenic variants (PVs) in *SMARCA4* and further established OC predisposition genes in early onset OC, we investigated a clinical cohort of 206 unrelated index patients using an extended panel of 24 (candidate) cancer predisposition genes. Patients were recruited between 2008 and 2020 by the Cologne Center for Familial Breast and Ovarian Cancer, German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC).

Results: PVs in established OC predisposition genes were most frequent in patients with high grade serous OC (21/62, 33.9%), rare in patients with other epithelial OC (5/74, 6.8%) or borderline ovarian tumours (2/39, 5.1%) and not detected in mucinous OC (0/27). In the overall study sample, 21 carriers of PVs in *BRCA1* and one carrier with a pV in *BRCA2* were identified (22/206, 10.7%). A positive *BRCA1* PV status was significantly associated with high grade serous OC.

Conclusion: The absence of PV in mucinous OC supports the notion that this entity represents a tumour phenotype not associated with PVs in established OC predisposition genes. PVs in *SMARCA4* were restricted to SCCOHT and unlikely predispose for early onset OC other than SCCOHT.

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P13.014.B Hereditary paraganglioma and pheochromocytoma: exploring the genotype and phenotype in a multi-ethnic adult population

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Background/Objective: Pheochromocytomas and paragangliomas (PPGLs) are neuroendocrine tumours which, although rare, are highly heritable (~40%). Studies describing the characteristics of hereditary PPGL syndromes are limited in large centres. Our aim is to describe a diverse hereditary PPGL adult population from the Princess Margaret Cancer Centre in Toronto, Canada.

Methods: We conducted a retrospective chart review of patients who had been diagnosed with PPGL and were seen in the cancer genetics program between 2000-2022.

Results: We ascertained 115 patients from more than 30 different ethnicities. Disease severity was varied; six patients were deceased and 17 patients had recurrence.

All patients were offered genetic testing via a PPGL panel (11-12 genes depending on the year). 107 patients underwent genetic testing, with 44 patients (41%) found to have a variant in a known cancer predisposition gene, and 63 patients (59%) had negative testing results. Of those with a variant found, 31/44 variants were categorized as likely pathogenic/pathogenic. Variants were identified in the SDHA, SDHB, SDHC, SDHD, RET, VHL, and NF1 genes.

Notably, SDHB protein expression in the tumour was lost in 25 patients, while a further two patients had deficient/inconclusive staining. Interestingly, 7 patients with loss of SDHB expression had negative genetic testing.

Conclusion: Our study demonstrates the heterogeneity of PPGL in a diverse population. We identified a subset of patients with loss of SDHB protein expression in the tumour despite negative germline testing. This indicates there may be further variants implicated in PPGL and broader genetic testing is merited.

Conflict of Interest: Sarah Redmond Postgraduate Medical Education, Division of Clinical and Metabolic Genetics, University

of Toronto, Kirsten Farncombe Toronto General Hospital Research Institute, University Health Network, Toronto, ON, Canada, Maia Norman University Health Network, Toronto, ON, Canada, Brittany Gillies University Health Network, Toronto, ON, Canada, Leslie Oldfield University Health Network, Toronto, ON, Canada, Raymond Kim University Health Network, Sinai Health Network

P13.015.C Improving the Diagnostic Yield of Genetic Testing for Hereditary Cancer Syndromes with RNA Sequencing

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Background/Objectives: Next generation DNA sequencing is routinely used for genetic testing for hereditary cancer syndromes. However, this method is accompanied by several technical limitations, including the failure to effectively detect hidden splice-site variants which might account for a significant part of potentially deleterious variants implicated in hereditary cancers. Our aim is to identify novel pathogenic transcript-altering variants and to re-evaluate known variants of unknown significance (VUS) in order to improve patient-relevant outcomes.

Methods: Patients that have met the criteria for genetic testing and that have been previously found to carry a splice-site variant of unknown significance using DNA sequencing and splice prediction tools have been selected. The RNA isolation from whole blood has been performed using three commercially available kits in order to maximise the RNA yield, followed by targeted RNA capture and sequencing of the 226 genes included in the CZECANCA panel.

Results: So far, 18 patients have been analysed. We have confirmed the suspected aberrant splicing for several previously unreported potentially pathogenic variants in the genes *MHS2*, *ATM* and *DICER1*, and have specified additional abnormal mRNA transcripts for variants with unavailable RNAseq data.

Conclusion: While our laboratory is still in the process of establishing RNA sequencing as a routine diagnostic tool, it is already evident that this method can increase the diagnostic yield of genetic testing and thereby improve the clinical outcomes of patients and of their relatives.

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Conflict of Interest: Adela Misove Masaryk Memorial Cancer Institute (full-time), Collaborator: MH CZ – DRO (MMCI, 00209805), Eva Machackova Masaryk Memorial Cancer Institute (full-time), Principal investigator: MH CZ – DRO (MMCI, 00209805), Jana Hazova Masaryk Memorial Cancer Institute, Petra Vasickova Masaryk Memorial Cancer Institute, Lenka Foretova Masaryk Memorial Cancer Institute, Principal investigator: MH CZ – DRO (MMCI, 00209805)

P13.016.D Development of a quantitative splice assay for the characterization of variants of uncertain significance using long-read sequencing

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Introduction: The characterization of the effect of potentially spliceogenic variants of uncertain significance (VUS) is challenging due to naturally occurring alternative splicing. Currently, most laboratories are using qualitative or semi-quantitative methods like RT-PCR for the analysis of such variants. These methods require many control samples due to the high inter-sample variability and often provide no quantitative information on the observed effect. Oxford Nanopore long-read sequencing enables the reconstruction of full isoforms, helping to differentiate aberrant splicing events from naturally occurring alternative splicing. This could improve the characterization and quantification of splicing events.

Methods: RNA was isolated from patient derived blood samples with known spliceogenic mutations and control individuals. Samples were converted into cDNA and amplified by long-range PCR using gene specific primers located in the first and last exon. Sequencing was performed on the MinIon. Basecalling was performed using Guppy and reads were mapped to GRCh38 using minimap2. Transcripts were reconstructed using FLAIR and quantified using salmon.

Results: We were able to reconstruct complete isoforms and identify previously described naturally occurring alternative splicing events in negative controls for *BRCA1* and *CHEK2*. Furthermore, aberrant splicing events were detected in positive controls previously characterized by Sanger sequencing.

Conclusion: Our results show that Oxford Nanopore long-read sequencing using RNA from blood is suitable for characterizing the effect of potentially spliceogenic VUS. In the future, phasing of variants could further improve the quantification of aberrant splicing events and enable the detection of allele specific effects.

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Conflict of Interest: None declared

P13.017.A SarcDBase: a tool for detection of genetic alterations in sarcoma

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Background: Sarcomas are a diverse group of malignant tumors arising in the bone and soft tissues. For an increasing number of subtypes, there are already pathognomonic genetic alterations described. For some sarcomas, such alterations remain to be identified. The latter group primarily contains sarcomas with a massive amount of genomic copy number and structural alterations. This extreme number of alterations makes it difficult to discriminate those of biological importance from background noise.

Methods: We have developed SarcDBase, a database-building method that match genetic variants detected in high-resolution genomic and transcriptomic data from tumour biopsies with information on established biomarkers, eliminating the need for prior knowledge and manual screening of data. This tool has been tested on a discovery cohort of osteosarcomas (n = 150), and its performance is currently being validated on cohort of a diverse spectrum of sarcomas (n = 120).

Results: SarcDBase was able to detect mutations that confirmed the diagnosis of some patients, such as *NAB2::STAT6* gene fusion, typical of solitary fibrous tumor, and *H3F3A* p.G35W, typical of giant cell tumor of bone. It was also able to identify new genetic alterations of likely biological significance, such as new combinations of partner genes in gene fusions.

Conclusion: SarcDBase is a useful tool to integrate information on genomic copy numbers, transcriptome, single nucleotide, and structural variants, and to extract previously reported alterations and new ones of likely biological importance. It also provides graphical visualization to help the user evaluate the findings.

Conflict of Interest: None declared

P13.018.B Understanding the molecular mechanisms of enzalutamide resistance in breast cancer cell lines

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Background: Breast cancer (BC) is the leading female cancer worldwide. The androgen receptor (AR) is emerging as a novel factor in breast cancer. Enzalutamide is a nonsteroidal antiandrogen commonly used in the treatment of prostate cancer, but patients receiving enzalutamide treatment will ultimately develop resistance. Thus, we aimed to evaluate molecular mechanisms of enzalutamide resistance in AR+ breast cancer.

Method: BT474 (AR+) cell line was employed. MTT and apoptosis assays were performed to test enzalutamide sensitivity. Enzalutamide-resistant cell line was generated by gradually increasing the concentration of the cytotoxic drug. We confirmed the establishment of enzalutamide resistant BT474 by repeating the MTT and apoptosis assays. Finally, we analyzed *AR-FL*, *AR-V7*, *DNAJC15*, and *ABCB1* genes expression by RT-qPCR.

Results: Our results show that we successfully established enzalutamide-resistant BT474 (BT474-EnzRes). We found that BT474 showed an increase in *AR-FL* and *AR-V7* expression, one of the most common enzalutamide-resistance processes in prostate cancer. Interestingly, BT474-EnzRes showed much higher *ABCB1* and *DNAJC15* expression induction than BT474 parenteral cell line. This suggests that enzalutamide resistance is partially induced by AR-related changes and partially mediated by multidrug resistance pumps in breast cancer cell lines.

Conclusion: Our study suggests that resistance to enzalutamide could be mediated through both AR-dependent and -independent pathways.

References: Feng T *et al*. Construction of enzalutamide-resistant cell model of prostate cancer and preliminary screening of potential drug-resistant genes. Exp Biol Med 2021 Aug.

Grants: This study was funded by FIS-FEDER PI20/01569.

Conflict of Interest: None declared

P13.019.C Analysis of miRNA profile in plasma and tissue samples of glioblastoma patients

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Background/Objectives: Glioblastoma multiforme (GBM) is the most common and lethal cancer of the adult brain, remaining incurable with a median survival time of only 15 months. Treatment options are limited, clinicians lack efficient prognostic and predictive markers. MicroRNAs – besides being important regulators of cancer developement – may have potential as diagnostic biomarkers.

Methods: In this study, we used two different methods for miRNA profiling: the Nanostring Technology, and the Next-Generation Sequencing (NGS). Nanostring Technology profiling of 798 human miRNAs was performed on plasma samples from 6 healthy and 6 patients with GBM. To validate our results, five miRNAs (hsa-miR-433-3p, hsa-miR-362-3p, hsa-miR-195-5p, hsa-miR-133a-3p and hsa-miR-29a-3p) were chosen for RT-qPCR detection. Using NGS we also analyzed 6 tissue samples from the control and GBM groups, as well as from the group of GBM patients with lung metastasis. Until now, our validation process only covered the control and GBM groups, so for RT-qPCR detection we selected four miRNAs (hsa-miR-196a-5p, hsa-miR-10b-3p, hsa-miR-383-5p and hsa-miR-490-3p) based on their LogFC values.

Results: In case of Nanostring analysis from the 5 selected miRNAs for RT-qPCR validation miR-433-3p, miR-195-5p and miR-29a-3p while as the result of NGS hsa-miR-196a-5p, hsa-miR-10b-3p, hsa-miR-383-5p and hsa-miR-490-3p were significantly deregulated.

Conclusion: We demonstrated that the differently expressed miRNAs selected on the base of the two different methods could be useful in developing miRNA panel which may help the diagnosis, and in the selection the adequate therapy of GBM.

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Conflict of Interest: None declared

P13.020.D Long-read genome sequencing and RNA sequencing resolve an intronic LINE-1 insertion in the APC gene in a so far unsolved adenomatous polyposis family

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Background/Objectives: Familial adenomatous polyposis (FAP) is caused by pathogenic variants in the tumor suppressor gene *APC*.

The genetic cause for a family with FAP across four generations could not be identified by cancer gene panel sequencing (94 genes), array-CGH, exome sequencing, and investigation of specific intronic variants.

Methods: Long-read genome sequencing (PacBio), short-read genome sequencing (Illumina), short-read RNA sequencing, and further validations were performed in different tissues of multiple family members.

Results: Long-read genome sequencing of one family member resolved a 6 kb insertion of a LINE-1 element between exons 7 and 8 of *APC* (NM_000038.6) that could be detected but not deciphered with short-read genome sequencing. RNA analysis revealed aberrant splicing. The variant segregates with the phenotype in several family members. Literature on pathogenic germline LINE-1 insertions was reviewed to place this variant into context.

Conclusion: This study supports the utility of long-read DNA sequencing techniques and complementary RNA approaches to tackle unsolved cases.

Conflict of Interest: None declared

P13.021.A Clinical importance of mutation types of FLT3-ITD in acute myeloid leukemia

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Introduction: Significance *FLT3*-ITD mutations is well known in in acute myeloid leukemia (AML), and prognostic significance of various aspects of *FLT3*-ITD is being revealed. In this study, the mutation types and dynamics of *FLT3*-ITD were studied along with other known factors.

Materials and Methods: Initial and follow-up samples from 45 AML patients with FLT3-ITD were analyzed by fragment length analysis (FLA), Sanger sequencing, and targeted next-generation sequencing (NGS).

Results: Diverse *FLT3*-ITD mutations were found. *FLT3*-ITD mutations were classified according to mutation types, including the duplication-only *FLT3*-ITD and *FLT3*-ITD with duplications and insertions (dup + ins) (48.1%). The dup + ins type *FLT3*-ITD variant was independently associated with poor prognosis. A high VAF (≥ 50%) and a longer *FLT3*-ITD length were also associated with poor prognosis. A total of 25% of patients exhibited *FLT3*-ITD measurable residual disease (MRD) at morphologic remission after the first cycle of induction chemotherapy. The VAF of *FLT3*-ITD was low when detected during morphologic complete remission (CR) after conventional chemotherapy; however, in two patients treated with gilteritinib after relapse, the VAFs of *FLT3*-ITD were much higher during morphologic CR.

Conclusions: The results of the FLA method reflecting the relationship with patient clinical indicators and prognosis were consistent with previous studies. The type of FLT3-ITD mutation is important for prognosis, and the dup + ins type of FLT3-ITD is a potential indicator of poor prognosis.

Conflict of Interest: None declared

P13.022.B Generation and characterisation of a BRCA2 knockout cell line for use in functional assays

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Background/Objectives: *BRCA2* is a tumour suppressor frequently mutated in hereditary breast and ovarian cancer syndrome. It is involved in homologous recombination (HR), an essential mechanism for the repair of DNA double-strand breaks (DSBs). Thousands of mutations in *BRCA2* are classified as variants of uncertain significance (VUS) because the effect on protein function is unknown. It is therefore necessary to develop functional assays that allow the reclassification of these VUS. The objective of this study is to build a human knockout cell line in *BRCA2* that can be used to determine the efficiency of DSB repair by HR.

Methods: The DR-GFP HR reporter cassete was introduced into HEK-293T cells and then *BRCA2* knockout clones were generated by CRISPR/Cas9 technology. Briefly, HEK-293T-DR was transfected with plasmids carrying 3 guideRNAs against *BRCA2* and the endonuclease Cas9. Single cells were then isolated by cell sorting. Gene inactivation was verified by PCR, Sanger sequencing and western blot. Proliferation and drug sensitivity were determined by MTT.

Results: 5 out of 48 clones analyzed did not express the BRCA2 protein. PCR and Sanger sequencing elucidated the specific modifications in two of them: a 108 bp insertion and a 2235 bp deletion. *BRCA2* KO clones exhibited lower proliferation and were hypersensitive to PARP1 inhibitors, as expected.

Conclusion: The generation of BRCA2 knockout cell lines is an important tool to develop functional assays that allow VUS reclassification. Complementation studies with different VUS are being carried out to analize their HR efficiency.

Grant: This project was funded by IP20

Conflict of Interest: None declared

P13.023.C Study of drug sensitivity of NOMO knockout cell lines

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Background: Colorectal cancer (CRC) is one of the most frequent and deadliest tumours worldwide. The early-onset subtype (EOCRC, patients under 50 years) has become especially relevant in the last decades. In the search for new EOCRC biomarkers, the region 16p13 which includes *NOMO* (Nodal modulator) was found to be more frequently deleted in patients below the age of 50. In this study we tested whether the loss of *NOMO* modifies cell response to the main drugs used in clinic.

Methods: *NOMO* knockout (KO) and wildtype (WT) HCT-116, HT-29 (CRC cell lines) and HS-5 (non-tumour cell line) were used. The effect of 5-Fluorouracil, Cisplatin, Oxaliplatin and Irinotecan was tested on both WT and KO cell lines by MTT, cell cycle and

apoptosis assays. Repair kinetics of DNA double strand break (DSB) induced by ionizing radiation (IR) were analyzed by the quantification of ɣH2AX foci at different times post-irradiation.

Results: Sensitivity to the 4 drugs analyzed was similar in WT and NOMO KO cell lines in HT-29 and HS-5. However, two out of three HCT116 KO clones analyzed showed increased resistance to Cisplatin which correlated to lower apoptosis and faster DSB repair kinetics after IR.

Conclusions: Loss of *NOMO* does not modify cell response to the tested drugs. Differences seen in the HCT16 KO clones could be explained by secondary alterations that may occur during clone generation. Analysis of several clones and different cell lines must be carried out to get clear biological conclusions.

Grants: Study funded by PI20/01569.

Conflict of Interest: None declared

P13.024.D The HIF-1-α inhibitor PX478 induce radio-sensibility in several solid tumours cell lines in hypoxic conditions

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Background/Objectives: Hypoxia is a common situation in the solid tumours' microenvironment. It is associated with resistance to radiotherapy and with poor clinical results. The activation of Hypoxia-inducible Factor (HIF) signalling pathway plays a pivotal role in the tumour adaptation to hypoxia. HIF proteins regulate the transcription of several genes implicated in angiogenesis, apoptosis, metastasis or tumoral growth and their overexpression correlates with a poor prognosis after radiotherapy. So, HIF inhibitors are used to improve the cell response to radiotherapy. Here, we study the effects of PX478, a first-generation HIF-1-alpha inhibitor, on tumoral cell lines under hypoxic and normoxic conditions.

Methods: HT29 (Colon), MCF7 (Breast), HCC1937 (Breast), VCAP (Prostate), CAL33 (Head and Neck) cell lines were used to test PX478 radio-sensibility through MTT, cell cycle and apoptosis assays. Western blot was used to analyse the expression of HIF-1-alpha.

Results: Western blot analysis showed that the expression of HIF-1-alpha was partially inhibited by PX478 in a dose-dependent manner. PX478 inhibited cell proliferation and decreased cell survival in normoxia but especially in hypoxic conditions.

Conclusion: PX478 inhibited the expression of HIF-1-alpha in a dose-dependent manner. This drug had a strong effect on cell survival and increased radio-sensibility, especially in hypoxic conditions.

References: Albadari N; Deng S, Li W. Expert Opin. Drug Discov. 2019. https://doi.org/10.1080/17460441.2019.1613370

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Conflict of Interest: None declared

P13.025.A Next generation sequence-based targeted somatic mutation analysis in thyroid nodules with pathologically diagnosed as "indeterminate cytology"

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Background/Objectives: Fine needle aspiration biopsy (FNAB) allows the examination of nodules' cytopathological characters. Molecular profiling has recently become one of the promising options for the further investigation of the nodules. This study aimed to help the clinical management of indeterminate nodules. To differentiate the nodule characters, somatic mutation analysis was performed.

Methods: Aspiration samples from 20 thyroid nodules with indeterminate cytology were included in the study. The specimens belonged to the patients who had undergone surgery. A next-generation sequencing panel containing 67 genes was used for the molecular profiling of the samples. The results were compared with the pathology of surgery material data, which is accepted as the gold standard. Sensitivity, specificity, PPV, and NPV values were calculated.

Results: Variants which were belonged to 6 different genes *(BRAF, NRAS, PTEN, TERT, PIK3CA, TP53)* were detected in 10 out of 20 samples. In the test, it was calculated as NPV: 40%, PPV: 90%, Specificity: 60%, and Sensitivity: 80%.

Conclusion: In all samples were able to obtain enough DNA for the study. According to the detected molecular markers, at least 9 patients could be managed correctly without the need for a repeat FNAB attempt. This study underlines the clinical practical utility of molecular tests in the management of nodules with indeterminate cytology. Hence it is the first comprehensive study about the molecular markers in thyroid nodules in Turkey, it is aimed to lead the next studies.

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Conflict of Interest: None declared

P13.026.B Pathogenic BRCA1/2 variation in a large Slovenian non-cancer cohort

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Background: Pathogenic variants in BRCA1 and BRCA2 genes are the most common genetic cause of breast and ovarian cancer susceptibility. The data on BRCA1/2 pathogenic variants prevalence in non-cancer national cohorts are lacking, and for those available, the numbers vary significantly. We have examined the prevalence of pathogenic BRCA1/2 variants in a large set of unaffected individuals representing 0,35% of the Slovenian population.

Methods: We performed a rigorous manual classification procedure, employing the ACMG criteria, to identify pathogenic variants in BRCA1 and BRCA2 genes in a cohort of 7091 individuals undergoing exome sequencing for various mendelian disorders, unrelated to cancer, from the Slovenian population.

Results: We have found the prevalence of BRCA1 pathogenic variants in the Slovenian population is 0.40 % and the prevalence of pathogenic variants in BRCA2 is 0.25 %. The most frequent pathogenic variant in BRCA genes in the Slovenian population is BRCA1 c.5266dup, and the most common BRCA2 pathogenic variant is BRCA2 c.7806-2A>G, a Slovenian founder variant. The prevalence of pathogenic variants in BRCA1 is statistically significantly enriched, compared to the gnomAD population ($p < 0.01$).

Conclusion: We report a high rate of pathogenic variation in BRCA1/2 genes in the non-cancer Slovenian population. The high burden is mostly due to the high occurrence of BRCA1 variants in the Slovenian population. A higher prevalence of BRCA1 pathogenic variants compared to BRCA2 is a unique feature of the Slovenian population since the BRCA2 pathogenic variants prevail in most populations.

Conflict of Interest: Urška Kotnik University Medical Centre Ljubljana, Clinical Institute of Genomic Medicine, Ljubljana, Slovenia, Borut Peterlin University Medical Centre Ljubljana, Clinical Institute of Genomic Medicine, Ljubljana, Slovenia, Luca Lovrecic University Medical Centre Ljubljana, Clinical Institute of Genomic Medicine, Ljubljana, Slovenia

P13.027.C Assessing the potential of miRNA biomarkers for the differential diagnosis of Wilms' tumor and diffuse hyperplastic perilobar nephroblastomatosis

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Wilms' tumor (WT) is the most frequently diagnosed renal tumor in children under the age of 5. Nephrogenic rests (NRs) are abnormalities consisting of embryonic metanephric cells classified based on their histology and location. In the case of diffuse hyperplastic perilobar nephroblastomatosis (DHPLN), a rarely seen condition considered as a precancerous stage of WT, perilobar NRs result in a bulky enlargement of the kidney.

WT and DHPLN are often indistinguishable based on their histological features. Molecular markers may improve differential diagnosis, but none are available at present. In our study, we investigated the potential of miRNAs as such biomarkers, and aimed to shed light on which of the described expression differences play a role in the initial steps of the pathogenesis (at a precancerous stage) and which ones occur later in WT.

Formalin-fixed, paraffin-embedded (FFPE) samples from DHPLN and adjacent healthy kidney tissue were acquired and used for miRNA extraction. Following reverse transcription, we performed expression analysis by qRT-PCR using a PCR array that contained primers for 84 miRNAs implicated in genitourinary cancer.

For many miRNAs, differences in the expression pattern were detected between DHPLN and WT samples. miR-135b-5p showed higher expression in DHPLN while no change was detected in WT. miR-146a-5p is a known tumor suppressor in WT, but the decrease in its expression may occur during malignization according to our findings. Several other miRNAs also show potential to differentiate between WT and DHPLN. More experiments are needed to confirm our observations and find new candidate markers.

Conflict of Interest: None declared

P13.028.D Birt-Hogg-Dubé syndrome: an update on clinical and genetic observations in the Dutch centre of expertise

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Background: Birt-Hogg-Dubé syndrome (BHD) is an autosomal dominant condition affecting skin, lungs and kidneys, caused by pathogenic variants in *FLCN*. Earlier reports suggested a potential increased risk of colorectal cancer in BHD, however this association remains unclear. Here we provide an update on clinical and genetic observations in our cohort. **1**. We evaluated renal surveillance compliance and outcomes in 199 patients. **2**. We examined available data on colorectal neoplasms in BHD patients and their unaffected relatives. **3**. We performed extensive clinical and genetic analysis in two families with an inherited BHD-like phenotype without an identifiable variant in *FLCN*.

Results: 1. Compliance to recommended renal surveillance was high. Of patients known to be under surveillance, 83% was screened annually and 94% at least bi-annually. **2**. No evidence for an increased prevalence of colorectal carcinoma was observed in our cohort. **3**. Two families with suspected BHD but without an *FLCN* variant were studied in detail. The first family presented with autosomal dominant trichodiscomas. The phenotype was linked to a locus on chromosome 5 including a predicted truncating variant in *FNIP1*. In the second family, multiple family members had fibrofolliculomas, lipomas and renal cell carcinoma. A heterozygous missense variant in *PRDM10* (p.Cys677Tyr) was found, co-segregating with the phenotype. We show that PRDM10Cys677Tyr loses affinity for a regulatory binding motif in the FLCN promoter, abrogating cellular FLCN mRNA and protein levels. Overexpressing PRDM10Cys677Tyr in renal epithelial cells altered the transcription of multiple genes, showing partial overlap with the effects of knocking out *FLCN*.

Conflict of Interest: arjan houweling Myrovlytis Trust MT21_6, Irma van de beek Myrovlytis Trust MT21_6, Lore van Riel Myrovlytis Trust MT21_6, Liselotte P van Hest: None declared, Iris Glykofridis: None declared, Hans Gille: None declared, Bart Boerrigter: None declared, Sylvie Mireille Franken: None declared, Patricia Zondervan: None declared, jeroen van moorselaar: None declared, Rob Wolthuis: None declared

P13.029.A A validated highly sensitive microsatellite instability assay accurately identifies germline biallelic PMS2 mutation carriers among Constitutional Mismatch Repair Deficiency individuals

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Constitutional MisMatch Repair Deficiency (CMMRD) is a rare and devastating childhood-onset cancer predisposition syndrome caused by germline biallelic inactivating variants in mismatch repair genes, being *PMS2* the most frequently mutated one. An accurate and rapid diagnosis is essential for patient management. We present the validation of a functional genomic tool for CMMRD diagnosis and the characterization of MSI patterns in blood and tumors.

Highly sensitive assessment of microsatellite instability using a custom NGS panel targeting 192 microsatellites (hs-MSI) was tested on 66 blood blinded samples and 24 CMMRD tumors. Hs-MSI scores were compared with whole genomic (LOGIC) scores. The correlation of hs-MSI scores in blood with age of cancer onset and the distribution of indel mutations were analyzed in a larger series of 68 CMMRD and 101 non-CMMRD individuals.

The hs-MSI approach identified CMMRD individuals with high sensitivity (98.4%) and specificity (100%). Hs-MSI and LOGIC scores in blood samples were highly correlated ($r = 0.89$, $p = 2.2e\text{-}15$). A phenotype-genotype correlation was observed between hs-MSI scores, and germline affected gene ($p = 0.03$). Age of onset was negatively correlated with hs-MSI scores ($r = -0.43$, $p = 0.011$). *PMS2* biallelic carriers showed a lower proportion of 1-bp deletions compared to other carriers. The accuracy in the identification of *PMS2* biallelic carriers using this proportion was 0.997.

Our study confirms the accuracy of hs-MSI assay for CMMRD diagnosis, also able to characterize MSI patterns in CMMRD-associated cancers. Hs-MSI is a powerful tool to pinpoint *PMS2* as the germline affected gene and potentially personalize cancer risk.

Grants: The Marató TV3 Foundation 202028-30

Conflict of Interest: None declared

P13.030.B MBD4-associated cancer predisposition

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Background/Objectives: Germline mutations in the *MBD4* gene, which, like *MUTYH* and *NTHL1*, encodes a glycosylase of the DNA based excision repair system, cause an autosomal recessive cancer syndrome characterized by increased risk to, mostly, acute myeloid leukaemia and colonic polyposis. Also, monoallelic germline *MBD4* mutations have been suggested to predispose to uveal melanoma (UVM). The aim of this study is to better define the phenotypic spectrum and tumour molecular features associated with biallelic and monoallelic MBD4-associated cancer predisposition.

Methods: Analysis of *MBD4* in >800 cancer patients with CRC, polyposis, UVM and other MBD4-suggestive phenotypes was performed, together with a comprehensive literature review and meta-analysis.

Results: Our results confirm the association of *MBD4* heterozygous pathogenic variants with UVM predisposition (>2,000 UVM cases vs. >134,000 controls; OR = 12; 95% CI: 6.5-20.7) and provide a better characterization of the recessive cancer syndrome. MBD4-deficient tumours, with complete inactivation of the two copies of *MBD4*, show high or relatively high tumour mutational burden (~10 mut/Mb) and a mutation spectrum characterized by CpG>TpG mutations (COSMIC mutational signature SBS96).

Conclusion: Our study, together with the data previously published, confirms that, with different risks and phenotypic features, germline *MBD4* biallelic and monoallelic pathogenic variants predispose to cancer. Recommendations for *MBD4* genetic testing, surveillance and clinical management will be presented, including the use of immune checkpoint blockade for the treatment of MBD4-deficient tumours.

Conflict of Interest: None declared

P13.031.C Characterization of heritable TP53-related cancer syndromes in Sweden - a retrospective nationwide study of genotype-phenotype correlations

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Background: Heritable TP53-related cancer syndrome (hTP53rc) is caused by germline alterations of TP53 and has a heterogenous clinical presentation. Increased understanding of genotype-phenotype relationships may improve clinical handling, diagnosis, and prognosis. Here we present the first nationwide delineation of the Swedish hTP53rc-cohort.

Methods: Genotype and phenotype data, including pedigrees, were retrieved for all TP53- variant carriers in Sweden up to March 2022 (91 families, 188 individuals). Families were classified according to classic Li-Fraumeni syndrome (classic LFS), Chompret or hereditary breast cancer (HBC) criteria and variants were reclassified using the newest ACMG guidelines.

Results: After reclassification a total of 83 families and 176 individuals were included. Among 176 variant carriers, 113 (64%) developed tumors among which 35 (31%) developed more than one primary tumor. Age at first tumor onset was higher in HBC families (42 years), compared to classic LFS (28 years, $p = 0.004$) and Chompret families (34 years, $p = 0.02$). The most prevalent tumor was breast cancer. Mean time between first and second primary tumor was 9 years. Patients with dominant-negative (DNE) missense variants had a lower age at first tumor onset (30 years) compared to all other variants (38 years, $p = 0.01$).

Conclusion: Patients with DNE missense variants and patients classified as classic LFS had an earlier age at first tumor onset. This study adds granularity to personalized risk modeling with potential implications for tailored screening regimes.

Funding: The King Gustaf V Jubilee Fund (201052).

Conflict of Interest: None declared

P13.032.D Predicting immediate colorectal cancer risk in a symptomatic cohort using a genetic risk score

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Background: Only 36% of colorectal cancer (CRC) cases are diagnosed at an early stage. Primary care consultations are an

important setting for identifying CRC earlier. We assessed whether a genetic risk score (GRS) for CRC could be used at the point of primary care consultation to improve triage of patients with bowel symptoms.

Methods: We identified 66,353 unrelated white European individuals with symptoms of CRC according to the NICE guidelines (e.g. abdominal pain, rectal bleeding) in the UK Biobank's primary care records. Of these, 598 (0.9%) had a CRC diagnosis within 2 years of first recorded symptom. We tested whether an integrated risk model including a 204-SNP GRS, age and sex could predict these immediate cancer diagnoses.

Results: The GRS associates with a CRC diagnosis following symptoms (OR per SD increase = 1.57 [1.45–1.7] $P = 7.2*10^{-28}$). An integrated risk model including the above covariates applied to symptomatic patients predicted CRC diagnosis (AUC 0.739 [0.721–0.758]). Individuals in the top 20% of predicted genetic risk had a 1.5% incidence rate (0.49% in the lowest 20%).

Conclusions: The integrated risk model using genetics at point of primary care presentation has modest power to predict colorectal cancer. The integrated risk model could help stratify individuals into high-risk and low-risk groups. Alongside test results, this could help improve the diagnostic pathway of symptomatic patients in primary care.

Conflict of Interest: None declared

P13.033.A CRISPR/Cas9 mediated generation of fusion transcript containing cell lines for Next Generation Sequencing based molecular analyses

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Background/Objectives: Next Generation Sequencing (NGS) has become an essential tool for research and diagnostics, especially for RNA-based analyses such as RNA sequencing (RNAseq). This study aimed to develop and research fusion transcript-based reference materials for RNAseq applications by CRISPR/Cas9 technology. Specifically, we intended to create a reference cell line that expresses a well-researched and frequently occurring fusion transcript, BCR-ABL1, caused by a chromosomal translocation involving the *ABL* and *BCR* proto-oncogenes.

Methods: CRISPR/Cas9 was used to create a fusion gene in both suspension and adherent cells by targeting the breakpoint region of the two introns of the genes involved in the rearrangement. Co-transfection of CRISPR guide RNAs and Cas9 induced double-strand breaks in the introns of both genes, leading to DNA misrepair and gene fusion.

Results: We created the BCR-ABL1 fusion gene in Jurkat cells which was confirmed by sequencing. Jurkat is a human cancer cell line derived from the peripheral blood of a T-lymphocytic leukemia patient lacking this fusion. Next, we expanded the study to human bladder cancer cells, which contain a fusion of *FGFR3* and *TACC3* on chromosome 4. By introducing the BCR-ABL1 fusion using the same CRISPR/Cas9 method, we generated a new cell line containing two different fusion transcripts per cell.

Conclusions: Having two cell lines with at least one or multiple known fusion transcripts will allow us to use them as reference materials for NGS-based RNA analysis and also to further study the molecular basis of acute lymphoblastic leukemia and chronic myeloid leukemia.

Grants: TBI-V-1-423-VBW-144 (Mecklenburg Vorpommern).

Conflict of Interest: None declared

P13.034.B MLH1 promotor methylation in colorectal and endometrial carcinomas from patients with Lynch syndrome

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Background/Objectives: The screening procedure for Lynch syndrome (LS) in colorectal cancer (CRC) and endometrial cancer (EC) patients generally involves immunohistochemical assessment of the mismatch repair (MMR) proteins. In the event of loss of MLH1 protein expression, *MLH1* promotor methylation (*MLH1*pm) testing is performed to indirectly distinguish germline *MLH1* variants from somatic epimutations. However, a growing number of recent studies suggest that *MLH1*pm and pathogenic germline MMR variants are not mutually exclusive.

Methods: The present study describes four new cases of LS patients with *MLH1*pm-positive CRCs/ECs and reviews previous reports from literature.

Results: A total of 103 *MLH1*pm-positive LS CRCs/ECs (98 CRCs and 5 ECs) originating from 70 *MLH1*, 14 *MSH2*, 8 *MSH6*, 2 *PMS2* and 10 undefined MMR variant carriers were identified. Median age at cancer diagnosis was 48 years (interquartile range 34.5-58).

Conclusion: Our findings provide further support for the argument that *MLH1*pm does not necessarily exclude a diagnosis of LS. Future prospective studies are needed to establish the significance of this clinical problem, amongst others by determining the prevalence of *MLH1*pm-positive CRCs/ECs in LS patients and vice versa. Clinicians should carefully consider whether or not follow-up genetic MMR gene testing should be offered, with age < 60-70 years and a positive family history amongst other factors being suggestive for a potential germline MMR gene defect.

Grant References: MLDS (FP16-06)

Conflict of Interest: None declared

P13.035.C Constitutional promoter methylation in familial and early-onset colorectal cancer patients

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Background/Objectives: Identifying the genetic causes of colorectal cancer (CRC) predisposition is a priority in the field of hereditary cancer. Research initiatives aimed to identify non-Lynch hereditary nonpolyposis CRC genes through genomic analyses have been mostly unsuccessful. In this study we explored the involvement of constitutional promoter methylation as a mechanism of gene inactivation in genetically unsolved familial and/or early-onset CRC cases.

Methods: Bisulfite-treated peripheral blood DNA from 46 CRC patients diagnosed before age 50 and/or with family history of CRC was analyzed with the Illumina Infinium MethylationEPIC array (935K CpGs). To identify patient-specific methylation patterns, CpGs beta-values of each patient were compared with those of the other analyzed individuals (PCA q<0.005). Only those genes with at least three consecutives differentially methylated CpGs were considered.

Results: Constitutional hypermethylation of the *BRCA1* promoter was identified in one CRC patient diagnosed at age 34. With beta-values of ~0.4, consistent with monoallelic methylation, constitutional promoter methylation of a new gene, of the BMP/TGF-β pathway, was detected in a patient diagnosed with CRC at 46 years and with family history of CRC. This gene region is found methylated, as a somatic event, in sporadic CRCs, but not in normal colon mucosa or blood.

Conclusion: Constitutional promoter methylation of known and yet-to-be-discovered hereditary cancer genes may explain some of the missing heritability in CRC. Particularly, we have identified promoter methylation of *BRCA1* as a potential cause of CRC predisposition, and a new candidate gene for hereditary CRC, involved in the BMP/TGF-β signaling pathway.

Conflict of Interest: None declared

P13.036.D Genetic testing of BRCA1- and BRCA2-associated hereditary breast and ovarian cancer at the University of Debrecen

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Introduction: BRCA1/BRCA2-associated hereditary breast and ovarian cancer syndrome is characterized by an increased risk mainly for breast (BC) and ovarian cancer (OC).

Patients and Methods: Between 2019-2022 BRCA1/2 genetic analysis was performed for 951 individuals by next generation sequencing. Retrospective analysis of genetic results, cascade testing and clinical and tumorpathological data was carried out.

Results: Altogether 146 BRCA1/2 variants were detected in 145 individuals, one patient harboured a variant in both genes. In the BRCA1 gene (95/145) 44.2% frameshift, 26.3% missense, 19% nonsense, 6.3% exonic deletions, 3.1% splicing mutations and 1.1% in-frame deletions, while in the BRCA2 gene (51/145) 60.8% frameshift, 15.7% splicing, 13.7% nonsense, 7.8% missense mutations and 2% in-frame deletions were detected. In half of the cases (74/145) family history was positive. Clinical data were available for 70/145 patients. Six individuals had OC, 56 persons BC, while 8 patients were diagnosed with both tumors. Among the 14 OC patients 13 carried a BRCA1 variant. Forty BRCA1+ and 24 BRCA2+ BC patients were diagnosed. BRCA1+ BCs were detected on average 5 years earlier and had more commonly triple negative hormon receptor status compared to BRCA2+ cases (65% vs. 19.23%). The proportion of cascade screening was very low (9/145), 19 family members tested positive for the familial mutation.

Conclusions: BRCA1 and BRCA2 variants were present in 2:1 ratio with four common mutations (37.8%). Clinical and tumorpathological characteristics were in good agreement with literature data. Based on the results number of cascade testing has to be improved greatly.

Grant References: ÚNKP-22-4-I.

Conflict of Interest: None declared

P13.037.A Results of whole-exome sequencing of germline DNA in pediatric patients with solid tumors

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Introduction: Pediatric cancers comprise only around 1% of all cancers. The majority, about 80% of cases, are sporadic, whereas, in 15-20%, a hereditary predisposition is assumed (compared to 5-10% in adults). Within published data from several international precision oncology programs, the percentage of patients with hereditary predisposition ranged between 6-35%.

Methods: We analyzed 259 patients from the Department of Children´s Oncology, University Hospital Brno, using whole-exome sequencing (Illumina TruSeq Exome Kit or Roche KAPA HyperExome). Genes associated with tumor predisposition syndromes and genes within the ACMG secondary findings list were evaluated. In case pathogenic or likely pathogenic variants were identified, the patient and his family were provided with a genetic consultation.

Results: Tumor predisposition was found in a total of 32 patients (12%). These were mainly autosomal dominant syndromes (most frequent mutations in the *NF1* (n = 6), *SMARCB1* (n = 4), and *BRCA2* (n = 4) genes). In 9% of patients, mutations in genes associated with predisposition syndromes inherited in an autosomal recessive manner were detected.

Conclusion: The identification of a genetic predisposition to the development of cancer is of fundamental importance for the clinical management of patients in terms of adjusting the treatment plan, monitoring of adverse effects of treatment, and the need for long-term dispensary due to the risk of developing other malignancies. Within the family, genetic testing of patients leads to the detection of persons at tumor risk and the initiation of their early preventive monitoring.

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P13.038.B Whole-Body MRI Surveillance - Baseline Findings in the Swedish Multicentre Hereditary TP53-Related Cancer Syndrome Study (SWEP53)

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Background*:* A surveillance strategy of the heritable *TP53*-related cancer syndrome (h*TP53*rc), commonly referred to as the Li–Fraumeni syndrome (LFS), is studied in a prospective observational nationwide multi-center study in Sweden (SWEP53). The aim is to evaluate whole-body MRI (WB-MRI) regarding the rate of malignant, indeterminate, and benign imaging findings and the associated further workup generated by the baseline examination.

Methods: Individuals with h*TP53*rc were enrolled in a surveillance program including annual whole-body MRI (WB-MRI), brain-MRI, and in female carriers, dedicated breast MRI.

Results*:* A total of 68 adults ≥18 years old have been enrolled to date. Of these, 61 fulfilled the inclusion criteria for the baseline MRI scan. In total, 42 (42/61 = 69%) individuals showed a normal scan, while in 19 (19/61 = 31%) the WB-MRI scan indicated findings that needed further workup. Among these three individuals (3/19 = 16%) were diagnosed with asymptomatic malignant tumors, *i.e*. thyroid cancer, disseminated upper GI cancer, and liver metastasis from a previous breast cancer, respectively. Forty-three participants were women, of whom 21 had performed risk-reducing mastectomy prior to inclusion. The remaining were monitored with breast MRI, and no breast tumors were detected on baseline MRI.

Conclusion: WB-MRI has the potential to identify asymptomatic tumors in individuals with h*TP53*rc syndrome. The challenge is to investigate all indeterminate findings adequately and efficiently in these individuals with extremely high cancer risks. Thus, a multidisciplinary team should be considered in surveillance programs for individuals with h*TP53*rc syndrome.

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Conflict of Interest: Svetlana Bajlica Lagercrantz Karolinska University Hospital, Karolinska Institutet, The Breast Cancer Theme Center (BRECT) at Karolinska Institutet and Karolinska University Hospital, The Cancer Society in Stockholm and The King Gustaf V Jubilee Fund (grant number: 201052), The Swedish Cancer Society (grant number: CAN 2016/775), Meis Omran Karolinska University Hospital, Karolinska Institutet, The Rare Disease Research Foundation, Yvonne Brandberg Karolinska Institutet, Emma Tham Karolinska University Hospital, Karolinska Institutet, Stockholm County Council (grant numbers SLL20180046 and SLL500306), Lennart Blomquist Karolinska University Hospital, Karolinska Institutet

P13.039.C Germline variants in patients with end-stage cancer

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Introduction: Cancer therapy can be guided by the genetic constitution of the neoplastic cells. In patients with a monogenic predisposition to cancer, knowledge of the predisposing variant(s) can be useful for both treatment and prevention.

Method: In a research project (NCT05695638), adult patients with end-stage cancer in the North Denmark Region are offered comprehensive genetic analysis to search for targets for experimental medication. The tumour is subjected to whole exome sequencing (WES). To aid interpretation, only variants not detected in blood are reported, with the exception of variants in *BRCA1, BRCA2, ATM, MLH1, MSH2, PMS1, MSH3, MLH2, MLH3, MSH6, PMS2, PALB2, RAD51C* and *RAD51D*.

Results: Among the first 190 patients with a solid tumour (predominantly breast, ovarian, lung, brain, and prostate cancer), germline variants were detected in 8 patients (4.2%): in *BRCA1 (*2 patients: bilateral breast, and prostate*)*, *BRCA2* (ovary), *PALB2* (prostate), *RAD51D* (tubal), *MSH2* (glioma), biallelic *MSH6* (glioma), *PMS2* (lung). For these patients, genetic work-up and counselling were performed.

For 3 of the 8 patients, the predisposing variants were already known from previous genetic work-up of the family. 3 of the remaining 5 patients did not have a family history suggestive of a monogenic predisposition to cancer.

Conclusion: For patients with cancer, medical history should include the question: "Do you, or a relative, have a predisposing gene variant?"

Systematic genetic analysis of patients with cancer can identify a monogenic predisposition to cancer, in families that are not identifiable by a significant family history.

Conflict of Interest: None declared

P13.040.D Molecular analysis of somatic and germline gene variants in thymic epithelial tumours using targeted next-generation sequencing

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Background/Objectives: The biology of thymic epithelial tumours (TETs), including thymomas (TMs) and thymic carcinomas (TCs), and particularly the extent of molecular dysregulation, is poorly understood. We evaluated somatic and germline variants in genes commonly mutated in solid tumours using next-generation sequencing (NGS).

Methods: In total, 53 (19 TMs and 34 TCs) archival tissue samples were analysed for nonsynonymous variants (SNVs, Indels) in 15 genes by targeted NGS (reference genome: hg19/GRCh37).

Results: Ten variants in *TP53* (G154V, R158P, L194H, R267fs, R273C, R306*, Q317*), *ERBB2* (V773M), *KIT* (L576P), and *KRAS* (Q61L) considered somatic and pathogenic/likely pathogenic were detected in 10 of 34 (29.4%) TCs. No somatic pathogenic/likely pathogenic SNVs were found in TMs. New and rare variants of uncertain clinical significance were found in *ERBB2* (S703R), *KIT* (I690V), and *FOXL2* (P157S) in 3 of 19 (16%) TMs. The most frequent germline SNVs were *TP53* P72R (94% TETs), *ERBB2* I655V (40% TETs), and *KIT* M541L (9% TETs). No significant difference in median disease-free survival (DFS) was found between TC patients with and without pathogenic variants ($p = 0.190$); however, a trend toward a longer DFS was observed in the latter (16.0 vs. 30.0 months, respectively).

Conclusion: NGS analysis of TETs revealed several somatic variants in genes related to the p53, AKT, MAPK, and K-Ras signalling pathways. TCs showed greater genetic dysregulation than TMs. *KIT* alterations in TCs have potential as therapeutic

targets. The germline and rare variants reported in this study increase the number of known genetic alterations in TETs.

Conflict of Interest: None declared

P13.041.A Genotype-phenotype correlation in heritable TP53-related cancers bearing truncating and non-truncating TP53 variants

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Background/Objectives: *TP53* germline pathogenic variants predispose to diverse cancers. Truncating variants produce loss-of-function, while non-truncating are often dominant-negative. Intensive surveillance of target organs reduces cancer-related mortality; however, it is difficult to prioritize high-risk organs for surveillance in different *TP53* variant types. We conducted a genotype-phenotype analysis considering truncating vs. non-truncating pathogenic *TP53* variant carriers.

Methods: We selected ClinVar *TP53* pathogenic/likely pathogenic variants reviewed by expert panels/consensual among multiple submitters to guide data collection from IARC *TP53* Database. Variants were classified as truncating (frameshift, nonsense, and out-of-frame splice-site) or non-truncating (missense or in-frame splice-site). Genotype-phenotype associations were analyzed using Mann-Whitney or χ^2 tests and multivariable logistic regression models.

Results: We included 2838 clinical data registries from 2125 patients belonging to 912 families carrying 144 distinct variants in exons 2-10. 546/2601 (21%) cancer-phenotypes occurred in truncating-variant, and 2055/2601 (79%) occurred in non-truncating variant carriers, the latter at significantly younger age of diagnosis (28.5 vs. 31.2y.o., $p = 0.002$). After multivariable regression analysis, breast (OR = 1.30, 95%CI: 1.05-1.60, $p = 0.014$) and hematopoietic system (OR = 1.71, 95%CI: 1.08-2.70, $p = 0.023$) cancers were associated with truncating variants, while adrenal gland cancer (OR = 2.11, 95%CI: 1.35-3.31, $p = 0.001$) with non-truncating. Distribution of truncating and non-truncating variants in families with Li-Fraumeni (LFS) criteria was similar, but truncating variants were significantly more frequent in LF-like criteria families ($p = 0.003$).

Conclusion: The higher estimated risks of breast and hematopoietic system cancers in truncating-variant carriers and adrenal gland cancer in non-truncating variant carriers supports, if further validated, privileged surveillance of specific organs/systems according to the *TP53* variant molecular type.

Conflict of Interest: None declared

P13.042.B Somatic alterations in prostate cancer: an analysis of TCGA data

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Prostate cancer is one of the most common cancers in men, however it can be difficult to predict disease aggressiveness and treatment outcomes. Here, we analysed clinical and genomic data collected by The Cancer Genome Atlas (TCGA) from 489 prostate tumour samples to determine whether somatic gene fusions, copy number variants (CNV) and/or exonic point mutations (SNPs/indels) are associated with tumour grade and biochemical recurrence. T tests and linear regression models were used to test association with continuous variables, Kruskal-Wallis rank sums tests for categorical variables, and Fisher-exact tests for binary variables. A higher burden of gene fusions ($p = 0.002$), CNVs ($p < 2.2 \times 10^{-16}$) and mutations ($p = 9.67 \times 10^{-8}$) were significantly associated with a higher International Society of Urological Pathologists (ISUP) tumour grade. A higher burden of gene fusions ($p = 0.02$) and CNVs ($p = 1.18 \times 10^{-4}$) were associated with biochemical recurrence. The most common deletion, *PTEN* (17.45%) and the most common mutation, *TP53* (11.5%) were associated with biochemical recurrence ($p = 0.003$, $p = 0.002$, respectively) and higher ISUP grade disease ($p = 0.0002$, $p = 0.0004$, respectively). The most common gene fusion, *TMPRSS2-ERG* (38%), was not associated with tumour grade or biochemical recurrence. Additionally, an increasing number of gene fusions was associated with an increasing number of CNV effected genes ($p = 1.08 \times 10^{-11}$). This comprehensive, publicly available dataset has provided an important avenue to assess associations between various types of somatic alterations and clinically relevant prostate cancer phenotypes/outcomes. These results suggest that the overall burden of somatic alterations may be just as important as specific alterations in determining tumour aggressiveness and predicting outcomes after primary treatment.

Conflict of Interest: None declared

P13.043.C Targeted next-generation sequencing as a diagnostic tool for identification of druggable molecular alterations in non-small cell lung cancer

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Background/Objectives: The growing number of druggable molecular alterations and targeted therapies in non-small cell lung cancer (NSCLC) require high-throughput and cost-effective diagnostic techniques to be implemented in daily practice. We evaluated the diagnostic usefulness of DNA and RNA-based targeted next-generation sequencing (NGS) for the detection of important genetic variants in different types of tumor specimens from advanced NSCLC patients.

Methods: To date, 51 tumor specimens (formalin-fixed, paraffin-embedded (FFPE) biopsies, surgically resected tissues, cytology specimens) were analyzed either for pathogenic variants (SNVs, Indels) and/or actionable gene fusions by targeted NGS assays (TruSight Tumor15, Illumina [TST15] and FusionPlexLung, Archer [FPLA], respectively; hg19/GRCh37) with reference to conventional In Vitro Diagnostic qPCR and fluorescence in situ hybridization (FISH) tests.

Results: In 32 NSCLC specimens, TST15 showed 100% concordance with qPCR results in *EGFR, KRAS, BRAF* gene variant detection at the Variant Allele Frequency (VAF) >5%. In 19 samples previously assessed for *ALK, ROS1* gene fusions by FISH, FPLA showed 100% concordance. Both NGS assays showed 100% concordance in detecting *EGFR, KRAS, BRAF* variants on DNA and RNA levels. The critical factors for successful NGS performance were

tumor cellularity >10%, tumor cell number >100 and quantity/quality of RNA/DNA, irrespectively from the specimen type.

Conclusion: Targeted NGS provides a robust diagnostic tool for sensitive detection and reliable identification of important gene variants in clinical specimens. NGS assays showed superior features over qPCR and FISH by exact identification of detected variants in over a dozen genes that might prove important in therapeutic approach. The study is ongoing.

Conflict of Interest: None declared

P13.044.D ATM c.7570G>C is a high-risk allele for breast cancer

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Background: *ATM* is generally described as a moderate-risk breast cancer susceptibility gene. However, some of the ATM variants might encounter even higher risk. *ATM* c.7570G>C, p.Ala2524Pro, (rs769142993) is a pathogenic Finnish founder variant causative for recessively inherited ataxia-telangiectasia. At cellular level, it has been reported to have a dominant-negative effect. *ATM* c.7570G>C has recurrently been described in Finnish breast cancer families and unselected case cohorts collected from different parts of the country, but the rarity of the allele (MAF 0.0002772 in Finns) and lack of confirming segregation analyses have prevented any conclusive risk estimates.

Methods: Here, we studied seven families from genetic counseling units with *ATM* c.7570G>C for co-segregation with breast cancer. We performed further mutation screening in the unselected breast cancer cohort from Northern Finland (n = 1822) by high-resolution melt analysis and compared the mutation prevalence to SISu database cancer-free controls from the same geographical region.

Results: *ATM* c.7570G>C co-segregated with breast cancer phenotype in the studied families. It also significantly associated with breast cancer in the case-control analysis, and the risk is estimated as high (odds ratio [OR] = 8.5, 95% confidence interval [CI] = 1.04–62.46, P = 0.018).

Conclusion: When combining the results of the performed case-control comparison and the genetic counseling unit family data, *ATM* c.7570G>C variant can be placed among the high-risk alleles for breast cancer, which should be taken into consideration in genetic counseling.

Conflict of Interest: None declared

P13.045.A The clinical utility of droplet digital PCR for detecting PIK3CA mutations in circulating tumor DNA in breast cancer patients – preliminary results

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Introduction: Breast cancer has the highest incidence among cancers and represents the leading cause of death from malignancy in women. Circulating tumor DNA can be used for identification of specific cancer-related mutations. Quantification of specific mutation by ddPCR provides not only unparalleled evaluations for target treatment and efficacy but also the ability to identify and monitor the patients who may be at risk for disease progression. The aim of this study is to identify and quantify PIK3CA mutations (E545K, E542K and H1047R) in patients with breast cancer.

Materials and methods: ctDNA were isolated from 50 patients with breast cancer (26 in adjuvant and 24 in metastatic stage). ddPCR was performed for detection and quantification of PIK3CA mutations. Patients who tested positive for PIK3CA mutations underwent serial monitoring of their ctDNA.

Results: PIK3CA mutations were identified in 5 (10%) of the patients. Upon serial monitoring, four of the patients showed an increase in the mutation quantity, which was consistent with poor response to treatment and fatal outcome. Conversely, one patient showed a reduction in the mutation quantity, which corresponded with a good response to treatment.

Conclusion: ctDNA represents an attractive, non-invasive tool for monitoring tumor evolution, treatment response and assessing patient prognosis. Serial monitoring of ctDNA could help to predict relapse in previously treated patients and to personalize the therapy. Larger clinical trials are necessary to fully establish the accuracy and clinical usefulness of ctDNA in the management of breast cancer.

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Conflict of Interest: None declared

P13.046.B Comparison of genomic profiles of primary and recurrent brain gliomas

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Background: Diffuse gliomas are heterogenous tumors with occurrence of recurrent lesions in majority of patients. During disease progression gliomas undergo cellular and genomic evolution with newly acquired genetic properties. The mechanism of this complicated process associated with treatment failure is poorly understood. We performed cytogenomic analyses of tumor cells of five patients with diffuse glioma who underwent surgical resections/biopsies of multiple recurrences.

Methods: Tumor samples were analyzed using combination of cytogenomic methods: I-FISH (Abbott, MetaSystems), aCGH/SNP (Agilent), MLPA, methylation-specific MLPA (MRC-Holland) and target NGS (Invitae).

Results: All patients experienced recurrence with newly acquired genetic/epigenetic changes with high frequency of CNAs. Moreover, several aberrations were not detected in recurrence despite being found in earlier samples. As a primary event we proved mutation R132H in *IDH1* gene. We detected methylation of *MGMT* promoter, *CDKN2A/B* homozygous deletion, and *RB1* deletion as later events associated with higher tumor grades. Besides the typical genomic changes, we detected aberrations with unknown/unclear prognostic relevance (inframe

deletion in *TP53*, p.Met243_Asn247del, etc.). The progression to a higher glioma grade occurred in four patients.

Conclusions: The evolutional patterns in glioma depend on clonal selection and/or the patient's treatment. Recurrence may arise from one major clone or from one or more subclones within the primary tumor. Integrated cytogenomic analyses of genetic/epigenetic profiles of primary and all recurrent tissues contribute to a better understanding of mechanisms responsible for these processes and to identification of alterations related to progression and/or treatment resistance.

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Conflict of Interest: None declared

P13.047.C Germline and somatic testing in a cohort of malignant mesothelioma (G-MESO)

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Background: Although asbestos exposure is the most common cause of malignant mesothelioma (MM), around 10-12% of patients harbor pathogenic or likely pathogenic germline variant (PGV) in cancer predisposition genes. The prevalence of PGVs in patients with MM in our country is unknown.

Methods: Sixty-six patients diagnosed with pleural (n = 60) and peritoneal (n = 6) MM were prospectively tested at the germline level using a custom NGS panel covering 164 cancer-predisposing genes. DNA and RNA from 32 tumors were sequenced using the TSO500+ gene panel (Illumina®).

Results: Most patients had history of asbestos exposure (67%). Personal and family history of cancer (1st degree relative) was recorded for 9 and 44 patients, respectively. Eight patients (12%) harbored PGVs in 6 genes: *BAP1* (n = 2), *BARD1* (n = 2), *FANCA* (n = 1), *RECQL4* (n = 1), SBDS (n = 1), *SDHC* (n = 1). Five patients (7.5%) were found in clinically actionable genes and were referred to Genetic Counselling (GC). At the tumor level, the most frequent somatic mutations were: *BAP1*, 28.1%; *NF2*, 25%; *TP53*, 12.5%; *LATS2*, 9.3% and *SETD2*, 6.2% and were present in the 62.5% (20/32) of MM tumors. Five suspicious PGVs in *PIK3C2G*, *FGFR3*, *ROS1*, *FGF5* and *EGFL7* detected in the tumor, were subsequently confirmed at the germline level.

Conclusion: In this series, 12% of patients with MM harbored PGVs and 7.5% were candidates to be referred to GC. Concurrent somatic and germline testing provides opportunities to discover putative predisposition cancer genes. Germline molecular testing should be considered in patients diagnosed with MM.

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Company: Bayer
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Company: Sanofi
Recipient: You
Company: Merck Serono
Recipient: You
Company: Janssen Oncology
Recipient: You, Travel, Accommodations, Expenses
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Recipient: You
Company: Roche
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Company: Lilly
Recipient: You

P13.048.D Functional analysis supports pathogenicity of a synonymous variant in MSH2 in a patient with suspected Lynch Syndrome

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Background/Objectives: Germline pathogenic variants of mismatch repair (MMR) genes are responsible for Lynch Syndrome

(LS), condition predisposing to colorectal cancer (CRC) and other tumours. For several MMR gene variants, including synonymous or intronic changes, the pathogenic effect is unclear. We aimed to investigate the functional significance of a synonymous *MSH2* variant presumably affecting splicing, to help risk assessment and management in a family with suspected LS.

Methods: Transcript analysis on RNA extracted from fresh blood samples was performed by RT-PCR with specific primers and direct sequencing. Western blot for MSH2 was performed on lysates derived from peripheral blood lymphocytes.

Results: A male patient with a family history of early-onset CRC (father died at 40 years) underwent three surgeries (first at 32 years) for CRCs characterized by MSH2/MSH6 deficiency. Germline MMR genes analysis detected a synonymous *MSH2* variant of unknown significance: c.1275A>G (p.Glu425 =). Transcript analysis in the patient's cDNA showed, in addition to the wild-type band, a shorter fragment, which was found to harbour a 48-bp deletion in exon 7, leading to a 16 amino acids in-frame deletion. Western blot on the patient's lysate revealed a strong decrease in MSH2 vs. controls.

Conclusion: Family and personal history of CRC were strongly suspicious for LS and MMR immunohistochemistry in neoplastic tissues was consistent with a variant in *MSH2*. Functional analysis demonstrated an effect on splicing, which, together with the reduction of the protein expression supports the pathogenicity of the c.1275A>G variant in *MSH2*.

Conflict of Interest: None declared

P13.049.A Reverse Mendelian randomisation separates causes from early proteomic biomarkers of glioma

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Background/Objectives: Glioma represents the largest entity of primary brain tumours in adults, with an overall survival of less than 20% over 5 years. Glioblastoma is the most frequent and aggressive glioma subtype. At present, there are few well-established risk factors for glioma. Our study aims to identify biological processes which could be associated with the genetic liability for glioma.

Methods: We generated a polygenic risk score (PRS) for all glioma cases (n = 12,496), glioblastoma (n = 6,191), and non-glioblastoma (n = 6,305). Combining reverse and forward Mendelian randomisation (MR) we examined the relationship between the genetic liability of glioma and 4,907 plasma pQTLs (deCODE study, n = 35,559).

Results: Reverse MR identified 87 proteins associated with the PRS of glioma, 42 proteins associated with the PRS of glioblastoma, and no proteins associated with the PRS of non-glioblastoma. PRS of glioma and glioblastoma were found to have 23 of the same proteins included. Enrichment analyses identified a proportion of plasma proteins to be associated with the PRS of glioma to be correlated with complement and coagulation cascades and the innate immune system. A cluster of plasma proteins linked to the PRS of glioma and glioblastoma were related to the extracellular region and extracellular space. Forward MR of the putative relationships were found to have little or no evidence of association on the causal pathway.

Conclusion: Our findings identify a high genetic liability to glioma is associated with the complement cascade and innate immunity. Non-causal plasma biomarkers identified through PRS associations could indicate novel biomarkers of early glioma development.

Conflict of Interest: None declared

P13.050.B Study of the response of ovarian cancer cell lines to enzalutamide

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Background: Ovarian cancer (OC) is the leading cause of death among gynaecological cancers in most developed countries. The heterogeneity of the tumours and the development of chemotherapy resistance transform an 80-90% response rate to first-line therapy to a <35% 5-year survival rate. Signalling via androgen receptor (AR) has been found to promote tumorigenesis and metastasis in several cancer types, including OC. Moreover, 43.5-86% of OCs express AR. In this project we assessed enzalutamide, an AR antagonist, as a possible alternative therapy for OC.

Method: OC cell lines (OCCLs): A2780, IGROV-1 and OVCAR-8 were used. Western blot and RT-qPCR were used to validate AR status. MTT, apoptosis and cell cycle assays were performed to test enzalutamide and dihydrotestosterone sensitivity. AR was transfected to AR- OCCLs using lentiviral vectors to asses possible differences in the response to enzalutamide when AR is or is not expressed.

Results: The results show that enzalutamide affects cell metabolism, inhibits cell growth, and induces apoptosis in AR + A2780 and OVCAR-8 and AR- IGROV-1 OCCLs.

Conclusion: We conclude that enzalutamide could be a potential therapeutical alternative in AR+ ovarian cancer.

References: Kossaï, M., Leary, A., Scoazec, J. Y., & Genestie, C. (2018). Ovarian cancer: a heterogeneous disease. *Pathobiology, 85*(1-2), 41-49.

Chung, W. M., Chen, L., Chang, W. C., Su, S. Y., Hung, Y. C., & Ma, W. L. (2021). Androgen/androgen receptor signaling in ovarian cancer: Molecular regulation and therapeutic potentials. *International Journal of Molecular Sciences, 22*(14), 7748.

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Conflict of Interest: None declared

P13.051.C Impact of germline variants in cancer susceptibility genes in sardinian patients with breast and ovarian cancer

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The Sardinian population represents a valuable resource for the study of complex diseases due to its unique genetic architecture. In fact, it offered an opportunity to search for genetic variants associated with breast and ovarian cancers, which are the leading causes of cancer-related deaths among women.

To identify genes implicated in carcinogenesis, we collected 975 individuals: 777 affected and 198 healthy people with a familial predisposition for breast or ovarian cancer. All the subjects were sequenced using a method able to extend the analysis to the whole DNA-coding region: the Whole-Exome Sequencing (WES). Variant pathogenicity was evaluated according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

The analysis revealed positive test results in 320 affected patients. In 127 patients, a single pathogenic variant was identified in one of the most common cancer-predisposition genes: 39 in *BRCA1* (15%), 22 in *BRCA2* (8%), 26 in *ATM* (10%), 30 in *CHEK2* (11%) and 10 in *PALB2* gene (4%). Additionally, 137 patients carried a variant in rarer cancer genes: 34 in *PRKN* (13%), 11 in *MLH1* (4%), 8 in *NBN* (3%), 7 in *MUTYH* and in *RAD51C* (3%), and others 29 genes with a frequency of less than 2%. Finally, 56 patients carried two different variants in these genes.

Genetically-homogeneous populations, such as that of Sardinia, could be useful to identify new high/low penetrant loci, which contribute to high or moderate risk of breast and ovarian cancer. These loci could represent new candidate susceptibility genes for these diseases.

Conflict of Interest: None declared

P13.052.D Complex structural variation patterns and mechanisms are shared across pediatric solid tumors

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Structural variants (SVs) and copy number (CN) alterations occur frequently in pediatric cancer and in specific cases are known to contribute to cancer initiation and progression. Yet, for most SVs in pediatric solid tumors, their precise role in carcinogenesis remains unknown and the presence of complex SVs is rarely considered. Previous molecular characterization efforts have focused on individual cancer types or on differences between subtypes. Nevertheless, the mutational mechanisms underlying cancer-type specific alterations could be very similar. Therefore, we analysed CN/SV patterns across pediatric cancers to determine the presence of shared underlying pan-cancer mutational mechanisms.

In a cohort of 120 patients representing six cancer types, we inferred CNs and SVs from paired tumor-normal whole genome sequencing data. Clustering tumors by CN/SV patterns supported the presence of pan-cancer mutational mechanisms. Although some tumor genomes have a relatively low burden of SVs, a significant proportion of seemingly CN neutral tumors often mask more complex SV patterns. More generally, complex SVs were identified in all cancer types, some suggestive of a single chromothripsis or chromoplexy event or the formation of double minute chromosomes. Furthermore, these complex SVs affected multiple oncogenes and tumor suppressor genes, indicating they are potentially pathogenic.

Altogether, we identified shared CN/SV patterns and mechanisms across pediatric solid cancers, including complex SVs with an unanticipated prevalence. In addition to CNs and SVs affecting cancer genes, associations to clinical characteristics further demonstrated the importance of CN/SV patterns in pediatric cancer.

Conflict of Interest: None declared

P13.053.A BRCA2, APC, and POLE are the genes most frequently affected by very rare germline variants in a European familial glioma cohort

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Background/Objectives: The genetic basis of brain tumor development, including gliomas, is poorly understood. However, pathogenic variants in a few cancer predisposition genes (CPGs), e.g. *NF1*, *NF2*, and *TP53*, are known to be associated with an increased brain tumor risk. Here, the CPG variant and tumor spectrum were determined in a European familial glioma cohort.

Methods: Leukocyte DNA of 85 glioma patients from 79 tumor families containing at least one glioma patient each were analyzed by whole-exome sequencing. Very rare (MAF<0.2%) variants in established CPGs (n = 114) were extracted and classified. The CPG-related tumor spectrum was assessed.

Results: Very rare variants were most frequently detected in *BRCA2* (8/79, 10%), *APC* (7/79, 9%), and *POLE* (4/79, 5%); all variants were heterozygous. Only one very rare *BRCA1* variant was found. The *BRCA2* variants included a known pathogenic splice site variant, i.e. *BRCA2*:c.316+5G>C. All *APC* variants were predicted to be deleterious. All *POLE* variants were previously described in colorectal or ovarian cancer patients. Of the 27 tumors in *BRCA2* families, 10 (37%) were breast carcinomas and one (4%) a prostate carcinoma. In 3/7 (43%) *APC* families and 3/4 (75%) *POLE* families, colorectal cancer cases were observed. The highest proportion of gliomas was observed in *POLE* families (50%), while in *BRCA2* and *APC* families gliomas made up 30% and 44% of the tumor cases.

Conclusion: Our data corroborate the notion that gliomas belong to the tumor spectrum of very rare *APC* and *POLE* variants, and suggest that *BRCA2* variants increase glioma risk more than *BRCA1* variants.

Conflict of Interest: None declared

P13.054.B CTNND1 is involved in germline predisposition to early-onset gastric cancer by affecting cell adhesion

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Background/Objectives: Germline predisposition behind gastric cancer is sometimes unclear, and it could be relevant in early-onset gastric cancer patients (EOGC;<50 years old). Besides *CDH1* and *CTNNA1*, there are no additional hereditary genes known so far for EOGC.

Methods: After germline whole-exome sequencing in a discovery cohort of 20 EOGC patients, a gene panel sequencing of 38 candidate genes was performed in an independent replication cohort of 164 EOGC patients. *CTNND1* stood out as an interesting new candidate gene for EOGC germline predisposition, since its protein product (Catenin delta-1/p120ctn) directly interacts with the *CDH1* protein (Cadherin-1). Two knock-out cellular models for *CTNND1* in NCI-N87 and hTERT-RPE1 cell lines were generated by CRISPR/Cas9, and genetic variants of interest were introduced using a lentiviral delivery system. Cell adhesion and Cadherin-1 localization were assessed by spheroids modelling. Cell-detachment assay and Wnt pathway (Catenin beta-1) were also studied.

Results: Three predicted pathogenic variants [c.28_29delinsCT (p.Ala10Leu), c.1105C>T (p.Pro369Ser), c.1537A>G (p.Asn513Asp)] were identified in our EOGC cohorts. Both cell adhesion capacity and spheroid generation were altered for all genetic variants. In addition, p.Pro369Ser variant, which is located in the Cadherin-1-Catenin delta-1 binding domain, showed mislocalization for Cadherin-1 protein.

Conclusions: Taking into account the close relationship with *CDH1* and *CTNNA1*, *CTNND1* stood out as an interesting candidate. Our findings suggest that *CTNND1* is involved in EOGC germline predisposition by affecting cell adhesion and Cadherin-1 localization.

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Conflict of Interest: None declared

P13.055.C Cancer prognosis in PTEN Hamartoma Tumor Syndrome - a European cohort study

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Introduction: PTEN Hamartoma Tumor Syndrome (PHTS) patients are at high hereditary cancer risk, e.g. with cancer risks up to 76% for breast cancer (BC) and 21% for thyroid cancer (TC). As cancer prognosis in PHTS is unknown, we aimed to provide this.

Methods: A European cohort study with medical file, registry and/or questionnaire data including adult PHTS cancer patients. The 5- and 10-year overall survival (OS) of the first primary cancer was estimated with left-truncated Kaplan-Meier analyses.

Preliminary results: Overall, 145 female BC patients were included (74% indexes). Mean BC age was 40 years (SD = 9), and BC stage 0-IV distributed as 31%, 28%, 24%, 13% and 4%. The median OS was 21 years. The 5y-OS was 93%(95%CI:86-100) and 10y-OS 79%(95%CI:68-92). The 5y-OS and 10y-OS ranged from 100%(95%CI:100-100) and 93%(95%CI:82-100) for stage 0 to 33% (95%CI:7-100) and 0%(95%CI:0-0) for stage IV. Furthermore, 58 TC patients were included (67% indexes/72% females). Mean TC age was 37 years (SD = 16). The median OS was not reached within current follow-up. The 5y- and 10y-OS was 89%(95%CI:78-100) both. TC was mainly stage I (85%) with 5y- and 10y-OS of 100% (95%CI:100-100) both. Number of events per stage-subgroup were limited.

Discussion and conclusion: Our results provide the-first-ever data on cancer-specific prognosis in PHTS. They suggest that OS is similar to sporadic cancer, however potential influencing factors (e.g. cancer characteristics and treatment) will follow. All results, including other cancer types, disease-free prognosis and standardized mortality ratios will be available soon.

Grant references: PTEN Research Foundation

Conflict of Interest: Linda Hendricks PTEN Research Foundation, Katja Verbeek: None declared, Hilde Brems: None declared, Robin de Putter: None declared, Lenka Foretova: None declared, Chrystelle Colas: None declared, Patrick Benusiglio: None declared, Claude Houdayer: None declared, Arne Jahn: None declared, Verena Steinke-Lange: None declared, Robert Hüneburg: None declared, Maurizio Genuardi: None declared, Giovanni Innella: None declared, Alessandra Renieri: None declared, Arvids Irmejs: None declared, Maran Olderode-Berends: None declared, Edward Leter: None declared, Daniëlle Bosch: None declared, Marianne Tveit Haavind: None declared, Siri Briskemyr: None declared, Kjersti Jørgensen: None declared, Juliette Dupont: None declared, Ana Blatnik: None declared, Joan Brunet: None declared, JUDITH BALMAÑA: None declared, Emma Tham: None declared, marc tischkowitz: None declared, Emma Woodward: None declared, Arjen Mensenkamp: None declared, Janneke Schuurs-Hoeijmakers: None declared, Nicoline Hoogerbrugge PTEN Research Foundation, Janet Vos PTEN Research Foundation

P13.056.D Exploring the chemopreventive effect of drugs on colorectal cancer associated gene expression in healthy colon mucosa: An observational and Mendelian randomization analysis

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Background: The use of some drugs has been related to colorectal cancer (CRC) risk. In this study, we wanted to determine the effect of drug intake on the expression of genes associated with cancer field-effect in healthy colon tissue.

Methods: We identified a robust set of 211 genes with upregulated expression in normal colon tissue adjacent to tumor, characterizing the cancer field-effect. We assessed the chemopreventive effect of intake of a total of 21 drugs applying a gene set enrichment analysis (GSEA) of differential expression for these genes over whole gene expression in the BarcUVa-Seq project colon healthy dataset (428 individuals), providing enrichment scores (ES). We also performed instrumental analyses using expression Quantitative Trait Loci (eQTLs) as instruments mimicking the effect of drugs on drug target genes. Instrumental results were validated in GTEx and CEDAR datasets.

Results: The intake of common used medication showed a significant downregulation of the cancer field-effect gene set, which included the intake of metformin (ES = -2.95, P = 0.0012), beta-blocking agents (ES = -2.84, P = 0.0013), and statins (ES = -2.82, P = 0.0023). Finally, the analysis of the corresponding eQTLs for the drug-targeted genes (rs17485664 for PRKAB1 - metformin, rs68122733 for ADRB1 – beta-blocking agents, rs4292 for ACE - renin-angiotensin system agents, and rs17238484 for HMGCR – statins), showed consistent results.

Conclusion: The results provided potential biological means by which the intake of specific drugs can help to reduce CRC incidence.

Conflict of Interest: None declared

P13.057.A Mismatch repair deficiency and Lynch syndrome among 1225 adult patients with glioma

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Introduction: The Lynch syndrome (LS)-glioma association is poorly documented. As for Mismatch Repair-deficiency (MMRd) in glioma, a hallmark of LS-associated tumors, there are few data available. We determined MMRd and LS prevalence in a large series of unselected gliomas, and explored the associated characteristics. Both have major implications regarding treatment, screening and prevention.

Methods: Somatic multigene sequencing was performed on 1225 treatment-naïve adult gliomas referred over 2017-2022. For gliomas with ≥1 MMR pathogenic variant (PV), MMR immunohistochemistry (IHC) was done. ≥1 PV and protein expression loss defined MMRd. Eligible patients had germline testing. To further explore MMRd specifically in glioblastomas, IDH-wild type (wt), we performed IHC, and complementary sequencing when indicated, in a series of tumors diagnosed over 2007-2021.

Results: Nine gliomas were MMRd (9/1225, 0.73%). Age at glioma diagnosis was <50 for all but one case. Eight were glioblastomas, IDH-wt, and one was an astrocytoma, IDH-mutant. LS prevalence was 5/1225 (0.41%). One 77-year old patient was a known LS case. Four cases had a novel LS diagnosis, with germline PV in MSH2 (n = 3) and MLH1 (n = 1). One patient had PMS2-associated CMMRD. Germline testing was negative in three MSH6-deficient tumors. In the series of glioblastomas, IDH-wt, MMRd prevalence was 12.5% in the <40-year age group, 2.6% in the 40-49 year group, and 1.6% the ≥50 year group.

Conclusion: Glioblastoma, IDH-wt, under the age of 50 is suggestive of MMRd and LS. MMRd should be sought systematically in this context, with downstream germline testing when indicated.

Conflict of Interest: Patrick Benusiglio AstraZeneca, MSD, BMS, Fikret Elder: None declared, Mehdi Touat: None declared, Alexandre Perrier: None declared, Marc Sanson: None declared, Chrystelle Colas: None declared, Léa Guerrini-Rousseau: None declared, Alex Duval: None declared, Florence Coulet: None declared, Franck Bielle: None declared

P13.058.B Identification of somatic Neurofibromatosis type 1 mosaicism using targeted next-generation sequencing of café-au-lait-macules

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Purpose: Detection of somatic mosaicism in blood can be challenging, even with a sensitive variant detection technique such as Next Generation Sequencing (NGS). For individuals with (segmental) neurofibromatosis type 1 (NF1), NGS-based analysis of tumors can be an alternative strategy for the identification of NF1 mosaicism, although this is not possible in individuals without syndrome-related tumors. Here we describe a patient with a segmental phenotype, presenting solely with café-au-lait macules (CALMs) and freckles, in whom no pathogenic *NF1* variant was identified in DNA isolated from peripheral blood.

Methods: DNA isolated from blood and four independent CALMs was investigated by targeted Ion Torrent-based NGS. In addition, in parallel, cells were cultured from a CALM and from normal skin, followed by RNA isolation and transcriptome analysis (RNAseq).

Results: Targeted NGS revealed that three of the four investigated CALMs showed the pathogenic variant NM_000267.3(NF1):c.3916C>T, p.(Arg1306*), which was absent in DNA derived from blood. RNAseq confirmed expression of the NM_000267.3(NF1):c.3916C>T, p.(Arg1306*) variant in RNA from the cultured fibroblasts from a CALM (variant allele frequency of 8%), while no expression of the variant was seen in RNA isolated from fibroblasts cultured from normal skin.

Conclusion: Our results indicate that targeted NGS of independent CALMs can assist in the detection of somatic mosaicism in patients with segmental NF1 for whom no pathogenic variant was identified in peripheral blood DNA.

Conflict of Interest: None declared

P13.059.C BRCA1/2 genotyping in liquid biopsy from metastatic prostate cancer patients

Gardenia Vargas-Parra[1], *Nuria Juanpere*[2], *Nikaoly Ciriaco*[2], *Montserrat Arumí*[2], *Concepción Fernández*[1], *Sergi Clavé*[1], *Laura Camacho*[1], *Gabriel Piquer Velasco*[1], *Andreu Escobar*[1], *Erica Torres*[1], *Blanca Fernández*[1], *Alex Corbera*[3], *Alejo Rodriguez-Vida*[3], *Beatriz Bellosillo*[1]

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Background/Objectives: PARP inhibitors have been approved for use in patients with metastatic castration-resistant prostate cancer (mCRPC) with BRCA1/2 pathogenic variants (PVs), which are present in 5-20% of mCRPC patients. Since prostate cancer tissue samples usually give low amounts of DNA, the use of ctDNA represents a scarcely studied alternative to genotype this tumor type. The aim of this study was to assess the viability of liquid biopsy for BRCA1/2 genotyping in a series of patients with mCRPC.

Methods: We analyzed BRCA1/2 coding regions by amplicon-based NGS with BRCAplus v2 (Qiagen) on a NextSeq platform (Illumina), in 104 ctDNA samples from 103 mCRPC patients and in available FFPE tissue samples. Variant detection sensitivity was calculated for each sample, based on the minimum detectable allele fraction (AF) in the 95% confidence interval given by the GeneGlobe software (Qiagen). Variant calling and annotation were done using VariantStudio (Illumina).

Results: Valid results were obtained in 96% (100/104) of ctDNA samples. The mean minimum AF found in valid ctDNA samples was 6% (1-20% interval). BRCA1/2 PVs were identified in 5 cases (5%): four individuals were carriers of *BRCA2* and 1 of *BRCA1* PVs. In two patients, variants were of somatic origin. Assessment in tissue was possible in 54 cases (52%), but only 28 provided valid results that were consistent with ctDNA.

Conclusion: Detected BRCA1/2 PVs in ctDNA from mCRPC patients correspond to the expected prevalence and show good correlation with tissue samples, validating liquid biopsy as a reliable option for BRCA1/2 genotyping.

Grant References: 2021SGR00628.

Conflict of Interest: None declared

P13.060.D Investigation of a fusion gene panel in paediatric solid tumours by next-generation sequencing

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Background/Objectives: The relevance of the research topic is determined by the high incidence of paediatric solid tumours (PST), their great malignancy potential, the high mortality rates and the possibility to offer the patients reliable therapy when timely and accurate diagnosis is made. PST have very common pathomorphological characteristics which make the accurate diagnosis based on routine light microscopy alone impossible. Characterization of chromosomal translocations has resulted in the discovery of several types of molecular abnormalities that are thought to contribute to tumourigenesis and are proofs of specific type of tumours.

Methods: Four rare childhood tumours were screened for the presence of fusion proteins using the FusionPlex® Sarcoma kit**:**(Archer) and next-generation sequencing. Total RNA was collected using a RNeasy FFPE Kit**:**(Qiagen).

Results: In 2 of 4 patients**:**(50%), a fusion protein was detected, helping to refine the histopathological diagnosis. One of these patients was a case of Ewing sarcoma with EWS1-FLI fusion variant proven by FISH method. Thanks to this investigation, a fusion variant (SS18-SSX1) typical of synovial sarcoma was discovered in one of the patients who has been diagnosed with differentiated EWS-like sarcoma. In one of the patients no variant was detected, and in one patient it was not possible to perform the analysis due to the low quality of the FFPE material.

Conclusion: For definitive diagnosis, genetic analyses were performed with NGS and they revealed fusion variants, which precise patients' diagnosis. The fusion proteins particularly affect

the cell growth, cell proliferation and chromatin remodeling mechanisms, contributing to sarcoma oncogenesis.

Grant References: MES/ContractsD01-395-18.12.2020,D01-278-14.12.2022,D01-302-17.12.2021/,MES,NSF/Contract-KP-06-H63/4-13.12.2022/;MU-Sofia/ContractNoD-82-04.06.2021/

Conflict of Interest: None declared

P13.061.A Cancer spectrum in monoallelic carriers of germline loss-of-function POLE variants: A case series

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Background: Germline missense variants in exonuclease domain of *POLE* are linked to autosomal dominant Polymerase Proofreading–associated Polyposis (PPAP), characterised by colorectal adenomas and carcinomas. Loss-of-function *POLE* variants are associated with autosomal recessive Facial dysmorphism, Immunodeficiency, Livedo, and Short-stature (FILS) syndrome but have not been linked to cancer risks. This case series describes germline monoallelic loss-of-function *POLE* variant carriers with young-onset cancers.

Methods: We reviewed patients who underwent clinical genetic testing for hereditary cancer syndrome in a Clinical Laboratory Improvement Amendments (CLIA) accredited lab and had a monoallelic pathogenic/likely pathogenic variant in *POLE*. We correlated clinical and family history with genetic variant data and current literature to report on the cases.

Results: Six monoallelic pathogenic/likely pathogenic variant carriers in *POLE* were identified from five unrelated families, four with breast cancer diagnosed age 29-60 years and two with endometrial cancer between age 55-56 years. Four unique variants were identified, all predicted to be loss-of-function. One variant, c.1191C>G (p.Tyr397*) occurred in two unrelated carriers, with metastatic endometrial cancer and mucinous breast cancer. Two protein-length altering variants (exon 19-23 deletion, c.720+1G>A) were identified in two breast cancer patients diagnosed under age 40 years. One initiator codon-disrupting variant, c.2T>C (p.Met1?) was found in two sisters with bilateral breast and endometrial cancers, respectively.

Conclusion: Our findings suggest that *POLE* loss-of-function variant carriers may harbour risk of cancers. Further investigations in larger cohorts of germline *POLE* variant carriers will help to clarify *POLE* genotype-phenotype correlations and to understand cancer risks among FILS syndrome carriers and families.

Conflict of Interest: Manasadevi Karthikeyan Full time employee at National cancer centre Singapore, Sock Hoai Chan Full time employee at National Cancer Centre Singapore, Joanne Ngeow Full time employee at National Cancer Centre Singapore

P13.064.D Analysis of germline variants in pediatric patients diagnosed with desmoids tumors and nuchal-type fibromas

Piedad Alba-Pavón[1], *Itziar Astigarraga*[1;2;3], *Lide Alaña*[1], *Isabel Llano Rivas*[4], *Blanca Gener Querol*[4], *Lorena Mosteiro González*[5], *Ricardo López Almaraz*[1;2], *Aizpea Echebarria-Barona*[1;2], ***Olatz Villate***[1]

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Background/objectives: Desmoid tumor is a fibroblastic proliferation arising in soft tissue characterized by localized infiltrative growth with an inability to metastasize but with a tendency to recurrence. Fibromas are soft tissue precursor lesions to the desmoid tumors. The development of these neoplasms can be associated to a hereditary cancer predisposition syndrome, mainly familial adenomatous polyposis caused by *APC* germline mutations. However, they could be associated with germline alterations in other genes related to colorectal cancer development.

Methods: Germline variants were analyzed in five pediatric patients diagnosed with desmoid tumors. *APC*, *MUTYH*, *POLE* and *POLD1* genes were sequenced using Custom Hereditary Cancer Solution Kit (Sophia Genetics) in MiSeq v3 (Illumina). Variants were classified according to international recommendations as pathogenic, likely pathogenic, benign, likely benign or of uncertain significance.

Results: We identified two pathogenic variants in *APC* gene in two different patients diagnosed with nuchal-type fibroma and desmoid tumor and two variants of uncertain significance in *POLD1* in two patients diagnosed with nuchal-type fibroma. Two patients had family history of colorectal cancer, however, only one of them showed an *APC* germline mutation.

Conclusion: The analysis of germline variants and the genetic counseling is essential for pediatric patients diagnosed with desmoid tumors or nuchal-type fibromas and their relatives.

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Conflict of Interest: None declared

P13.065.A Extensive blood trait-cancer risk pleiotropy identifies the influence of RNY pseudogenes

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The immune system has a central role in restraining carcinogenesis. Thus, systemic alteration of immune cells may facilitate cancer development. Here, to address this question, we use summary statistics from genome-wide association studies of 28 cancer types and 27 immune cell traits to uncover genetic variants with pleiotropic effects between them, unveiling several underlying cellular and molecular alterations. The analysis identified thousands of common genetic variants with pleiotropic effects, involved in a broad range of biological processes. We detected pleiotropy hotspots mapping to genes *TERT*, *HLA-B*, *HLA-DQA1*, *HLA-DQB1*, *ATM*, *CRHR1* and *APOBEC3*, highlighting the role of telomere length and immune system related pathways. Additionally, we observed an overrepresentation of genes involved in haematopoiesis regulation in our list of genes harbouring pleiotropic variants. Surprisingly, we detected a significant excess of pleiotropic variants influencing the differential expression and regulation of a subset of RNY pseudogenes, which are a class of noncoding RNAs that bound and regulate Ro60, a protein involved in cellular response to stress and identified as an autoantigen of several autoimmune diseases. Our study successfully identifies pleiotropic variants in a genome-wide manner, and provides a detailed catalogue of variants associated to immune system with potential functional impact in carcinogenesis. Characterization of the levels of certain systemic immune cell types and/or related molecules might be useful to develop or complement existing cancer prevention algorithms.

Conflict of Interest: None declared

P13.066.B The application of miR-30d-5p mimic sensitizes ovarian cancer cells to cell death inducing agents

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Background/Objectives: MicroRNAs have key role in cancer progression due to the fact that they can function as oncomirs or tumor supressors. Furthermore, they are considered to be promising therapeutic candidates in cancer. Our aim was to characterize the role of miR-30d-5p in ovarian cancer.

Methods: We applied the PEO1 (ERα expressing) and A2780 (ERα non-expressing) cell lines. The expression of miR-30d-5p was studied by qPCR. Functional characterization was performed by bioinformatic analysis and by the miRNA mimic method.

Results: The expression of miR-30d-5p was higher in the estrogen sensitive PEO1 cell line than in A2780 and was increased in response to cell death inducing treatments. This suggest its role in the cell death process of ovarian cells that was confirmed by our bioinformatic analysis that revealed several genes involved in the regulation of cell proliferation and apoptosis among the targets of miR-30d-5p. The application of miR-30d-5p mimic suppressed cell proliferation in both PEO1 and A2780 that was accompanied with decreased *SOX4* expression, which gene is involved in the regulation of the PI3K/AKT pathway. Furthermore, miR-30d-5p mimic sensitized the PEO1 cells to cell death induced by high dose estrogen or hydrogen-peroxide as well as to tamoxifen. The application of AZD8835 (PI3K inhibitor) led to the same results that suggests that miR-30d-5p might exert its role by the inhibition of this pathway.

Conclusions: MiR-30d-5p mimic might be a promising candidate in the therapy of both ER+ and ER- ovarian cancer either administered alone or in combination with other agents.

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Conflict of Interest: None declared

P13.067.C Identification of fusion genes in childhood hematological malignancies

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Introduction: Hematological malignancies are neoplasms that develop in blood-forming or immune system tissues and are one of the most common types of tumors in pediatric oncology. Identification of fusion genes, as one of the most characteristic structural aberrations in childhood malignancies, contributes to the development of precision medicine and, thus, effective methods for treating the disease.

Materials and methods: The longitudinal study enrolled 34 patients, aged 0 to 18 years, with a diagnosis of hematological malignant tumor. RNA was isolated from whole blood, bone marrow, or tissue samples, and complementary DNA libraries were prepared. DNA-seq was performed on the MGI™ DNBSEQ-G400 sequencer. For bioinformatics analysis, QIAGEN CLC Genomics Workbench v.22.0, DAVID v.2021, Ensembl VEP, and RStudio tools were used.

Results: In total, 23 459 potential fusion genes were identified, among them gene fusions that are strongly related to childhood malignancy initiation, progression, and relapse (ETV6-RUNX1, KMT2A-AFDN, NTRK3 gene fusion). Identified fusion genes consisted of 9714 unique genes; most of them were connected to cancer hallmarks, such as sustaining proliferative signaling, genome instability, tumor-promoting inflammation, and others.

Conclusion: The largest number of fusion genes is observed in the group of patients diagnosed with AML. Most genes included in fusions were responsible for the "cell division" Gene Ontology process, thus falling into the "Sustaining proliferative signaling" hallmark category.

Grant References: Funded by the patronage of MikroTik Ltd. within the framework of the University of Latvia Foundation project Nr.2242 "Investigation of precision medicine strategies in the treatment of pediatric malignancies".

Conflict of Interest: None declared

P13.068.D Plasma sequencing versus FFPE in endometrial cancer: a pilot study

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Background: Endometrial cancer (EC) is the most common gynaecological malignancy in the developed world. Many genetic changes have been described as valuable diagnostic and prognostic markers. Our pilot study aims to evaluate liquid biopsy as a potential diagnostic approach.

Materials/methods: The plasma and tumour nucleic acids of 21 patients with EC were processed using TruSight™ Oncology 500 panel, sequenced on NovaSeq 6000, and analysed on DRAGEN pipelines. Detected variants were annotated and evaluated using PierianDX and Ingenuity Variant Analysis.

Results: We identified pathogenic mutations in 73 EC-related genes. In 67 % of cases, at least 1 driver mutation was shared in both plasma and tumour DNA. Remarkably, for tumours in the 3a stage, such correlation was observed in only 2/5 cases. Interestingly, mutations in genes DNMT3A and TET2 were detected only in plasma DNA with 7-fold and 2-fold higher frequencies, respectively, than expected. These gene mutations are known by their association with the age-related clonal haematopoiesis.

Conclusions: The pilot study showed the moderate potential of liquid biopsy as a diagnostic method for EC-associated cancer gene changes. Even with a small cohort size, we were able to identify the mutations of clinical significance in the majority of ctDNAs, even for early-stage and low-grade tumours. Moreover, methylation changes related to gene mutations were detected and their role as potential disease biomarkers is up to future studies.

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P13.069.A Lifestyle factors and breast cancer risk in females with PTEN Hamartoma Tumor Syndrome

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Introduction: Females with *PTEN* Hamartoma Tumor Syndrome (PHTS) have a genetic increased lifetime risk for breast cancer (BC) of about 54% to 76%. This study assessed the association between BC risk and lifestyle such as physical activity, body mass index (BMI), alcohol consumption and tobacco smoking in PHTS.

Methods: A European cohort study of female adult PHTS patients including lifestyle data from questionnaires and baseline information from medical files. Hazard ratios (HRs) were calculated using uni- and multivariate Cox regression, including *PTEN* variant because risks differ by location and type of variant. Ascertainment bias was corrected by excluding index patients with BC before PHTS diagnosis (BC-index).

Results: Between July 2020 and November 2022, 116 patients (including 17% BC-index) completed the questionnaire at 44 years (SD = 13). Of these, 37 patients had BC at 43 years (SD = 9). Low physical activity, less than 2 times/week, (79%) was associated with 2.6-fold (95%CI = 0.99-6.5) increased risks. Overweight or obesity (73%) was associated with a 1.2-1.4-fold ($HR_{total} = 1.4$, 95% CI = 0.6-3.1; $HR_{non\text{-}BC\text{-}index} = 1.2$, 95%CI = 0.4-4), and alcohol consumption of at least 1 glass/day (13%) with a 1.4-2.1-fold increased risk ($HR_{total} = 1.4$, 95%CI = 0.6-3.3; $HR_{non\text{-}BC\text{-}index} = 2.1$, 95%CI = 0.6-6.9). Most patients (77%) reported no smoking history and a slightly increased risk was observed for smoking ($HR_{non\text{-}BC\text{-}index} = 1.3$, 95%CI = 0.4-3.7). Results were similar for univariate and multivariate analyses including all lifestyle and genetic factors.

Conclusion: Among women with PHTS the highest BC risk increase of 2.6-fold is associated with low physical activity. Therefore, lifestyle counseling in patients should focus on high physical activity levels.

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P13.070.B Combined genome and transcriptome sequencing in families suspicious for hereditary breast and ovarian cancer

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Objective: Multi-gene panels or exome sequencing are the current standard for HBOC testing. Pathogenic or likely pathogenic variants in HBOC genes are found in only 15% to 25% of high-risk families. We sought to increase the diagnostic yield with a combination of genome and transcriptome sequencing (GS, TS).

Methods: We included 135 patients with breast or ovarian cancer (BC, OC), fulfilling criteria for HBOC. All patients gave consent for participating in the Ge-Med study (NCT04760522). GS and TS of blood-derived DNA and RNA was performed and analyzed in our diagnostic laboratory. Integrated GS and TS data analysis and interpretation was performed using the clinical decision support system GSvar.

Results: Our cohort included 122 BC and 13 OC patients. One was male. Genomes were sequenced to an average depth of 39.7x (33.11-58.76). Pathogenic variants were found in 21/135 patients (15.6%); as well as 17 variants of uncertain significance. TS confirmed exon skipping for two splice variants. Three cases of allelic imbalances and one additional splice variant warrant follow-up studies. High-risk Polygenic Risk Scores (PRS) for BC were found in 21 patients (one additionally carrying a pathogenic variant), resulting in a total of 41/135 cases with a molecular finding.

Conclusion: GS allows the comprehensive detection of pathogenic variants and PRS associated with HBOC and combined GS and TS increased the overall diagnostic rates. Hereby TS can serve as an orthogonal validation for GS-findings, especially splice-variants. However, for TS low expression in blood limits its value for some HBOC genes.

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Illumina, Professor Riess owns a patent, Kalms Consulting, Professor Riess created a brochure for:
AstraZeneca
Professor Riess is:
President-elect/President/Vice-President of the European Society of Human Genetics (ESHG)
External Advisory Board (EAB) of the E-Rare-3 funding program
Coordinator of the diagnostic task force of the European Joint Programme Committee
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P13.072.D Identification of novel candidate genes for familial 'non-RET' thyroid cancer by exome sequencing

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Background/Objectives: Thyroid carcinomas (TC) can be sporadic or familial. Depending on the cell of origin, familial forms may be classified in medullary thyroid carcinoma (MTC) and non-medullary thyroid carcinoma (NMTC) accounting for less than 1% and 3–9% of all TC cases, respectively. Mutations in *RET* are found in more than 95% of familial MTC, whereas familial NMTC shows a high degree of genetic heterogeneity. The genetic underlying familial 'non-*RET*' TC is still poorly understood. Herein, we aimed to identify susceptibility genes for familial 'non-*RET*' NMTC and MTC cases.

Methods: Whole exome sequencing (WES) was performed in 58 affected and unaffected individuals belonging to 18 Spanish families with these neoplasms. After bioinformatics analysis and the application of filtering and prioritization steps, family co-segregation of detected variants was performed in all available family members (a total of 109 individuals).

Results: This approach resulted in the identification of 56 rare candidate variants showing co-segregation with MTC and NMTC phenotypes in 12 out of 18 families.

Conclusion: Our strategy provides clues to possible molecular mechanisms underlying familial forms of MTC and NMTC. These new molecular findings, together with clinical data of patients, is fundamental for early detection, the development of tailored therapies and optimizing patient management.

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Conflict of Interest: None declared

P13.073.A Clinical evaluation of a low-coverage whole-genome sequencing test for homologous recombination deficiency detection in ovarian cancer

Céline Gottin[1], *Jiri Ruzicka*[1], *Nicolas Duforet-Frebourg*[2], *Denis Bertrand*[1], *Romain Boidot*[3], *Marie-Pierre Wissler*[4], *Nicolas Philippe*[2], *Michael Blum*[2]

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Background/Objectives: The PAOLA-1 trial showed that advanced ovarian cancer patients with Homologous Recombination Deficient (HRD) tumors benefit from PARP inhibitor olaparib plus bevacizumab as maintenance treatment. Using PAOLA-1 data, we report a retrospective clinical evaluation of the SeqOne HRD solution based on shallow whole genome sequencing (sWGS).

Methods: SeqOne HRD solution combines genomic instability and CCNE1 gene amplification features extracted from sWGS, and pathogenic mutations in BRCA 1/2 genes. We conducted biological and computational experiments to evaluate the lower limit for sequencing coverage and tumoral content. The clinical evaluation on 368 patients from PAOLA-1 included Progression-Free Survival (PFS) data and comparisons with Myriad MyChoice® CDx test.

Results: The SeqOne solution provides consistent HRD status for at least 0.1X sequencing coverage and a minimum tumoral cells content of 20%. A concordance above 90% was found between MyChoice test and SeqOne HRD solution. The Hazard Ratio measuring olaparib PFS benefit in PAOLA-1 data was 0.38 (0.27-0.55) and 0.34 (0.24-0.50) in HRD positive patients for SeqOne solution or MyChoice test respectively, and was 0.98 (0.68-1.41) and 0.99 (0.67-1.46) in HRD negative patients respectively.

Conclusion: The SeqOne HRD solution brings a cost-effective and clinically validated WGS approach to detect HRD signature and select ovarian cancer patients to be treated with olaparib plus bevacizumab maintenance. This solution is efficient with a low tumoral content and low coverage sequencing.

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P13.074.B Identification of co-expression modules associated with platinum resistance in ovarian cancer patients using WGCNA

Markus Riedl[1], ***Karolina Seborova***[2;3], *Viktor Hlavac*[2;3], *Martin Hruda*[4], *Lukas Rob*[4], *Jiri Bouda*[5], *Jiri Spacek*[6], *Iva Sedlakova*[6], *Pavel Soucek*[2;3], *Thomas Mohr*[1], *Radka Vaclavikova*[2;3]

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Background/Objectives: The development of platinum resistance in ovarian cancer (OC) patients is a tremendous problem as still ~ 80% of patients develop drug resistance within 5 years after adjuvant chemotherapy completion, even despite progress in new drugs development (e.g., PARP inhibitors) and a better understanding of molecular mechanisms of drug resistance. There is still ongoing demand for novel therapeutic targets and drugs.

Methods: Co-expression analysis was performed in two datasets, i/ training cohort of 60 OC patients with varying responses to adjuvant chemotherapy (37 platinum-sensitive and 23 platinum-resistant patients) and ii/ validation TCGA OV cohort ($n = 426$). Gene co-expression networks were developed using the WGCNA method by Langfelder and Horvath and visualized by the Cytoscape platform.

Results: In the testing cohort, ~30 gene modules were significantly associated with overall survival (OS) and platinum-resistant status. One of the most significantly correlated modules showed higher expression of immune-related genes (mainly T-lymphocyte activation) in patients with platinum-sensitive status. Module correlating with OS was associated with cell adhesion and migratory pathways. Gene expression of *NOTCH3* was most correlative to platinum-resistant status modules. Major constructed gene expression modules were validated in the TCGA-OV cohort, namely immune and migratory genes enriched modules.

Conclusion: WGCNA analysis discovered ~30 gene modules associated with the platinum-resistance status. These modules were enriched with immune-related genes and associated with the *NOTCH3* gene. The module associated with OS was enriched with cell-adhesion genes.

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P13.075.C Genetic and chromatin characterization to unveil the underlying cause of primary constitutional MLH1 epimutation

Paula Climent-Cantó[1], *Mireia Ramos-Rodríguez*[2], *Marc Subirana-Granés*[2], *Estela Dámaso*[1], *Fátima Marín*[1], *Covadonga Vara*[1], *Beatriz Pérez-González*[2], *Helena Raurell*[2], *Megan Hitchins*[3], *Marta Pineda*[1], *Lorenzo Pasquali*[2], *Gabriel Capellá*[1]

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Background: Lynch syndrome (LS), characterized by an increased risk for cancer, is mainly caused by a germline pathogenic variant in a mismatch repair gene (MLH1, MSH2, MSH6, PMS2). Occasionally, LS is caused by a constitutional MLH1 epimutation (soma-wide methylation of one allele of the MLH1 promoter), which arise de novo without an underlying cis-genetic cause and are reversible between generations. We aimed to investigate the molecular mechanism underlying primary MLH1 epimutations.

Methods: Four primary MLH1 epimutation carriers heterozygous for a promoter SNP and 2 relatives carrying the methylation-associated allele in a non-methylated state were included. Genetic alterations were analyzed by WGS. Transcriptome (RNA-seq), chromatin landscape (ATAC-seq, H3K27ac CUT&Tag) and 3D chromatin structure (UMI-4C), were studied in lymphoblastoid cell lines. Bioinformatic tools were used to scan transcription factors binding sites.

Results: The presence of rare variants in the differentially methylated region or shared variants within the MLH1 locus were ruled out. DNA methyltransferases were not differentially expressed in epimutant cells. MLH1 epimutant alleles presented a closed chromatin conformation and decreased levels of H3K27ac, as compared to the unmethylated allele. Moreover, the epimutant MLH1 promoter exhibits a differential 3D chromatin landscape including loss and gained interactions with distal regulatory elements. In one case genetic variants inside contact regions were predicted to create new transcriptional repressor binding sites.

Conclusions: Primary MLH1 constitutional epimutations present allele-specific differential interaction patterns with neighboring genes and regulatory elements. Further investigation is needed to elucidate the role of 3D chromatin changes in the establishment of MLH1 epimutation.

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P13.076.D Salivary gland basal cell adenocarcinomas – a novel manifestation of attenuated familial adenomatous polyposis

Fiona Chan-Pak-Choon[1], *Catherine Beaumont*[2], *Josianne Leblanc*[3], *Sonja Dahlum*[4], *Reiner Siebert*[4], *Francois Thuot*[2], *Marc Pusztaszeri*[5], *Jacinthe Chênevert*[6], *Tania Cruz Marino*[1;3], *Barbara Rivera*[7;8], *William Foulkes*[1;8]

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Attenuated familial adenomatous polyposis (AFAP) is a disorder caused by germline pathogenic variants in *APC* and is characterized by the presence of <100 colonic polyps and a high lifetime risk of developing colorectal cancer (CRC). Extracolonic tumours such as osteomas and desmoid tumours occasionally occur in AFAP patients. Conversely, salivary gland (SG) tumours have rarely been associated with AFAP.

We report a 40-year-old male AFAP patient presenting with colonic polyps, malignant bilateral parotid gland tumours and a malignant submandibular gland tumour. The proband inherited *APC* germline deletion, c.4910_4923del; p.(Asp1637Valfs*4), from his mother who died of CRC. A mix of intercalated duct hyperplasia (IDH), basal cell adenomas (BCAs) and basal cell adenocarcinomas (BCACs) was observed in the SG tumours. Immunohistochemical staining showed abnormal nuclear localization of β-Catenin in abluminal cells. Targeted sequencing detected loss of heterozygosity at the *APC* variant locus in the bilateral parotid tumours and OncoScan copy-number-variant analysis confirmed the extended loss of Chromosome 5q in the right parotid tumour. However, no second hit was detected in *APC* in the submandibular lesion. Instead, whole-exome-sequencing revealed a somatic *CTNNB1* variant (I35T) seen in ~45% of BCAs and ~15% of BCACs.

This is the first reported case of malignant SG tumours caused by *APC* and *CTNNB1*. The mixed histology and involvement of two key components of the Wnt signaling pathway suggest a potential malignant progression event from IDH to BCA to BCAC. We also highlight salivary gland BCAC as a possible new phenotype of AFAP.

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P13.078.B Tumour profiling frequently informs the interpretation of germline variants arising from paired analysis in children and young people

Eliza Courtney[1;2;3], *Kate Hetherington*[2;4], *Pamela Ajuyah*[1], *Ann-Kristin Altekoester*[1], *Megan Rumford*[1], *Kimberly Dias*[1], *Dianne Sylvester*[1;5;6], *Noemi Fuentes-Bolanos*[1;2;7], *Meera Warby*[7], *Chelsea Mayoh*[1;2], *Judy Kirk*[8;9], *Ashleigh Sullivan*[1;10], *Marie Wong*[1], *Loretta Lau*[1;2;7], *Dong Anh Khuong Quang*[1;11;12], *Paul Ekert*[1;2;12], *Mark Cowley*[1;2], *Claire Wakefield*[2;4], *Vanessa Tyrrell*[1;2], *Michelle Haber*[2;13], *David Ziegler*[2;7;13], *Katherine Tucker*[7;14;15], *Paulette Barahona*[1], *Mark Pinese*[1;2]

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Background/Objectives: Paired tumour-germline analysis is increasingly being utilised in precision oncology. However, it remains unclear what impact tumour findings have on germline variant interpretation. We evaluated the utility of comprehensive tumour profiling to inform the interpretation of germline whole-genome sequencing in children and young people.

Methods: We identified 57 participants with germline pathogenic/likely pathogenic variants (P/LPV) and/or variants of uncertain significance categorised as suspicious (VUS+) from the Zero Childhood Cancer Precision Medicine Program. A retrospective review was performed to determine how often tumour profiling was cited as evidence for germline interpretation.

Results: Tumour profiling had high utility for germline variant interpretation: 63.0% of participants with germline P/LPV had tumour features supportive of pathogenicity; in 6.5% of cases tumour findings led to an upgrade in classification. Examples include mismatch repair deficiency in a leukaemia sample from a participant with a heterozygous *MSH2* germline variant (VUS to LP) and altered splicing for a germline *NF2* variant (VUS to P) in a participant with schwannomas. Tumour features were referenced as evidence of causality in 65.2% of participants, including 4 atypical findings. They were cited as evidence against causality in 37.0% of cases, including the absence of a second hit (30.4%) or mutational signature (10.9%). Fifteen percent of VUS+ were categorised based on tumour features.

Conclusion: Review of 57 cases from an active precision medicine study demonstrates high utility of tumour profiling to inform germline variant interpretation for both cognate and atypical findings.

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P13.080.D Development of a liquid biopsy pan-cancer comprehensive genomic profiling assay with MSI and TMB immunotherapy biomarkers for therapy selection

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Background/Objectives: Precision oncology and cutting-edge technologies enable the identification of actionable targets to guide cancer therapy. To overcome the limitations of FFPE samples, including limited availability of tissue biopsy material, poor sample quality due to formalin fixation and the need of an assay that captures the complete molecular profile of the disease we developed a novel liquid biopsy (LB) assay. The assay is able to detect single nucleotide variants (SNVs), small insertions and deletions (Indels), copy number alterations (CNAs), rearrangements and complex biomarkers for immunotherapy treatment [microsatellite instability (MSI)] and tumour mutational burden (TMB) in a single test.

Methods: Circulating cell-free DNA derived from plasma was subjected to library preparation and hybrid capture enrichment with a panel of 221 clinically significant genes for a wide spectrum of solid-tumour malignancies. Enriched libraries were subjected to next generation sequencing on an Illumina sequencing platform. The data were analysed using proprietary analysis pipeline. Reference materials, contrived samples and clinical samples were used to validate the TMB and MSI thresholds.

Results: The assay demonstrated high sensitivity and specificity for all types of genomic alterations including SNVs, Indels, CNAs, translocations as well as exceptional performance for immuno-oncology biomarkers MSI and TMB.

Conclusion: We have developed and validated a novel, highly accurate non-invasive, comprehensive NGS-based test for the assessment of genetic alterations and complex biomarker status in a single assay for guidance of therapy selection. primary and metastatic disease, and **treatment re-evaluation** for therapy resistance

Conflict of Interest: Kyriakos Tsangaras Full-time employment at Medicover Genetics, Alexia Eliades Full-time employment at Medicover Genetics, Chrysa Soteriou Full-time employment at Medicover Genetics, Stephanie Constantinou Full-time

employment at Medicover Genetics, Achilleas Achilleos Full-time employment at Medicover Genetics, Christos Lemesios Full-time employment at Medicover Genetics, Christodoulos Savva Full-time employment at Medicover Genetics, Chrystalla Havadjia Full-time employment at Medicover Genetics, Chrysovalando Sotiriou Full-time employment at Medicover Genetics, Louisa Constantinou Full-time employment at Medicover Genetics, Haris Kkoufou Full-time employment at Medicover Genetics, Michalis Spyrou Full-time employment at Medicover Genetics, Stelia Pissaridou Full-time employment at Medicover Genetics, Antonia Matsentidou Full-time employment at Medicover Genetics, Christos Prokopi Full-time employment at Medicover Genetics, Melina Vaki Full-time employment at Medicover Genetics, Styliana Georgiou Full-time employment at Medicover Genetics, Elena Kypri Full-time employment at Medicover Genetics, Marios Ioannides Full-time employment at Medicover Genetics, George Koumbaris Full-time employment at Medicover Genetics, Philippos Patsalis Full-time employment at Medicover Genetics

P13.081.A MUTYH gene phenotype-genotype correlation: one center experience

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Background/Objectives: MUTYH is a base excision repair enzyme which plays a key role in repairing DNA damage due to guanine oxidation. Biallelic MUTYH variants may cause MUTYH-associated polyposis syndrome. However, cancer predisposition of monoallelic variants is controversial, and the relationship between the effect on protein structure and non-polyposis diseases is yet to be clarified. We aimed to investigate the spectrum of monoallelic and biallelic MUTYH variants in Turkish population.

Methods: In this study, 36 cancer patients with MUTYH variants that were detected in hereditary cancer susceptibility panel between 2014-2023 were analyzed for malignancy types and their variant spectrum.

Results: The most common malignancy among these patients was colorectal cancer and it was observed in 16 patients. In the study, 24 different variants(8 pathogenic, 3 likely pathogenic, 13 variant of uncertain significance) have been found in 36 patients. 3 out of 24 variants were novel. 11 patients had biallelic and 25 had monoallelic variants. Isolated monoallelic MUTYH variants were detected in 11 patients, of which 8 had breast cancer. We detected variants in other cancer predisposing genes in 17 patients and most frequently accompanied variants were in MSH2 and MSH6.

Conclusion: Even though the most common variants in North America and Europe are Tyr151Cys and Gly368Asp, the most common variant in our study was Pro267Leu. Our study contributes to the literature with the evaluation of novel variants detected in the MUTYH gene and phenotype-genotype correlation.

Conflict of Interest: None declared

P13.082.B Make every cell count: a novel and versatile approach to single cell RNA-Seq

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Background/Objectives: The adoption of single-cell methods is leading to rapid progress in our understanding of complex biological systems. However, challenges in current single-cell approaches remain. We recently developed several new innovating multi-omics technologies to obtain a richer and more complete picture of the events occurring in each cell. In a single assay, we interrogated single cells from lung cancer biopsies with a combination of three different tools simultaneously: transcriptomics, enrichment of oncogenes, and assessment of cell surface proteins.

Methods: Tissue from the core needle biopsies were dissociated into single-cell suspensions. The cells were labeled using a panel of tagged antibodies (BD AbSeq Immune Discovery Panel, cat #625970). The cells were partitioned and barcoded using FocuSCOPE Single Cell Multiomics Lung Cancer Druggable Mutation Analysis Kit (Singleron cat# 4122111). The chemistry allows to interrogate transcriptomics and enrich for key actionable targets for somatic variant detection, both at the single cell level. The libraries generated from 6000 cells per samples were sequenced on Illumina Novaseq.

Results: Combined data from single cell transcriptomics and proteomics were used to annotate cancer cells and immune cells. We also identified somatic and germline variants in 5 key genes (KRAS, EGFR, PIK3CA, BRAF and TP53) previously identified in lung cancer. Our finding highlighted the heterogeneity of the tumor samples. It also allowed us to examine proteins that were previously not measurable by transcriptomics but revealed by a targeted protein assay.

Conclusion: New transforming approaches in single-cell sequencing overcome previously encountered limitations related to multi-omics assessment.

Conflict of Interest: julie laliberte Singleron Biotechnologies, Tijda Argun Singleron Biotechnologies, Eva Maleckova Singleron biotechnologies, Bruno Monschau Singleron Biotechnologies, Nan Fang Singleron Biotechnologies

P13.083.C Targeted predictive genetic testing for colorectal cancer syndromes in southern Sweden

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Objectives: To describe the pattern of targeted predictive genetic testing in patients at risk of hereditary colorectal cancer syndromes in southern Sweden.

Methods: From 1996 to 2019, data from patients registered for targeted predictive genetic testing for hereditary colorectal cancer syndromes were retrieved including gender, age, year of analysis and genetic test result.

Results: Of 520 patients, 5 were excluded due to lack of genetic test result. The analyzed cohort (n = 515; 286 females, 229 males; 56 % and 44 %, respectively) had a median age at test result of 40,5 years (42 years for females, 38 years for males). In total, 225/515 (44 %) tested positive (42 % of all females; 46 % of all males). Lynch syndrome was the predominant reason for testing (n = 453/515; 88 %).

Conclusion: The cohort contained more females. Possibly, this represents a gender bias due to the tumor panorama of Lynch syndrome, which also includes endometrial and ovarian cancer, or that women more often are offered, or seek, predictive testing. Gender distribution among those testing positive was less skewed. However, less than half of the cohort tested positive, conceivably because distant relatives to known carriers may have been included in the cohort. Also, a proportion of carriers could have escaped our cohort due to previous symptomatic testing. Indeed, age at testing in our cohort was significantly higher than the

recommended age for cancer surveillance, e.g., from 25 years or earlier.

Grant References: Southern healthcare region (grant no. 14714), Swedish cancer research foundation (grant no. 2020-1107).

Conflict of Interest: None declared

P13.085.A Effect of Origanum onites L. essential oil on RARB promoter hypermethylation in gastric cancer

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Background/Objectives: *Origanum onites* essential oil (OOEO), known as oregano oil extracted from *Origanum onites* L., is widely used by people, especially in the Mediterranean region for digestive system problems. Many biological effects of OOEO have been intensively investigated, and its anticancer effect has been shown in vitro and in vivo before. However, studies on its anticancer effects are limited to its antiproliferative, antimigratory, and antiapoptotic effects. In this study, we aimed to investigate the epigenetic effects of OOEO which can be used orally.

Methods: OEOO was extracted from *Origanum onites* L. Components of the OEOO were analyzed by GC/MS. AGS cell line was used as in vitro model of human intestinal-type gastric cancer (GC). Methylation analyses were performed with methylation-specific real-time PCR.

Results: Promoter hypermethylation of the *RARB* gene is common in GC, and is associated with poor prognosis and tumor size. Here we showed that OOEO administration to the AGS cells resulted in a 33-fold decrease in *RARB* gene promoter methylation.

Conclusion: Considering that promoter hypermethylation of *RARB*, a tumor suppressor gene, has an important role in the pathogenesis of GC, detected methylation change induced by OOEO may have a crucial role in its anticancer effect. Although more studies are needed, our results detail the molecular mechanisms of OOEO's anticancer effects and indicate the potential of OOEO to be used as a preventive or therapeutic epigenetic agent against GC.

Grant References: This project was funded by Ege University Office of Scientific Research Projects with "TOA-2021-22453" ID number.

Conflict of Interest: None declared

P13.086.B Harnessing the Power of Multiomics from a Single Sample for Advancing Immuno-oncology Research

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Background: The omics era has expanded the repertoire of available approaches for researchers and clinicians to unravel the complexity behind human cancer onset. Next Generation Sequencing (NGS) solutions can characterize genomes, epigenomes, transcriptomes, and proteomes to provide insight into immune cells mediating anti-tumor responses in cancer patients.

Methods: Here, we detail a workflow using a single blood draw to rapidly produce a diverse set of multiomics data. Germline and somatic mutations can be detected using whole exome or whole genome sequencing. Methylation, ATAC, or ChIP sequencing can be used for epigenetic characterization of the same sample. Bulk expression offers a high-level transcriptomics profile, single-cell transcriptomics facilitates detection of gene expression changes in each individual cell type, allowing for analysis of rare cell types including circulating tumor cells. Olink proteomic assays can be used for biomarker discovery and validation, with highly targeted or broad-spectrum panels.

Results: Our workflow starts with automated sample handling and processing of the primary blood draw, along with simultaneous plasma separation and collection. In this study, samples were processed for whole exome sequencing, single-cell RNA sequencing, methylation sequencing and an Olink targeted proteomics assay.

Conclusion: With this robust workflow, these datatypes can be produced within days of primary sample collection using minimal sample amounts. Our high-throughput integrative omics workflows described are useful in gaining a multidimensional view of cancer and advance immunotherapies by characterizing immune cell modulation in tumor progression and can be expanded for use in tumor/normal analysis, evaluation of metastases and exploration of tumor microenvironment.

Conflict of Interest: None declared

P13.087.C DICER1 syndrome – the importance of symptom awareness

Michal Barzily Rokni[1], *Michael Weintraub*[2], *Iris Fried*[2], *Omri Weiss*[1], *Mirav Sabag*[1], *Rachel Beeri*[1], *Reeval Segel*[1;3], *ephrat lahad*[1;3]

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Background: *DICER1* syndrome is a relatively newly defined pediatric cancer predisposition syndrome caused by heterozygous *DICER1* germline mutations[1]. The syndrome is characterized by an increased risk for benign and cancerous neoplasms[2].

Objectives: Increase awareness to *DICER1* syndrome as a leading cause of pediatric cancer.

Results: Since launching a dedicated clinic for pediatric oncology genetic counselling two years ago we examined 47 patients. Genetic testing was recommended for 41 patients, of these 23 results were obtained. *DICER1* germline mutations were identified in 3 out of 8 solved families (~40%), making *DICER1* syndrome the leading genetic cause of pediatric cancer in our center.

In total, we identified 11 *DICER1* carriers among 3 families. Pleuropulmonary blastoma (PPB) was diagnosed in two probands and medulloblastoma in the third. We identified 8 *DICER1* carriers among probands' relatives. Of them 3 developed neoplasms related to *DICER1* syndrome (PPB and Wilm's tumor, thyroid cancer, cystic nephroma).

In one of these families, in which two siblings presented with PPB, an intronic variant of unknown significance was detected in *DICER1* (c.2040+6T>A). As PPB is highly suggestive of *DICER1* syndrome, we analyzed cDNA, demonstrating that this variants results in skipping of exon 12, predicted to result in a frameshift. Coupled with variant segregation, this led to reclassification of the variant as likely pathogenic.

Conclusion: Our data suggests that *DICER1* syndrome is one of the leading causes of pediatric cancer. Therefore, it is crucial to raise awareness to *DICER1*-related conditions among clinicians in the field of pediatric oncology and geneticists.

Conflict of Interest: None declared

P13.088.D Comprehensive analysis of TP53 in a Spanish cohort of hereditary cancer patients assessing mutational spectrum, clonal hematopoiesis and mosaicism

Paula Rofes[1;2], *Mireia Menéndez*[1;2], *Àlex Teulé*[1], *Jesús del Valle*[1;2], *Silvia Iglesias*[1], *Mireia Ramos*[1;2], *Marta Pineda*[1;2], *Lidia Feliubadaló*[1;2], *Joan Brunet*[1;2;3], *Conxi Lázaro*[1;2]

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Background: Germline *TP53* pathogenic variants (PVs) cause Li-Fraumeni syndrome (LFS). Previous studies analyzing the genomic landscape of *TP53* identified high lifetime cumulative risk for a wide spectrum of tumors and for developing multiple primary malignancies. Moreover, *TP53*-mediated clonal hematopoiesis (CH) and *TP53*-mosaicisms have also been described and challenge genetic testing and counseling.

Aim: To characterize the mutational spectrum of *TP53* in a Spanish cohort to elucidate genotype-phenotype correlations and to assess the prevalence of CH and mosaicism.

Methods: 6,891 individuals were tested using our in-house panel. Four different patient cohorts were established (Table1): C1 comprises patients fulfilling LFS or Chompret criteria;C2 includes early-onset colorectal or breast cancer patients;C3 comprises secondary findings involving *TP53*;C4 includes patients harboring *TP53* PVs at unusual VAFs. Clinical features were carefully revised, and somatic testing was performed in C4 to test whether *TP53* PVs could be attributable to CH or mosaicism.

Results: *TP53* mutational rate is summarized in Table1. Preliminary results of somatic testing in 8 patients from C4 identified 6 cases of *TP53*-mediated CH and 2 mosaicisms. These findings highlight the importance of assessing CH and mosaicism in carriers of low-VAF *TP53* PVs, considering their impact on the clinical management and follow-up.

Cohort	Total individuals	*TP53* PV carriers	% *TP53* PVs
C1	270	19	7.04%
C2	1,531	7	0.46%
C3	5,074	4	0.08%
C4	16		

Conclusions: Our study represents the largest *TP53* analysis in a Spanish cohort, which further reinforces the importance of comprehensive *TP53* testing.

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P13.089.A The potential of cfDNA sequencing for routine somatic diagnostics and treatment decisions - experience from a real-life cohort

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Background/Objectives: Cell-free tumor DNA (cfDNA) is easy to obtain as a starting point for somatic diagnostics using liquid biopsy. Nevertheless, cfDNA is not yet widely used in routine diagnostics. We present the results of cfDNA sequencing of a large real-live cohort of tumor patients and evaluate the potential utility for diagnostics and as a basis for therapy decisions.

Methods: We sequenced more than 500 cfDNA samples from patients with >45 tumor entities using an UMI (unique molecular identifier) based gene panel, custom made standard gene panels or whole exome sequencing (ongoing project).

Results: We detected somatic variants in 228 of all samples analysed (44.1%). The tumor content within the cfDNA samples ranged from 0-95% (median 10%). Out of 228 cases with somatic variants 205 (90%) cases presented with at least one variant linked to an approved therapeutic option (FDA/EMA). We present direct comparison of sequencing results from FFPE material with those from cfDNA in individual patients. We discuss cfDNA sequencing results in clinical context considering the course of therapy in selected patients.

Conclusion: The results of the cfDNA sequencing approach have revealed a potential therapeutic relevance for 39.65% of all cases in the cohort. The method is minimally invasive and can provide a more complete picture of the genetics of the tumor than sequencing a single biopsy, especially in the metastatic setting. It should thus be considered as a standard approach in routine somatic diagnostic testing.

Conflict of Interest: None declared

P13.090.B Transcriptomic identification of a novel FH-deficient-like papillary renal cell carcinoma molecular subtype

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Introduction: Papillary renal cell carcinoma (pRCC) is a heterogeneous kidney cancer histologic subtype, which historically included FH-deficient tumors. *FH* mutation causes fumarate accumulation leading to pseudohypoxia through HIF-1a/2a stabilization, and DNA hypermethylation through TET inhibition. While *FH* mutations lead to aggressive renal tumors potentially sensitive to anti-angiogenics, other mechanisms altering FH activity remain unexplored. Here, through transcriptomic analyses we identify a subgroup of pRCC tumors with reduced FH function and distinct molecular and clinical characteristics.

Methods: Somatic and germline mutations, RNA-seq and clinical data were obtained from pRCC cases: 289 from TCGA-KIRP and 64 from metastatic patients in Spanish hospitals. Optimal K-means clustering was performed using a HIF-targets expression signature and the molecular and clinical features of resulting groups were compared. Samples with RCC drivers were excluded from the analysis.

Results: Mutational re-analysis of KIRP uncovered previously unidentified *FH*-mutated cases, raising the number of FH-deficient tumors from 5 to 10. K-means = 3 clustering identified a group containing all *FH*-mutated cases plus samples classified as "FH-deficient-like". Similarly to *FH*-mutated tumors, FH-deficient-like cases had low *FH* mRNA, distinct methylation profile, enhanced angiogenic tumor microenvironment (TME), worse tumor stages and shorter overall survival. In the Spanish metastatic cohort, including *FH* (n = 4) and *SDHB* (n = 2) mutated tumors, we identified a similar FH-deficient-like cluster with reduced *FH* expression and distinct TME.

Conclusions: We propose that a novel subgroup of pRCCs with tumor and TME features similar to *FH*-mutant cases can be identified through expression profiling. The impact on therapeutic response will be determined in future studies.

Conflict of Interest: None declared

P13.091.C Heterogeneity in chromatin states define a disease spectrum in synovial sarcoma

Jakob Hofvander[1], *Alvin Qiu*[1], *Kiera Lee*[1], *Annaick Carles*[1], *Rachel Cao*[1], *Michelle Moksa*[1], *Jonathan Steif*[1], *Angela Goytain*[1], *Sam Singer*[2], *Irene Andrulis*[3], *Jay Wunder*[3], *Torsten Nielsen*[1], *Martin Hirst*[1]

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Synovial sarcoma (SyS) is an aggressive soft-tissue malignancy characterized by a chromosomal translocation leading to the formation of the SS18-SSX fusion oncoprotein. Previous research shows that SS18-SSX associates with BAF, a chromatin remodelling complex, suggesting deregulation of the chromatin architecture as the oncogenic driver. SyS are genomically stable but the clinical and histological presentation vary. Here, we performed comprehensive multi-omics analysis on 52 SyS tumors using RNA-seq, WGS, WGBS and ChIP-seq. Epigenomic analysis revealed an unexpected level of heterogeneity at sentinel genes. We found that SyS display abnormally broad H3K4me3 regions, particularly at SS18-SSX binding sites. The H3K4me3 expansion was associated with striking DNA hypomethylation, particularly apparent in promoter regions in which SyS display the lowest methylation level of any sarcoma subtype. Finally, we identify that the number of bivalent promoters, dually marked by the repressive H3K27me3 and activating H3K4me3 marks, has strong prognostic value and outperforms tumor grade in predicting patient outcome.

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Conflict of Interest: None declared

P13.092.D PALB2 and ATM germline pathogenic mutations in a case of very early onset of Pancreatic adenocarcinoma (PDAC)

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Pancreatic adenocarcinoma (PDAC) is the seventh leading cause of cancer death worldwide, and its incidence has been increasing. Modifiable (tobacco smoking, alcohol, high BMI) and not modifiable (cystic fibrosis, pancreatitis, family history of PDAC) risk factors confer an increased risk, as well as germline pathogenic variants in several cancer predisposing genes (CPGs) and in chronic pancreatitis genes. No effective prevention strategies are currently available except for high-risk individuals. A 38-year-old man comes to our observation after CT finding of pancreatic tail neoformation, with hepatic multinodular formations; later confirmed as pancreatic adenocarcinoma. No specific risk factors emerge, except for first-degree obesity (BMI 33.74) and daily alcohol consumption of 0.28 units. *BRCA1* and *BRCA2* NGS analysis was uninformative; therefore, given the young age of onset and the complex family history, a NGS panel of cancer predisposition genes (*ATM, BARD1, BRIP1, PTEN, PALB2, TP53, NBN, RAD51C, RAD51D, STK11, CDH1, and CHEK2*) was performed. It highlights the presence of two pathogenic variant: c.550_562dup, p. (Ala188GlufsTer6) in the *PALB2* gene and c.9047_9057del, p. (Lys3016SerfsTer43) in the *ATM* gene, both in heterozygosity. He was later recruited in MK0482-001 clinical trial evaluating the safety and tolerability of MK0482 combined to Pembrolizumab; successive CT scans show reduction of the primary and secondary masses. Up to date, this is the first case of PDAC described in literature with two germline pathogenic variants in CPGs; further effort is needed to elucidate the interaction of *ATM* and *PALB2* germline variants and its role in cancer predisposition and potential therapeutic application.

Conflict of Interest: None declared

P13.093.A Genome-first approach to characterize the prevalence and associated cancer phenotypes of pathogenic or likely pathogenic germline TP53 variants

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Background/Objectives: Pathogenic or likely pathogenic (P/LP) heterozygous germline *TP53* variants are the primary cause of Li-Fraumeni syndrome (LFS), a cancer predisposition disorder. The population prevalence of P/LP germline *TP53* variants is estimated to be approximately one in every 3,500-20,000 individuals. However, these estimates are likely impacted by different ascertainment biases and lack of clinical and genetic data to account for potential confounding factors, such as clonal hematopoiesis. Genome-first approaches can further refine population prevalence estimates by identifying individuals with variants of interest and then assessing their phenotypes. Our aim was to determine the prevalence of P/LP germline *TP53* variants and associated cancer phenotypes via a genome-first approach.

Methods: This study evaluated P/LP germline *TP5*3 variants (variant allele fraction ≥30%) in three cohorts with available electronic health record data: UK Biobank (UKB, n = 200,590), Geisinger (n = 170,503), and Penn Medicine Biobank (PMBB, n = 43,731).

Results: The prevalence estimates of P/LP germline *TP53* variants were 1:10,439 in UKB, 1:3,790 in Geisinger, and 1:2,983 in PMBB after removing related individuals and heterozygotes who had ever developed a hematologic cancer.

Conclusion: Our genome-first approach identified a high prevalence of P/LP germline *TP53* heterozygotes. In health-care contexts, the range was from 1:2,983 to 1:3,790 individuals and 1:10,439 in population-based settings mostly comprised of healthy individuals. Prospective studies of diverse, young cohorts are required to better understand population prevalence of germline *TP53* variants and their associated cancer penetrance.

Grant References: Not applicable

Conflict of Interest: Kelvin Cesar De Andrade Unpaid member of the ClinGen TP53 Variant Curation Expert Panel, Natasha T. Strande: None declared, Jung Kim: None declared, Jeremy S. Haley: None declared, Jessica N. Hatton Unpaid member of the ClinGen TP53 Variant Curation Expert Panel, Megan N. Frone Co-developer of CancerGene Connect, Unpaid member of the ClinGen TP53 Variant Curation Expert Panel, Payal P. Khincha: None declared, Gretchen M. Thone: None declared, Uyenlinh L. Mirshahi: None declared, Cynthia Schneider: None declared, Heena Desai: None declared, James T. Dove: None declared, Diane T. Smelser: None declared, Arnold J. Levine: None declared, Kara N. Maxwell: None declared, Douglas Stewart: None declared, David J. Carey: None declared, Sharon A. Savage Unpaid member of the ClinGen TP53 Variant Curation Expert Panel

P13.094.B Somatic genetic rescue in a patient carrying a MECOM LoF variant

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We present a unique case of clonal hematopoiesis of indeterminate potential (CHIP) where a copy neutral loss-of-heterozygosity (CN_LOH) on the long arm of chromosome 3 results in a rescue mechanism for MECOM loss-of-function (LoF). This patient is a 20y old woman diagnosed in 2020 with 'radioulnar synostosis with amegakaryocytic thrombocytopenia-2 (RUSAT2) syndrome', caused by a heterozygous de-novo nonsense variant in the MECOM gene. Since diagnosis her mild cytopenias remain stable (hemoglobin 7.0-8.4 mmol/L; leukocytes 3.2-5.1 × 10^9/L; platelets 127-161 × 10^9/L). Routine bone marrow (BM) analysis showed a decreased cellularity. Additional routine surveillance for BM failure, using single nucleotide polymorphism (SNP) array, revealed a mosaic CN-LOH on chromosome 3q in approximately 10% of the BM cells. Other genomic aberrations were absent, as tested by standard karyotyping and FISH analysis for inv(3q).

Since MECOM is has an important role in hematopoiesis, our hypothesis is that the expanding clone in the BM cells has undergone mitotic recombination resulting in two wild-type MECOM alleles. This rare phenomenon is called somatic genetic rescue, and confers selective advantage over cells carrying one pathogenic MECOM allele in the germline. A SNP array from 3 years earlier, indeed shows the presence of the CN-LOH event in ~5% of the BM cells. This confirms our hypothesis that we are, for the first time, witnessing somatic genetic rescue for MECOM LoF by clonal expansion of BM cells carrying an acquired chromosome 3q CN-LOH. Clinical follow-up of this patient will show if this results in a good prognosis regarding her cytopenias

Conflict of Interest: None declared

P13.095.C A 26-year-old woman with solely extra-endocrine features of MEN2B and classical MEN2A genotype

Kristin Bosse[1], *Joohyun Park*[1], *Stephan Waldmueller*[1], *Ulrike Faust*[1], *Antje Stäbler*[1], *Cristiana Roggia*[1], *Simone Olivieri*[1], *Ute Grasshoff*[1], *Andreas Dufke*[1], *Tobias Haack*[1], *Olaf Riess*[1], *Christopher Schroeder*[1], *Kathrin Grundmann-Hauser*[1]

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Background: Pathogenic variants in the *RET* proto-oncogene have been associated with two clinically distinct subtypes of multiple endocrine neoplasia (MEN), MEN2A and MEN2B, and isolated familial medullary thyroid cancer (MTC). While MEN2A is characterized by MTC, pheochromocytoma, and parathyroid disease, MEN2B presents with MTC, marfanoid habitus, and submucosal tumors. There is evidence of a strong genotype-phenotype correlation.

Case report: A 26-year-old woman from Russia presented to our out-patient clinic with joint laxity, cutis laxa, and arachnodactyly. Her major complaint was alacrima, and her phenotype was suggestive of a clinical diagnosis of Ehlers-Danlos Syndrome (EDS). Family history was unremarkable. Genome sequencing showed a pathogenic missense variant (NM_020975.6:c.2410G>A, p.Val804Met; ClinVar ID: 37102) in *RET*, with no evidence of additional genetic variants possibly responsible for EDS. The American thyroid association (ASA) classifies this *RET* variant as "moderate risk variant" with large intra- and interfamilial variability in the penetrance of MTC. So far reported, heterozygous carriers of the c.2410G>A variant manifest with features of the MEN2A spectrum. Rarely, patients with classical MEN2B phenotype carry combinations of double *RET* germline missense variants on the same allele, which was not the case in our patient. However, her skeletal phenotype and ophthalmologic features were consistent with the typical MEN2B phenotype. Although no parental samples were available to confirm that this alteration occurred de novo in our proband, the majority of MEN2B cases are known to be due to de novo variants.

Conclusion: The data broaden the phenotypic spectrum associated with the missense change c.2410G>A, p.Val804Met.

Conflict of Interest: Kristin Bosse part-time, Joohyun Park full, Stephan Waldmueller full, Ulrike Faust part-time, Antje Stäbler: None declared, Cristiana Roggia full-time, Simone Olivieri full-time, Ute Grasshoff part-time, Andreas Dufke full-time, Tobias Haack full time, Olaf Riess full-time, Professor Riess declares research grants from:

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SOLVE-RD
SIMPATHIC
CanHeal

genomDE, Professor Riess declares to have participated as a speaker for:

Pfizer
Shire
AstraZeneca

Illumina, Professor Riess owns a patent, Professor Riess is consultant/advisor for following organizations/companies:

President-elect/President/Vice-President of the European Society of Human Genetics (ESHG)
External Advisory Board (EAB) of the E-Rare-3 funding program
Coordinator of the diagnostic task force of the European Joint Programme Committee
Member of the "Unsolved" Task Force of IRDiRC

UDNI Board of Directors (Undiagnosed Disease Network Initiative)

Member of the European Board of Medical

Genetics, Branch of Medical Genetics and Genomics (BMGG)

Advisory board of the BMG on Genome Diagnostics of Rare Diseases

Member of the Senat of the University of Tübingen (Senator)

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P13.096.D Solving complex karyotypes in leukemia samples using long-read sequencing

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Background/Objectives: Highly complex karyotypes represent an adverse prognostic marker in leukemia samples. Their detailed study is substantive to identify specific genomic defects contributing to the deterioration of the disease course. Methods of classical and molecular cytogenomics are widely used in laboratory diagnostics to detect chromosomal aberrations, but their resolution is limited. We aim to employ Oxford Nanopore whole genome long-read sequencing to decipher cancer genome in difficult and ambiguous cases of chronic lymphocytic leukemia with complex karyotypes.

Methods: Complex karyotype cases were identified and characterized using classical (IL-2/CpG-stimulated chromosomal banding) and molecular (24xCyte Multicolor FISH, CytoScan HD Array) cytogenomics. For long-read sequencing, high molecular weight DNA was isolated using chloroform-isopropanol extraction, fragmented using an injection needle, and short DNA fragments were eliminated (SRE XS Kit). The sequencing libraries were prepared using Ligation Sequencing Kit and sequenced on the MinION or PromethION sequencer. Sequences were aligned to the hg19 human genome reference, and structural variants were identified using the split read method, filtered, and annotated.

Results/Conclusion: For each patient, we obtained sequencing data enabling 10× (MinION), or >20× (PromethION) average coverage of the genome. We performed a comprehensive comparison of long-read sequencing results with those of classical and molecular cytogenomics and assessed the benefits and shortcomings of long-read sequencing in deciphering the structure of genomic rearrangements in the tested chronic lymphocytic leukemia cases. Long-read sequencing provides more accurate characterization of breakpoints and majority of genome rearrangement events.

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Conflict of Interest: None declared

P13.097.A High Concordance between ctTSO500 NGS Results from Tissue and Plasma Samples for Detection of Tumor-Specific Genomic Alterations

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ctTSO500 from Illumina is a next-generation sequencing (NGS) assay that is used for the detection of tumor-specific genomic alterations in circulating tumor DNA (ctDNA) extracted from plasma. In recent research, the concordance between ctTSO500 NGS results from tissue and plasma samples was investigated. The study included patients with various cancer types, including lung, breast, stomach, and colon cancer, among others.

The results showed a high level of concordance between the ctTSO500 NGS results obtained from tissue and plasma samples, often >80% for SNV's but nearer 70% for indels. The study found that ctTSO500 NGS had a high sensitivity and specificity for detecting tumor-specific genomic alterations in plasma samples. Additionally, the researchers found that ctTSO500 NGS was capable of detecting additional genomic alterations in plasma samples that were not detected in tissue samples. Although no fusions were observed in the cohort of 70 patient samples, the pipeline is capable of recognizing and reporting DNA-based chimeric or fusion constructs.

These findings suggest that ctTSO500 NGS can be a valuable tool for monitoring disease progression and treatment response in cancer patients. Furthermore, the high concordance between ctTSO500 NGS results from tissue and plasma samples suggests that ctDNA analysis using ctTSO500 NGS can be a reliable and non-invasive alternative to traditional tissue biopsies for the detection and monitoring of cancer.

Conflict of Interest: None declared

P13.098.B Germline DNA repair pathways deficiency in pancreatic cancer and prospective for target therapy

Elena Pasquinelli[1;2], *Viola Bianca Serio*[1;2], *Simona Innamorato*[1;2], *Debora Maffeo*[1;2], *Mirjam Lista*[1;2], *Samantha Minetto*[1;2], *Loredaria Adamo*[1;2], *Giulia Rollo*[1;2], *Lorenzo Loberti*[1;2;3], *Enrica Antolini*[1;2], *Jasmine Covarelli*[1;2], *Michele Carullo*[1;2], *Pietro Ilardi*[1;2;3], *Rossella Tita*[3], *Mirella Bruttini*[1;2;3], *Maria Antonietta Mencarelli*[3], *Margherita Baldassarri*[1;2;3], *Anna Carrer*[1;2;3], *Anna Maria Pinto*[3], *Caterina Lo Rizzo*[3], *Francesca Mari*[1;2;3], *Francesca Ariani*[1;2;3], *Alessandra Renieri*[1;2;3]

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Background/Objectives: Pancreatic Cancer (PC) continues to be a hard to treat cancer with a 5-year survival rate of approximately 11%. Classically, germline variants in *BRCA1/2, PALB2, CDKN2A, CDK4, MLH1, MSH2, MSH6, PMS2 and PRSS1* are associated with a high-risk for PC. Here, we employed exome sequencing to investigate genetic variants in PC patients.

Methods: Exome sequencing was performed on 33 patients with a suspect of familial pancreatic cancer enrolled in the Medical Genetics unit of AOUS.

Results: Fifteen patients carry at least one germline mutation in DNA repair genes (DRGs) and in 4 unrelated patients simultaneously two pathogenic germline variants were observed.

The Homologous recombination (HR) genes (*PALB2, CHEK2, ATM, SLX4, RECQL4)* were found to be the most frequently mutated (37%), followed by Fanconi anemia (FA) genes (*FAN1*, *ERCC4, NBN, FANCM) (21%)* and mismatch repair (MMR) genes (*MSH2, MSH3, MSH6) (21%)*. Of note, Fanconi anemia genes were always mutated paired with a second germline mutation in other DRGs.

Conclusion: A hallmark of many cancers is an inactivation of DRGs meanwhile their upregulation is found to be associated with resistance to common chemotherapies. In fact the disruption of one pathway frequently enhances cell dependency on compensatory pathways explaining why a combination therapy might be more efficient. HR and FA deficiency is primarily targetable with PARP and histone deacetylase inhibitors whilst MMR deficiency tumors are sensitive to immunotherapy with checkpoint inhibitors. Early-detection of germline susceptibility variants in targetable genes could open a new personalized combined therapeutic era for PC patients.

Conflict of Interest: None declared

P13.099.C Functional characterization of the novel c.2468G>T variant in DICER1 in a family with multinodular goiter recurrence

Eliana Zacchi[1;2], *Giovanni Innella*[1;2], *Antonio De Leo*[3;4], *Elisa Albertini*[2;4], *Thais Maloberti*[2;4], *Sara Coluccelli*[2;4], *Dario De Biase*[4;5], *Simona Ferrari*[1], *Giovanni Tallini*[3;4], *Elena Bonora*[1;2], *Daniela Turchetti*[1;2]

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Background: *DICER1* syndrome is a rare cancer predisposing disorder: carriers of heterozygous *DICER1* variants are at increased risk of developing tumors, which usually display "second-hit" mutations.

Methods: After genetic counselling and sequence analysis of *DICER1* in constitutional DNA, transcript analysis was performed to assess the effect of a missense variant detected. RNA was extracted from whole blood and cDNA was obtained by reverse transcription. RT-PCR amplification of the region spanning exons 14-18 of the transcript was performed and the RT-PCR products sequenced.

Results: A 16-year-old boy underwent thyroidectomy due to multinodular goiter, with detection of a microcarcinoma. Tissue characterization showed the c.5429A>T pathogenic mutation in *DICER1*. Family history collection revealed that the older brother, the mother and several relatives in the mother's side had undergone thyroidectomy for goiter (thyroid cancer in one case). *DICER1* germline testing in the proband showed the novel variant c.2468G>T in exon 16, which was also found in his mother and brother. Amplification from patient's cDNA resulted in a wild-type fragment and a smaller fragment, the latter due to the use of a cryptic acceptor splice site inserted by the mutation in exon 16, resulting in a 32 base pairs deletion in exon 16, as detected by Sanger sequencing. This alteration in the open reading frame is predicted to induce a frameshift.

Conclusion: The demonstrated effect on splicing, the co-segregation with thyroid disease in the family, and the presence of a somatic mutation consistent with a second-hit suggest that the c.2468G>T variant is causative of DICER1-syndrome.

Conflict of Interest: None declared

P13.100.D Spectrum of pathogenic and likely pathogenic gene mutations detected in BRCA-negative breast cancers and their pathologic evaluation

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Background/Objectives: Next-generation sequencing allows the investigation of mutations in cancer patients with a suspected history of hereditary cancer syndromes. 5-10 percent of breast cancer are related to a known inherited gene mutation. *BRCA1* and *BRCA2* genes are the most common and most investigated genes among known genetic breast cancers, and there are few studies on other causative genes. This study aims to reveal the spectrum of non-*BRCA* cancer-related genes mutations and evaluate the pathologic characteristics of patients' tumors with a single center based in Izmir, Turkey.

Methods: Peripheral blood samples were collected from breast cancer patients with an indication for genetic analysis. A targeted next-generation sequencing panel containing 58 hereditary cancer predisposition genes was used for the detection of mutations. Patients with *BRCA1* and *BRCA2* mutations were excluded. Pathogenic or likely pathogenic variants in other cancer predisposition genes were examined. After evaluating the pathological findings of the tumor tissues, relationship between the mutated gene and its pathological characteristics were evaluated.

Results: 1136 patients were evaluated and 42 of them had a pathogenic/likely pathogenic non-*BRCA* mutation in 17 different genes (*APC, ATM, PALB2, CHEK2, RAD51D, BRIP1, CDKN2A, ERCC2, ERCC5, FANCA, GALNT12, MLH1, MUTYH, NBN, NTHL1, POLE, POLH)*.

Conclusion: This study is the first comprehensive study that investigate the spectrum of non-*BRCA* mutations and the correlation between pathologic characteristics of tumors and causative genetic background in breast cancer patients in Turkey. It is aimed to lead to further studies.

Conflict of Interest: None declared

P13.101.A Reclassification of germline variants of uncertain significance identified in breast/ovarian cancer predisposition genes other than BRCA1/BRCA2 in a single genetic laboratory over a 6-year period

Fulvia Brugnoletti[1], *Nicolas Molnarfi*[2], *Aurélie Ayme*[1;2], *Gaëlle Benais-Pont*[2], *Isabelle Gauchat-Bouchardy*[2], *Amandine Cadénes*[1], *Anne-Laure Rougemont*[2], *Thomas McKee*[2], *Pierre O. Chappuis*[1;2]

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Background: Identification of variants of uncertain significance (VUS) in breast/ovarian cancer (BOC) predisposition genes is common in the era of gene panel testing. This study aimed to describe data on reclassification of VUS other than *BRCA1/2* from a single clinical laboratory over 6 years.

Methods: We reviewed 147 distinct non-*BRCA1/2* VUS identified in the Laboratory of Molecular Oncology, University Hospitals of Geneva (Switzerland) between 2017-2022. Variant assessment included review of distinct lines of evidence (functional, genetic, population, computational, etc.) according to current American College of Medical Genetics (ACMG) recommendations by two investigators. Once reclassification was confirmed, physicians in charge of index cases were informed and, if management modification occurred, a follow-up consultation was suggested.

Results: In total, 46/147 (31%) non-*BRCA1/2* VUS were reclassified, distributed as follow: *ATM*: 17/47, *PALB2*: 9/16, *CDH1*: 5/9, *CHEK2*: 3/17, *MSH2*: 3/11, *BRIP1*: 2/5, *PMS2*: 2/9, *MLH1*: 1/1, *MSH6*: 1/13, *NBN*: 1/3, *RAD51D*: 1/3, *TP53*: 1/5. Overall, 38/46 (83%) of reclassified VUS were downgraded to likely benign/benign variants, 8/46 (17%) were upgraded to likely pathogenic/pathogenic (LP/P) variants, leading to updated reports for 85 index cases. To date, this reviewing process resulted in 10 follow-up consultations, with possibility of targeted testing among 44 first-degree relatives.

Conclusions: Approximately one third of non-*BRCA1/2* VUS identified in BOC syndromes were reclassified with 5% (8/147) upgraded to LP/P variants. This study highlights the importance of periodic reevaluation of VUS to appropriately manage BOC patients and families. This process is complex and represents an underestimated additional workload for laboratories and clinical geneticists.

Conflict of Interest: None declared

P13.102.B Clinical pathways in cancer screening of PTEN hamartoma tumor syndrome

Celia Azevedo Soares[1;2;3], *Gabriela Soares*[4], *Ana Rita Soares*[1], *Marta Soares*[5], *Marcia Rodrigues*[5], *Juliette Dupont*[5], *Patricia Dias*[5], *Mariana Soeiro e Sá*[5], *Ana Berta Sousa*[5], *Sofia Pérez*[6], *Diana Antunes*[6], *Margarida Venancio*[6], *Ana Maria Fortuna*[1], *Natalia Tkachenko*[1]

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PTEN hamartoma tumor syndrome (PHTS) is associated with various phenotypes including neurodevelopmental disorders, macrocephaly, and malignancy. In this study, we investigated the cancer screening strategies employed in three Medical Genetics departments in Portugal for PHTS patients to understand who is performing cancer screening and plan professional training on this rare disorder.

Our study included 24 patients from 17 families, of which 12 were adults. Two adult patients were diagnosed with cancer, one with thyroid cancer at the age of 26 years and the other with bilateral breast cancer and papillary thyroid cancer by the age of 38 years. Our data shows that cancer screening for PHTS patients varied widely among medical centers and even by patients in the same department. In Center I, five patients between the ages of five and 13 years did not initiate cancer screening. Seven minors in the other two centers had already begun cancer screening, all for thyroid cancer. All adults were participating in cancer screening programs. In Center I, cancer screening was performed by family doctors and non-oncologist hospital-based specialists for three patients, and by non-oncologist hospital-based specialists for five patients. In Center II, one patient was only screened by a family doctor, and six patients were screened by non-oncologist hospital-based specialists. In Center III, all patients were screened by an oncologist.

These findings highlight the need for multidisciplinary collaboration and the training of multiple medical professionals on PHTS. Improved education on PHTS could lead to better management of these patients.

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P13.103.C Rare case of a constitutional primary epimutation of MLH1 in a female patient with metachronous colon cancer

Simone Olivieri[1], *Kristin Bosse*[1], *Irina Bonzheim*[2], *Christopher Schroeder*[1], *Cristiana Roggia*[1], *Silja Gauß*[1], *Tobias Haack*[1], *Antje Stäbler*[1], *Olaf Riess*[1], *Ulrike Faust*[1]

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Background: Constitutional epimutations in *MLH1* are a rare cause for Lynch syndrome. Whereas primary epimutations occur without any detectable underlying genetic alteration and are not thought to be inheritable, secondary epimutations are due to cis- or trans-acting genetic lesions and may follow autosomal dominant transmission.

Case-Report: The presented female patient was diagnosed with caecal carcinoma at the age of 28 years and developed a metachronous transverse colon carcinoma by age 50. Family history was positive for colon cancer and revised Bethesda criteria were fulfilled. Tumor tissue demonstrated loss of MLH1 expression at immunohistochemistry and MSI- high phenotype. A *BRAF*-Hotspot mutation had been excluded. Diagnostic genomic sequencing showed no evidence of a germline pathogenic variant in the MMR genes. An aberrant methylation of the *MLH1* promoter was detected in tumor as well as in normal tissue and led to diagnosis of Lynch syndrome. No known cis-acting genetic lesion was identified. *MLH1* promoter methylation was excluded in blood samples of both healthy children.

Discussion: As there is no evidence of an underlying germline variant, e.g. in the *MLH1* promoter, we consider the described constitutional epimutation of *MLH1* as primary in our patient, most probably being the result of environmental or cellular perturbation in the gametogenesis or early embryogenesis. Epigenetic reprogramming events make inheritance highly improbable. We proposed intensified surveillance concerning Lynch syndrome related tumors in our proband.

Conclusion: Constitutional epimutations of *MLH1* should be considered as an alternative pathogenic mechanism for Lynch syndrome.

Conflict of Interest: Simone Olivieri Institute of Medical Genetics and Applied Genomics, University of Tübingen Full-time, Kristin Bosse Institute of Medical Genetics and Applied Genomics, University of Tübingen Part-time, Irina Bonzheim Institute of Pathology and Neuropathology, University Hospital Tübingen Full-time, Christopher Schroeder Institute of Medical Genetics and Applied Genomics, University of Tübingen Full-time, Dr. C. Schroeder reports an institutional grant from Illumina and research grants from BMS Stiftung Immunoonkologie, Cristiana

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Part-time, Tobias Haack Institute of Medical Genetics and Applied Genomics

Full-time, Antje Stäbler Institute of Medical Genetics and Applied Genomics

Part-time, Olaf Riess Institute of Medical Genetics and Applied Genomics

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GIF

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SOLVE-RD

SIMPATHIC

CanHeal

genomDE, Professor Riess declares to have participated as a speaker for:

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Illumina, Professor Riess owns a patent, Professor Riess is consultant/advisor for following organizations/companies:

President-elect/President/Vice-President of the European Society of Human Genetics (ESHG)

External Advisory Board (EAB) of the E-Rare-3 funding program

Coordinator of the diagnostic task force of the European Joint Programme Committee

Member of the "Unsolved" Task Force of IRDiRC

UDNI Board of Directors (Undiagnosed Disease Network Initiative)

Member of the European Board of Medical

Genetics, Branch of Medical Genetics and Genomics (BMGG)

Advisory board of the BMG on Genome Diagnostics of Rare Diseases

Member of the Senat of the University of Tübingen (Senator)

President of the German Society of Human Genetics, Ulrike Faust Institute of Medical Genetics and Applied Genomics

Part-time

P13.104.D Molecular characterization of patients with gliomas using a multi-gene next-generation sequencing panel

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Background/Objectives: Gliomas are the most common primary intracranial tumour, characterised by poor prognosis. Therapy and prognosis are driven partly by molecular characterisation of the disease. The present study provides a comprehensive understanding of the molecular profile and clinical features of 32 patients with glioma.

Methods: DNA was extracted from FFPE specimens and subjected to library preparation and in solution hybridisation with a 80-gene panel followed by NGS on n Illumina platform. A subset of samples were also analysed with a 55-gene Ampliseq gene panel.

Results: In total, 129 genetic alterations including 33 structural variants were identified in 38 distinct genes. Among 96 variants, 38 were pathogenic and 58 variants of unknown clinical significance. *TP53* was the most frequently mutated gene, followed by *PTEN* and *IDH1* genes. Glioma patients with *IDH1* mutant tumours were younger and had significantly longer overall survival compared to patients with wild-type *IDH1* tumours. Subsequently, a comparison of the mutational profiles of samples analysed by 2 assays was also performed. The comprehensive 80-gene pan-cancer panel identified 24 additional variants, 22 of which were in regions that were not targeted by the 55-gene panel.

Conclusion: Overall, the present study demonstrated the use of an extended tumor profile assay instead of a glioma-specific tumor panel identified additional genetic changes that may be taken into consideration as potential therapeutic targets for glioma diagnosis and molecular classification.

Grant references: This work was supported by an internal HeCOG research grant (grant no. HE R_17/15), by Medicover Genetics and by a HeSMO Grant.

Conflict of Interest: Alexia Eliades Full employment at Medicover Genetics, Kyriakos Tsangaras Full employment at Medicover Genetics, Achilleas Achilleos Full employment at Medicover Genetics, Christos Lemesios Full employment at Medicover Genetics, Ourania Romanidou HeCOG research grant (grant no. HE R_17/15) and by a HeSMO Grant, Paraskevi Apostolou: None declared, Chrysovalando Sotiriou Full-time employment at Medicover Genetics, Louisa Constantinou Full-time employment at Medicover Genetics, Haris Kkoufou: None declared, Michalis Spyrou Full-time employment at Medicover Genetics, Stelia Pissaridou Full time employment at Medicover Genetics, Antonia Matsentidou Full-time employment at Medicover Genetics, Elena Kypri Full-time employment at Medicover Genetics, Marios Ioannides Full-time employment at Medicover Genetics, George Fountzilas: None declared, George Koumbaris Full-time employment at Medicover Genetics, Philippos Patsalis Full-time employment at Medicover Genetics

P13.105.A From comprehensive cancer genome profiling to hereditary cancer predisposition syndromes

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Tumor-only genomic profiling is an important tool in the clinical care of cancer patients, through the identification of actionable somatic mutations.

Cancer genome profiling (CGP) may additionally reveal germline variants of clinical relevance for patients and their at-risk family members, having a major impact in terms of preventive healthcare.

In January 2022, Fondazione Policlinico Universitario Agostino Gemelli IRCCS started a monocentric interventional CGP prospective study (ID: FPG500). The study includes patients with 11 different cancer types. The patients are enrolled by clinicians, based on the availability of a target therapy according to national and international guidelines in specific clinical settings. DNA and RNA from patient's formalin-fixed paraffin embedded (FFPE) and cytological specimens are profiled using TruSight Oncology 500 assay (Illumina).

To date, a total of 1585 patients have been enrolled in the study. Of all the patients referred to genetic counseling, 179 underwent genetic evaluation.

Among them: 3 (1.67%) patients refused consent to germline genetic analysis, 13 (7.26%) patients were not considered suitable for germline genetic analysis after accurate clinical evaluation, 49 (27.37%) patients resulted negative to genetic testing, 58 (32.40%) patients have at least one genetic test ongoing.

The number of patients diagnosed with a hereditary cancer predisposition syndrome is 54 (30.16%), including a patient with a MINAS. The germline variants were found in BRCA1 (26/54), BRCA2 (14/54), RAD51C (3/54), BRIP1 (2/54), ATM (2/54), RAD51D (1/54), MLH1 (1/54), MSH2 (1/54), LZTR1 (1/54), RET (1/54), PALB2 (1/54) genes. Two MUTYH biallelic variants were found in a patient with MAP.

Conflict of Interest: None declared

P13.106.B Identification and characterization of a new germline mutation in CDKN2A in a pediatric patient with osteosarcoma

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Background/Objectives: Genetic predisposition is an important risk factor for cancer in children and adolescents but detailed associations of individual genetic mutations to childhood cancer are still under intense investigation. The association of sarcomas with these syndromes is often missed, due to the rarity and heterogeneity of sarcomas and the limited search of cancer genetic syndromes. The objective was to identify germline variants in sarcoma patients and to analyze the pathogenicity of these variants.

Methods: Sequencing results from NGS tumor profiling of 43 pediatric patients with sarcomas were reviewed for potential germline alterations in clinically relevant genes associated with cancer predisposition syndromes. Selected variants were confirmed in the germline. Functional assays including proliferation assay and cell cycle analysis were done by ectopic expression of the variant in the U2OS human osteosarcoma cells.

Results: We identified the germline *CDKN2A* c.350del; p.(Leu117ArgfsTer29) likely pathogenic variant in a patient with osteosarcoma. We have shown that this variant causes an increase in cell proliferation and cell cycle deregulation, specifically in the G1 phase. These results suggest that *CDKN2A* c.350del variant results in a loss of functionality of the p16INK4 protein.

Conclusion: We have shown that the results of somatic testing can be used to identify germline pathogenic variants in sarcomas. The *CDKN2A* c.350del variant is involved in cell replication.

Grant References: Navarra government (Ref. 54/2018), Asociación Pablo Ugarte APU (APU-osteosarcoma), La Cuadri del Hospi (BC/A/17/008), EITB media AND BIOEF, SAU (BIO20/CI/015/BCB and BIO20/CI/011/BCB), Basque government (2021111030) and Fundación La Caixa with Niños Contra el Cáncer.

Conflict of Interest: None declared

P13.107.C Liquid biopsy for early tumor relapse detection

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Ultrasensitive and specific methods for rare allele detection are essential in order to fully exploit the potential of identifying a single genetic variant that might be poorly represented in a biological mixture (liquid biopsy, for instance). In this context, several methods have been described that use unique molecular identifiers (UMIs) to analytically remove NGS errors. Among them, Duplex Sequencing (DS) has been shown to be highly-effective by leveraging the sequence complementarity of the two DNA strands. Nevertheless, the described DS adaptors' production methodology leads to a low ligation efficiency, which hinders their capability to work with limited amounts of input DNA such as cell-free DNA (cfDNA) samples. Moreover, DS needs a much higher sequencing depth and is a costly approach together with large panels.

Here, we have devised an efficient and cost-effective approach to produce sequencing adapters with a double-stranded 12 bp UMI that can be used with cfDNA inputs as low as 2 ng. This, together with the capacity to efficiently produce mixtures of enrichment probes that are able to deliver very high on-target metrics, are key to any personalized medicine strategy. Within the pediatric oncology context, promising preliminary results demonstrate that we can detect circulating tumor DNA (ctDNA) at frequencies down to one in one thousand with extreme accuracy.

In summary, we are laying the foundations for a robust personalized medicine solution that will allow an extremely accurate detection of ultra rare mutations by using small custom panels that may be strictly personalized in different clinical settings.

Conflict of Interest: Pau Rodriguez-Sodupe full, full, Jairo Rodríguez full, LLuís Armengol intellectual property, Luis Pérez-Jurado: None declared, Marco Telford: None declared

P13.108.D Low-level constitutional mosaicism in breast cancer patients

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Background/Objectives: The development of next-generation sequencing (NGS) technologies has revealed a significant contribution of mosaic variants to cancer predisposition genes. In this study we describe the occurrence of very low-level constitutional mosaicism in breast cancer predisposition patients.

Methods: A cohort of patients diagnosed with breast cancer and referred to our laboratory for genetic testing using a NGS panel of 52 genes was evaluated for the occurrence of very low-level constitutional mosaicism in breast cancer predisposition genes. In all cases suspected of mosaicism, DNA was isolated from the buccal swab to confirm the state of mosaicism.

Results: NGS analysis of 7 patients diagnosed with breast cancer in peripheral blood and buccal swab DNA revealed low-level constitutional mosaicism variants in four genes. More specifically, we identified a gross deletion of the genomic region the full coding sequence of the *BRCA2* gene. In the *NF1* gene the following variants were detected c.2294_2295del, p.(Arg765Hisfs*2) and c.3197+1G>A at 31% and 32%, respectively. Also, the variant c.1117G>T, p.(Glu373*) was identified in *PTEN* at 25%. Finally, in *TP53* mosaicism was identified in variants: c.814delinsCTT, p.(Val272Leufs*74) (32%), c.916C>T, p.(Arg306*) (32%) and c.722C>G, p.(Ser241Cys) (31%).

Conclusion: In this study we report cases of low-level constitutional mosaicism in breast cancer predisposition genes and emphasize the importance of deep sequencing in breast cancer patients. Clinical laboratories should establish procedures to ensure the detection of mosaic variants and strategies for the verification of the results from additional material (buccal swabs, saliva or fibroblasts).

Conflict of Interest: None declared

P13.109.A Germline heterozygous deletion containing CDKN2A/CDKN2B as genetic substrate for rare familial neural and skin tumor syndrome

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Background/Objectives: The genes *CDKN2A/CDKN2B*, located on 9p21.3 are part of the vital cell cycle pathway often disturbed in human tumours. Somatic deletions of this gene cluster are often detected in a myriad of tumours. Germline heterozygous deletions containing *CDKN2A/CDKN2B* have been described in only six families to date, presenting a complex familial cancer syndrome.

Clinical data: We describe an adult brother and sister presenting atypical neurofibromas, lacking any skin or ocular stigmata of neurofibromatosis type 1. In addition the sister developed a pleomorphic xanthoastrocytoma, the brother a suspected low grade glioma in the brainstem and also smaller white matter lesions in the frontal, parietal, temporal lobes and the cerebellum. Additional imaging showed multiple peripheral nerve tumours in the brothers limb.

Methods: Blood and tumour tissue of both siblings and blood of the mother was analysed through NGS, MLPA and array-CGH.

Results: NGS analysis of the brothers neurofibroma exhibited a NF1 loss-of-function mutation. Additionally, analysis of tumour samples showed a homozygous deletion of *CDKN2A/CDKN2B* in each sibling's tumour. The xanthoastrocytoma showed a BRAF V600E mutation. The patient is responding well to treatment with both BRAF and MEK inhibitors. A maternally inherited heterozygous deletion of 1.25Mb was confirmed in both siblings. There was no evidence for mosaicism in the mother. Remarkably, at 55 years of age, the mother did not show any tumoral complications.

Conclusion: Germline heterozygous deletions containing CDKN2A/CDKN2B are a rare cause of a familial tumour syndrome with increased risk for neurofibromas, skin melanomas and several types of brain tumours.

Conflict of Interest: None declared

P13.110.D Cancer susceptibility genes in triple-negative breast cancer: Prevalence of reported variants in a tertiary hospital in Northern Portugal

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Background: Germline *BRCA1* Pathogenic (P) or Likely Pathogenic (LP) variants are identified in 4.4% to 16% of patients with triple negative breast cancer (TNBC). This study characterizes the 17-year genetic approach to TNBC patients in one Center.

Methods: A retrospective observational transverse study was performed. Patients referred to genetics with TNBC in one Center between 2006 and 2022 were included. Personal history, family history (according to NCCN testing criteria) and germline genetic tests were analyzed. Descriptive analysis, chi-square and logistic regression were performed.

Results: We identified 318 patients. Of these 278 (87.4%) underwent genetic testing with 43 (15.5%) carrying P/LP variants and 35 (12.6%) carrying variants of unknown significance (VUS). P/LP variants were identified in *BRCA1*(23), *BRCA2*(13), *PALB2*(1), *CHEK2*(1), *ATM*(1), *RAD51C*(2), *RAD51D*(1) and *MSH2*(2) genes. In 185 patients only *BRCA1* or *BRCA1/BRCA2* were studied, whereas in 91 a multi-gene panel was used. The diagnostic rates increased with larger panels (13.5% to 18.6%), as did VUS rates (8.1% to 22.0%, $p = 0.002$), increasing the VUS:P/LP ratio from 0.6 to 1.18. An association was found between the presence of family history of cancer fulfilling NCCN criteria and the presence of a P/LP variant (OR 2.68, $p = 0.04$).

Conclusion: The overall diagnostic rate and the prevalence of *BRCA1* variants were consistent with the literature. We conclude that:

- Multi-gene panels slightly increase the diagnostic rate in TNBC, at the expense of a significant increase in VUS:P/LP ratio.
- NCCN family history criteria correlated with the likelihood of positive results, corroborating its clinical utility.

Conflict of Interest: Alice Porto Vasconcelos: None declared, Luzia Garrido: None declared, Renata Oliveira: None declared, Sérgio Castedo Director in Genetic Diagnostics Laboratory

P13.111.C PTEN hamartoma tumor syndrome (PHTS): further expanding the PTEN-mutations phenotypic spectrum

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Background/Objectives: The *PTEN* hamartoma tumor syndrome (PHTS) comprises a group of diseases that includes Cowden (CS) and Bannayan-Riley-Ruvalcaba (BRRS) syndromes as well as *PTEN*-related Proteus (PS) and Proteus-like Syndromes. *PTEN* is an autosomal dominant tumor suppressor gene of the PI3K/AKT/mTOR pathway involved in the cell cycle regulation and the mutations of which predispose to the development of organ solid tumors and autistic type neurodevelopmental disorders. PI3K/AKT/mTOR gene pathway mutations as a whole, give rise to overlapping clinical phenotypes. Three pediatric patients with *PTEN* mutations and common features of macrocephaly and developmental disorders fulfilling some criteria of Cowden Syndrome are described with the aim of stressing the importance of the emergence and investigation of the *PTEN*-mutations clinical phenotypes as it is shaping with the contribution of the contemporary methods of next generation sequencing

Methods:. Detailed clinical genetic examination and follow-up of the patients was performed, followed by genetic molecular analysis with whole exome sequencing (WES).

Results: The de novo missense mutations c.277C>A (p.His93Asn), c.202T>C (p.Tyr68His) and the intronic pathologic change c.1027-2A>G (intron 8) were detected respectively.

Conclusions: Given the oncogenic action of the *PTEN*-mutations and the great spectrum of overlapping phenotypes, the elaborate based on criteria clinical genetic examination and application of the newest diagnostic tools such as WES, allows rapid diagnosis, follow-up and proper genetic counseling of the patients

Conflict of Interest: stavroula psoni part-time, Maria Moschovi full-time, Irene Tsoutsou full-time, Stella Amenta part-time, George Papadopoulos full-time, HELEN FRYSSIRA part-time

P13.113.A Long non-coding RNA signature in esophageal squamous cell carcinoma in Kazakh patients

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Background/Objectives: Esophageal squamous cell carcinoma (ESCC) is the prevalent histological subtype of EC in middle Asian region, including Kazakhstan. ESCC is characterized by poor prognosis worldwide with a 5-year survival rate of less than 25%. LncRNAs play an important role in the cancer proliferation, metastasis, and therapy resistance.

Methods: Whole transcriptome sequencing was performed following the Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus, Protocol on Illumina NovaSeq6000 platform. STAR software and DESeq2 package have been used for mapping and defining differentially expressed genes. Functional analysis of DEGs was performed using various R packages.

Results: We examined 34 genes that were consistently abnormally expressed.

Long non-coding RNAs (lncRNA, lncRNA), transcripts over 200 nucleotides in length, play an important role in oncogenesis, while lncRNA and miRNA are also involved in the development and progression of esophageal cancer. Among DEGs, we found 61 unique lncRNAs (lncRNAs) and microRNAs (microRNAs) including 59 highly expressed and 2 low expressed genes that were identified in tumor samples compared to normal samples. The number of long non-coding RNAs specific to each stage of the tumor was also determined.

Conclusion: The identified lncRNAs are potentially new and have not been previously described; therefore, the involvement of these lncRNAs in the pathogenesis of ESCC will be studied using functional and accumulative analysis methods. Analysis of these lncRNAs showed that 20 lncRNAs were common for all tumor stages of ESCC.

Grant References: NU CRP grant 021220CRP2222 and grant MES RK #AP09058660.

Conflict of Interest: None declared

P13.114.B Prognostic Value of Next Generation Sequencing in the Evaluation of Minimal Residual Disease in Pediatric Acute Lymphoblastic Leukemia

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Background: The assessment of minimal residual disease (MRD) is essential for pediatric acute lymphoblastic leukemia (ALL) management. We compared the prognostic role of MRD evaluation at two different time-points using two DNA-based methodologies.

Methods: A total of 30 patients (27 with B- and 3 with T-cell ALL) aged 0-11 were analyzed at diagnosis and on days 33 and 78 after treatment initiation. MRD was evaluated by assessment of the presence/absence of patient-specific immunoglobulin and/or T-cell receptor gene rearrangements detected at diagnosis using two methods: PCR/capillary electrophoresis (CE) and next generation sequencing (NGS) of IGH-FR3 and TCRG genes with a target sensitivity of 10^{-4}. The median follow-up time was 38 months.

Results: Overall, 14/30 (47%) of the MRD results on day33 and 22/30 (73%) on day78 were concordant between the two methods. In samples that were negative by CE, the NGS analysis detected MRD in 15/28 (54%) on day33 and 7/29 (24%) on day78.

All NGS-MRD-negative patients on day33 (13/13) and 91% (20/22) on day78 are in clinical remission. A total of 3 patients had a relapse; all of them were NGS-MRD-positive on day33, whereas only 1 of them remained NGS-MRD-positive on day78. Currently, 14/17 (82%) and 7/8 (88%) of the NGS-MRD-positive patients (days 33 and 78, respectively) are still in remission.

Conclusion: NGS should be considered for routine clinical application as a prognostic marker in pediatric ALL patients with excellent negative predictive value at day33. The reason for NGS-MRD-positive results of patients that are still in remission warrants further investigation.

Conflict of Interest: None declared

P13.115.C A multi-omics approach on hereditary colorectal cancer

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Introduction: High-penetrant pathogenic variants in established genes associated with colorectal cancer (CRC) explains the disease in approximately 5% of the patients. However, a suspected genetic component is expected to contribute to the disease in 20%-30% of all CRC cases. To increase the diagnostic yield, omics approaches have recently been introduced in variant detection pipelines.

Materials and methods: The study involves 112 index patients (Linköpings University Hospital, Linköping, Sweden) and one large family with hereditary CRC (Sahlgrenska University Hospital, Gothenburg, Sweden). The large family includes 24 affected and unaffected family members. Variant screening was performed using a panel containing 28 CRC genes. Whole-genome sequencing (WGS), transcriptomics (RNA-seq) and epigenomics (methylation assay) were performed on the large family. The same multi-omics approach was stepwise applied on the 112 patients. A data-driven AI tool was used for variant filtering and ranking of the WGS data.

Results: Fourteen of the 112 patients had variants in established CRC genes, in addition, potential candidate genes and genomic regions were found.

Conclusions: The introduction of multi-omics together with AI data-driven filtering, ranking and prioritization of variants are contributing to increased diagnostic yield for patients with hereditary CRC.

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Conflict of Interest: None declared

P13.117.A Variants causing splicing defects – challenging cases

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Background/Objectives: Mis-splicing is a well-documented mechanism of hereditary cancer syndrome development and it is estimated that 15–25% of pathogenic DNA variants cause splicing abnormalities. Increasing number of laboratories are implementing RNAseq for the discovery of RNA splicing defects in a routine diagnostic setting. However, the interpretation of RNAseq results can often be challenging due to leaky splice site variants or lack of exonic tag SNP.

Methods: We have bioinformatically evaluated all VUS obtained from 1037 consecutive patients who have undergone genetic testing for hereditary cancer syndromes. 17 VUS were selected for RNA analysis using in-house developed RNAseq protocol.

Results: RNAseq analysis revealed that 3 (18%) variants showed complete splicing alteration, 6 (35%) variants caused partial or uncertain splicing aberration, 6 (35%) variants showed minimally expressed splicing aberration and 2 (12%) variants had no impact on mRNA splicing.

Implementing RNAseq results into variant classification, we were able to reclassify 35% of variants to likely pathogenic and 30% of variants into likely benign. Nevertheless, 35% of variants were not reclassified and remained categorized as VUS.

Conclusion: A great proportion of variants caused partial (leaky) or uncertain splicing aberration. Such variants are especially difficult to interpret, since there is no agreed threshold for classifying leaky splice site variants as pathogenic. Leaky splicing variants in genes with incomplete penetrance and without a characteristic phenotype are particularly problematic. Therefore, RNAseq data obtained from patients with hereditary cancer predispositions should be interpreted cautiously, and many variants remain classified as VUS.

Grant References: /

Conflict of Interest: None declared

P13.118.B Structural and functional insights from single-cell transcriptional profiles of pituitary tumors

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The pituitary gland is a main component of the endocrine system and a master controller of hormones production and secretion. Unlike neoplastic formation in other organs, Pituitary Neuroendocrine Tumors (PitNETs) are frequent in the population (~15%) and are almost exclusively benign. Here, we present the single cell transcriptome analysis on 10 independent tumors for a total of ~64000 single cells: 3 bi-hormonal (GH-PRL) tumors, 2 corticotropic macro-adenoma, 4 non-secreting adenomas and one prolactinoma. Characterization of all tumors showed heterogeneous cell populations. We discover that *GATA2*, a transcription factor for early gonadotrophs differentiation is exclusively expressed in non-secreting adenoma. GATA2 inhibits GNRH receptor, leading to a decrease in LHB and FSHB production possibly explaining the non-secreting phenotype. Furthermore, *GATA2* expression profile in single nuclei RNAseq from healthy pituitary glands shows restricted transcription in prepubescent individuals suggesting a come-back to a pre-differentiated state for gonadotroph cells in non-secreting adenomas. In three tumors we identified an unexpected small population of proliferative cells (*MKI67*+, *TOPA2*+, *BIRC5*+, *PBK*+). Intriguingly, *IQGAP3*, a gene already known to be a bad prognostic marker in different type of

carcinomas, was highly expressed in this cluster, suggesting a correspondence between proliferative markers in PitNETs and malignant adenomas. Moreover, we identified in all tumors non-overlapping clusters of cells specifically expressing mitochondrial and ribosomal proteins, suggesting a recurrent structural organization to optimize energy balance and transcriptional activity.

Our results give a new perspective on the comprehension of the structural composition and the dynamic progression of pituitary tumors.

Conflict of Interest: None declared

P13.121.A Physical activity as a modifying factor for prostate cancer risk associated with genetic risk

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Background: Both genetic and lifestyle factors are known to be associated with prostate cancer (PCA) risk. However, it is unclear whether the association between physical activity (PA) and PCA risk depends on the individual's PCA-related genetic risk. Additionally, it is necessary to quantify how much genetically determined risk could be compensated by active exercise.

Methods: We conducted GWAS using 2,908 PCA patients from multi-centers hospitals and 17,376 controls from 3 community-based cohorts in Korea. Polygenic risk score (PRS) was constructed by aggregating the estimated effect of PCA-associated genetic variants. We categorized target participants (N = 7056) as low, intermediate, or high based on PRS.

Results: In the target participants (103 PCA patients), PRS was associated with PCA risk. Participants with high genetic risk and low PA had more than a 2-fold higher PCA risk than those with low genetic risk. However, participants with high genetic risk and high PA had a 52% higher PCA risk compared to those with low risk. High PA was associated with a 35% decreased PCA risk. Among men in the high PRS group, moderate PA for more than 420 min/week was associated with decreased risk of PCA.

Conclusion: Regular moderate PA of more than 420 min/week (or 1h/day) may help reduce the risk of PCA. In particular, for individuals with a genetic predisposition to PCA, increasing PA can have a more positive effect on risk reduction. Our findings have important implications for targeted strategies for PCA prevention.

Conflict of Interest: Jeeeun Kim part-time, Yu Jin Jung full, Joohon Sung full, Seok-Soo Byun full

P13.122.B Methylation and expression profiling for Sex Cord-Stromal Tumors of the ovary subtype characterization

Carla Roca[1], *Anne-Sophie Chong*[1], *Eduard Dorca*[2], *Nairi Tchrakian*[3], *Gulisa Turashvili*[4;5], *Susana López-Agulló*[6], *Colin Stewart*[7], *David Hardisson*[8], *Xavier Matias-Guiu*[9;10], *Glenn McCluggage*[11], *William Foulkes*[12;13], *Blaise Clarke*[14], *Barbara Rivera*[1;12]

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Background/Objectives: Sex cord-stromal tumors (SCSTs) are rare ovarian neoplasms comprising entities ranging from benign to malignant tumors. Some subtypes are associated with a genetic alteration, while in others like steroid cell tumors (SCTs), the genetic driver remains unknown. Pathological diagnosis is difficult due to their rarity and overlapping immunohistochemical features, therefore leading to potential misclassification. Recently, molecular analysis combined with histological review has shown to increase diagnostic precision. Here we analyze methylation data combined with WES and expression data to characterize a set of SCSTs.

Methods: 84 SCSTs, 37 non-SCSTs and 5 normal ovarian tissue samples were included in the study. DNA methylation array and targeted gene expression profiling data were used for performing the analyses.

Results: Initial pathology review classified the cohort in 32 SCTs, 3 signet ring stromal tumors, 4 microcystic stromal tumors (MSTs), 6 granulosa cell tumors (GCTs), 24 sertoli-leydig cell tumors (SLCTs). Methylation analysis defined distinct clusters, which matched with their diagnostic categories. Within tumor clusters, segregation was observed according to mutational status (wild-type or mutated: FOXL2 in GCTs, DICER1 in SLCTs, APC or CTNNB1 in MSTs). For SCTs, with no genetic driver described yet, methylation CNVs were studied to assess whether a profile could be defined. Differential expression and enrichment analyses where then performed, revealing the over-representation of several genes and pathways.

Conclusion: The combination of multiple molecular analysis holds potential for understanding the pathogenesis and classification of non-epithelial ovarian tumors and could provide an aid to diagnosis in histologically problematic cases.

Grant References: FIS-PI20/01721

Conflict of Interest: Carla Roca IDIBELL, Anne-Sophie Chong: None declared, Eduard Dorca: None declared, Nairi Tchrakian: None declared, Gulisa Turashvili: None declared, Susana López-Agulló: None declared, Colin Stewart: None declared, David Hardisson: None declared, Xavier Matias-Guiu: None declared, Glenn McCluggage: None declared, William Foulkes: None declared, Blaise Clarke: None declared, Barbara Rivera IDIBELL, FIS grant PI20/01721 Miguel Servet Fellow

P13.123.C Surveillance outcomes in Hereditary Mixed Polyposis Syndrome

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Background: Hereditary Mixed Polyposis Syndrome (HMPS) is caused by a 40kb duplication upstream of *GREM1*- a founder pathogenic variant in Ashkenazi Jews, which predisposes to colorectal cancer (CRC). Colon surveillance is recommended but there are few data on effectiveness and long-term outcomes.

Methods: Patients are *GREM1*-dup carriers from 11 medical centers in Israel and UK. Clinical data was retrospectively collected.

Results: 63 carriers were identified. Colonoscopy surveillance data was available for 46/63, comprising 529 patient year follow-up. 287 surveillance colonoscopies were performed, 3.5 (1-23) colonoscopies per patient.

Nine patients had 11 CRC, median diagnosis age 42y (36-50y). Two were diagnosed at index colonoscopy, one developed CRC under surveillance, and others were diagnosed prior screening. 4/4 CRC were microsatellite stable (MSS).

Fourteen patients developed 23-advanced adenomas (AAs), 6 contained high dysplasia. Median age at first AA was 41y (31-64). 6/23 (27%). Patients with AAs were more likely to have more polyps (11.7 vs. 2.7, $p < 0.001$) and CRC (5/14 vs. 2/27, $p = 0.016$). Median age at first adenoma was 38y (12-93y), median number of adenomas per patient was 2.5 (0-37).

Four patients had extra-colonic cancers: BCC, melanoma, prostate cancer and metachronous duodenal and prostate cancer.

Conclusions: CRC in HMPS appear to be MSS. There is no apparent excess risk of extra-colonic tumours.

AAs/cancer arising on surveillance are uncommon, but patients with AAs appear to be a subgroup at particular risk for multiple polyps at CRC.

We suggest starting colonoscopy at age 25-30y, and personalizing surveillance intervals based on colonoscopy findings, especially AAs.

Conflict of Interest: sari Lieberman a speaker honorarium from AstraZeneca Company, menna hawkins: None declared, kalaikshiga kengadaran: None declared, Menachem Schechter: None declared, naim abu-freha: None declared, Ido Laish: None declared, rinat Bernstein Molho: None declared, Alon basevitch: None declared, Rakefet Chen-Shtoyerman: None declared, Elizabeth E Half: None declared, lior H Katz: None declared, Sofia Naftaly-Nathan: None declared, Gili Reznick Levi: None declared, Nadra Samra: None declared, Sharon Simchoni: None declared, Revital Bruchim: None declared, Chana Vinkler: None declared, Amit Weinstein: None declared, Rachel Gingold-Belfer: None declared, Zohar Levy: None declared, Kevin Monahan: None declared, Ian Tomlinson: None declared, Huw Thomas: None declared, Yael Goldberg: None declared, Andrew Latchford: None declared

P13.124.D Germline mutations in cancer predisposition genes in South African paediatric cancer patients

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Background: The incidence of childhood cancer incidence is increasing gradually in low-middle income countries, such as South Africa (SA). In SA it is estimated that ~ 50% of the cases do not survive longer than 5 years. NGS technologies have been key in determining the genetic contribution of germline variants in paediatric cancers.

Objective: The aim of this study was to design and evaluate a candidate gene panel of inherited cancer-predisposing genes and to provide insight into the contribution of germline variants to childhood cancer.

Methods: 64 paediatric patients diagnosed with solid tumours were included. DNA was sequenced using custom Ion Ampliseq 50 candidate gene panel. Variants were called using Ion Torrent Suite software and subsequently annotated using Ion Reporter and Ensembl's VEP. Variant prediction tools such as MutationTaster, SIFT-INDEL and VarSome were used to prioritize potential disease associated variants. Putative identified candidate variants were validated via Sanger Sequencing.

Results: The patients included in the study had a variety of cancers, the most common being nephroblastoma, osteosarcoma and astrocytoma. Analysis has identified 21 pathogenic variants. Further validation analysis is still under way. Of the confirmed variants some were known, previously reported, while some are novel.

Conclusions: We expected ~10% of our cases to harbour pathogenic germline variants and thus far we identified germline putative disease variants in ~30%. Of interest is that only 5 of the individuals had a family history suggestive of inherited cancer syndrome. This study further highlights the challenge in identifying inherited cancers syndromes in paediatric cases.

Conflict of Interest: Lindiwe Lamola Full time, Principal investigator

P13.125.A Taxane resistance and therapeutic alternatives in head and neck squamous cell carcinoma

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Background: Locally advanced head and neck squamous cell carcinoma (HNSCC) current standard treatment is chemo-radiotherapy with taxanes, cisplatin and 5-fluorouracil. However, some patients have poor prognosis due to therapy resistance. Currently, there are no response biomarkers for therapy optimisation. This study aims to characterize taxane-resistant cell lines and to identify effective alternative therapies in these cell lines.

Methodology: We generated paclitaxel-resistant cell lines from two HNSCC cell lines by gradually increasing drug concentration. We confirmed the establishment of taxane-resistant HNSCC cell lines by MTT viability assay and apoptosis assay. We characterized these cell lines by karyotyping, aCGH, expression microarrays and qPCR. Subsequently, response to different therapeutic agents, including cisplatin, 5-fluorouracil and a patent-pending antimitotic agent, was tested by MTT. Finally, response to radiotherapy was determined by apoptosis assay.

Results: We successfully established two taxane-resistant HNSCC cell lines. We found a significant *MDR1* overexpression in resistant cell lines and a pronounced decrease in *MCJ* expression, a *MDR1* negative regulator, caused by a deletion of this gene. Moreover, taxane-resistant cell lines remained sensitive to cisplatin, 5-fluorouracil and radiotherapy. Additionally, the anti-mitotic agent was effective in both parental and resistant cell lines, while having the same cellular effect as taxanes but a different mechanism of action.

Conclusions: Our results suggest the main taxane-resistance mechanism in HNSCC is *MDR1* overexpression probably caused by *MCJ* deletion. This alteration could be a taxane response biomarker in HNSCC. Furthermore, the antimitotic agent could be an effective therapeutic alternative in taxane-resistant HNSCC.

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Conflict of Interest: None declared

P13.126.B Advantages of cDNA analysis in Neurofibromatosis type 1 diagnosis

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Background/Objectives: Neurofibromatosis type 1 is caused by inactivating variants in *NF1* gene. Although clinical diagnosis can be established by medical evaluation, molecular diagnosis is of major importance to allow a proper follow up and genetic counselling. It is estimated that about 30% of *NF1* causal variants affect splicing of which 10% are not detected by conventional gDNA techniques. In this context, our aim was to implement a full-length *NF1* cDNA sequencing method to improve the detection rate of the *NF1* splicing variants.

Methods: The entire *NF1* cDNA sequence was sequenced in 8 partially overlapping amplicons to identify *NF1* splicing variants in 28 suspected NF1 patients.

Results: Direct sequencing of *NF1* cDNA allowed the detection of 3 splicing variants that would have been missed through conventional gDNA based approaches. In 2 patients, it was observed the retention of intronic sequences in the transcript, particularly the formation of a pseudoexon in intron 3, and the retention of the last portion of intron 14. In another patient, exon 6 skipping was detected, caused by an Alu insertion at the gDNA level.

Conclusion: Sequence of cDNA enables not only the detection of deep intronic causal variants but also the identification of the functional consequences of splicing variants, detected by an gDNA based approach, which in some cases is indispensable to classify them as likely pathogenic and therefore suitable for clinical actionability. These results emphasize the advantages of cDNA analysis screening as a first approach for *NF1* genetic testing in an integrated, multi-step cDNA/gDNA protocol.

Conflict of Interest: None declared

P13.127.C Genotype-phenotype correlations in retinoblastoma

JESSICA LE GALL[1;2]*, Meriam Mahmoudi[1;2], Sarah Mezghani[3], Yassine Bouchoucha[4;5], Alexandre Matet[5;6], Liesbeth Cardoen[3], Hrant Ghazelian[7], Jennifer Carrière[1;2], Nicolas Fort[1;2], Marion Gauthier-Villars[1;2], Dominique Stoppa-Lyonnet[1;5], Clément Hua[2;8], François Radvanyi[2;8], Paul Fréneaux[7], Hervé Brisse[3], Nathalie Cassoux[5;6], François Doz[4;5], Golmard Lisa[1;2]*

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Introduction: Retinoblastoma is a pediatric malignant tumor of the retina, mainly caused by *RB1* gene inactivation, with germline *RB1* pathogenic variant in 45% cases. Recently, retinoblastoma subtypes were described: subtype 1, with few tumor genetic alterations other than the initiating *RB1* inactivation, and subtype 2, more aggressive, with frequent recurrent genetic alterations. Other genetic factors were also described as retinoblastoma biomarkers, such as *MYCN* amplification. The aim of this study was to investigate associations between tumor genetic factors and histological or clinical criteria in retinoblastoma.

Methods: A cohort of 90 patients with unilateral enucleated retinoblastoma was selected. Tumor DNA was analysed with a gene panel using SureSelect XT-HS2 enrichment (Agilent) and sequencing on NextSeq 500 (Illumina). Subtypes were determined by the genomic alterations detected. Associations of genetic alterations with histological and clinical features were tested for genetic biomarkers separately and for the two subtypes.

Results: Exophytic growth was associated with subtype 1 (13[65%] vs. 5[29.4%] in subtype 1 or 2, respectively; $p = 0.031$), and vitreous invasion with subtype 2 (23[50%] vs. 34[77.2%] in subtype 1 or 2, respectively; $p = 0.008$). *MYCN* oncogene amplification was associated with earlier age at diagnosis (median[Q1-Q3] = 9.5[6.2-14.0] vs 29.7[17.6-47.9] months, for amplified [n = 6] or non-amplified *MYCN* [n = 84], respectively; $p = 0.009$).

Conclusion: This study confirmed on an independent cohort the association of subtype 2 with a more aggressive phenotype and *MYCN* amplification with lower age at diagnosis. Imaging may

help identifying additional biomarkers. Prospective studies are needed to evaluate these biomarkers as outcome predictors.

Grant: Ligue contre le cancer

Conflict of Interest: None declared

P13.128.D Molecular and clinical features of adrenocortical tumors in Beckwith-Wiedemann spectrum (BWSp)

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Background: Adrenocortical tumors (ACTs), including adrenocortical adenoma (ACA) and carcinoma (ACC), are rare in children (0.3-0.4% of pediatric tumors). Up to 50% of ACTs develop in children with a cancer predisposition syndrome, such as Beckwith-Wiedemann spectrum (BWSp). ACC is the fifth most common tumor in BWSp.

Methods: we reviewed data from one newly reported and 50 published patients with BWSp-ACT, including those with a positive molecular or clinical diagnosis (score ≥ 4).

Results: Twenty-seven ACC (52% with paternal Uniparental Disomy of chromosome 11p15.5 (patUPD11) and 19% with Imprinting Center 2 Loss-of-methylation (IC2-LoM)) and 24 adenomas (21% with patUPD11, 17% with IC2-LoM) were reviewed. Thirty-one were diagnosed after symptoms onset (15 ACA and 16 ACC, 4 metastatic) and 9 through screening (7 ACA and 2 metastatic ACC). The mean BWSp clinical score was 3.6±2.3 for ACA and 2.6±2.0 for ACC ($p < 0.01$), 16 (59%) ACC had a score of 4. Six out of 27 (22%) ACC were metastatic, two of whom had histological features classified as benign/uncertain.

Conclusion: BWSp-ACT patients mostly carried patUPD11, followed by IC2-LoM. Almost 60% of the patients reached the score for clinical diagnosis only after ACC onset, suggesting that the BWSp score has limited value for the early diagnosis in such a setting. Tumor histology did not correlate with respective clinical malignancy in two cases, highlighting limitations of the current histopathological classification. Ultrasound screening failed identifying the ACC before metastasis in two cases, indicating an urgent need to develop new strategies for screening of ACTs in BWSp.

Conflict of Interest: None declared

P13.129.A Distinct cell composition and gene expression patterns predispose an organ to develop primary cancer or metastasis

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Background/Objectives: Prevalence of primary cancers and metastases is highly variable across different organs, with some organs having preference either for primary cancers or for metastases. This raises a possibility that certain characteristics of the healthy tissue may predispose such tissue for the malignant growth. With this study we aim to determine whether the likeliness of an organ to develop either primary cancer or metastasis is linked with its basic features – cell composition and gene expression.

Methods: In this in-silico study we determined the correlations between the prevalence of primary cancers or metastases and the healthy-state cell composition or gene expression for each of the 39 organs.

Results: We identified dramatic differences in cell composition and gene expression landscape between organs susceptible to primary cancers and those susceptible to metastases. Our most striking finding was that the abundance of immune cells and high expression of immune genes had a strong positive correlation with the likeliness of an organ to host metastasis. Specifically, overexpression of interleukins, cytokines and platelet activation signalling genes were associated with the organ-specific prevalence of metastases, whereas overexpression of TNF signalling and low expression of interleukins signalling genes were associated with the prevalence of primary cancers.

Conclusion: Our results show that an organ may be predisposed to host primary cancer or metastasis due to its healthy-state cell composition and gene expression pattern, which could be the foundation for predictive and early diagnostics strategies to prevent or delay the onset of cancer.

Conflict of Interest: None declared

P13.131.C Molecular gene profiling of multiple primary neoplasms

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Background: Multiple Primary Neoplasms (MPN) are defined as more than one synchronous or metachronous cancer that occurs in the same individual. The leading causes of the disease are genetic, hormonal factors, lifestyle, environmental factors, etc. The mechanisms hidden in MPN occurrence remain unknown. The current study aims to study molecular profiling and explore the role of genetics in MPT development.

Material and Methods: FFPE samples from six patients with multiple tumors in the penis/kidney (MPN016), breast/endometrium (MPN021), ovary/GIST (MPN024), breast/colorectal (MPN030), bladder/prostate (MPN034) and colorectal/lung (MPN046). The NGS was performed with an Agilent V6 exome kit on NovaSeq 6000 platform. We used the DRAGEN somatic pipeline for alignment and variant calling. After that, we extracted the mutation signatures of analysed tumors and performed gene ontology analysis and signaling pathway analysis.

Results and Discussions: We assessed the somatic mutation spectra in the different MPNs and extracted the mutation signatures of each tumor. We found several SNPs, specific for the first tumor and absent in the second tumor and vice versa for each MPN patient. These uncommon SNPs are enriched in different biological processes, molecular functions and affect various signaling pathways.

Conclusion: This pilot study showed different profiles of somatic mutations in patients with MPN in the Bulgarian population. The biological behaviour and molecular mechanism underlying MPN were different. The obtained results will help elucidate the specific molecular genetic profiles and the influenced signal-transduction pathways and networks leading to the development of MPT.

Grant References: MES:D01-395/18.12.2020,D01-278-14.12.2022,D01-302/17.12.2021,D01-165/28.07.2022;KP-06-OPR03/1719.12.2018/NSF

Conflict of Interest: None declared

P13.132.D Toward poligenic inheritance in familial cancer

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Background/Objectives: Increasing evidence supports the thesis of a more complex inheritance in familial cancer than a simple classic Mendelian inheritance but the exact model is still elusive.

Methods: In order to address this issue, Exome Analysis (EA) was performed in 50 families in which the DNA of at least two affected members was available and in 115 unrelated affected patients. Cancers included breast, pancreas, prostate, colon and melanoma. Furthermore, we have used The Mann-Whitney U test to compare the differences between the number of rare potentially clinically relevant variants (MAF<0,01) in cancer driver genes in cancer patients (50 patients) and in controls (50 subjects).

Results: In a relevant percentage of families (30%) the pathogenic variant (P) identified in one cancer gene did not segregate with the disease. We found instead another variant classified as VUS (variant of unknown significance) in another cancer driver gene segregating with the disease. Furthermore in a significant fraction of unrelated patients (10%) we identified at least two P in different cancer driver genes in each patient. Finally, we demonstrated that cancer patients present a significant enrichment (2.8 versus 1.6) of P/VUS number in cancer driver genes compared to the control cohort ($p\text{-value} = 0{,}0008$).

Conclusion: These data suggest that cancer susceptibility is not inherited according to a single-gene Mendelian pattern but is the result of the combination of at least two genes belonging to different pathways, including different DNA repair pathways or others.

Conflict of Interest: None declared

P13.133.A Identification of germline variants in 546 breast/ovarian cancer families: Complementary testing with multigene NGS and MLPA panels

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Introduction: Hereditary breast and ovarian cancers(HBOC) occur due to germline pathogenic variants in various tumour suppressor genes, with *BRCA1* and *BRCA2* being the two most significant. Individuals with HBOC benefit from germline molecular testing, which can lead to personalised treatments, follow-up protocols, and cascade testing to identify at-risk family members.

Material&Methods: The cohort consists of 546 unrelated index cases with breast and/or ovarian cancer, referred between 2015-2022. All ovarian cancers were included regardless of age. Inclusion criteria for breast cancer were: age below 60 years, male sex, presence of family history, triple-negative, and locally advanced HER-2 negative breast cancer. Next-generation sequencing(NGS) using Illumina TrusightCancer(94 genes) was performed, followed by gene-specific or multigene(30 genes) MLPA analyses in the absence of a likely-pathogenic/pathogenic(LP/P) variant.

Results: 447(81.9%) cases had positive family history for cancer. 102(102/546;18.7%) had one or more LP/P variants detectable by panel testing, such as point mutations, small indels. The genes with the most frequent mutations were *BRCA1*(26/102;25.5%), *BRCA2*(24/102;23.5%), *ATM*(7/102;6.9%), and *CHEK2*(6/102;5.9%). Out of the remaining 444 mutation-negative cases, nine (2.0%) had large deletion/duplications(del/dups): five in *BRCA1*, two in *CHEK2*, one in *MSH2*, and *POLE* each.

Conclusion: *BRCA1/2* variants accounted for half of the LP/P variants identified in our cohort. *BRCA1* had the highest number of large del/dups, accounting for 15.2% of all *BRCA1* variants. Identification of large deletions in *CHEK2*, *MSH2* and *POLE* was only feasible by multigene MLPA testing. Our findings support multigene NGS panels followed by multigene MLPA as the gold standard for germline testing of HBOC.

Conflict of Interest: None declared

P13.134.B Do EPCAM full deletions have a role in Lynch Syndrome?

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Background/Objectives*:* Pathogenic variants of the *EPCAM* gene are associated with different clinical conditions. Loss of function variants usually cause autosomal recessive congenital tufting enteropathy, while partial deletions of the 3' exons are involved in 1%-3% of Lynch Syndrome (LS) cases. The latter association is due to a secondary effect of the *EPCAM* deletion on the function of the downstream LS gene *MSH2*, which is silenced by hypermethylation.

Methods: We studied 3 unrelated subjects with a complete *EPCAM* deletion using high-resolution oligonucleotide Array-CGH (Agilent 2x400k), SALSA MLPA® P003 *MLH1/MSH2* D1-0718 and SALSA MLPA® P072- *MSH6/MUTYH* D1-0120.

Results: The *EPCAM* deletion was ascertained incidentally in the 3 subjects, who were 74, 49, and 38 years old, respectively. The first proband underwent NGS analysis (SOPHiA® HCS panel) because of breast cancer. The second subject discovered the deletion incidentally following prenatal array-CGH. The third also performed array-CGH following the finding of a deletion in his son with intellectual disability. MLPA showed that in all cases the deletion included the upstream region of *MSH2* but didn't contain the core promoter region of the *MSH2* gene. All patients underwent colonoscopy, with negative results, and had negative cancer family history. One patient had a history of chronic diarrhea and malabsorption.

Conclusion: These findings support the notion that monoallelic deletions of the entire *EPCAM* gene are unlikely to cause LS. Further confirmation can be provided by follow-up of these patients and molecular studies of bowel tissue, in *MSH2* methylation analysis.

Conflict of Interest: None declared

P13.135.C PTEN Hamartoma Tumor Syndrome (PHTS) - genotype–phenotype correlation in Polish patients

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Background/Objectives: This is a first study aiming at characterizing mutation spectrum and genotype-phenotype correlations in PHTS Polish patients.

Methods: During the last 4 years we identified 7 patients with PHTS. They were evaluated by the same clinical geneticist. Diagnostic NCCN criteria for PHTS were used. The probability of finding *PTEN* mutation was calculated using The Cleveland Clinic Adult Clinical Scoring System.

DNA was extracted from PBL using QIASymphony QIAGEN technique. For the first 3 patients we used Illumina Platform NextSeq500, for the last 4 Ion AmpliSeq On-Demand DNA NGS Panel by Thermo Fisher. The pathogenicity of the variants was assessed according to the Recommendations from the ClinGen PTEN Expert Panel.

Results:

Conclusion: We found 7 different pathogenic/likely pathogenic mutations in our unrelated 7 patients. Unexpected diagnoses were: thyroid cancer at the age of 5 years, schwannoma, neurofibroma, lung carcinoid. Our study confirms value of the established clinical criteria and online tools for identifying PHTS. Routine head circumference assessment in hereditary cancer clinics seems to be a good recommendation.

Conflict of Interest: None declared

ID	mutation	pathogenicity	tumor spectrum	head circumference >95 percentile
07365	NM_000314.8: (PTEN)c.697C>T (p.Arg233Ter)	pathogenic	multiple intestine polyps	yes
15710	NM_000314.8(PTEN):c.182A>G (p.His61Arg)	pathogenic	Breast Cancer 48 y. Colon cancer 58 y.	yes
18299	NM_000314.8(PTEN):c.1026+1G>A	pathogenic	Thyroid cancer 5 y.	yes
19134	NM_000314.8(PTEN):c.370T>C (p.Cys124Arg)	pathogenic	Breast Cancer 53y. Endometrial and Ovarian Cancer 37y.	yes
20161	NM_000314.8(PTEN):c.802-2A>T	pathogenic	benign neurofibroma,	yes
15568	NM_000314.8(PTEN):c.70G>T (p.Asp24Tyr)	pathogenic	thyroid adenoma 19y,intestine polyps	yes
21308	NM_000314.8(PTEN):c.1111delG	likely pathogenic	Breast Cancer 52y. Lung Carcinoid 54y.	yes

P13.136.B Frequency of CHEK2 variants in a Romanian cohort of non-BRCA cancer patients

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Background: The protein encoded by CHEK2 gene (checkpoint kinase 2, OMIM #604373, cytogenetic location: 22q12.1) is a cell cycle chepoint regulator and tumor suppressor. It is activated as a response to DNA damage, preventing the cell to enter mitosis, stoping the cell cycle. Mutations in one copy of CHEK2 gene are linked to Li-Fraumeni syndrome, predisposition to breast cancer, colorectal cancer, prostate cancer, sarcomas and brain tumors.

A pathogenic variant in CHEK2 gene can, according to new studies, double the lifetime risk of breast cancer.

Methods: We have investigated 37 patients with confirmed CHEK2 variants in order to look for the most frequent variant. Our results were obtained using Next-Generation Sequencing (NGS), ILLUMINA Dragen Bio-IT platform, U.S.A., and the identified variants were compared with the reference sequence for the analyzed genes (Human Gene Mutation Database Professional hg38). For the interpretation we used the VarSeq (GoldenHelix) software.

Results: Although the number of the analyzed patients is still low at the moment, we have identified that the NM_007194.4:c.470T>C / NP_009125.1:p.Ile157Thr (I157T) / rs17879961 variant is the most frequent found in our studied group.

Conclusions: Our work provides valuable information about CHEK2 variants identified in high-risk cancer patients. We need to increase the number of patients with CHEK2 gene variants in order to establish a correlation between this variant and a high risk for breast cancer.

Conflict of Interest: None declared

P13.137.A Analysis of rare disruptive germline mutations in 2,135 enriched BRCA-negative breast cancer cases excludes additional high-impact susceptibility genes

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To better understand breast cancer susceptibility, we undertook germline whole exome sequencing (WES) using of 2,135 BRCA-negative cases of female breast cancer. Our series was highly enriched for early-onset, bilateral, and familial disease, and concomitant ovarian cancer, substantially boosting power.

We used gnomAD WES data from 51,377 ethnicity-matched controls for comparison. Parallel variant annotation, QC, per-site coverage normalisation and GATK QualByDepth calibration, were applied to reduce confounding effects from differences in datasets. Burden testing was performed on damaging variants (protein truncating, damaging missense, and ClinVar pathogenic) at MAF ≤ 0.5% for targeted gene sets (known cancer susceptibility genes, DNA repair genes, oncogenes) and exome wide, using Fisher's exact test and Bonferroni corrected significance thresholds.

Excluding known breast cancer susceptibility genes, no gene demonstrated significant association with breast cancer in any analysis after correction for multiple testing.

Our study was well powered to identify additional major high-penetrance breast cancer susceptibility genes. We had 90% power to detect a gene, should one have existed, of PALB2-like effect (odds ratio = 5) down to a population mutational frequency of 1 in 1475 (less than half that of PALB2). Multiple breast cancer susceptibility genes of extremely low mutational frequency and/or very modest effect (odds ratio ≤ 2) are likely to exist, but studies much larger than ours are required to identify them. In concert with findings of GWAS, our data support the architecture of residual inherited susceptibility to breast cancer as being highly polygenic, with limited prospect regarding existence of additional genes relevant to clinical testing.

Conflict of Interest: Chey Loveday Full, Alice Garrett Part, Philip Law Full, Diana Eccles Full, D Gareth Evans Full, Katie Snape Full, Helen Hanson Full, Richard S Houlston Full, Clare Turnbull Full

P13.139.C TPD52L1 Gene Silencing Hinders Migration and Invasion Potential of Chordoma Cells in Vitro

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Chordoma is a rare, slow growing, bone sarcoma that originates from embryonic notochord remnants. Determining the factors that contribute to the pathogenesis of chordoma is of great importance to develop new targets for treatment. Previous studies suggested the prognostic role of TPD52L1 in several cancers along with the cell proliferation colony formation and wound healing, but its role on metastasis and invasive features on chordoma has never been investigated.

In this study, differential expression profiles of tumorspheres derived from UM-Chor1 and MUG-Chor1 chordoma cell lines were determined via total transcriptome microarray. Expression of TPD52L1 gene was altered by using small interfering RNA in the chordoma cell lines, one of the common differentially regulated genes in both tumorsphere groups that identified in the transcriptomic analysis. Differential expression in tumorspheres and subsequent inhibition of gene expression in UM-Chor1 and MUG-Chor1 cell lines were validated via RT-qPCR. Downstream functional effects on migration and invasion capacity were assessed via boyden chamber assay, in vitro.

Whole transcriptomic profiling chordoma tumorspheres and parental cell lines revealed differential expression of several genes including TPD52L1 as a potential target. siRNA-mediated silencing of TPD52L1 gene expression significantly impaired both invasion and migration capacity of UM-Chor1 and MUG-Chor1 cell lines.

Our findings revealed the role of TPD52L1 in regulating invasive and migration characteristics of chordoma, in vitro. The effect of TPD52L1 on chordoma progression and therapeutic potential requires further investigation.

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Conflict of Interest: None declared

P13.142.B A rare initial presentation of Li-Fraumeni syndrome: mesothelioma

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Introduction: Li-Fraumeni Syndrome (LFS) is a rare cancer predisposition syndrome associated with germline pathogenic variants in the *TP53* gene and has high risks for various early onset malignancies. Although 10% of patients with malignant mesothelioma (MM) carry a germline variant, pathogenic *TP53* mutations are rarely reported. Here, a case diagnosed with peritoneal mesothelioma in childhood, carrying germline heterozygous *TP53* mutation, was reported.

Case Description: A 14 years old female patient was admitted to the hospital due to persistent fever despite antibiotic treatment. Personal and family history was irrelevant for a causative disease. Physical examination revealed hepatosplenomegaly. Diffuse thickening of the omentum was detected in imaging studies. After omental biopsy, the patient was diagnosed with peritoneal MM. The patient was investigated for possible cancer susceptibility

syndromes. NGS was performed using a custom panel of 36 cancer predisposing genes. A novel de novo pathogenic *TP53* (NM:000546.6;c.417_420delGACC;p.Lys139fs*30) variant was detected in peripheral blood sample of the patient and confirmed by Sanger sequencing. FISH anaylsis on paraffin-embedded tissue demonstrated monoallelic *TP53* deletion in 30% of neoplastic cells, suggesting second hit. In immunohistochemical study, mutant staining with TP53 and BAP1 nuclear positivity was observed in tumor tissue.

Discussion: MM is a very rare clinical presentation in LFS and there are only three cases previously reported in the literature. In conclusion, we report a rare association of LFS with peritoneal MM in a patient without a known asbestos exposure.

References:
PMID: 35032816
PMID: 21464421

Conflict of Interest: Can Berk Leblebici Ankara University, Sule Altıner Ankara University, Halil Gürhan Karabulut Ankara University, Ezgi Serbes Ankara University, Berna Savaş Ankara University, Emel Cabi Ünal Ankara University

P14 Genome Variation and Architecture

P14.001.A Inheritance of rare modifiers causing severe clinical phenotype in carriers of β-thalassemia; consequences for counseling

Cornelis Harteveld[1], *Ahlem Achour*[1;2], *Sandra Arkesteijn*[1], *Jeanet ter Huurne*[1], *Maaike Verschuren*[1], *Sharda Bhagwandien-Bisoen*[1], *Rianne Schaap*[1], *Linda Vijfhuizen*[1], *Hakima el-Idrissi*[1], *Tamara Koopmann*[1]

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Abstract

Background/Objectives: Beta-thalassemia Major is a severe transfusion dependent hemolytic anemia due to a severe reduction in hemoglobin synthesis. This autosomal recessive disease is caused by DNA variants in the HBB gene leading to a severely reduced or absent gene expression. As most hemoglobinopathies, beta-thalassemia shows recessive inheritance and carriers are usually clinically silent. However, a small group of β-thalassemia carriers present with features of β-thalassemia intermedia, in spite of having a single HBB gene defect, due to modifiers increasing disease severity. A variety of unexpected mechanisms were identified.

Methods: With the development of genetic tools such as Array analysis and Next Generation Sequencing in addition to functional studies at the hematologic, biochemic and genetic level, have contributed to the discovery of a number of novel chromosomal rearrangements, disease genes and mechanisms influencing the disease severity over the recent years.

Result: This study shows rare cases expressing disease mechanisms involving CNV's of segmental duplications and triplications of the entire α-globin gene cluster, mosaic partial Uniparental Isodisomy of chromosome 11p15.4 and a novel disease gene *SUPT5H* in addition to a regular HBB gene variant expressing in contrast to what is expected in carriers a severe beta-thalassemia intermedia phenotype.

Conclusion: This abstract summarizes the importance of genotype-phenotype correlation and how this may lead to the discovery of exceptional interactions causing a clinically more severe phenotype in otherwise asymptomatic carriers. Co-inheritance of modifying factors has profound implications for preconception and antenatal screening programs and genetic counselling.

Conflict of Interest: None declared

P14.002.B The relationship between gene copy number and gene expression

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Background/Objectives: Copy number variants (CNVs) are a class of structural variants that are implicated in various human phenotypes. However, the underlying mechanisms of these associations are still being explored. Gene dosage effects may contribute to the functional mechanisms of CNVs that span entire genes. This study aims to investigate the relationship between gene expression and copy number variation through an analysis of expression levels of genes spanned by CNVs.

Methods: We analysed whole genome sequencing and gene expression data from 908 individuals in the GTEx project. We identified genes with a variable copy number and performed correlation analyses to explore the relationship between gene copy number and expression levels.

Results: The relationship between gene-spanning CNVs and expression could be examined in a total of 84 genes. We observed significant correlations in the expected direction between CNV status and gene expression in 57% and 40% of genes, in the case of deletions and duplications, respectively. Interestingly, we also observed a small number of cases in which copy number and expression were strongly correlated in the opposing direction.

Conclusion: Gene dosage effects may explain some contribution of CNVs to phenotypic variation. However, our results suggest that these effects are not always consistent with expectations, suggesting that CNVs can impact gene expression through complex mechanisms beyond simple dosage effects. A potential future direction for this project is to explore whether gains in copy number for certain genes or gene sets can confer robustness to some disease phenotypes.

Grant References: Science Foundation Ireland [18/CRT/6214]

Conflict of Interest: None declared

P14.004.D Characterising HLA diversity in the Gambian Genome Variation Project

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Background: Human Leukocyte Antigens (HLA) play a vital role in immunity. Previous studies have shown that *HLA* genes are under strong selective pressure, with high levels of variation observed both between and within continental populations. However, the extent of *HLA* genetic diversity within African populations is still under-explored.

Methods: Here, we used whole-genome sequence data to explore *HLA* diversity among four ethnic groups (Mandinka, N = 99, Fula, N = 95, Jola, N = 100, and Wolof, N = 100) included in the Gambia Genome Variation Project (GGVP). We inferred *HLA* alleles using the HLA-LA algorithm and characterised their diversity. We compared *HLA* frequencies observed in GGVP to other global populations (N = 21,546).

Results: We observed a total of 476 distinct *HLA* alleles. As expected, *HLA* frequencies in the GGVP samples showed highest correlation with African populations ($R^2 = 0.49$), and lowest with East Asian populations ($R^2 = 0.05$). We also observed significant variations in *HLA* allele frequency among GGVP ethnic groups. For example, DRB1*13:02 had the highest frequency in Jola ($f = 0.21$), and lower in other ethnic groups ($f_{Mandinka} = 0.10$, $f_{Fula} = 0.06$ and $f_{Wolof} = 0.08$, Pchi-square $= 1.89 \times 10^{-5}$). Previous studies showed that DRB1*13:02 allele is associated with protection against Hepatitis B, an infectious disease with higher prevalence in West Africa. Interestingly, we saw a lower frequency of DRB1*13:02 in East Africa ($f_{Kenya} = 0.07$), and globally ($f_{EastAsian} = 0.05$, $f_{European} = 0.04$ $f_{Latino} = 0.02$, $f_{SouthAsian} = 0.03$).

Conclusion: This study examines *HLA* allele diversity in the Gambia as well as in comparison to global populations, highlighting the potential impact of population-specific *HLA* alleles on immune traits.

Grant references: KENN202109

Conflict of Interest: None declared

P14.005.A LINE-1 retrotransposon insertion in RPS6KA3 as a cause of Coffin-Lowry syndrome

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Background: Transposable elements are mobile repeated DNA sequences representing about half of human genome. LINE-1 are autonomous retrotransposons still active today, amplifying themselves via a "copy/paste" mechanism. Despite their key role in genome evolution, they can induce human disease depending on their insertion site.

Methods/Results: In a patient suspected of Coffin-Lowry syndrome (CLS), RPS6KA3 coding exons, exome and short reads genome sequencing failed to detect any causal variant. Using two different approaches, we identified the de novo insertion of a 6081-bp LINE-1 in a deep intronic position of RPS6KA3. Firstly, targeted RNAseq analysis detected an abnormal splice site in intron 10, then trio genome data at this genomic position and long-range PCR precised the causal variant. Using the LINE-1 polymorphic 5'UTR sequence, we identified a potential precursor of this event, located on chromosome X, which could have retrotransposed during maternal meiosis or at an early stage of embryonic development. This retrotransposon probably activated a cryptic splice site through its regulatory sequences by promoting the recruitment of splicing enhancer proteins. Blood mRNA molecules incorporated an intron 10 fragment and a LINE-1 fragment, resulting in a premature stop codon and a truncated protein. The absence of normal remaining mRNA molecules explained the CLS displayed by the patient.

Conclusion: RNAseq and DNAseq data integration allowed us to resolve an undiagnosed CLS by identifying the insertion and the partial exonization of a LINE-1 in RPS6KA3. Thus, the multi-omics approach is necessary to confirm the pathogenicity of these rare mutational events.

Grant: CREGEMES

Conflict of Interest: None declared

P14.006.B Impact of an intronic variant in RAD50 on RNA splicing due to inefficient branch point recognition

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Background: Intronic variants do not change a gene coding sequence but they can impact gene function via aberrant RNA splicing. The creation of a new AG dinucleotide in the AG exclusion zone of the acceptor splice site typically causes its utilization, or exon skipping. This kind of alteration was revealed in intron 15 of *RAD50* gene (NM_005732.4:c.2525-13T>A). RAD50 gene is involved in sensing and repairing DNA damage.

Methods: The computational prediction for the RAD50 variant's potential impact on RNA splicing was predicted by SpliceAI and acceptor splice site strengths were calculated using MaxEntScan. Branch-point predictions were processed by LaBranchoR. The transcript profile was determined using two-step PCR followed by capillary electrophoresis. The quantification of mutated allele ratio was performed by a minigene assay.

Results: Contrary to expectations, this variant activated two very weak cryptic splice sites within the downstream exon. We demonstrated that this unusual pattern was probably caused by weakening an authentic branch-point located 15 nucleotides upstream of the mutated position, and subsequently favouring cryptic branch-points. These cryptic branch-points supported near cryptic splice sites even though they were part of extremely weak acceptor splice sites with no polypyrimidine tract.

Conclusion: It is clear from our study that variant c.2525-13T>A disturbs correct *RAD50* splicing, which leads to the frameshift and subsequent NMD or production of nonfunctional protein. It represents a very interesting case, when authentic 3′ss usage is suppressed due to inefficient branch point recognition.

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Conflict of Interest: None declared

P14.007.C Exploring the complex spectrum of dominance and recessiveness in genetic cardiomyopathies

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Background: Mendelian disorders have classically been described as having recessive or dominant modes of inheritance. However, this is increasingly recognised as an oversimplification of the complex genotype-phenotype relationships in these diseases. Cardiomyopathies are relatively common and medically important cardiac diseases which are genetically heterogeneous and typically present in adulthood with autosomal dominant inheritance.

Objectives: Investigate the genetic landscape of recessive cardiomyopathy and explore the putative spectrum of dominance and recessiveness in cardiomyopathies.

Methods: Published genetic studies of cardiomyopathies caused by biallelic genotypes were systematically analysed. For established recessive cardiomyopathy genes, monoallelic associations with relevant cardiac phenotypes were investigated using the UK Biobank.

Results: After appraisal, we characterised 17 genes that are robustly associated with recessive cardiomyopathy based on genome-wide approaches across multiple pedigrees. The recessive cases were characterised by early age of onset (mean (SD) 12.5±15.1 years), poor outcomes (42.8% reported deceased) and predominance of dilated cardiomyopathy (11/17 genes). Studies in understudied groups, particularly bottleneck populations and regions of high consanguinity, were particularly informative for novel gene discovery. For several of the recessive genes, monoallelic variants were associated with cardiac phenotypes in the UK BioBank: *ALPK3* and *SLC30A5* with hypertrophic cardiomyopathy and *LMOD2* with dilated cardiomyopathy, the latter two are novel monoallelic associations.

Conclusions: The genetic architecture of cardiomyopathy is increasingly found to be complex. Moving beyond the dichotomous dominant/recessive paradigm enabled a more accurate understanding of the complexities of these gene-disease relationships and yielded novel genotype-phenotype associations—an approach likely to be applicable to other seemingly Mendelian diseases.

Conflict of Interest: Alex Lipov: None declared, Sean J Jurgens: None declared, Francesco Mazzarotto: None declared, Mona Allouba: None declared, James P Pirruccello: None declared, Yasmine Aguib: None declared, Massimo Gennarelli: None declared, Magdi H. Yacoub: None declared, Patrick T Ellinor P.T.E. has received sponsored research support from Bayer AG, IBM Health, Bristol Myers Squibb, and Pfizer, P.T.E. has consulted for Bayer AG, Novartis and MyoKardia, Connie R. Bezzina: None declared, Roddy Walsh: None declared

P14.008.A A systematic assessment of the impact of rare canonical splice site variants on splicing using functional and in silico methods

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Background/Objectives: Canonical splice site variants (CSSVs) are often presumed to cause a loss-of-function (LoF) and assigned very strong evidence of pathogenicity (PVS1). However, the exact nature and predictability of splicing effects of unselected rare CSSVs in blood-expressed genes are poorly understood.

Methods: A total of 199 rare CSSVs in blood-expressed but otherwise unselected genes were identified by genome sequencing in 125 individuals from 100 families, and their impact on splicing was interrogated manually in RNA sequencing (RNAseq) data. Blind to these RNAseq data, we also attempted to predict the precise impact of CSSVs using in silico tools and published guidelines.

Results: There was no evidence of a frameshift nor of reduced expression consistent with nonsense-mediated decay for 25% of CSSVs: 18% had wildtype splicing only and normal read depth, 3.5% resulted in cryptic splice site usage and in-frame indels, 3% resulted in full exon skipping (in-frame), and 0.5% resulted in full intron inclusion (in-frame). The predicted impact on splicing using (i) Alamut's Splicing Prediction Module with ClinGen Sequence Variant Interpretation Working Group's 2018 guidelines for applying PVS1 criterion, and (ii) AutoPVS1, was concordant with the RNAseq analyses for 63% and 54% of CSSVs, respectively.

Conclusion: A significant minority of CSSVs may not cause LoF, based on analysis of RNAseq data. Predictions from in silico methods were often discordant with findings from RNAseq. More caution may be warranted in applying PVS1-level evidence to CSSVs in the absence of functional data.

Conflict of Interest: None declared

P14.009.D Identifying pathogenic non-canonical splicing variants

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Background/Objectives: Proper intron recognition and removal rely on consensus sequences at the intron-exon boundaries and on branch points. Genomic variants that affect the canonical splice site in known disease-associated genes are often pathogenic. However, interpretation of variants beyond the canonical splice site (i.e, more than two base pairs upstream or downstream from the intron-exon boundary) is challenging. Numerous in-silico tools predict the effect of such variants, yet dedicated transcriptomic experiments are still required for validation.

Methods: Following informed consent, we collected several cases in which a potential non-canonical mis-splicing variant was identified in an affected individual by either exome or genome sequencing. Expression of the relevant gene was checked in control cDNA derived from three accessible tissues - whole blood, EBV-transformed lymphoblastoid cell lines (LCLs), and fibroblasts. The effect of the variant on cDNA was examined using reverse transcriptase (RT)-PCR and sequencing in the relevant tissue.

Results: Potentially disruptive variants in known disease-associated genes as well as in candidate genes were investigated. We identified cases of exon skipping as well as intronic retention. Out-of-frame insertions of intronic sequence, leading to a frameshift, were deemed likely pathogenic. In-frame insertions require functional assays to determine pathogenicity, especially when

found in a candidate gene not previously associated with human disorder.

Conclusion: Although useful, the accuracy of in silico prediction tools in foreseeing the pathogenicity of non-canonical splice site variants is limited. In cases where gene expression is restricted to inaccessible tissues, functional tools such as minigene-based assays are extremely useful.

Conflict of Interest: None declared

P14.010.B The Telomere-to-Telomere genome build reduces the proportion of ClinVar variants with mismatching gene annotation information compared to current genome builds

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Background: The Telomere-to-Telomere (T2T) Consortium aims to close known gaps in previous builds of the human reference genome. As part of their recent release, they include well-known databases lifted over to their new genome build. However, due to their re-structuring of transcripts, it is not known how consistent gene-level annotation will be for variants in known databases lifted over from older genome builds.

Methods: We examined gene-level VEP annotation information for single nucleotide variants and indels from the ClinVar database on both GRCh38 and the new T2T build. We pre-filtered the GRCh38 variants, excluding those where the VEP gene was inconsistent with the ClinVar records. We then calculated the proportion of the remaining variants with different gene IDs, different transcript IDs, and different predicted functional consequences between GRCh38 and the T2T build.

Results: One of the main causes of mismatching is inconsistency between the gene annotation from ClinVar submissions and the VEP annotated gene at that position on either build. Following quality control, approximately 0.5% of ClinVar variants had mismatching annotation information between GRCh38 and the T2T build. When a similar analysis was performed from GRCh37 to GRCh38, the proportion of variants with mismatching VEP annotation information was markedly higher than between GRCh38 and the T2T build.

Conclusion: The new T2T genome build appears to reduce the proportion of variants with inconsistent gene-information. This will allow researchers higher confidence in the fidelity of variants converted to the new build.

Conflict of Interest: None declared

P14.011.C Structural variation detection in a Black Crested Gibbon genome by single-cell template strand sequencing

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A wide variety of approaches have been developed to investigate structural variants (SVs). However, none of these allowed an exhaustive assessment of SVs due to their inherent complexity, especially for the copy-neutral variants. A NGS technology based on the creation of directional libraries, the single-cell template strand sequencing (Strand-seq), promises to revolutionize the study of SVs. This technique recognizes Watson and Crick strands in each homologous chromosome thus detecting SVs that fail to be identified with conventional approaches.

Here we used Strand-seq to integrate the complex picture of genomic rearrangements characterizing the gibbon karyotype. In detail, we sequenced 144 single cell directional libraries from the genome of a *Nomascus concolor* individual. The libraries were then mapped against the gibbon GGSC Nleu3.0/nomLeu3 reference and against the human reference genome (GRCh38/hg38) by considering each of the human–gibbon synteny blocks as a separate chromosome. A composite file was uploaded on UCSC Genome Browser (GRCh38/hg38) to manually analyze changes in the directionality of the reads.

Our analysis identified numerous small-scale simple and nested inversions that enrich the large and varied set of rearrangements of the *Nomascus Concolor* genome. These findings indicate that inversions occur frequently in primate chromosomal evolution, supporting the hypothesis that they play an important role in speciation.

Through Strand-seq it is now feasible to complete the map of SVs across the human and non-human primate genomes, providing interesting insights to reconstruct the history of their speciation and the role of SVs in human disease and genome architecture.

Conflict of Interest: None declared

P14.012.D Genome sequencing enables base-pair resolution of structural variation in known and candidate disease genes

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Objective: Exome sequencing has enabled resolution of numerous diagnostic odysseys. However, it is limited in its ability to recognize structural variation (SV). In order to identify and characterize SVs, we undertook whole genome sequencing (WGS) in exome-negative cases and in cases where previous studies suggested SVs that required confirmation.

Methods: Following informed consent, WGS was undertaken on genomic DNA. Split read analysis with subsequent PCR amplification and Sanger sequencing was used to confirm breakpoints.

Results: Low-coverage short-read WGS and breakpoint sequencing allowed for resolution of simple rearrangements in novel candidate disease genes, such as a homozygous *Alu-Alu* mediated recombination within *PHF20* in patients with syndromic developmental delay, and a de novo intragenic breakpoint in *PDS5B* mediated by nonhomologous end joining in a patient with microcephaly. *PHF20* encodes a component of the non-specific lethal (NSL) complex, which regulates gene expression through its histone acetyltransferase activity, and PDS5B encodes a cohesin-associated protein. On the contrary, short-read WGS could not resolve all the breakpoints of a complex rearrangement involving an inverted triplication in *TBC1D4*, in an individual with monogenic diabetes. This was ultimately resolved by long-read sequencing. *TBC1D4* encodes a Rab-GTPase activating protein implicated in insulin-stimulated glucose transporter 4 (GLUT4)

translocation, and has been previously implicated in susceptibility to type 2 diabetes.

Conclusions: WGS can identify SVs in exome-negative cases and can shed light on mechanisms leading associated with the formation of SVs underlying potential genomic disorders.

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P14.013.A Processed pseudogene insertion in GLB1 causes Morquio B disease by altering intronic splicing regulatory landscape

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Background/Objectives: Morquio B disease (MBD) is an ultra-rare lysosomal storage disorder associated with pathogenic variants in the *GLB1* gene. Object of this study is the unique case of "pure" MBD caused by insertion of mobile genetic element (MGE) from the class of retrotransposons.

Methods: Whole genome sequencing (WGS), mRNA analysis and experiments on co-expression of minigenes and antisense splice-modulating oligonucleotides (ASMOs) in HEK293T cells were performed to identify the MGE and characterize its effect.

Results: Analysis of patient's WGS data allowed us to identify the *NPM1* processed pseudogene insertion deep in the *GLB1* intron 5. mRNA analysis revealed 18 bp insertion of intron 5, representing a cryptic exon, located 36 bp upstream of the MGE's integration site. To unveil the underlying molecular-genetic mechanism of pathogenesis we reproduced the altered splicing pattern of patient's *GLB1* in minigene system and co-expressed a number of ASMOs targeting predicted functional motifs (exonic splicing enhancers) in MGE's sequence. We demonstrated that at least ~123 bp of proximal MGE's sequence significantly contributes to the cryptic exon activation by altering splicing regulatory landscape of the *GLB1* intron 5. We also designed ASMOs targeting the cryptic exon itself and almost completely restoring the wild-type splicing (up to 85%) in minigene system.

Conclusion: We demonstrated ultra-rare type of both mutation and molecular mechanism of pathogenesis. WGS plays a unique role in identification of such variants, while co-expression of minigenes and ASMOs provide a powerful tool for characterizing their effects and making first steps towards splice-modulating personalized genetic therapies.

Conflict of Interest: None declared

P14.014.B Benchmarking genotype accuracies of structural variant merging tools

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Increasing number of genetic studies are leveraging whole genome sequencing to discern association between disease and a spectrum of genomic variants including structural variants (SVs). Methods to genotype SVs across multiple samples from a cohort to improve accuracy have been proposed. However, SVs are often called separately in individual samples, and it is extremely challenging to merge them. While several tools have been developed to merge SVs, performance of these tools in terms of genotype accuracies has not been evaluated.

Here, we performed systematic evaluation of three SV merging tools (BCFtools, SURVIVOR, Jasmine) and one SV merging and genotyping tool (Graphtyper) and compared the results to within-family joint calls. To determine genotype accuracies, we estimated Mendelian inconsistency (MI) rates for each merging tool using short read whole genome sequencing data from UK Biobank. SVs were called using DRAGEN SV in each sample independently as well as jointly within families, and single-sample SVs were merged for each trio.

Preliminary analysis of four trios showed that the tools that perform only SV merging show very high MI rates (23.10% – 35.60%). Filtering for PASS SVs did not improve the MI rate. Within-family joint SV calling using DRAGEN SV reduced the MI rate to 9% when filtered for PASS SVs. The Graphtyper showed a low MI rate (5.45%) across all SVs, which was further reduced to 3.40% for PASS SVs. Our results suggest that merging and genotyping using Graphtyper provide highest genotype accuracy for Dragen SV calls amongst the tools tested in this study.

Conflict of Interest: Santosh Atanur Author is employee of AstraZeneca Plc, Author is share holder of AstraZeneca Plc, Katherine Smith Author is employee of AstraZeneca Plc, Author is share holder of AstraZeneca Plc

P14.015.C A transgenerational mutational signature from ionizing radiation exposure

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Effects of prolonged radiation exposure on the human germline remains a topic of medical interest. Transgenerational signatures of ionizing radiation exposure are primarily implicated to be clustered de-novo mutations (cDNMs, multiple de-novo mutations within 20bp).

We analyzed the whole genome of 1.515 offspring and their parents. Parents of 1.275 children were not exposed to ionizing radiation. Fathers of 240 children were exposed to ionizing radiation prior to conceiving their child: 110 children were born to German soldiers (Radar cohort) and 130 are offspring of liquidators (Chernobyl cohort). Dosage estimates ranged between 0-353 mSv in the Radar cohort and between 0-4,080 mSv in the Chernobyl cohort.

In total, we observed 1.475 cDNMs with a median of two cDNMs per offspring in both exposed cohorts. This was a significant increase ($p < 0.005$) in the number of cDNMs compared to age matched controls. Furthermore, we showed that the number of cDNMs increased with increasing paternal exposure to ionizing radiation ($\beta = 0.0005$, $p < 0.001$). Since error rates for cDNMs are expected to be high, we validated all cDNMs. The positive predictive value (PPV) for cDNMs is estimated at 0.24. In simulations accounting for the PPV, the difference in cDNM rates remains significant in all cases.

In conclusion, this is the largest trio cohort to date that was analyzed for the transgenerational effect of ionizing radiation. Due to its size, we were able to increase the evidence that signatures for parental exposure exist in the genome of offspring and should be characterized further.

Conflict of Interest: None declared

P14.016.D Characterizing a recent germline retrotransposition event involving POMGNT1 mRNA in the human genome

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Background: Genome evolution is an ongoing process wherein LINE-1 mediated retrotransposition of host mRNA may lead to the emergence of multiple (pseudo)genes, resulting in the continued evolution of the human genome.

Methods & Results: In this report, we describe a recent germline retrotransposition event involving POMGNT1 mRNA (1p34.1) at chromosome 15 (15q13.3), which was identified during a routine clinical whole-genome analysis unrelated to the event. Subsequently, the identical rearrangement was confirmed in the parent of the index case. Examination of the transposed element sequences revealed the entire transcript (including UTRs) NM_017739.4 had been reintroduced into the genome. We identified the specific location (chr15: 32.785.340 [GRCh37]) on chromosome 15 where retrotransposition occurred by analysing the soft-clipped sides of sequencing reads at the 5′ and 3′-ends of a potential transcript.

Conclusion: The exact time of origin of this novel (pseudo)gene cannot be determined due to the lack of genetic material from the ancestors. However, we can confirm that it was successfully passed down to at least one subsequent generation, thus representing a novel element in the evolution of the human genome.

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Conflict of Interest: None declared

P14.017.A Functional characterization of CLOCK gene SNPs using in-silico and in-vitro experimental approach

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Background/Objectives: Circadian rhythm generates rhythmic changes in behavior and physiology based on the daily light-dark cycle around 24 h. In mammals, the molecular circadian clock is controlled by two transcriptional factors, CLOCK and BMAL1, and two repressors, CRY and PER. Epidemiological studies revealed that CLOCK gene variations are associated with adverse effects in daily life and lead to diseases. Little is known about the effect of these variations at molecular level on circadian rhythm and phenotypes.

Methods: We aimed to develop in-vitro assay to investigate CLOCK gene variations. We investigated CLOCK SNPs which were selected from the Ensembl database based on their homology conservation, functional positions, and in-silico pathogenicity scores.

Results: Cell-based assays revealed that p.Leu118Arg, p.Asp119Val, and p.Phe121Cys CLOCK had reduced transactivation activity, while p.Gly120Val CLOCK had increased transactivation along with BMAL1. p.Leu118Arg, p.Asp119Val, and p.Gly120Val CLOCK variants had reduced affinity to BMAL1 while p.Phe121Cys CLOCK had increased affinity. Molecular dynamics simulations showed that despite the locations of SNPs in a row, their effects on CLOCK function are different due to their R chain positions' effects on the hydrophobicity of PASA domain.

Conclusion: Although the functional consequence of these variations with disease remains unclear, the investigation of the effects of SNPs helps us to understand the diseases related to CLOCK disruption and give insights into molecular mechanisms of the circadian clock. Using both in-silico and experimental analysis markedly improves prediction of effect of variants on the protein's structure and function.

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Conflict of Interest: Seden Nadire Efendi Student`, PhD, TÜBİTAK-KBAG 121Z862, Şeref Gül Full, TÜBİTAK-KBAG 121Z862, İbrahim Halil Kavaklı Full, TÜBİTAK-KBAG 121Z862, Ibrahim Barış Full, PI, TÜBİTAK-KBAG 121Z862

P14.018.B Accessing Human Genome Structural Variation Consortium data via EMBL-EBI resources

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Background/Objectives: Accurate identification of structural variants (SVs) is essential for genomic research and clinical variant interpretation. The Human Genome Structural Variation Consortium (HGSVC) is identifying reference sets of precisely defined SVs using modern genomic technologies. Robust methods for data discovery, sharing, and intuitive visualisation are needed to maximise the value of these data.

Methods: We have integrated HGSVC data into the International Genome Sample Resource (IGSR), a FAIR resource, and the Ensembl genome browser to enable data access and visualisation.

Results: The IGSR data portal enables filtering of HGSVC data based on population, sample, and data types to identify sets of interest. Sample information and sequence data can be easily downloaded.

The Ensembl genome browser displays SVs from specific HGSVC publications in genomic context, alongside genes and regulatory elements and SVs from other sources. To aid interpretation, individual SV pages show the predicted effect on transcripts and regulatory regions, calculated by the Ensembl Variant Effect Predictor.

Conclusion: In combination, Ensembl and IGSR facilitate discovery, access and visualisation of HGSVC data, enabling improved reuse and interpretation of these valuable data.

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from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825575 (EJP RD) and the European Molecular Biology Laboratory., S. Nakib Hossain EMBL-EBI Full time, Ensembl receives majority funding from Wellcome Trust [WT222155/Z/20/Z] with additional funding for specific project components. Research reported in this publication was supported by National Human Genome Research Institute of the National Institutes of Health under award number 2U24HG007497-05. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. 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P14.019.C Australia's largest primary ciliary dyskinesia cohort: exploring the genetic background

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Primary ciliary dyskinesia (PCD) is a genetically heterogenous, autosomal/X-linked recessive disease. ~50% PCD patients have homozygous/compound heterozygous (CompHet) pathogenic variants in one of 72 known PCD genes. Australia's largest PCD cohort to date (n = 56 unrelated; n = 4 siblings) underwent whole exome sequencing (WES), to identify causal variants using a virtual gene panel comprising known PCD and ciliopathy genes (316 genes). Filtering WES data retained good quality, nonsynonymous, rare variants (minor allele frequency <0.005). 21/60 individuals carried n = 35 rare, pathogenic/likely pathogenic/variants-of-unknown-significance in 11 PCD genes (n = 26/35 were pathogenic/likely pathogenic, n = 21/35 were loss-of-function (LOF)). Of these, n = 3 homozygotes (*RPGR*-novel, *DYX1C1* and *OFD1*-novel), and n = 17 CompHet for which n = 7 carried 1x novel and 1x previously reported variant, and n = 2 carried novel LOF variants. While *DNAH5* accounts for the greatest number of reported international cases in literature, *DNAH11* was greatest for this Australian cohort (n = 7, two were siblings). Eleven individuals carried a single rare LOF and/or pathogenic/likely pathogenic variant in a known PCD gene, but a second variant could not be identified. Expanding the filtering to include known ciliopathy genes (not associated with PCD), identified n = 21 rare, variants of

likely functional significance across nine genes in 10/39 individuals. *CFTR* was the most frequently mutated gene in this subset (n = 7 variants across n = 3 individuals) though there were no cases of previously reported biallelic *CFTR* variants. This study of an Australian PCD population identified novel/rare variants in known PCD and ciliopathy genes. Further elucidation of genes involved in disease pathogenesis may inform the development of targeted therapies.

Conflict of Interest: None declared

P14.020.D The role of start-stops elements in rare disease

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Background: 5′ untranslated regions (UTRs) may contain upstream start sites (uAUGs) which can recruit the ribosome and reduce downstream coding sequence translation. Start-stops are uAUGs that are immediately followed by a stop codon. These cause unusual scenarios where the ribosome is simultaneously initiating and terminating with the P-site occupying the start codon and the A-site occupying the stop codon. Start-stops are thought to cause ribosome pausing but little is known about them.

Aims: We aimed to systematically identify start-stops in human genes and variants that create or disrupt them in disease variant databases.

Methods: We investigated occurrence of start-stops in human genes and frequency in genes intolerant to loss-of-function (LoF). We assessed 5′UTR variants in the ClinVar and the Genomics England datasets.

Results: Start-stops occur in 5% of human genes. Genes intolerant to LoF significantly contain more start-stops than genes tolerant to LoF (6.8% vs 4.5%; $P = 8.5 \times 10^{-05}$). By shuffling 5′UTR sequences, we show that start-stop elements are depleted compared to what would be expected. In ClinVar, 16 variants create a start-stop and 11 variants disrupt native start-stops. Of these, 18 were classified as variants of unknown significance. One was classified as conflicting; this creates a start-stop 148 bps upstream of the coding sequence of *BTD*.

Conclusion: Start-stops are a little known and unexplored 5′UTR regulatory feature that can decrease downstream protein translation. When these elements are created or disrupted, this could cause or increase risk of disease.

Grant: 220134/Z/20/Z; PGL19-2/10025

Conflict of Interest: Nechama Wieder: None declared, Elston D'Souza: None declared, Nicola Whiffin Full time, Principal Investigator

P14.021.A Looking beyond coding mutations: the role of CDH1 regulatory elements in early onset gastric cancer

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Background: Missing heritability in hereditary diffuse gastric cancer (HDGC), caused mainly by *CDH1* inactivation, accounts for >50% of cases. Absent genetic diagnosis hampers disease prevention through gastrectomy and/or mastectomy offered to *CDH1* carriers. We hypothesized that defects in *CDH1*-regulatory elements contribute to HDGC missing heritability.

Methods: We called single-nucleotide/copy-number variants (SNV/CNV) from 19 HDGC probands whole-genome sequencing data, performed gene-ontology analysis, 4C-seq and ATAC-seq in normal stomach epithelia and CRISPR-Cas9, RT-PCR and flow cytometry in cell lines.

Results: No relevant coding variants were found except for a *MLH1* 2,7kb deletion. From stomach ATAC and 4C-seq data, we extracted 46249 accessible chromatin regions and 370 promoter *CDH1* interactor regions that were overlapped with 1882 rare CNVs. CNVs overlapping *CDH1* promoter interactions revealed a 39bp-intergenic deletion downstream of *CDH1* and a 20kb *CDH3* deletion. CRISPR-Cas9 mimicking each deletion (homozygous) revealed 30% and 50% *CDH1* mRNA downregulation, respectively for the intergenic region and the *CDH3* CNV, respectively. The pattern of deleted accessible chromatin regions per patient revealed a HDGC group bearing downregulated immune-associated pathways.

Conclusion: We identified two novel hypomorphic *CDH1*-regulatory regions contributing for *CDH1* downregulation that may partially explain the missing heritability in two HDGC families, and a *MLH1* deletion in a family without classical Lynch Syndrome. Gastric-specific regulatory elements within the *MLH1* CNV support the DGC bias in this family. Affecting the stomach germline-regulatory landscape may create a predisposing immune-suppressive phenotype contributing to HDGC.

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Conflict of Interest: None declared

P14.022.B Use of genome sequencing to hunt for cryptic second-hit variants: analysis of 31 cases recruited to the 100,000 Genomes Project

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United Kingdom; [12]Nottingham University Hospitals, Nottingham, United Kingdom; [13]NHS Grampian, Aberdeen, United Kingdom; [14]Radiology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom; [15]North West Thames Regional Genetics Service, Middlesex, United Kingdom; [16]Department of Radiology, Sheffield Children's Hospital, Sheffield, United Kingdom

Background/Objectives: Current clinical testing methods have inherent limitations, often leading to pathogenic variants remaining undetected. A heterogeneous cohort of 49 families were recruited to the rare-disease arm of the 100,000 Genomes Project with strong clinical suspicion for a specific autosomal-recessive disorder, but with only one suspected pathogenic variant identified through standard-of-care testing. This study aimed to identify molecular diagnoses for 39/49 cases which remained unsolved by Genomic England's clinical tiering pipeline.

Methods: For 31/39 unsolved cases, information about the 'first-hit' gene/variant was retrieved. SVRare was used to aggregate/prioritise structural variant calls. Small variants were assessed using SpliceAI and population allele frequency data. Literature searches and publicly-available online databases were used for further assessment of pathogenicity. Statistical enrichment was assessed using aggregate data for 78k individuals.

Results: Using these strategies, 8 additional cases were solved, increasing the overall diagnostic yield from 10/49 (20.4%) to 18/49 (36.7%). Exemplar cases include cystic fibrosis patient harbouring a novel exonic LINE1 insertion in CFTR in trans with p.(F508del). Another patient with generalized arterial calcification of infancy harboured interlinked duplications involving exons 2-6 of ENPP1; Bionano optical-mapping using >100kb molecules proved critical to resolving the structure and confirming the diagnosis. A deep intronic c.3874-4522A>G CFTR variant was also shown to be strongly enriched in this cohort.

Conclusion: This study highlights the importance of direct interaction between clinicians and data-analysts. Systematic investigation of cryptic variants across a multi-disease cohort with presumed autosomal-recessive disorders and prior identification of a single heterozygous variant can achieve a significant diagnostic uplift.

Conflict of Interest: None declared

P14.023.C POSTRE: a tool to predict the pathological effects of human structural variants

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Understanding the pathological impact of non-coding genetic variation is a major challenge in medical genetics. Accumulating evidences indicate that a significant fraction of genetic alterations, including structural variants (SVs), can cause human disease by altering the function of non-coding regulatory elements, such as enhancers. In the case of SVs, described pathomechanisms include changes in enhancer dosage and long-range enhancer-gene communication. However, there is still a clear gap between the need to predict and interpret the medical impact of non-coding variants, and the existence of tools to properly perform these tasks. To reduce this gap, we have developed POSTRE (Prediction Of STRuctural variant Effects), a computational tool to predict the pathogenicity of SVs implicated in a broad range of human congenital disorders. By considering disease-relevant cellular contexts, POSTRE identifies SVs with either coding or long-range pathological consequences. Furthermore, POSTRE not only identifies pathogenic SVs, but also predicts the disease-causative genes and the underlying pathological mechanism (e.g, gene deletion, enhancer disconnection, enhancer adoption, etc.). POSTRE is available at https://github.com/vicsanga/Postre.

Conflict of Interest: None declared

P14.024.D Spatial interaction affects the formation of CRISPR/Cas9-induced large deletions and inversions in the human genome

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Background: Copy number variations (CNVs) and chromosome rearrangements contribute to genetic diversity, evolution, and carcinogenesis and are, as such important for human health. Recurrent CNVs are typically formed during meiosis and driven by repetitive DNA sequences that result in an uneven crossing-over event. However, we know little about factors that drive the formation of non-recurrent CNVs, such as large deletions that are often hallmarks of carcinogenesis, including chromothripsis.

Methods: We use HiC and ChIA-PET data to identify two highly interacting and stable loci on six different chromosomes. CRISPR/Cas9-sgRNA ribonucleoproteins against these targets are used on both K562 cells and H9 human ESCs. The target regions correlate with the borders of mega-base-sized topologically associated domains (TADs) in the chromosomal landscape. DNA is extracted at different time points after nucleofection to determine repair speed and at 72h for end-point analysis. Digital droplet PCR (ddPCR) is used to quantify the resulting recombination events. Next-generation sequencing (NGS) is used to analyse the chimeric junctions.

Results: We find deletions and inversions to be significantly more frequent outcomes when breakpoints interact prior to cutting with CRISPR/Cas9. We find a tendency towards faster and less error-prone repair in deletions and inversion with prior interaction of breakpoints.

Conclusion: Spatial interaction between breakpoints is an important, yet undescribed, predictor of CRISPR/Cas9-driven large-scale deletion and inversion frequency. This finding may have implications for understanding the formation of non-recurrent CNVs.

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Conflict of Interest: Mikkel Dahl-Jessen Research Year Studen at Aarhus University, Grant References: Independent Research Fund Denmark, grant 1149-00024B (Grant holder PI Uffe Birk Jensen), Thorkild Terkelsen PhD student at Aarhus University, Grant References: Independent Research Fund Denmark, grant 9039-00337B. (Grant Holder PI Uffe Birk Jensen), Rasmus O Bak Associate Professor at Aarhus University, Uffe B Jensen Clinical Professor and Chief at Aarhus Univesity Hospital, Grant References: Independent Research Fund Denmark, grant 9039-00337B. and Grant References: Independent Research Fund Denmark, grant 1149-00024B.

P14.025.A Telomere Oxidation Status (TOS) is Correlated With Relative Telomere Length (RTL) Across Different Mouse Tissues But Not With Nicotinamide Nucleotide Transhydrogenase (Nnt) Status

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Background: Numerous studies implicate oxidative stress with TL but rarely directly assay the oxidative damage in telomeric DNA. We used a mouse model to demonstrate telomere specific oxidative stress and its effects on TL, in a range of tissues. These mice have a naturally occurring *Nnt* deficiency. Previous reports suggest that absence of *Nnt* results in high levels of cellular reactive oxygen species (ROS). We investigated whether *Nnt* deficient mice have reduced TL and of high TOS compared to wild type and rescued mice and whether this was tissue specific.

Methods: DNA was isolated from tissues (kidney, spleen, pancreas, heart, brown adipose tissue, white adipose tissue and testes) of wild type, *Nnt*-deficient and *Nnt*-rescue mice. mmQPCR was used to assess RTL and a formamidopyrimidine DNA glycosylase enzyme-based qPCR (FPG-qPCR) method, to directly determine TOS. 137 samples were analysed.

Results: Analysis of RTL and TOS revealed no significant difference ($p > 0.05$) between the mouse genotypes. However, RTL and TOS analysis comparisons between tissues, revealed significant differences between each tissue type ($p < 0.001$).

Conclusion: We have demonstrated that tissues with the shortest telomeres (in our study, the testes) showed the highest amount of oxidative damage and vice versa for tissues with the longest telomeres (kidney tissue). This work provides compelling evidence for the potential harmful relationship between oxidative stress and TL. Surprisingly, in our hands, murine *Nnt* status is not associated with TL or telomere specific oxidative stress suggesting that other ROS reducing pathways may be important with respect to TL attrition.

Conflict of Interest: None declared

P14.026.B Dominantly pathogenic cis D4Z4 repeat duplications in FSHD

richard lemmers[1], Russell Butterfield[2], Patrick van der Vliet[1], Jan De Bleecker[3], WL Van Der Pol[4], Corrie Erasmus[5], Marc D'Hooghe[6], Kristof Verhoeven[6], Judit Balog[1], Baziel van Engelen[5], Jeffrey Statland[7], Enrico Bugiardini[8], Nienke van der Stoep[1], Evangelista Teresinha[9], Chiara Marini Bettolo[10], Peter Van den Bergh[11], Rabi Tawil[12], Nicol Voermans[5], John Vissing[13], Bob Weiss[2], ***Silvère M. van der Maarel***[1]

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Facioscapulohumeral dystrophy (FSHD) is caused by partial chromatin relaxation of the D4Z4 macrosatellite repeat on 4qter. This facilitates inappropriate myogenic expression of the transcription factor DUX4, a retrogene located within D4Z4. The D4Z4 repeat ranges between 8-100 units (U) in the population and in FSHD, D4Z4 chromatin relaxation is mostly caused by a repeat contraction to 1-10U (FSHD1) or by a digenic mechanism requiring pathogenic variants in D4Z4 chromatin modifiers, most often SMCHD1, combined with a D4Z4 repeat of 8-20U (FSHD2).

Recently, FSHD2 patients were identified with an SMCHD1 mutation and DUX4 expression originating from a D4Z4 cis duplication allele encompassing two D4Z4 arrays with a spacer in between. However, there is inconsistent evidence for the necessity of a SMCHD1 mutation for these cis-duplication alleles to become pathogenic as independently affected D4Z4 cis-duplication individuals were identified without SMCHD1 mutation.

To address the pathogenic nature of these alleles we compared cis duplication alleles that are dominantly pathogenic ($n = 9$) with those that become only pathogenic in combination with an SMCHD1 mutation ($n = 9$). For both groups we showed duplication-allele-specific DUX4 expression. We studied these alleles in detail using Southern blotting, molecular combing and optical mapping, emphasizing the challenges in the characterization of these rearrangements. Nanopore sequencing was instrumental to study the methylation of the duplicated D4Z4 repeats and to identify the breakpoints and the spacer sequence between the repeats By comparing the repeat composition of cis duplication alleles in both groups we uncovered the criteria that determine their pathogenicity.

Conflict of Interest: richard lemmers: None declared, Russell Butterfield: None declared, Patrick van der Vliet: None declared, Jan De Bleecker: None declared, WL Van Der Pol: None declared, Corrie Erasmus full, Marc D'Hooghe: None declared, Kristof Verhoeven: None declared, Judit Balog: None declared, Baziel van Engelen: None declared, Jeffrey Statland: None declared, Enrico Bugiardini: None declared, Nienke van der Stoep: None declared, Evangelista Teresinha: None declared, Chiara Marini Bettolo: None declared, Peter Van den Bergh: None declared, Rabi Tawil: None declared, Nicol Voermans: None declared, John Vissing: None declared, Bob Weiss: None declared, Silvère M. van der Maarel: None declared

P14.027.C Detection of chromosomal aberrations by next generation sequencing (NGS): possibilities, challenges and limitations

Claudia Funke[1;2], *Christine Froehlich*[1;2], *Martin Schulze*[1;2], *Heinz Gabriel*[1;2], *Florian Battke*[1;2], *Saskia Biskup*[1;2]

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Background/Objectives: In the past, the detection of structural chromosomal aberrations was limited to classical karyotyping, FISH analysis and microarray analysis. Nowadays, copy number variations (CNVs) can also be reliably detected using next-generation sequencing (NGS) and in some cases even structural events can be identified. This advancement in NGS data analysis increases the diagnostic yield in exome diagnostics.

Methods: Diagnostic exome sequencing was performed using the Illumina NovaSeq6000 system. Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth). CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage.

Results: We present different examples, which underline the power of our NGS-based CNV-calling approach. We are able to detect copy number imbalances, which are indicative for structural chromosomal aberrations such as marker chromosomes or ring chromosomes. Moreover, the simultaneous analysis of CNVs and SNVs enables the diagnostic detection of pathogenic CNVs and SNVs within the same gene responsible for autosomal recessive disorders. Nevertheless, structural chromosomal aberrations have to be still confirmed by karyotyping, FISH analysis or optical genome mapping.

Conclusion: The method is limited in detecting balanced complex rearrangements, uniparental disomy, or low-level mosaicism. Deletions and duplications indicative of large structural chromosomal aberrations but also small events, such as single exon CNVs, can be detected using high quality NGS data.

Therefore, exome sequencing should be considered as the first diagnostic step for patients with critical conditions.

Conflict of Interest: Claudia Funke part-time, Christine Froehlich full-time, Martin Schulze full-time, Heinz Gabriel full-time, Florian Battke full-time, Saskia Biskup full-time

P14.028.D Direct long-read RNA sequencing uncovers functional genetic variation affecting transcripts expression

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Background: Our knowledge about how an individual's genetic background may affect the splicing process is limited due to the lack of full transcript measurements.

Methods: To directly measure the impact of genetic variation on transcript abundance, we produced long-read native RNA-seq data for 60 genetically different lymphoblastoid cell lines (LCLs) from the GEUVADIS project.

Results: We identified 11,929 genes expressed in >50% of the samples. Of the 44,993 transcripts identified, 61% were novel, of which 14% were expressed in all samples, while that was true for 35% of the annotated. A genome-wide QTL analysis identified 105 variants associated with specific transcripts (trQTLs; FDR 5%) and 34 associated with total expression of the gene (eQTLs). As many as 92 trQTLs were not identified as eQTLs using a larger published short read dataset of LCLs (317 samples). Genes with eQTLs had a significantly lower number of annotated transcripts than genes with trQTLs (p-value = 5.23e-05), suggesting that eQTLs were missed on genes with higher transcript diversity. Conditional analysis identified 52 secondary trQTLs with 57.7% showing an opposite direction of effects on different transcripts, explaining the inability to detect eQTL. For example, rs4796398 was significantly associated with 5 of the transcripts for *EIF5A1*, while no eQTLs was identified with long or short-read data.

Conclusion: Overall, we identified new trQTLs whose effect on expression was missed using short-read technology. Current work aims to identify RNA modifications and genetic factors associated to them.

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P14.030.B Mosaicism in germline NGS diagnostics - Findings from routine diagnostics

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Background: Postzygotic mosaic variants are a common cause of genetic disease. Although next-generation sequencing (NGS) has improved mosaic detection, molecular genetic diagnostics encounters several challenges when dealing with mosaic variants. Crucial factors are the choice of tissue, the application of appropriate enrichment and sequencing technologies, creating a suitable bioinformatic pipeline to detect potential low-grade mosaicism, and the appropriate interpretation of mosaic variants with regard to a patient's symptoms.

Methods: Exome sequencing using an inhouse customized enrichment kit, which includes also clinically relevant non-coding regions (CeGaT ExomXtra®) was performed on patient´s DNA using the Illumina NovaSeq6000 system. NGS data were analysed using an in-house bioinformatic pipeline.

Results: Over the last few years, our team has identified many diagnostically relevant mosaic variants through germline panel and exome diagnostics, and we present a selection of these variants here. The cases cover a large spectrum of genetic diseases. We have identified mosaic variants for disorders that are classically associated with mosaicism, as well as diseases for which mosaic variants have not yet been described. We also present a mosaic variant, which is consistent with a somatic rescue mechanism, in a patient affected by *IL2RG*-associated X-linked combined immunodeficiency.

Conclusion: Given the large spectrum of somatic variants that are detected through NGS, mosaic variants should be routinely considered in molecular genetic diagnostics, and not only for indications that are classically associated with mosaicism. Correct interpretation of mosaic variants remains challenging but is of utmost importance for the patient.

Conflict of Interest: None declared

P14.031.C Resolving ring chromosomes with long read sequencing

Kristine Bilgrav Saether[1], *Marlene Ek[1;2], Anna Lindstrand[1;2], Jesper Eisfeldt[1;2;3]*

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Ring chromosomes are circular DNA molecules whose ends have fused together to form a ring. Often, ring chromosomes form due to double-stranded breaks occurring on each chromosome arm, followed by fusion. Such events can lead to loss or silencing of genetic material, which is associated with clinical phenotype. Telomeres, which are often involved in ring formation, are regulated by epigenetic marks that can spread and repress gene expression up to several kb from the telomere[1]. DNA methylation is a key epigenetic mark that influence gene expression, where methylation of gene promoters is known to downregulate their expression. Long read whole genome sequencing (LR WGS) has recently emerged as a more precise way to resolve structural variants[2], while also providing DNA methylation information. Herein, we have studied derivative ring chromosome structures as well as methylation patterns in 8 rings involving chromosomes 5, 7, 8, 9, 10, 13, 20, 21. Overall, we were able to perform a characterization of the ring chromosomes, pinpointing exact breakpoints. Finally, by comparing the methylation patterns in the rings with a normal methylation in-house database from 30 individuals, we observed specific epigenetic patterns associated with ring formation. In conclusion, our LR WGS analysis allowed for a comprehensive multiomic analysis of ring chromosomes, increasing our understanding of ring chromosome formation.

References: Peron, A. *et. al*. https://doi.org/10.3389/fneur.2020.613035 Schuy, J. *et. al*. https://doi.org/10.1016/j.tig.2022.06.003

Grants:

Conflict of Interest: Kristine Bilgrav Saether: None declared, Marlene Ek: None declared, Anna Lindstrand Illumina Honoraria, Consultant for Oxford Nanopore Technologies and Pacific Biosciences, Jesper Eisfeldt: None declared

P15 Cytogenetics

P15.001.A Improving the accuracy of reporting diagnostic genomic tests

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Background/Objectives: Describing cytogenomic abnormalities accurately can be challenging, requiring an understanding of both the nomenclature and aetiology of chromosomal structural variation.

In order to clearly communicate abnormal genomic results to clinicians and accurately record them in databases, a standardised nomenclature is required that transcends language barriers. Human Genome Variation Society (HGVS) nomenclature describes genomic abnormalities at a nucleotide level while the International Standard for Cytogenomic Nomenclature (ISCN) describes structural and copy number variation detected using cytogenetic and molecular cytogenomic techniques.

Methods: GenQA is a global accredited external quality assessment (EQA) provider that delivers EQA and offers training and competence programmes when unmet training needs are identified. Since 2021 the ISCN EQA has provided exercises for reporting ISCN for karyotyping, FISH, arrays and region-specific results.

Results: The EQAs have shown wide variation in the application of ISCN when reporting diagnostic results, many of which would result in incorrect patient management. Common misconceptions and misinterpretation of the ISCN will be presented.

This presentation will also discuss the tools, webinars and EQAs available that laboratories can use to train staff or to check their competence on a laboratory or individual basis. Use of these resources results in an improvement in genomic reporting that benefits both the clinician and the clinical management of the patient.

Conclusion: There is a need for more EQAs with supporting training programmes enabling centres to understand the aetiology of structural chromosomal variation within the context of the patient referral and the application of variant classification and ISCN systems.

Conflict of Interest: Ros Hastings NHS Lothian, Melody Tabiner OUH NHS Foundation Trust, Mark Sales NHS Lothian, Fiona Morgan NHS Lothian, Zandra Deans NHS Lothian

P15.002.B FISH or Chip: what is the preferred test after detection of a pathogenic copy number variation in the family?

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Background/Objectives: When detecting a pathogenic copy number variation (CNV), parental testing is recommended to determine inheritance, for recurrence estimation and family planning options. Parental testing can be done using either Fluorescent in-situ hybridization (FISH) or Chromosomal microarray analysis (CMA). FISH is capable of detecting structural variants (SV), but is more labour intensive than CMA and requires specific probes for each finding. Little is known about the prevalence of balanced SVs in families harboring a pathogenic CNV.

The purpose of the study is to establish the preferred method of testing family members, both for analysis and the ensuing genetic counselling aspects.

Methods: 10-year retrospective (01/2013-01/2023) analysis of in-house 85 parental FISH tests consisting of 46 cases, in post (28 cases) and prenatal (18 cases) settings.

Results: In 12 of the 46 families the pathogenic CNV was due to the presence of a chromosomal finding in a parent. Eight CNVs were inherited, four (4/46, ~9%) originated from a SV in a parent (two balanced translocations, one inversion and one complexed SV).

Conclusion: Although CMA has become the preferred method for CNV testing, with a short turn-around-time and a higher-resolution whole genome view, it would have missed all SVs detected in our cohort. Further, in ~10% of CMA tests, incidental findings (pathogenic and variants of uncertain clinical significance) are detected. However, FISH allows the detection of SVs as well as low-level mosaicism, enabling correct genetic counseling. In addition, the anxiety caused by incidental findings, especially during pregnancy, can be avoided.

Conflict of Interest: None declared

P15.003.C Apparently benign cryptic complexity in an affected carrier of a de novo translocation

Constantia Aristidou[1], *Maria Paraskevopoulou*[1], *LUDMILA KOUSOULIDOU*[1], *athina theodosiou*[1], *Mana M. Mehrjouy*[2], *Violetta Anastasiadou*[3], *Georgios Tanteles*[4], *Niels Tommerup*[2], *carolina sismani*[1]

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Background/Objectives: The majority of apparently balanced translocation (ABT) carriers are phenotypically normal. However, reproductive complications are often reported, while a ~27% morbidity risk has been estimated in de novo ABT carriers due to dosage-sensitive gene disruption, cryptic complexity, and long-range position effect. Therefore, detailed breakpoint mapping is essential to identify causal genes, underlying molecular mechanisms, genotype-phenotype correlations, and accurate reproductive risks.

Methods: We report a follow-up of a prenatal case with right-sided aortic arch and a de novo ABT [46,XX,t(6;19)(q13;p13.3)dn]. Postnatally, whole-genome mate-pair sequencing (WG-MPS), followed by Sanger sequencing, were performed to further characterize this rearrangement.

Results: WG-MPS mapped one breakpoint on der(19) and at least five on der(6). The resulting fragments were repositioned in a random order and orientation, while a ~6.5kb chr19 segment was apparently deleted. Microhomology and small indels were identified at the breakpoints, while no causal genes were found within the disrupted topologically-associated domains.

Conclusion: Our study successfully applied a combination of WG-MPS and Sanger sequencing to reveal cryptic complexity in a two-way reciprocal ABT. The breakpoint signatures suggested non-homologous end joining as the mechanism underlying this chromothripsis event. Preliminary WG-MPS results did not provide an obvious cause for the patient's phenotypes; thus, further investigation is needed, including whole-exome sequencing, to decipher the aetiology of right-sided aortic arch. The identified cryptic complexity provides useful insights into the meiotic segregation of derivative chromosomes and the associated increased reproductive risk, therefore appropriate genetic counselling is recommended.

Conflict of Interest: Constantia Aristidou: None declared, Maria Paraskevopoulou: None declared, LUDMILA KOUSOULIDOU: None declared, athina theodosiou: None declared, Mana M. Mehrjouy: None declared, Violetta Anastasiadou: None declared, Georgios Tanteles: None declared, Niels Tommerup Supported 2015-2020 - Independent Research Fund Denmark Grant to International Breakpoint Mapping Consortium Project (NT), carolina sismani: None declared

P15.004.D 1q duplications due to inherited translocations reveal phenotypic similarities

Priscila Soares Rodrigues[1], *Bruna Burssed*[1], *Vera Ayres Meloni*[1], *Ana Beatriz Alvarez Perez*[1], *Marco Antonio Ramos*[1], *Fernanda Teixeira Bellucco*[1], *Maria Isabel Melaragno*[1]

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Background: Duplication of distal regions of the long arm of chromosome 1 cause different phenotypic features, including growth retardation, dysmorphisms, congenital malformations, heart defects, and intellectual disability. 1q partial trisomy generally involves partial monosomy of another chromosome segment due to unbalanced translocations, causing a wide clinical variability and hampering phenotype characterization.

Methods: We described four patients (P1-P4) with chromosome 1 duplications characterized by karyotype and chromosome microarray.

Results: Unbalanced translocations inherited from balanced rearrangements present in the parents were found: P1: 46,XX,der(4)t(1;4)(q41;q34.2)dmat, P2: 46,XX,der(4)t(1;4)(q41;q34.3)dmat, P3: 46,XY,der(18)t(1;18)(q32.1;q23)dmat, P4: 46,XY,der(11)t(1;11)(q32.3;q25)dpat. The 1q41q44 region is involved in the rearrangements of P1 and P2, which share phenotypic characteristics, including congenital anomalies, heart defects, neuropsychomotor developmental delay, and intellectual disability. P3 and P4 had different breakpoints in 1q32.1 and 1q32.3, and both present pulmonary stenosis, dysmorphisms, and ocular abnormalities. The 1q32.1 breakpoint region involves a segmental duplication present also in chromosome Y and a SINE repetition element, as does the breakpoint for P2. Also, the breakpoints revealed that there is no concordance between A and B compartments.

Conclusion: The phenotype-genotype correlation is hampered by the variable size of the involved segments and the associated partial monosomy, which causes more deleterious effects and is more compatible with the phenotype than the 1q duplication. We conclude that the patient's phenotypic variability was caused mainly by the concomitant imbalances and different breakpoints, but the four patients still share the described main phenotypic features.

Grant Reference: Financial support: São Paulo Research Foundation (FAPESP), Brazil.

Conflict of Interest: None declared

P15.005.A Complex rearrangement involving a three-way balanced translocation detected by optical genome mapping

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Background: Complex chromosomal rearrangements are defined as structural genome variations that involve at least three breakpoints in one or more chromosomes and result in exchanges of chromosomal segments. These rearrangements may be de novo or inherited from a parent, and different phenotypic consequences must be present.

Methods: We studied a 4-year-old female patient with neuropsychomotor developmental delay, hypotonia, and speech delay, through karyotype and chromosome microarray. In order to investigate genetic alterations not precisely described by the previous techniques, we performed optical genome mapping (OGM), which is a high-resolution technique that can detect structural chromosome alterations.

Results: Karyotype analysis showed a possible alteration on chromosome 12 while array analysis revealed a copy number variation in chromosome 4q. OGM revealed a complex rearrangement: a three-way balanced translocation between chromosomes 12, 14, and 21, an inversion in chromosome 12, and a 2.3 Mb chromosome 4q deletion.

Conclusion: The use of a high-resolution technique such as optical genome mapping allowed for the resolution of the patient's balanced complex rearrangement that could not be detected by karyotype and chromosomal microarray. The rearrangement appears to be balanced and the determination of the breakpoints at nucleotide level may indicate the most probable pathogenic factor in order to perform a genotype-phenotype correlation.

Grant Reference: Financial support: São Paulo Research Foundation (FAPESP), Brazil.

Conflict of Interest: None declared

P15.007.C Mutations in splicing factor genes and their diagnostic impact on patients with myelodysplastic syndromes (MDS)

Tatiana Aghova[1], *Halka Lhotska*[1], *Karla Svobodova*[1], *Lucie Hodanova*[1], *Libuse Lizcova*[1], *Lenka Pavlistova*[1], *Karolina Luksikova*[1], *Slavka Sotakova*[2], *Lubomir Minarik*[2;3], *Tomas Stopka*[2;3], *Anna Jonasova*[2], *Zuzana Zemanova*[1]

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Background: The splicing factor genes mutations (SF3B1, SRSF2, U2AF1, ZRSR2) are found in 60% of patients with myelodysplastic syndromes (MDS) and lead to the accumulation of R-loops and associated DNA damage, resulting in activation of the ATR pathway in affected cells. We assessed the frequency and prognostic value of splicing genes mutations and their association with karyotype and mutations of other genes.

Methods: We examined 243 MDS patients (40% normal karyotype, 21% isolated del(5q), 30% complex karyotype) with a combination of cytogenomic methods (G-banding, I-FISH, mFISH/mBAND, aCGH/SNP) and next-generation sequencing (NGS) using the Archer Myeloid VariantPlex gene panel (Invitae) which allowed us to sequence 75 genes associated with myeloid malignancies.

Results: We detected mutations in splicing factor genes in 35% of patients. The most frequently mutated were SF3B1 (20%), SRSF2 (9%), U2AF1 (4%) and ZRSR2 (1,6%). The SF3B1 and U2AF1 mutations were mostly associated with complex karyotypes (10,2% and 30%, respectively). SRSF2 mutations were often found together with del(7q) (13,6%) and ZRSR2 with trisomy 8 (25%). The splicing genes mutations were often co-occurred with ASXL1, DNMT3A, RUNX1, STAG2 and TET2 mutations.

Conclusion: The identification of splicing genes mutations is an important part of diagnostic of MDS. Mutations in the SF3B1 gene have in general more favorable prognostic score, according to IPSS-M, compared to SRSF2 or U2AF1. However, the prognostic significance of individual variants may differ. NGS may contribute to a more accurate diagnosis and to the development of new therapeutic approaches targeted directly at the mutated genes.

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Conflict of Interest: None declared

P15.008.D Long-read genome sequencing required to resolve complex chromosomal rearrangements involving both the short and the long arm of chromosome 21

Jakob Schuy[1], *Kristine Bilgrav Saether*[2;3], *Jasmin Lisfeld*[4], *Marlene Ek*[1;5], *Christopher M. Grochowski*[6], *Susanne Rudolph*[7], *Sigrid Fuchs*[4], *Kornelia Neveling*[8], *Maja Hempel*[7], *Alexander Hoischen*[8;9], *Alex Hastie*[10], *Claudia Carvalho*[6;11], *Jesper Eisfeldt*[2;3;5], *Anna Lindstrand*[3;5]

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Chromosome (chr) 21, although the smallest human chromosome, is highly relevant in disease, with trisomy 21 (Down syndrome) being the most common chromosomal anomaly in humans. In addition, cases with rare structural variants (SVs) of chr 21 have been reported. Such events vary in size and include gross chromosomal events such as ring chromosomes as well as small partial aneuploidies (deletions or duplications). Chromosome 21 is an acrocentric chromosome devoiced of reference genomic sequence of the short arm until the recent T2T release, which often hampered our ability to investigate mechanism of formation of pathogenic SVs. We hypothesize that repeats play an important role in the formation of complex SVs involving chromosome 21.

Here, we describe three cases with complex SVs on chr 21. Utilizing a combination of short-read genome sequencing (SR-GS), long-read GS and optical genome mapping (OGM), we were able to resolve 100% of BPJs (7, 11 and 26 BPJs respectively). Importantly, by using the T2T assembly on integrated multiomics data, we narrowed down the p- and q-arm breakpoint of a ring chromosome 21 forming the full ring derivative. In all three cases, we found satellite DNA at several junctions suggesting an important role in the mutational process. By comparing the here identified BPJs with cases from the literature, we pinpoint regions on the q-arm which are more likely to be involved in chr21 rearrangements. Taken together, our results give further insights into the architecture and underlying mechanisms of complex rearrangements on acrocentric chromosomes.

Conflict of Interest: Jakob Schuy: None declared, Kristine Bilgrav Saether: None declared, Jasmin Lisfeld: None declared, Marlene Ek: None declared, Christopher M. Grochowski: None declared, Susanne Rudolph: None declared, Sigrid Fuchs: None declared, Kornelia Neveling: None declared, Maja Hempel: None declared, Alexander Hoischen Salaried employee of Bionano Genomics Inc, Alex Hastie Salaried employee at Bionano Genomics Inc, Claudia Carvalho: None declared, Jesper Eisfeldt: None declared, Anna Lindstrand - Oxford Nanopore

- **PacBio**
- **Illumina**

P16 New Technologies and Approaches

P16.001.A Clinical validation of optical genome mapping (OGM) for constitutional structural variation (SV) detection

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Background/Objectives: SVs are a major source of human genetic variation and a frequent cause of geneticdisorders. Conventional and molecular cytogenetic approaches including karyotype, FISHand chromosomal microarray analysis(CMA) have been the gold standard for SV detection,albeit with limitations. OGM is an emerging technology that can, in theory, detect allclasses of SVs with high resolution, sensitivity, and specificity, while providing location and orientation information for aberrant segments. Here we present preliminary data on the ability of OGM to detect known constitutional SVs.

Methods: 5 patients with known aberrations ranging from small CNVs to complex rearrangements and various phenotypic features were included in the study. Ultra-high-molecular-weight DNA was extracted from peripheral blood samples, labeled and imaged with the Saphyr instrument following the manufacturer's instructions (Bionano Genomics Inc.). From the data obtained, a de novo genome assembly was performed on which SV and CNV detection algorithms were applied, followed by annotation against hg19 (Bionano Access v.1.7.1.1). Results were compared to previous testing.

Results: A 100% concordance to karyotype and CMA was observed for the first three patients for which results have been obtained (2 complex rearrangements, 2 deletions and 1 duplication).

Conclusion: The data presented herein suggest that OGM is a promising tool for the detection of all typesof SVs that overcomes most limitations of existing cytogenetic approaches. Validation in

alarger set of clinical samples is needed before a routine application is considered.

Grant References: Part of the reagent costs for this study was covered by Bionano Genomics.

Conflict of Interest: None declared

P16.002.B A multi-site cohort analysis of telemedicine and in-person visits during the COVID-19 pandemic in clinical genomics practice

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Introduction: COVID-19 pandemic has led to adoption of telemedicine by genomic practices.

We aimed to study and compare patient experience data from April 2020- December 2022 between traditional clinical genomics practice and tele genomics practice at Mayo clinic three sites.

Methods: We utilized a standard version of the Press Ganey® outpatient medical practice survey. Questions were focused on patient perceptions of access, moving through the visit, nurse/assistant, care provider, personnel issues, technology, and their overall assessment and requests. Responses were measured using a Likert scale ranging from very poor (1) to very good (5).

Results: A total of 2036 visit surveys were completed at all three sites in this time period. Of these 876 were for person visits while 1160 were for tele visits.

Overall satisfaction, measured by likelihood to recommend the practice in top box score, was found to be equal between in-person visits and all telemedicine visits.

Patients rated access and easy of scheduling higher in tele visits (70.5%) vs face to face visits (71%). No differences were found in care provided by the team moving through the visit, nurse/assistant, care provider, personnel issues and their overall assessment and requests.

Conclusion: Patient experience with telemedicine is comparable to traditional, face-to-face visits. Despite the inherent differences, top level patient experience is attainable with telemedicine. Telemedicine offers opportunities for enhanced access to Genomic Medicine, including to those in areas with fewer trained providers.

Conflict of Interest: None declared

P16.003.C Low-coverage genotyping-by-sequencing with accurate long HiFi reads and optimized imputation

Michael Eberle[1], ***George Busby***[2], *Jen Kintzle*[3], *Paolo Di Domenico*[4], *Gregory Conception*[5], *Geoff Henno*[1], *Giordano Bottà*[6]

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Background/Objectives: PacBio's HiFi technology generates highly accurate long reads (~15-20 kb) from native DNA, allowing to sequence the most challenging regions of the genome and provide direct information about chromosomal phasing. Our objective is to achieve high-quality genotyping calls leveraging imputation from low coverage (<5x) HiFi data and outperform current sequencing/array-based methods.

Methods: We used sequencing data from 43 samples with diverse genetic ancestries as part of the Human Pangenome Project and the Genome in a Bottle Initiative. The full sequencing files from Illumina and PacBio were down sampled to a range of coverages from 0.1x to 30x and compared. To generate genotypes with low coverage (<5x) long reads, we developed a new and optimized pipeline for variant calling and imputation. The resulting genotypes were used to assess the quality of imputation at different coverage levels, PRS accuracy and small variant detection.

Results: At matched low coverages, PacBio genomes capture a significantly larger amount of highly concordant SNPs and Indels. This finding led us to observe higher imputation accuracies (r^2) with smaller confidence intervals across all low coverages and samples. We also observed high concordance when cancer PRS scores were applied to the resulting genotypes and compared to a "gold standard PRS", calculated at 30x coverage.

Conclusion: Low-coverage HiFi data, leveraging haplotype phasing and accurate base calling, allows for improved genotyping and variant discovery across diverse ancestries, resulting in more accurate PRS calculations. This provides a new and improved sequencing-based genotyping solution for research, population genetics and health screening.

Conflict of Interest: Michael Eberle PacBio, PacBio, George Busby: None declared, Jen Kintzle: None declared, Paolo Di Domenico: None declared, Gregory Conception PacBio stock, Geoff Henno PacBio and Illumina stock, Giordano Bottà: None declared

P16.004.D Inferring microsatellite mutation rates from allele frequencies obtained from (non-)isothermal DNA amplification approaches using thirty-two genetic models

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Microsatellites, also known as Short Tandem Repeats (STRs), are sequences composed of 1 to 6 nucleotides repeated in tandem. Their high degree of polymorphism is due to the "Polymerase Slippage" mechanism, which allows the gain or loss of repetitions during DNA replication. In our study, we aimed to estimate microsatellite mutation rates by analyzing allele frequencies obtained from in vitro PCR and iso-thermal RPA (recombinase polymerase amplification) experiments and to identify the underlying genetic model for polymerase slippage.

We developed a method that infers microsatellite mutation rates using 32 genetic models. This model differs in the relationship between mutation rates and microsatellite allele length, as well as the number of steps allowed to be inserted or deleted during a slippage event. Our approach is based on the minimization criteria of the RMSE (Root Mean Square Error) between observed and simulated microsatellite allele frequencies using the Simulated Annealing algorithm (a stochastic optimization algorithm) combined to a Grid Search, to infer mutation rates and identify the genetic model best explaining the observed microsatellite mutation profiles.

Our method was successfully tested on experimental data generated with PCR and RPA experiments using 4 synthetic mono-nucleotide repeat microsatellites (A15, A19, A20 and A24) and 4 di-nucleotide repeat microsatellites (AC15, AC19, AC20 and AC24). This method should be a valuable tool for the study of microsatellite mutational mechanism.

Conflict of Interest: None declared

P16.005.A Long read revealed a complex structure of an extra dic(21;21) chromosome of a patient and its biological changes

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Complex congenital chromosomal abnormalities are rare but often cause adverse phenotypes. However, their structures and biological impacts has seldom analyzed at the molecular level. We have previously reported a patient with severe developmental defects (Takano et al. EJMG (2019)). The patient had an extra dic(21;21) chromosome, which has two centromeres and an oscillation of copy numbers. In this study, we used whole genomic, transcriptional and methylome analysis, coupled with novel bioinformatic approaches, to reveal the complex structure of the extra chromosome and its transcriptional and epigenetic changes. Long-read sequencing revealed accurate structures of junctions of copy number changes in the extra chr21 and suggested that chromothripsis caused the complex structural changes. Allele-specific transcriptome analysis revealed an overexpression of genes in the extra chr21. Allele-specific methylome analysis using long reads suggested that the centromeric region of the extra chr21 was hypermethylated, which may cause inactivation of a centromere in the extra chromosome. Our comprehensive analysis provides implications into molecular mechanism leading to the extra chromosome and its biological impact to the patient.

Conflict of Interest: None declared

P16.006.B Fully automated DNA extraction solution from stabilized saliva using Omega Bio-tek's reagents

Massimiliano Memo[1], *Stefan Schmidt*[1], *Claire McClain*[1], *Julie Baggs*[1], *Travis Butts*[1], *Kiranmai Durvasula*[1]

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Background/Objectives: One of the biggest challenges facing scientists is the difficulty of sample collection, transportation, and other logistics for nucleic acid extraction to obtain high quality DNA. For a non-invasive collection method, saliva is an excellent biospecimen for molecular analyses, supporting applications such as diagnostics, pharmacogenomics, and biomarker discovery.

Methods: Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399) offers a magnetic bead-based solution for high quality, high throughput DNA extraction from saliva stored in collection devices. Here, DNA was extracted from stabilized saliva using the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399) after being transferred to a 96-well deep-well plate and placed on the Hamilton Microlab® STAR™.

Results: DNA yield was quantified, integrity was analyzed, and real-time PCR was used to assess the suitability of the purified DNA for downstream applications.

Conclusion: These analyses show that Omega Bio-tek has developed a rapid, reliable, fully automatable solution that can extract DNA from 96 samples of stabilized saliva in less than 90 minutes.

Conflict of Interest: Massimiliano Memo Employed by Omega Bio-tek, Stefan Schmidt Employed by Omega Bio-tek, Claire McClain Employed by Omega Bio-tek, Julie Baggs Employed by Omega Bio-tek, Travis Butts Employed by Omega Bio-tek, Kiranmai Durvasula Employed by Omega Bio-tek

P16.007.C Comprehensive de novo mutation discovery with HiFi long-read sequencing

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Background/Objectives: Long-read sequencing (LRS) techniques have been very successful in identifying structural variants (SVs) but have traditionally not been used for small variant detection. Given recent advances in LRS accuracy, we evaluated the ability of highly accurate HiFi long-reads to detect de novo mutations (DNMs) of all types as compared to short-read sequencing (SRS).

Methods: We performed PacBio HiFi LRS and Illumina SRS for eight patient-parent trios. We called de novo substitutions, small indels (1-20bp), short tandem repeats (STRs) and SVs (>20bp) in both datasets and compared these DNMs to each other. We also performed phasing of small DNMs (substitutions and small indels).

Results: We identified a total of 672 and 859 small DNMs, 28 and 126 STRs, and 24 and 1 SVs in LRS and SRS respectively. The concordance between LRS and SRS for the small DNMs was 92% (618/672). For both the STRs and SVs only one variant overlapped between the two technologies. We confirmed 11 LRS-unique and 8 SRS-unique small DNM calls as true DNMs. Validation of 18 LRS-unique and SRS-unique STRs confirmed none as true DNM, while 10 LRS-unique SVs were true de novo events. Furthermore, we phased 96% of the small DNMs with LRS, as opposed to 33% with SRS.

Conclusions: HiFi LRS allows for the detection of small DNMs on par with SRS. In addition, LRS has improved detection of STRs and SVs and allows for variant phasing. We conclude that HiFi LRS can now produce a comprehensive variant dataset containing all variant types.

Conflict of Interest: Erdi Kucuk: None declared, Bart van der Sanden: None declared, Luke O'Gorman: None declared, Michael Kwint: None declared, Ronny Derks: None declared, Aaron Wenger Pacific Biosciences, Pacific Biosciences, christine lambert Pacific Biosciences, Pacific Biosciences, shreyasee chakraborty Pacific Biosciences, Pacific Biosciences, primo baybayan Pacific Biosciences, Pacific Biosciences, William Rowell Pacific Biosciences, Pacific Biosciences, Zev Kronenberg Pacific Biosciences, Pacific Biosciences, Han Brunner: None declared, Lisenka Vissers: None declared, Alexander Hoischen: None declared, Christian Gilissen: None declared

P16.008.D Contribution of CRISPRable DNA to human complex traits

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CRISPR-Cas is a powerful genome editing tool for various species and human cell lines, widely used in many research areas including studying the mechanisms, targets, and gene therapies of human diseases. Recent developments have even allowed high-throughput genetic screening using the CRISPR system. However,

due to the practical and ethical limitations in human gene editing research, little is known about whether CRISPR-editable DNA segments could influence human complex traits or diseases. Here, we investigated the human genomic regions condensed with different CRISPR Cas enzymes' protospacer-adjacent motifs (PAMs). We found that Cas enzymes with GC-rich PAMs could interfere more with the genomic regions that harbor enriched heritability for human complex traits and diseases. The results linked GC content across the genome to the functional genomic elements in the heritability enrichment of human complex traits. We provide a genetic overview of the effects of high-throughput genome editing on human complex traits.

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P16.009.A Adaptive Nanopore Sequencing as a preeminent tool to elucidate structural variants in cancer predisposition genes

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Background / Objectives: Detection of germline alterations is a standard practice in oncogenetics. Since the advent of Next Generation Sequencing, the detection of nucleotidic variants is fast and standardized. However, the detection and characterization of structural variants (SV) is a challenge and requires additional time-consuming analyses. We report our experience to bring the proof of concept that Nanopore adaptive sequencing is a suitable tool to explore SV with a direct impact on clinical practice.

Methods: Each DNA library is prepared in half a day without complex preparation steps. By detecting ionic potential changes as the DNA molecule passes through a biological nanopore, it enables real-time long-read sequencing. With adaptive sampling, the bioinformatics selection of genomic regions of interest using the MinKnow interface allows significant reading enrichment on those targeted regions. Downstream analyses are performed using the NanoClid pipeline, developed by the bioinformatics unit of the Institut Curie and available on GitHub (https://github.com/InstituteCurieClinicalBioinformatics/NanoCliD).

Results: Through the presentation of few clinical cases, we demonstrate that Nanopore adaptive sequencing allowed the germline SV characterization in cancer predisposition genes, such as *BRCA1*, *RAD51C*, *MSH6* or *SMARCB1*. The speed of response (below 15 days) offered by Nanopore technology has a major impact on the immediate patient management.

Conclusion: Nanopore sequencing unique features allow adaptive sampling. It therefore appears as a simple and fast technique to resolve SV that conventional short read sequencing techniques were unable to characterize, in a timeframe compatible with clinical decisions.

Conflict of Interest: None declared

P16.010.B Large scale end-to-end target discovery using UK Biobank Research Analysis Platform

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Objectives: Large scale omics and phenotypic collections enable rigorous and reproducible research, especially in the drug discovery area. Here, we present a use case of the end-to-end target discovery pipeline in the UK Biobank Research Analysis Platform (UKB RAP).

Methods: Ischaemic heart diseases (I20-I25) cases and controls cohorts were created using Cohort Browser. For this analysis UK Biobank array data and imputed data were used. Samples and variants were quality controlled using PLINK and custom python code. We performed GWAS using Regenie with array data in Step 1 and imputed data in Step 2. Significantly associated variants were filtered using the LD clumping approach. Remaining significant variants are used to test association with other phenotypes by performing PheWAS analysis.

Results: We were able to run successfully the whole pipeline on UKB RAP. We validated our results by comparing it with already published loci that are significantly associated with ischaemic diseases.

Conclusion: Our research demonstrates how using a trusted research environment to perform large scale genomic analyses makes research efficient, secure and scalable.

Conflict of Interest: None declared

P16.011.D PacBio HiFi WGS identifies potential causal variants not found by short read sequencing

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Background/Objectives: PacBio HiFi reads (99.9% accuracy, 15-20 kb) enable comprehensive variant detection in human genomes, extending to repetitive regions of the genome not accessible with short-read WGS (srWGS) or WES (srWES). HiFi reads match or surpass srWGS for single nucleotide variant and small indel (<50 bp) detection while also improving detection of structural variants (SVs, ≥50 bp), with recall far exceeding that of srWGS. Here we apply HiFi-WGS to 10 probands with unexplained hearing loss who had previously undergone srWES and srWGS with a negative result.

Methods: We prepared SMRTbell libraries for 10 probands and sequenced libraries to a depth of 24-32x on the Sequel II system. We analyzed HiFi reads with an internally developed workflow to align reads to the reference (pbmm2), call small variants (DeepVariant) and structural variants (pbsv, trgt, HiFiCNV), phase

variants (HiPhase), filter and annotate variants (slivar, svpack, bcftools), and generate de novo assemblies (hifiasm).

Results: We identified a median of 4,505,589 SNVs, 981,037 small indels, and 22,682 SVs per sample. Variants of phenotypic interest were identified in 7 cases, with 3 cases explained: 1) a compound heterozygous ~104 kb deletion and G>A stop-gain variant in *STRC*, 2) a compound heterozygous 706 bp deletion and A>G missense variant in *OTOA*, and 3) a copy number neutral 403 kb inversion interrupting *MITF*.

Conclusion: HiFi-WGS increases the ability to explain rare disease cases by allowing for the detection of a broad range of variants, especially in regions that are difficult to map with srWGS.

Conflict of Interest: William Rowell Pacific Biosciences, Pacific Biosciences, Shelby Redfield: None declared, Cillian Nolan Pacific Biosciences, Pacific Biosciences, J. Matthew Holt Pacific Biosciences, Pacific Biosciences, Cairbre Fanslow Pacific Biosciences, Pacific Biosciences, Eirini Maria Lampraki Pacific Biosciences, Pacific Biosciences, christine lambert Pacific Biosciences, Pacific Biosciences, Chris Saunders Pacific Biosciences, Pacific Biosciences, Margaret A. Kenna: None declared, Eliot Shearer: None declared, Michael Eberle Pacific Biosciences, Pacific Biosciences

P16.012.C Interpretation of variant of uncertain significance (VUS) in genetic disorders by rapid generation of VUS-mimicking mice by i-GONAD

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Background: The drastic development of genetic analysis in the last two decades has identified a great deal of pathogenic variants in genetic disorders. However, such high-throughput genetic analyses have also found enormous variants of uncertain significance (VUS) concurrently and it remains unsolved problems how to interpret etiological meaning of VUS. Although in vitro analysis, *e.g*. functional analysis of mutant protein, or in silico estimation may provide an evidence to understand VUS, they are sometimes inadequate to clarify a correlation between VUS and phenotype.

Methods: To resolve the problem, we have generated transgenic mice precisely mimicking the target VUS precisely rapidly using the i-GONAD method [Takahashi *et al*. Sci Rep. 2015]. The method generates a transgenic mouse in 19 days at the earliest by intraoviductal inducing of genome-editing reagents into fertilized ovum directly.

Results: We have already generated 14 strains of intended transgenic mice in ten genes including missense variants, microdeletion, or tag insertion. For example, we generated a mouse precisely reproducing a variant at a splice site of *TENM4* identified in a pedigree of epilepsy and intellectual disability. The transcript and the protein from the mouse brain showed a shorter form of Tenm4 with exon 10-skipping, and the mice showed increased seizure susceptibility to pentylenetetrazole. Primary culture of the neural cells showed less growth of the neurite. These findings provided good evidence to explain the probands' etiology.

Conclusion: Our current strategy is useful in understanding VUS in genetic disorders.

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Funding Agency: Japan Society for the Promotion of Science
Role: PI

P16.013.A 28 years of genetic screening for Leber's Hereditary Optic Neuropathy at LBioMiT

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Many cases of optic atrophies, particularly Leber's Hereditary Optic Neuropathy (LHON), remain without an identified genetic cause[1,2]. LHON's diagnosis includes screening of mitochondrial DNA (mtDNA) variants: m.3460G>A, m.11778G>A, and m.14484T>C (TOP3)[4]. In the last 28 years, 141 cases (206-333 LHON patients estimated in Portugal) were assigned to our laboratory. Recent technological advances bring additional difficulties in validating novel mutations and biochemical/functional studies are crucial[3]. GenEye24[4] ensures TOP3 screening within 24h. The results of LHON screening at LBioMiT are presented.

Genetic screening of both genomes was performed by NGS and bioinformatics' analysis[3]. Biochemical evaluation of OXPHOS complexes' activities was done by double wavelength spectrophotometry[5]. The GenEye24 relies on real-time PCR with High-Resolution Melting[4].

Whole mtDNA screening grouped: 124 patients unidentified mtDNA cause (88%); 17 patients with mtDNA variants (12%) – 59% m.11778G>A; 17% m.14484T>C; 24% m.3460G>A. Additionally, a case of dominant optic atrophy with the variant c.635_636delAA (p.Lys212Argfs – OPA1) was found. In GenEye24, 108 samples were genotyped: 64 wild-type, 36 m.11778G>A, 6 m.14484T>C and 2 m.3460G>A, with high confidence values (Mode = 100%). Values for sensitivity and specificity: 1.

Results reveal the necessity of a profound genetic screening. The GenEye24 (fast, robust, simple, cost-effective) represents an improved alternative for TOP3 screening; its fast response increases probabilities of treatment efficacy and visual function rescue. The project "Translational Epidemiological, Bigenomics and Functional Research in Optic Atrophies" (approved in 11 hospitals) allows centralization of the bigenomic study in Portugal.

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Conflict of Interest: None declared

P16.014.B A weights-based variant ranking pipeline for complex familial disorders

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Background/Objectives: Identifying susceptibility factors in complex familial disorders remains challenging. A weights-based approach was developed to detect susceptibility factors by prioritizing variants and genes in collections of small and large families where genetic heterogeneity is likely, but biological commonalities are plausible.

Methods: The weights-based pipeline retains variants shared among the cases in each family and then prioritizes the variants by weighting them on incidence rate, number of cases in a family, proportion of genome shared amongst cases in a family, variant allele frequency, and variant deleteriousness. All weights except allele frequency weights are normalized from 0 to 1. The five weights are combined multiplicatively to produce a family-specific variant weight. For each variant, the family-specific variant weights are averaged across all families in which the variant is observed to produce a multifamily variant weight. Multifamily variant weights are ranked for further investigation. The pipeline was validated on exome sequence data from a UK familial melanoma study deposited at the European Genome-phenome Archive (EGAS00001000017).

Results: 4 out of 13 families (31%) revealed causal variants in known germline melanoma genes *POT1*, *MITF* and *BAP1*. Analysis of the remaining 9 families identified several interesting genes; some, such as *BRCA1* or *DOT1L*, play a putative role in melanoma.

Conclusion: The pipeline provides a systematic approach to perform a combined analysis of small and large pedigrees to empower the detection of disease-predisposing genes. Identifying genetic factors enables understanding of biological processes involved in complex heritable disorders.

Conflict of Interest: None declared

P16.015.C Reporting method for variants of uncertain significance in targeted gene panels

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Background: Clinical variant interpretation refers to the process of determining the probability that a variant is contributing to a patient's clinical phenotype. In case there is insufficient evidence to classify a variant as pathogenic or likely pathogenic (LP/P), the variant is classified as a variant of uncertain significance (VUS). The posterior probability that a VUS is pathogenic ranges from 10-90%, depending on the number of supporting criteria.

Methods: One hundred gene panels for 5 different phenotypes were analyzed and classified based on the ACMG guidelines for variant classification. Three rules were applied to define a VUS as clinically reportable: (1) Panels were divided into "core" or "extended" categories if they had high or limited phenotypic association, respectively. (2) All LP/P variants, regardless of phenotypic association, were reported. (3) A VUS in the core or extended categories were reported only if it had 50% or 32.5% posterior probability of pathogenicity, respectively.

Results: Fifty P/LP were detected, 26% (13/50) affected 'extended' genes. Causative P/LP variants were identified in 23 cases, 2 of which (8.7%) affected 'extended' genes. The reporting strategy significantly reduced the number of reported VUS per case (0.96 vs 3.54). Overall, 40% (74/182) and 12% (22/172) of VUS were reported in core and extended genes, respectively ($P < 0.05$).

Conclusion: Including genes with lesser phenotypic association is crucial for gene-panel analyses. Our approach improves the balance between sensitivity and specificity when analyzing gene panels by expanding the number of candidate genes while limiting the amount of identified VUS.

Conflict of Interest: None declared

P16.016.D MetDecode: a novel methylation-based method for cell-free DNA tissue-of-origin assignment

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Plasma cell-free DNA (cfDNA) originates mainly from dying cells throughout the body and preserves the (epi)genetic characteristics, like the nucleosome-guided cfDNA fragmentation patterns and the DNA methylation profiles of its cellular origin. Therefore, cfDNA can be a valuable non-invasive biopsy to pinpoint the main contributor to a cfDNA mixture and hence support the identification of suspected pathological conditions. We developed a novel deconvolution method, MetDecode, that estimates the relative proportion of each contributor to the cfDNA sample relying on a multi-tissue reference atlas of specific methylation markers. Using in-silico mixtures of tumour genomic DNA spiked into cfDNA from healthy individuals, MetDecode accurately estimated the tumour proportion ($r = 0.929$) and correctly assigned the tissue-of-origin in mixtures with as low as ~4% tumour fraction. As proof-of-concept, we tested MetDecode on 16 samples from patients with advanced cancer stages (breast, ovarian and colorectal), assigning 14 to the correct tissue-of-origin (accuracy: 87.5%). Among the different cancer types, colorectal cancer cases were the most accurately classified (100%), followed by ovarian cancer cases (85.71%). After validation on cfDNA samples from cancer patients with known high contribution of tumour-derived cfDNA, we are now evaluating MetDecode on cfDNA samples with unexplained abnormal copy number profiles ($n = 24$), aiming at identifying the cellular origin of the detected alterations. To conclude, we developed a novel deconvolution algorithm, MetDecode, that can efficiently estimate the contribution of multiple tissues in cfDNA samples, and we are now testing its potential in interpreting different physio-pathological conditions.

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Conflict of Interest: None declared

P16.017.A An automated, low-cost library preparation protocol for low-coverage whole genome sequencing-based genotype imputation

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Background/Objectives: Complex diseases with a major impact on public health result from the interaction of many independent genetic variants. The complete characterisation of these variants is crucial to understand and treat the clinically heterogeneous forms of many diseases. The relatively high cost of standard-coverage Whole Genome Sequencing (WGS) restricts its use as a method for large-scale calling of genetic variants. Low-coverage WGS (lcWGS) combined with genotype imputation has been shown to outperform conventional SNP microarrays for genome-wide variant calling. We evaluated the feasibility of implementing an automated, low-volume library preparation method and lcWGS for imputation-based genome-wide variant calling.

Methods: Illumina DNA PCR-free library preparations were performed on Hamilton handling robots at 50% of the normal manual protocol volume. Following lcWGS, variant calling and imputation was evaluated using an implementation pipeline integrating the GLIMPSE algorithm. Evaluation of precision/recall were assessed against reference genome (e.g. GIAB and additional references). The impact of reducing the sequencing coverage was also evaluated.

Results: Replicate libraries were prepared and sequenced to an average read depth for all experiments of 2x (SD = 0.4), yielding an average variant calling precision of 0.9925 (SD = 0.0009) and recall of 0.9711 (SD = 0.0016). Read rarefaction analysis showed that sequencing depths as low as 0.5X provide excellent and usable variant call accuracy.

Conclusions: While maintaining quality required for genotyping common variants, this automated protocol reduces sample preparation costs by 2x compared to the standard protocol and will thus facilitate the use of lcWGS for genome-wide variant calling applications.

Conflict of Interest: None declared

P16.018.B The influence of MECP2 mutation type and the polygenic risk scores in the clinical variability of Rett syndrome

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Background/Objectives: De novo variants in the X-linked dominant *MECP2* gene lead to a wide range of neurodevelopmental disorders in females, under the Rett syndrome's umbrella, ranging from severe non-speaking classic to preserved speech form. Notable modulating factors such as the percentage of skewing X-inactivation and mutation type are insufficient to be used in clinical practice.

Methods: We tested the contribution of *MECP2* mutation type and polygenic scores (PRS) of epilepsy and intelligence using two separate generalized linear models (GLM) in 220 Rett patients. Additionally, the two PRS were assessed in subgroups stratified by mutation type. The severity score elaborated by the Rett Network database was used.

Results: The findings show that *MECP2* mutation type (gene deletion/early-truncating versus missense/late-truncating) can separate with statistical significance ($p = 0.006$) patients with lower (<40) and higher (>40) IQ scores, but not epileptic from non-epileptic patients ($p = 0.294$). In contrast, neither epilepsy PRS nor intelligence PRS made any additional significant contributions to separating the patients ($p = 0.477$, $p = 0.216$, respectively).

Conclusion: The results indicate that epilepsy is independent of both mutation type and PRS, consistent with epilepsy absence in any mouse models (regardless of their background), and the hypothesis that the primary modulators could be rare variants in channel genes. Partial MeCP2 preservation is instead relevant for cognitive function. Further refinement of the GLM is ongoing, combining the effects of common functional variants in the proximity of *MECP2*, rare variants in different genes, and PRS for other disease traits.

Grant References: INTERVENE to AG and AR & ANTICIPATED to AR

Conflict of Interest: None declared

P16.019.C A novel reference architecture for multi-party federation: enabling joint analysis of large-scale health data across distributed trusted research environments

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Enabling secure access to clinical-genomic data is crucial to accelerate medical research. Organisations frequently use secure computing environments, known as Trusted Research Environments (TREs), to keep data secure while allowing researcher access, but TREs at different organisations are often restricted from working together, preventing the optimal use and collaboration across valuable datasets. Multi-party federation solves this problem by enabling TREs to securely communicate with each other. Here, we present what we believe to be the first UK test case of multi-party federation. We formed a consortium including the University of Cambridge, NIHR Cambridge BRC, Genomics England, Eastern AHSN, Cambridge University Health Partners, and Lifebit to develop a novel reference architecture to demonstrate multi-party federation by bridging the TREs of NIHR Cambridge BRC and Genomics England. We successfully deployed the Lifebit Platform to bridge these TREs, demonstrating this in a live test case showing joint analysis of data from both TREs without the data leaving the respective locations. We developed novel Application Programming Interfaces to enable TRE communication, in addition to a scalable airlock system for secure export into and out of the TRE. All novel technology for this project is open-source, developed in alignment with GA4GH standards. This ground-breaking study has provided novel insights for future

multi-party federated analysis and demonstrates the best practice reference architecture for bridging TREs.

Grant Reference: Funded by UK Research & Innovation (#MC_PC_21026) as part of the Phase 1 Data and Analytics Research Environments UK programme, in partnership with HDR UK and ADR UK.

Conflict of Interest: None declared

P16.020.D Optimisation of low-coverage sequencing approach

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Background/Objectives: Low-coverage sequencing is a novel genotyping technique combining whole genome sequencing to an average depth of 1× with genotype imputation to identify genetic variants. Low-coverage sequencing is a cost-effective alternative to genotyping arrays to identify genetic variants. In this study, we compare different low-coverage sequencing methods.

Methods: Sequencing libraries were prepared from a number of DNA reference standards, including an Ashkenazi Jewish trio, using five miniaturized assays adapted from different commercial suppliers (Illumina, Agilent, New England Biolabs and Tecan) that have been optimized in our laboratory. Libraries were pooled to obtain low coverage and sequenced on a NovaSeq (Illumina). Coverage metrics were assessed using our in-house bioinformatics pipeline.

Results: We obtained homogeneous results for all libraries. Coverage was relatively homogeneous across all chromosomes. The "Illumina DNA Prep" process shown best results with a mean depth of coverage 1.5× (interquartile range: IQR = 0.13×) and mean breadth of coverage 76.4% (IQR = 2.89%). We also showed that the protocol significantly improved the homogeneity of breadth of coverage. The Illumina process is now fully automated in our laboratory, with a seven-fold miniaturization.

Conclusion: This is a comparative study to evaluate our low-coverage sequencing approach. The new miniaturized process has been optimized for automation to be compatible with high-throughput sequencing platforms.

Grant References: LabEx GenMed (grant number ANR-10-LABX-0013)

Conflict of Interest: None declared

P16.021.A Genetic atlas of membrane protein complexes in human plasma

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Proteins serve as the building blocks of biological functionality and are encoded in the human genome. Unraveling the genetic underpinnings of the human proteome is critical in gaining insights into the regulation of human complex traits and diseases. Integrating this information with existing genomic data from genome-wide association studies (GWAS) is especially beneficial. While various assays have been developed to measure protein abundance in human plasma samples, they lack the capability to assess the functional interactions between these protein molecules. In this study, utilizing a novel technology, we analyzed plasma from 1,000 individuals and detected and quantified millions of membrane protein complexes (MPCs) in each individual. Our genetic atlas of these MPCs identified 377 MPCs with significant cis-regulatory loci and specific protein-protein combinations. The mapped trans-regulatory loci provide insights into the genetic mechanisms of extracellular vesicles in biological processes, such as neurodegeneration, cell signaling and communication. By integrating with established genome-wide association study (GWAS) findings, we were able to draw inferences regarding the potential causal links between specific membrane protein complexes (MPCs) and complex diseases, particularly autoimmune disorders like asthma, ulcerative colitis, inflammatory bowel disease, systemic lupus erythematosus, among others. This discovery positions MPCs as a highly promising area of focus for future medical research.

Grant References: National Natural Science Foundation of China (NSFC) grant, Natural Science Foundation of Guangdong Province grant, National Key Research and Development Program grant, Medical Research Council Human Genetics Unit program grant "Quantitative Traits in Health and Disease".

Conflict of Interest: Ting Li: None declared, Ranran Zhai: None declared, Lucija Klaric: None declared, James F. Wilson J.W. was in receipt of a Medical Research Council Human Genetics Unit program grant "Quantitative Traits in Health and Disease" (U. MC_UU_00007/10)., Di Wu Vesicode AB, D.W. has filed a patent application (PCT/SE2014/051133) describing the PBA technique. D.W. holds shares in Vesicode AB commercializing the PBA technology., Xia Shen X.S. was in receipt of a National Natural Science Foundation of China (NSFC) grant (No. 12171495), a Natural Science Foundation of Guangdong Province grant (No. 2114050001435), and a National Key Research and Development Program grant (No. 2022YFF1202105).

P16.022.B The role of DNA methylation for disease severity in patients with heterozygous mutations in the Mediterranean Fever Gene MEFV

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Familial Mediterranean Fever (FMF) is an autosomal dominant disease with mutations in the MEFV gene that encodes for Pyrin, an important innate immunity regulator. However, some heterozygous individuals also show an FMF phenotype, which leads to the assumption that other modifying factors lead to a manifestation of the phenotype[1]. In recent years, DNA methylation has demonstrated its potential for disease diagnosis by several studies.

The main goal of this study was to evaluate DNA methylation in patients carrying heterozygous mutations in the MEFV gene but show different phenotypes. We hypothesis that alterations in DNA methylation can add important and valuable information about the disease etiopathogenesis.

The study included a total of 55 patients: 23 homozygous FMF patients, 12 heterozygous mutation carriers presenting with an FMF phenotype, 9 heterozygous without FMF phenotype and 12

healthy controls without any mutation in the MEFV gene. We performed a genome wide DNA methylation analysis using Illumina's EPIC BeadArray

We revealed over 30000 significant cpgs ($p < 0.05$) where between 28 and 75 CpG sites showed at least 15% differences in mean methylation between the groups. Using these features, the patient groups separated well in hierarchical clustering. More specifically, group comparisons between heterozygous disease vs heterozygous healthy revealed 71 differentially methylated sites ($p < 0.05$, difference ≥ 15%), indicating that these two groups can be separated well using DNA methylation data. We show that the identified CpG sites can help diagnose patients and provide valuable information to the etiopathogenesis of FMF.

Conflict of Interest: Klemens Vierlinger AIT, Collaborator, Julie Krainer AIT, Collaborator, Walter Pulverer AIT, Seza Ozen University, PerSAIDs, Novartis and SOBI, Dirk Föll University, PerSAIDs, Andreas Weinhaeusel AIT, INSAID

P16.023.C External assessment of germline copy-number variation calling using short-read sequencing

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Background: Analysis of copy number variants (CNVs) by short-read Next Generation Sequencing (NGS) is increasingly offered by genomic clinical diagnostic laboratories. Analysis of CNVs is much more challenging than for single nucleotide variants (SNVs) and therefore external quality assessment (EQA) is essential to determine that the methodology employed is fit for purpose.

Methods: EMQN CIC and Genomics Quality Assessment (GenQA) have delivered EQA for germline SNV (and small indel) NGS testing since 2016. An optional genomic DNA sample for CNV analysis was included in the 2022 EQA, containing at least one expected deletion covering two consecutive exons in BRCA1. The submitted CNV calls were assessed and (exon level) feedback was provided to each participant laboratory.

Results: A VCF file containing variant calls and a BED file describing the targeted genomic region were submitted by 104 laboratories; 78 of the participants submitted a file that contained CNV calls, 18 performed Whole Genome Sequencing (WGS), while others used targeted sequencing. The expected deletion in BRCA1 was correctly identified by 61 (78%) of the participants. Analysed results have demonstrated that the proper usage of the VCF standard format and appropriate variant calling continues to be problematic.

Conclusion: NGS has become the standard method of characterising sequence variants in clinical diagnostics. CNV analysis using short read NGS is challenging, and thus external assessment is of crucial importance. The genomics community, including variant calling tool vendors, needs more awareness that the correct usage of VCF file format provides an appropriate way for presenting CNV calls.

Conflict of Interest: None declared

P16.024.D Applications of long-read sequencing in clinical Neurology

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Background/Objectives: Nanopore sequencing is a technique that can directly read long (>20kb) DNA or RNA molecules. It provides a scalable sequencing method from targeted sequencing of small regions (~a few kb) to the whole genome/transcriptome. In addition, reading long, continuous sequences is useful for deciphering repetitive regions that make up more than 50% of the genome. Our goal is to translate this technology into clinical applications. Neurogenetic diseases are a broad spectrum of disorders caused by changes in genes or intergenic regions of the genome, affecting the brain, spinal cord, nerves, and muscles. There are many neurogenetic diseases known to be caused by mutations in repetitive regions of the genome which provides the rationale for using nanopore sequencing.

Methods: We developed two bioinformatic tools; *tandem-genotypes* to detect alteration of repeat copy number changes and *dnarrange* to find genomic rearrangements. These tools were used in diagnostic workflows to elucidate genetic causes of a variety of Neurogenetic diseases using nanopore sequencing.

Results: Nanopore long read sequencing successfully detected small base changes, tandem repeat copy number changes, complex chromosomal rearrangements, retroelements, mitochondria genome and integrated viral genome.

Conclusion: Our approaches can be applied to molecular diagnostics in clinical practice for patients with neurogenetic diseases.

Conflict of Interest: None declared

P16.026.B RNA-binding protein MEX3A acts as a PPARG direct regulator with functional impact in colorectal carcinogenesis

Ana Rita Silva[1], *Dalila Mexieiro[1], Vanessa Machado[1], Morgana Russel[1], Ana Luisa Amaral[1], Bruno Cavadas[1], Raquel Almeida[1;2;3], Bruno Pereira[1]*

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Background/Objectives: RNA-binding proteins (RBPs), as part of the post-transcriptional regulatory machinery, play a key role in diverse cellular processes. Recently, we described the role of MEX3A RBP in maintaining LGR5+ intestinal stem cells identity and epithelial renewal by repression of the PPARγ pathway. This work aimed to characterize MEX3A functional impact in colorectal cancer (CRC) and uncover MEX3A targets.

Methods: We characterized the molecular expression profiles of CRC mouse models and a cohort of CRC cases ($n = 172$). Mouse CRC tissues were used for the establishment of tumoroids (MDTs), and MEX3A CRISPR/Cas9-mediated knockout was performed in patient-derived tumoroids (PDTs) to further understand its biological relevance. Simultaneously, we implemented the high-throughput technique HyperTRIBE to uncover MEX3A RNA targets.

Results: Intestinal adenomas from $Apc^{+/fl}$ mice have increased expression of *Mex3a* and low PPARγ expression. $Apc^{+/fl}$;$Mex3a^{+/-}$ animals presented a significant reduction in tumour burden. $Apc^{+/fl}$;$Kras^{+/G12D}$;$Mex3a^{+/-}$ compound mice presented reduced tumour area, and MDTs exhibited reduced growth ability and enhanced differentiation potential mediated by PPARγ signalling. MEX3A overexpression was observed in 85% of human CRC cases, while 72% presented PPARγ downregulation, with a statistically significant inverse correlation ($P = 0.039$). Accordingly, MEX3A-depleted PDTs showed decreased *Lgr5* expression, accompanied by increased PPARγ expression. HyperTRIBE results revealed a direct interaction between MEX3A and *PPARG* transcripts.

Conclusion: Our results suggest that MEX3A overexpression has an important role in CRC carcinogenesis by repressing the PPARγ differentiation pathway.

Grant references: Funded by FEDER through the COMPETE 2020 Operational POCI and by FCT: grant PTDC/BIA-CEL/0456/2021; ARS - 2021.06919.BD; BP - CEECINST/00131/2021.

Conflict of Interest: None declared

P16.027.C A microfluidics-free approach for simultaneously profiling the whole transcriptome and TCR repertoire of 1 million cells in a single experiment

Efi Papalexi[1], Vuong Tran[1], Peter Matulich[1], Grace Kim[1], Sarah Schroeder[1], Daniel Diaz[1], Charlie Roco[1], Alex Rosenberg[1], Bryan Hariadi[1], Ajay Sapre[1], ***Alex Stretton***[1]

[1]*Parse Biosciences, Seattle, United States*

T cells recognize and eliminate a wide variety of immunologic threats while maintaining self-tolerance. This pathogen recognition and clearance activity is managed through a process called V(D)J recombination, during which a T cell obtains a unique set of V, D and J gene segments for all the chains (α and β or γ and δ) that make up its T cell receptor (TCR). Each recombined TCR detects a specific disease-associated antigen peptide, which triggers the appropriate adaptive immune response.

The diversity of possible TCRs in the immune repertoire is enormous, yet the low throughput of existing tools have limited the ability to capture this complexity at high resolution. To overcome these limitations, we have extended Parse Biosciences' combinatorial barcoding technology (originally based on Split Pool Ligation-based Transcriptome sequencing or SPLiT-seq) to simultaneously characterize the TCRs alongside the full transcriptomes of up to 1 million T cells.

Using this approach, we fixed and prepared 1 million isolated Pan T cells from the PBMCs of 8 donors. Evercode TCR enabled detection of at least one TCR chain in 88% of all T cells together with their corresponding transcriptome. We found that clonotype detections were highly accurate as a cell cluster with known semi-invariant TCR alpha chains matched the TCR assignments in our analysis. We identified nearly half a million unique beta chain clonotypes across multiple samples with the Evercode TCR kit resulting in the most comprehensive immune repertoire detection from a single experiment to date.

Conflict of Interest: Efi Papalexi full, stock options, Vuong Tran Parse Biosciences, Stock options, Peter Matulich: None declared, Grace Kim Parse Biosciences, Stock options, Sarah Schroeder Parse Biosciences, Stock options, Daniel Diaz Parse Biosciences, Stock options, Charlie Roco Parse Biosciences, Stock options, Officer of the company (CTO), Alex Rosenberg Parse Biosciences, Stock options, Officer of the company (CEO), Bryan Hariadi Parse Biosciences, stock options, Ajay Sapre Parse Biosciences, Stock options, Alex Stretton: None declared

P16.028.D Cell fixation enables flexible, scalable single cell RNA sequencing

Sarah Schroeder[1], Vuong Tran[1], Joey Pangallo[1], Peter Matulich[1], Ryan Koehler[1], Daniel Diaz[1], Alex Sova[1], Grace Kim[1], Lauren Kenyon[1], Charlie Roco[1], Alex Rosenberg[1], ***Jorge Arturo Zepeda Martínez***[1]

[1]*Parse Biosciences, Seattle, United States*

In scRNA-sequencing, sample size is critical to confidently detect rare cell types and low-expressed transcripts. Accommodating large sample sizes enables multi-sample and time-course experiments. But with a large sample size come technical concerns, such as storing multiple samples until use without compromising their integrity and avoiding batch effect.

Parse Evercode™ Whole Transcriptome (WT) Mega can profile up to 96 samples across up to 1 million cells in a single experiment. A fixation procedure separates sample preparation from cell barcoding, enabling the pooling of different samples into one experiment. The Parse combinatorial barcoding strategy yields hundreds of thousands of uniquely labeled cells with a verified low doublet rate.

For this study, we collected 24 PBMC samples from donors over a three-week period, fixed them using the Evercode Fixation kit, and stored at -80C until use. We processed all 24 samples with a single Evercode WT Mega kit and generated more than 1 million individually barcoded cells that were used to generate a sequencing library. The FASTQ files resulting from sequencing were analyzed using the Parse data analysis pipeline.

After sequencing the transcriptome for 1 million cells, we could detect distinct sub-types of cells in each sample. With > 27,000 cells captured from each sample, we were able to generate gene expression profiles for low-frequency cell types such as classical and plasmacytoid dendritic cells.

Parse Evercode WT Mega showed high-resolution immune cell profiling and low-frequency cell subtypes identification.

Conflict of Interest: Sarah Schroeder Parse Biosciences, Stock options, Vuong Tran Parse Biosciences, Stock options, Joey Pangallo Parse Biosciences, Stock options, Peter Matulich Stock Options, Ryan Koehler Parse Biosciences, Stock options, Daniel Diaz Parse Biosciences, Parse Biosciences, Alex Sova Parse Biosciences, Stock options, Grace Kim Parse Biosciences, Stock options, Lauren Kenyon Parse Biosciences, Stock options, Charlie Roco Parse Biosciences, Stock options

patent, Officer of the company (CTO), Alex Rosenberg Parse Biosciences, Stock options

Patent, Officer of the company (CEO), Jorge Arturo Zepeda Martínez: None declared

P16.029.A High-sensitivity detection of specific ultra-low frequency somatic mutations for minimal residual disease monitoring

Tong Liu[1], *Michael Bocek[1], Patrick Cherry[1], Shawn Gorda[1], Jean Challacombe[1], Derek Murphy[1], Esteban Toro[1]*

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Background: Minimal residual disease (MRD) monitoring tracks the abundance of any malignant cells remaining in the body after therapeutic intervention. Currently, circulating tumor DNA (ctDNA) is a promising biomarker for MRD diagnosis. Due to low abundance of ctDNA and unique somatic variants developed from each individual, MRD diagnosis requires personalized NGS assays with high sensitivity and specificity.

Methods: Twist Bioscience has developed MRD Rapid 500 Panels, enabling customers to design, manufacture and ship fully personalized MRD panels (up to 500 targets) in as little as six days. To investigate the detection sensitivity of this product, we designed five custom MRD panels targeting either the reference or variant allele for a set of hundreds of variants, and tested them on a contrived sample of NA12878 with spiked-in synthetic mutations, at low allele frequencies.

Results: Variant calling results revealed clear separation between WT and 0.01% VAF samples, with an average of 20/200 SNV targets detected at 80000x sequencing depth. Our results highlight the benefits of targeting a larger set of sites when trying to detect very low tumor fractions. Further, we show that variant calling accuracy increases 10%-20% when the alternate allele is targeted in samples with less than 1% VAF abundance.

Conclusion: The performance of the Twist MRD Rapid 500 Panels showed high detection sensitivity and specificity for MRD monitoring.

Conflict of Interest: Tong Liu The author is a shareholder of Twist Bioscience, Michael Bocek The author is a shareholder of Twist Bioscience., Patrick Cherry The author is a shareholder of Twist Bioscience., Shawn Gorda The author is a shareholder of Twist Bioscience., Jean Challacombe The author is a shareholder of Twist Bioscience., Derek Murphy The author is a shareholder of Twist Bioscience., Esteban Toro The author is a shareholder of Twist Bioscience.

P16.031.C Long-read sequencing including DNA methylation detection enables detailed characterization of the C9orf72 locus within ALS patients

Martin Elferink[1], *Maria Zwartkruis*[1;2], *Paul Hop*[2], *Ramona Zwamborn*[2], *Ivo Renkens*[3], *Joke van Vugt*[2], *Leonard van den Berg*[2], *Patrick Vourc'h*[4;5], *Véronique Danel*[6], *Philippe Corcia*[5;7], *Hanneke van Deutekom*[1], *WL Van Der Pol*[2], *Gijs van Haaften*[1], *Ewout Groen*[2], *Jan Veldink*[2]

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Recent advances in long-read single-molecule sequencing enables detailed characterization of complex loci in the genome. Besides resolving the chromosomal haplotypes of these loci, direct detection of basemodifications such as 5-methylcytosine can be performed with high accuracy. Long-read sequencing could therefore provide new insights in the underlying mechanisms of complex loci and their possible contribution to genetic diseases.

In this study we used Oxford Nanopore sequencing to characterize the C9orf72 locus in ten amyotrophic lateral sclerosis (ALS) patients with distinct phenotypes regarding disease onset, penetrance, and progression. All patients were heterozygous carriers of the hexanucleotide repeat expansion. We performed amplification-free CRISPR/Cas9-targeted enrichment on DNA isolated from blood. The C9orf72 promoter region, including the repeat site and both adjacent CpG islands, was used as target. Sequencing was performed using the Minion flowcell (R9.4.1) followed by basecalling with Guppy (v6.1.2) using the SUP-model including direct 5-methylcytosine detection. Repeat counts were determined using STRique (v0.4.2).

In agreement with previous studies the expanded allele showed extreme heterogeneity regarding the number of repeats, near complete hypermethylation within the expansion, and increased hypermethylation of both CpG islands. In addition, the methylation status within single DNA molecules allowed for a detailed analysis of the locus within and between patients. Initial analysis revealed a strong positive correlation between the repeat count and degree of hypermethylation of adjacent CpG islands. Our study gains new insights in understanding the etiology of ALS and could improve diagnostic testing including enhanced prediction of disease progression in patients.

Conflict of Interest: None declared

P16.032.D gMendel® Test-SCAN, a novel Decision-Supporting tool for Mass Screening of Aneuploidies, based on Oxford Nanopore technology

Anne Kristine Schack[1;2], *Carmen Garrido Navas*[2;3;4], *David Galevski*[5], *Aleksandar Nikov*[5], *Gjorgji Madjarov*[2;5;6], *Chris Kyriakidis*[2], *Zoran Velkoski*[2], *Lukasz Krych*[1]

[1]*University of Copenghagen, Department of Food Science, Copenghagen, Denmark;* [2]*gMendel, Copenhagen, Denmark;* [3]*GENYO Centre for Genomics and Oncological Research, Liquid Biopsy and Cancer Interception group, Granada, Spain;* [4]*ConGen, Genetic Counselling, Granada, Spain;* [5]*Netcetera, Zurich, Switzerland;* [6]*University Ss Cyril & Methodius, Skopje, Macedonia*

Background: Turner Syndrome (TS) and Triple X Syndrome (TXS) are two common sexual chromosome aneuploidies which are rarely detected and only discovered by coincidence or due to heart failures. TS is the only monosomy (45,X0) that humans can survive, affecting 1 in 2000 female births. TXS involves females born with an additional X chromosome (47,XXX) and has a frequency of 1 in 1000 female births. Despite its frequency, fewer than 10% TXS individuals are aware of their condition. Previous studies suggest that early diagnosis benefits both disorders within the neurodevelopmental outcome (for TXS) and malfunctions of the cardiovascular system (TS), hence we postulate that both diseases should be included in standard postnatal screening programs.

Methods: gMendel® Test-SCAN is a cost-effective, end-to-end assay with diagnostic utility for postnatal detection of TS and TXS based on Oxford Nanopore Technologies.

Results: With gMendel® Test-SCAN hundreds of samples can be analyzed simultaneously on a single MinION/GridION Flow Cell in less than 24h with high specificity and sensitivity. The data analysis process (as a port of the decision supporting platform, Phivea®) is fully automated and is performed in real-time using cutting-edge machine learning approaches. Finally, it also detects different mosaicisms (45,X0/46,XX and 47,XXX/46,XX or 47,XXX/45,X0 for TS and TXS respectively).

Conclusion: gMendel® Test-SCAN can be used as a standard postnatal screening tool for detecting potentially any chromosomal aneuploidy in a rapid, economic, and accessible manner.

Conflict of Interest: Anne Kristine Schack Employed by gMendel as industrial PhD student, Carmen Garrido Navas Consultant at gMendel, David Galevski: None declared, Aleksandar Nikov: None declared, Gjorgji Madjarov Consultant at gMendel, Chris Kyriakidis Employed by gMendel, Owner of gMendel technology and Phivea platform, Zoran Velkoski Employed by gMendel, Owner of gMendel and Phivea platform, Lukasz Krych: None declared

P16.034.B RNA-seq identifies diagnostically relevant splicing abnormalities in patients without candidate variants

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Background: RNA-seq is increasingly being used to aid rare disease diagnosis. We previously described its utility in

characterising splicing to help interpret variants of uncertain significance. Here, we illustrate how RNA-seq can be used to identify novel clinically relevant splicing abnormalities in patients without candidate variants.

Methods: 87 blood RNA samples from patients with suspected or confirmed genetic conditions (49 with candidate variants, 33 without candidates, 5 with known diagnoses) underwent total RNA-seq with ribodepletion with 150bp paired-end reads and 70 million reads per sample. FASTQ files were aligned to GRCh38 using STAR. Splicing events were analysed using rMATS, LeafCutter and MAJIQ and also via direct analysis of STAR output files. RT-PCR and qPCR were used to confirm specific splicing events detected by RNA-seq and to independently assay splicing associated with candidate variants.

Results: From 33 undiagnosed cases without a pre-existing candidate variant, at least two cases were identified to have abnormal alternative splicing events in genes that could explain the patients' presenting phenotypes. At least one other case originally had a candidate variant but was instead found to have an abnormal splicing event in a different gene that could explain the phenotype. Two cases involved cryptic exons created by deep intronic variants that would ordinarily be missed by conventional analysis.

Conclusion: RNA-seq can identify clinically relevant splicing abnormalities in patients without candidate DNA variants. Some cases of this type may be suitable candidates for personalised antisense oligonucleotide therapy development to block and/or manipulate splicing.

Grant References: NIHR RP-2016-07-011

Conflict of Interest: None declared

P16.036.D Comprehensive multiparametric analysis of cell-free circulating nucleic acids from a single extraction in healthy subjects

Nicolas Tessier[1], *Lise M. Hardy*[1], *Antoine Daunay*[1], *Jean-François Deleuze*[1;2], *Alexandre How-Kit*[1]

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Circulating cell-free nucleic acids in plasma (ccfNAPs) are released into the bloodstream by cells from various tissues and organs through mechanisms such as apoptosis, secretion or necrosis. Circulating cell-free DNA (ccfDNA) and microRNAs (ccfmiRNAs) from plasma are the two components of ccfNAPs that have been most studied to date in many pathologies, while very few studies have focused on ccfNAPs in healthy individuals.

We here study the variations of a set of molecular parameters of circulating blood plasma nucleic acids (ccfNAPs) in a cohort of 140 healthy donors from the French Blood Bank (EFS) aged between 19 to 66 years old. We used a single extraction of all ccfNAPs, including DNA (nuclear and mitochondrial) and RNA (messenger, ribosomal and micro-RNA) combined to high resolution analysis methods. PCR assays targeting high copy number genomic elements (Line-1/Kpn, mtDNA and rRNA) were used to minimize the volume of ccfNAPs required for all analysis. They included real-time quantitative PCR, ultra-sensitive high-resolution capillary electrophoresis and pyrosequencing. These methods have been used to quantify and assess the integrity of ccfNAPs as well as quantification of global DNA methylation and of expression of a few candidate tissue-specific mRNAs and age-associated miRNAs.

Our study provides a workflow for multiparametric analysis of all types of ccfNAPs from a single extraction. These methods allowed to highlight the age-related molecular changes occurring in ccfNAPs as well as their inter-individual variability.

Conflict of Interest: None declared

P16.037.A Evaluating a novel multi-omics automation platform for input reduction in transcriptomic studies

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Background/Objectives: Total RNA is predominantly rRNA. The remaining subpopulation is diverse, with an expanding non-coding/isoform repertoire. Depicting this diversity requires bolstering the robustness of protocols with technologies favoring retrieval of all relevant molecules.

We tested whether automation of an RNA-seq library preparation application using reduced inputs would preserve transcriptomic trends.

Methods: Brain total RNA was treated for sequencing using Illumina's Stranded Total RNA Prep Ribo-Zero Plus kit to evaluate Magelia®, a multi-omics platform combining technologies for miniaturization/precise bead handling.

Libraries were prepared in Magelia® using 1, 10 and 100 ng of RNA. Manual preparation and a different automation solution were used for comparison, starting with 100 and 500 ng of RNA, respectively. The latter are recommended inputs for these treatments.

Results: High quality reads (>97% at Q>30), high mapping rates (>87%), efficient ribosomal depletion (<0,9% rRNA reads) and negligible adapter dimers, were shown across samples/conditions.

The number of transcriptionally active genes showed minimal variation for all treatments. Significantly differentially transcribed genes ranged from 73 to 217 when comparing Magelia® treated samples to manual and automated references. This suggested that despite an input reduction of up to 500x, Magelia® treatment reflected the expected transcriptional profiles. Analysis of Magelia® treated replicates highlighted reproducibility, as no more than 15 significantly differentially transcribed genes were identified. GSEA showed that 92% of pathways reported in the literature as altered in brain tissues were detected through Magelia® treatment, underlining biological concordance.

Conclusions: Automation of a transcriptomic application allowed for considerable input reduction while preserving biological meaning.

Conflict of Interest: Sebastian Aguilar Pierle Inorevia, manufacturer of the tested platform., Stock options at Inorevia., Aino Palva: None declared, Camille Soucies Inorevia, manufacturer of the tested platform., Stock options at Inorevia., Harri Kangas: None declared, Pirkko Mattila: None declared, Amel Bendali Inorevia, manufacturer of the tested platform., Ownership and stock options at Inorevia.

P16.038.B Non-invasive prenatal determination of the risk for aneuploidies, and autosomal recessive and X-linked conditions. Our experience with 134 couples

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Background/Objectives: The incidence of recessive single gene disorders (SGD) is higher than the incidence of chromosomal anomalies at birth. This study aims to improve the detection of high-risk pregnancies combining cell-free fetal-DNA in maternal blood analysis (NIPT) and the screening of highly prevalent monogenic diseases in the couples.

Methods: A total of 134 couples were included. Segmental and complete aneuploidies were screened by NIPT strictly following the manufacturer procedure (Veriseq NIPT Solution V2, Illumina Inc., San Diego, CA, USA). Matching for recessive SGD in couples was perfomed by NGS sequencing using a panel of 112 genes, followed by bioinformatic analysis. Quality parameters implies that more than 99.7% of the analyzed variants must have a minimum reading depth of 7x. For some genes and mutations, complementary tests were performed by an alternative method.

Results: Screening for aneuploidies showed a high-risk for a complete trisomy (T) in three pregnancies (a T13, a T20 and a T21) (3/134). The analysis for SGD revelled mutations in the same gene in 4 couples (4/134). Two of this couples had mutations in the CFTR gene, one couple had mutations in the HBA gene, and the last couple had mutations in both OTC and GJB2 genes. A total of 264 mutations have been detected in all the individuals analyzed in this study.

Conclusion: The proposed procedure has doubled the prenatal screening yield, compared with the classical NIPT analysis. This new approach improves the understanding of non-classically screened genetic conditions, allowing patients to make informed reproductive decisions.

Conflict of Interest: Emilia Mateu-Brull Full time, Nuria Balaguer Full time, Arantxa Hervas Full time, Miguel Millán Full time

P16.039.C Nanopore adaptive sampling for rapid gene fusion detection in hematological malignancies

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Background/Objectives: Recurrent chromosomal rearrangements (translocations, inversions, deletions) that generate gene fusions and/or activate oncogenes facilitate the diagnosis and prognostication of patients with hematological malignancies and play a role in selection of treatment strategies. Standard-of-care methods that are used for detection of clinically relevant chromosomal rearrangements are laborious, time-consuming, show low sensitivity and may require prior knowledge about genes/regions involved in rearrangements.

Methods: Here we have used a targeted long-read sequencing method, adaptive sampling from Oxford nanopore technologies (ONT), to detect gene fusions at high resolution from DNA samples suitable for a convenient clinical workflow. Adaptive sampling is solely software-controlled and requires no wet lab-based enrichment. It therefore enables a rapid workflow that is easy to customize by including new regions for genetic analysis.

Results: With ONT's adaptive sampling we were able to detect chromosomal rearrangements in well-characterized cancer cell lines, including a *MYC::IGH* rearrangement in the OCI-LY7 cell line and a *FUS::ERG* rearrangement in the YNH-1 cell line. Importantly, and in contrast to FISH and karyotyping, the exact chromosomal breakpoints were identified. Analyses of patient samples are currently on-going.

Conclusion: ONT's adaptive sampling has a potential to replace standard-of-care methods that are used for detection of gene fusions in hematological malignancies. This method provides a simple laboratory workflow and is easy to adapt to include any target regions. Furthermore, by identifying patient-specific breakpoints, sensitive assays (e.g., ddPCR) may easily be designed for tumor burden monitoring in patients undergoing treatment or following stem-cell transplantation.

Grant references: SciLifeLab Technology Development Funding

Conflict of Interest: None declared

P16.040.D Simultaneous measurement of genetics and epigenetics enables new biological insight

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DNA comprises molecular information stored in genetic and epigenetic bases, both of which are vital to our understanding of biology. The interaction of genetics with the DNA epigenome plays a causal role in cell fate, ageing, response to environment and disease development. Methods widely used to detect epigenetic DNA bases do not distinguish unmodified cytosines and thymine, therefore fail to capture common C-to-T mutations and thus capture incomplete genetic information. Five-letter seq is a single base-resolution sequencing methodology that sequences complete genetics and cytosine modification in a single workflow.

Five-letter seq generates high quality genetic and epigenetic information, even from low DNA input, enabling the identification of genetic variants and quantification of modified cytosine levels in a single experiment. The phased nature of the technology, whereby genetic and epigenetic information is available jointly at read-level, enables the study of genetic and epigenetic co-variation. For example, allele-specific methylation (ASM), whereby differential methylation patterns are observed between heterozygous variants. We identify ASM across the genomes of all 7 Genome-in-a-bottle samples. We go on to show that a substantial increase in the degree of ASM is associated with successful "maturation phase transient reprogramming" (MPTR) whereby the transcriptome and epigenome of fibroblasts from middle-aged donors are rejuvenated about 30 years. No such increase in ASM is associated with fibroblasts that were treated by MPTR but failed to rejuvenate. This work demonstrates not only that ASM can be directly identified using five-letter sequencing, but that ASM is associated with cellular ageing and function.

Conflict of Interest: Nicholas Harding Cambridge Epigenetux, Páidí Creed Cambridge Epigenetix, David Currie Cambridge Epigenetix, Sabri Jamal Cambridge Epigenetix, David Morley Cambridge Epigenetix, Fabio Puddu Cambridge Epigenetix, Casper Lumby Cambridge Epigenetix, Jack Monahan Cambridge Epigenetix, Jamie Scotcher Cambridge Epigenetix, Rosie Spencer Cambridge Epigenetix, Jean Teyssandier Cambridge Epigenetix, Michael Wilson Cambridge Epigenetix, Jens Fullgrabe Cambridge Epigenetix, Aurel Negrea Cambridge Epigenetix, Alexandra Palmer Cambridge Epigenetix, Audrey Vandomme Cambridge Epigenetix, Shirong Yu Cambridge Epigenetix, Philippa Burns Cambridge Epigenetix, Daniel Brudzewsky Cambridge Epigenetix, Diljeet Gill Altos Labs, Aled Parry Altos Labs, Wolf Reik Altos Labs, Cambridge Epigenetix, Cambridge Epigenetix, Joanna Holbrook Cambridge Epigenetix

P16.041.A Optical Genome Mapping aids better understanding and classification of structural variants

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Background: Structural variants (SVs) cause changes to the genomic landscape. These changes can shape genome evolution at the population-level, but also lead to genetic diseases in individuals. The current standard-of-care cytogenetic tools, along with next generation sequencing methods enable the detection of such SVs, albeit with limitations. Optical genome mapping (OGM) overcomes many of these limitations by allowing genome-wide detection of unbalanced and balanced SVs at a resolution of a few hundred base pairs.

Methods: We present two cases, where standard cytogenetic analysis led to the identification of a rare translocation (t(3;5)(p13;q22)) and a rare duplication (~60 kb on 11q23.3) variant, respectively. These variants, however, could not provide a clear genotype-to-phenotype correlation for the patients. We therefore, went on to perform OGM, to obtain additional information on the detected variants.

Results: Through OGM analysis, both variants could be identified. Fine-mapping using OGM-data revealed that the breakpoint of the translocation-event lies in the *FOXP1* gene (3p13), whereas the duplicated region on 11q23.3 lies within the *KMT2A* gene. These events lead to a disruption of the respective genes, and can adequately explain the clinical phenotype of the patients.

Conclusion: These cases illustrate that OGM data can provide additional detailed information, such as the breakpoint of translocation-events and the positional information of duplication-events, which assists in the better understanding and classification of SVs, and hence allowing for a better diagnosis, prognosis, and genetic counseling of the patient.

Conflict of Interest: None declared

P16.042.B One-step library preparation for next generation sequencing

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Abstract Title: One-step library preparation for next generation sequencing

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Background/Objectives: Many genetic diseases are caused by mutations in a single nucleotide affecting genes implicated in biological procedures. The current technology for its detection is the next generation sequencing, that requires the generation of genetic libraries. In amplicon based libraries, two consecutive PCR reactions are generally required. This work attempts to simplify the process of library generation by optimizing the technology easy one-step amplification and labeling (EOSAL) for generation of next generation sequencing libraries in only one PCR reaction. The procedure will also reduce the risk of contamination and handling errors.

Methods: To achieve the goal, genes implicated in angioedema hereditary were selected and primers for these regions were designed. Then, the library preparation for these genes was optimized using the conventional technology in two steps and one-step technology, analyzing the coverage means for both techniques. A validation of easy one-step amplification and labeling EOSAL was performed in a population of 28 individuals suspected of hereditary angioedema and the results were compared with the two-step procedure.

Results: Via analyzing the coverage obtained and the mutations detected in the individuals studied using both techniques, the results obtained with EOSAL technique provide the same genetic information as those obtained with the two-step procedure.

Conclusion: The easy one-step amplification and labeling technique is valid for faster and simpler genetic libraries preparation. This technique, which has been validated in a hereditary angioedema population, could be applied simplify library generation for genetic studies.

Grant References: FDGENT 2019, Generalitat Valenciana. Valencia, Spain. PI21/00506 (ISCIII and FEDER)

Conflict of Interest: Soraya Garcia-Sorribes FDGENT/2019/006 Generalitat Valenciana., Maria Dolores Olivares: None declared, Francisco Lara-Hernandez: None declared, Elena Quiroz: None declared, Ana Barbara Garcia-Garcia FDGENT/2019/006 Generalitat Valenciana, Javier Chaves: None declared, Carmen Ivorra: None declared

P16.043.C Whole genome sequencing methods with low input DNA

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Background/Objectives: With the recent drop in sequencing price, whole genome sequencing (WGS) becomes the method of choice to interrogate human genome for medically relevant causal variants. However, for some applications such as cancer genetics or rare diseases in newborns, the current input requirement of 1µg genomic DNA (gDNA) for PCR-Free WGS is not always attainable. In this study, we compared different PCR-Free WGS approaches compatible with low input gDNA.

Methods: NGS libraries were prepared from human DNA reference standard HG002 using five commercial kits of low input WGS PCR-Free library prep. Both enzymatic and mechanical fragmentation were represented. The input ranged from 50ng to 500ng gDNA. We compared the library yield and quality control profiles for different inputs. We sequenced all libraries on NovaSeq (Illumina) at 30×. We assessed the depth, breadth and uniformity of genome coverage among the different kits. We evaluated their capacity to detect SNVs and indels correctly compared to known GIAB reference sequence of HG002 individual.

Results: We observed that enzymatic fragmentation provides more homogeneous results than mechanical fragmentation. We noted that the lowest input usually yields libraries with the shortest insert size, sometimes resulting in coverage below 30×. Consequently, the lowest input libraries require further optimisation to obtain longer fragments, or a decrease in multiplexing to reach target coverage.

Conclusion: All methods tested in this study yielded exploitable sequencing results with low input gDNA. The coverage metrics were similar for input around 200ng, while the performance varied for input below 100ng.

Grants: LabEx GENMED (ANR-10-LABX-0013).

Conflict of Interest: None declared

P16.044.D Genetic and Epigenetic study of Formalin-damaged DNA

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DNA comprises molecular information stored in genetic and epigenetic bases, both of which are vital to our understanding of biology. Here we apply five-letter seq, which sequences at base resolution complete genetic sequence integrated with modified cytosine (modC). Five-letter sequencing has previously been demonstrated for genomic and cell free DNA and here we show application to formalin fixed paraffin embedded (FFPE) samples.

FFPE samples represent an important resource for studying genetic and epigenetic information from archived tissues. However, DNA damage induced by formalin fixation can lead to decreased data quality from next generation sequencing (NGS).

Formalin damage to DNA results in lower library yields and insert sizes, as has previously been observed when using NGS approaches. It was also observed in this five-letter sequencing study. Also as previously observed for orthologous techniques, higher relative duplication and lower coverage rates were associated with increasing DNA damage. However, the genetic accuracy of five-letter sequencing was largely preserved and no effect was observed on variant allele frequency (VAF) calling for all formalin compromised DNA standards even with severe damage (DIN ≤ 2.0). Five-letter sequencing data for formalin compromised DNA shows slightly (~5%) diminished modC levels at CpG sites, consistent with that observed using other epigenetic sequencing technologies and likely due to formalin induced damage to the DNA prior to sequencing.

In conclusion we demonstrate the compatibility of our technology with formalin-compromised DNA, producing high accuracy genetic and epigenetic information from FFPE samples even at severe levels of DNA damage.

Conflict of Interest: Robert Crawford Cambridge Epigenetix, stock options in Cambridge Epigenetix, Jamie Scotcher Cambridge Epigenetix, stock options Cambridge Epigenetix, Fabio Puddu Cambridge Epigenetix, stock options in Cambridge Epigenetix, Daniel Brudzewsky Cambridge Epigenetix, stock options in Cambridge Epigenetix, Jane Hayward Cambridge Epigenetix, stock options in Cambridge Epigenetix, Andrada Tomoni Cambridge Epigenetix, stock options in Cambridge Epigenetix, David Morley Cambridge Epigenetix, stock options in Cambridge Epigenetix, Páidí Creed Cambridge Epigenetix, stock options in Cambridge Epigenetix, Philippa Burns Cambridge Epigenetix, stock options in Cambridge Epigenetix, Joanna Holbrook Cambridge Epigenetix, stock options in Cambridge Epigenetix

P16.045.A Towards implementing Nanopore sequencing for routine molecular diagnosis of germline cancer predisposition

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Background/Objectives: Molecular diagnosis can be used for patient management, from detection of germline predisposition to the choice of treatment. New challenges arose to generate more comprehensive data and to reduce sequencing time. In this context, routine molecular diagnosis laboratories have to explore and implement new technologies.

Methods: We have explored Nanopore sequencing on a GridION. We are performing technological assessments on 24 clinical germline cancer samples carrying abnormalities on BRCA1, BRCA2, PALB2 and MLH1 genes to test methylation status, large scale rearrangements (LSR), and single nucleotide variants (SNV). We have used adaptive sampling to enrich our target region of more than 150 cancer predisposition genes and used R10.4.1 flowcells and the Q20 chemistry for this experiment.

Results: After setting up the critical pre-analytical processes (DNA extraction, library prep), and optimizing the experimental workflow, the initial results show that we were able to enrich our region of interest and that adaptive sampling did not induce any bias in the read depth among the different chromosomes.

Conclusion: Nanopore sequencing for molecular diagnosis of germline cancer predisposition is a potential solution for routine clinical testing.

Grant references: The project has been partially funded by Oxford Nanopore Technologies

Conflict of Interest: Marie Mille SeqOne, SeqOne SO, Denis Bertrand SeqOne, SeqOne SO, Michael Blum SeqOne, SeqOne SO, Corentin Richard: None declared, Sandy Chevrier: None declared, Nicolas Philippe SeqOneGenomics, SeqOne stock, Romain Boidot Boehringer Ingelheim, Takeda, Oxford Nanopore Technologies, Conference travel: Takeda, Oxford Nanopore technologies, New England Biolabs, Agilent Technologies, SeqOne Genomics, SeqONE, Astra Zeneca, MSD, GSK, Myriad Genetics

P16.046.B Scalable single cell pooled CRISPR screening

Joey Pangallo[1], ***Anastasia Potts***[1], *Charlie Roco*[1], *Lauren Kenyon*[1], *Alex Sova*[1], *Alexa Suyama*[1], *Ryan Koehler*[1], *Alex Rosenberg*[1]

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Single cell CRISPR screens enable pairing of individual gene perturbations with rich whole transcriptome expression phenotypes. This approach has greatly expanded understanding of gene function and regulatory networks across mammalian cells. In particular, it has made it possible to distinguish the unique responses of different cell subpopulations to perturbations. However, pooled single cell CRISPR studies have been limited by the throughput of droplet-based single cell RNA-seq technology. With limited throughput, the number of perturbations has primarily been constrained by the feasibility of processing sufficient cells for large CRISPR guide libraries and the focus on sample types to those with limited cell type diversity.

With Evercode combinatorial barcoding technology, up to a million cells and 96 samples can be analyzed in a single experiment. When combined with CRISPR specific enrichment, Evercode technology makes it possible to analyze high plex guide libraries and complex samples with multiple cell types of interest.

In this study, we demonstrate the performance of Evercode technology in a CROP-seq pooled CRISPR screen. CRISPR

sequencing libraries mapped to the vector in >95% of reads, and single guide RNAs were confidently assigned to >85% of cells. We also demonstrated highly sensitive whole transcriptome analysis, enabling robust correlation of guide to gene expression output.

Conflict of Interest: Joey Pangallo Full time at Parse Biosciences, Stock options, Anastasia Potts Full time at Parse Biosciences, stock options, Charlie Roco Full time at Parse Biosciences, Stock options patent, Officer of the company (CTO), Lauren Kenyon Full time at Parse Biosciences, stock options, Alex Sova Full time at Parse Biosciences, stock options, Alexa Suyama Full time at Parse Biosciences, stock options, Ryan Koehler Full time at Parse Biosciences, stock options, Alex Rosenberg Full time at Parse Biosciences, stock options patent, Officer of the company (CEO)

P16.047.C Compatibility of non-human primate plasma with Olink® proteomics technology

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Background/Objectives: Animal models play a pivotal role in medical research especially larger species such as dogs, pigs, and non-human primates (NHPs). Due to their close phylogenetic relationship to humans, NHPs are often chosen as disease models in preclinical and translational studies, where developing therapeutics using other models like rodents have failed. This has been observed in disease research, such as for tuberculosis, in studies aiming to improve human vaccines, and in drug development for HIV infections and other conditions. NHPs serve to bridge the translational gap between small animal models and humans, where the large size of NHPs make sample collection and diagnostic assays easier. The similarity between the genomes and exomes of different NHP species and humans ranges between 93 %- 98.8 %, and this is translated at the protein level. Thus, antibodies targeting human proteins will potentially recognize those of NHP origin.

Results/Conclusion: In this study, we examined the detectability of proteins in *Macaca fascicularis* plasma using Olink's Explore 3072 panel, which measures ~3,000 human proteins using the Proximity Extension Assay (PEA). In addition to favorable data quality, proteomics signals obtained from individual animal plasma samples were consistent with expected biological changes including inflammatory responses. We conclude that Olink's PEA panels designed to target human proteins can serve as valuable tools for proteomics studies of NHP disease models.

Conflict of Interest: Tarif Awad Olink Proteomics, Mariana Fontes Olink Proteomics, Showgy Ma'ayeh Olink Proteomics, Simon Forsberg Olink Proteomics

P16.048.D Detection of 5-hydroxymethylcytosine at single base resolution

*Daniel Evanich[1], Vaishnavi Panchapakesa[1], Ariel Erijman[1], Matthew Campbell[1], Nan Dai[1], Bradley Langhorst[1], Romualdas Vaisvila[1], Chaithanya Ponnaluri[1], **Louise Williams**[1]*

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DNA methylation is an epigenetic regulator of gene expression with important functions in development and diseases such as cancer. NEBNext® EM-seq™ or bisulfite conversion are frequently used to detect 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). However, these methods cannot differentiate between 5mC and 5hmC. Methods such as oxBS-seq and TAB-seq have been used to identify 5mC and 5hmC respectively, however these methods are based on modifications of bisulfite conversion and have similar issues such as DNA fragmentation and loss of DNA. Here we describe an enzymatic method that enables specific detection of 5hmC.

5hmC is detected using two enzymatic steps. Initially, 5hmCs are glucosylated, which protects them from subsequent deamination by APOBEC. In contrast, cytosines and 5mCs are deaminated to uracil and thymine, respectively. During Illumina sequencing 5hmC are represented as cytosine whereas cytosine and 5mC are represented as thymine.

5hmC data were generated for 0.1 ng - 200 ng of DNA isolated from E14 mouse embryonic stem cells and human brain. The 5hmC libraries had similar characteristics to EM-seq libraries, including expected insert sizes, low duplication rates and minimal GC bias. 5mC and 5hmC levels were also profiled during E14 cell differentiation for a period of 10 days. Interestingly, 5hmC levels decreased whereas 5mC levels increased particularly during the first five days of differentiation. LC-MS/MS quantification of this same DNA mirrored the changes observed by sequencing. The ability to discriminate between 5mC and 5hmC will provide key insights into the role of these cytosine modifications in development and disease.

Conflict of Interest: Daniel Evanich Industry (NEB), Vaishnavi Panchapakesa Industry (NEB), Ariel Erijman Industry (NEB), Matthew Campbell Industry (NEB), Nan Dai Industry (NEB), Bradley Langhorst Industry (NEB), Romualdas Vaisvila Industry (NEB), Chaithanya Ponnaluri Industry (NEB), Louise Williams Industry (NEB)

P16.049.A What more can be done? A new approach to supporting hospitals and the health sector to achieve safe and effective implementation of genomics

***Clara Gaff**[1;2;3], Cate Kelly[1;2], Katie Arkell[1;3], Luisa Clucas[1;4;5;5], Claire Harris[1;6], Maira Kentwell[1;5], Melissa Martyn[1;2;3], Belinda McClaren[1;2;3]*

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Background/Objectives: There is a need for organisational systems to ensure safe and effective delivery of genomic medicine This requires healthcare organisations to consider how best to apply existing clinical governance frameworks to genomics while changing systems, processes and practices.

Hospitals are increasingly recognising the major challenge of mainstreaming genomics but there is a paucity of evidence-based resources to support this. We developed a suite of interdependent action research programs to inform organisation-level change.

Methods: Three key programs form a multi-pronged approach:

A systematic review of existing hospital implementation and clinical governance frameworks

Co-design (with hospital leaders) of a clinical genomics capability framework

'Change projects' to test a range of implementation science methodologies to support genomic testing in clinical practice.

Results: Nine papers describing frameworks for implementation have been identified but none comprehensively addressed hospital-level clinical governance of genomics.

A capability model addressing key clinical governance domains has been iterated with hospital leaders and key resources identified. A clinical change model has been developed and implemented in six Change Projects. Details will be presented.

Conclusion: Evidence for the benefit of genomic testing is not itself sufficient to ensure that genomic testing is embedded in routine clinical care where that is appropriate. This innovative

research brings new knowledge to the field of genomic medicine by testing approaches to the safe and effective implementation of genomics and through creation of co-designed resources to enhance hospitals' capability to embed genomics in routine clinical care.

Conflict of Interest: None declared

P16.050.B Single-cell RNA phenotyping of a mouse model for hypothyroidism reveals a pivotal role of thyroid hormone receptor alpha for hypothalamic development

Varun Sreenivasan[1;2], *Riccardo Dore*[3], *Julia Resch*[3], *Julia Maier*[3], *Carola Dietrich*[4], *Jana Henck*[1;2;4], *Saranya Balachandran*[1;2;5], *Jens Mittag*[3], *Malte Spielmann*[1;2;4;5]

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Background/Objectives: The thyroid hormone is a key regulator of physiological processes, including growth and brain development. Congenital hypothyroidism due to genetic causes or due iodine deficiency results in severe mental retardation. Mice harbouring mutations in the thyroid hormone receptor TRα1 display abnormalities in several autonomic functions, which was partially attributed to a developmental defect in hypothalamic parvalbumin neurons. However, whether other cell types in the hypothalamus are similarly affected remains unknown. We aimed to use single-nucleus RNA sequencing to obtain an unbiased view on the importance of TRα1 for hypothalamic development, in terms of the cellular diversity and phenotypes that cause mental retardation.

Methods: We studied the effect of hypothyroidism in mice heterozygous for the TRα1R384C mutation, which reduces the affinity of the receptor to the thyroid hormone by 10-fold.

Results: From a cell composition level, the mutation had a surprisingly little effect, with the cell numbers of all major cell types and neuronal subtypes unaffected by the mutation. However, the transcriptome of the hypothalamic oligodendrocytes was significantly altered, with the misexpression of ~100 genes. Using selective reactivation of the mutant TRα1 during specific developmental periods, we found that early postnatal thyroid hormone action is crucial for proper hypothalamic oligodendrocyte development.

Conclusions: Taken together, our findings underline the well-known importance of postnatal thyroid health for brain development and provide an unbiased roadmap for the identification of cellular targets of TRα1 action in mouse hypothalamic development.

Grant References: German Research Council, Max-Planck-Gesellschaft, and Deutsches Zentrum für Luft- und Raumfahrt

Conflict of Interest: None declared

P16.051.C Monitoring of long-term cultured induced pluripotent stem cells by Optical Genome Mapping (OGM) confirms sustained fine-structural genomic stability across more than 60 in vitro passages

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Genetic integrity testing of induced pluripotent stem cells (iPSCs) is crucial for their safe use in human disease modelling and drug therapy studies.

The conventional approach is by karyotyping and genome-wide SNP array, while submicroscopic genetic changes in iPSCs and their dynamics through passaging are poorly documented. In this study, Optical Genome Mapping (OGM), a next-generation platform for all structural variant types with a 1000-fold resolution vs. karyotyping, was used to track genomic events through early, intermediate and late passages of a fibroblast-derived iPSC line. Concurrently, cells were monitored by karyotyping and short-read sequencing.

Using the Bionano Genomics Gen 2.3 Saphyr instrument, molecules with at least 280 kbp N50 and 300x effective coverage were obtained for de novo-assembly. After removing assembly artefacts from the output, only variants <800 kbp size remained, supporting unremarkable karyograms in all three passages. Variant tracking across passages revealed only minimal changes in the temporal profile of cultured iPSCs. All 24 unique variants, absent in both the Bionano- and our own collective of 170 OGM-characterized genomes, were shared between passages, including one variant of clinical interest.

Of variants not shared between passages, several showed low allele frequencies in single-molecule data, indicating mosaicism as driver of inter-passage variability. However, low-level variants showed a bias towards early passage stages, suggesting that such cell fractions were selected against during in vitro-cultivation.

While OGM data can be useful to bridge blind spots in quality control of iPSCs they may require elaborate interpretation strategies complementary to the system´s standard variant calling.

Conflict of Interest: None declared

P16.052.D Optical Genome Mapping finds its place in a paediatric hospital: results of a validation

Barbara Fernández Garoz[1], *Ana Martín Martín*[1], *Laura Rodero Jurado*[1], *Beatriz Ruiz Gil*[1], *Manuel Ramirez Orellana*[1], *Ana Isabel Quinteiro García*[1], *Nelmar Valentina Ortiz Cabrera*[1]

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Background: The development of new cytogenetic techniques, such as optical genome mapping (OGM), could replace conventional cytogenetic/molecular techniques by combining them in a single test.

Methods: Here we present data of the validation study to include OGM in clinical practice in a paediatric hospital; this study was done from March 2022 to January 2023.

A retrospective and ongoing validation has been done. In table 1, we show the number of samples validated.

Table 1

Event	Retrospective samples		Ongoing samples	
	Normal	Abnormal	Normal	Abnormal
CNV	29	19	10	9
Structural variants*	27	21	19	6
>200 fragile X repeats	43	5	6	0

*aneuploidies, ring chromosomes, translocations and inversions

Results: In the retrospective validation, we obtained a 100% concordance in all events but structural variants, where we had 90% concordance due to a Robertsonian translocation and ring chromosomes not detected. In the prospective validation, we obtained 100% concordance in all events but CNVs, where we obtained 98%, observing a false positive deletion in cytoband 15q11-q13 with an allelic frequency of 30%, which was not confirmed neither by arrayCGH nor by FISH.

Conclusion: The good concordance data allow us to establish a pioneering technique in the routine of a paediatric hospital, improving turnaround time and detecting variants that we would not be able to with conventional techniques. Variants in telomeres, centromeres and satellites are undetectable because gaps in the current reference genome. Knowing the limitations of the OGM, if there is a specific suspicion, those specific tests can be requested.

Conflict of Interest: Barbara Fernández Garoz: None declared, Ana Martín Martín: None declared, Laura Rodero Jurado: None declared, Beatriz Ruiz Gil: None declared, Manuel Ramirez Orellana: None declared, Ana Isabel Quinteiro García: None declared, Nelmar Valentina Ortiz Cabrera part-time: NIMGenetics

P16.053.A Genetic diagnosis of repeat expansion disordersusing targeted nanopore sequencing

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Background: Sequencing using longer reads overcome many of the limitations of short read sequencing, such as the ability to reliably produce sequences of long repetitive regions and large segmental duplications. In addition, the ability to enrich for regions of interest (ROI) prior to sequencing is for many applications advantageous. In a clinical setting this means a cost-effective way to sequence only disease loci relevant for a specific patient.

Methods: We used two methods of targeted enrichment, firstly adaptive sampling using 'Read Until' API and secondly a Cas9 enrichment-based approach. Adaptive sampling: We designed a Read Until target covering the entire gene, including the repeat loci, for the most well-defined disorders associated with repeat expansions. Cas9 enrichment: We designed an ataxia panel (ONT Cas9 Sequencing kit) targeting the repeat loci in genes involved in ataxia.

Results: Sequencing of a clinical sample generated data with an estimated read length (N50) of 8.73 kb, compared to 19.83 kb from the high-quality DNA. The alignments and coverage of the regions was compared to an available PacBio WGS of the HEK293 cell line.

Conclusion: Using nanopore sequencing, we can reliably generate reads spanning the entire repeat region for most repeat expansion disorders, allowing for a more reliable repeat length estimation compared to current diagnostic tools. Furthermore, a targeted approach using adaptive sampling allows for a cost-efficient method. Lastly, the high coverage generated using Cas9 enrichment opens for the possibility to pool and sequence several samples on a single MinION flow cell using barcoding.

Conflict of Interest: None declared

P16.054.B Rapid trio whole genome sequencing in critically ill children – preliminary results of the first German multicenter study ("Baby Lion")

Bernd Auber[1], *Gunnar Schmidt*[1], *Chen Du*[1], *Alexander von Gise*[2], *Bettina Bohnhorst*[3], *Michael Sasse*[2], *Harald Köditz*[2], *Michaela Losch*[1], *Bernd Haermeyer*[1], *Benedikt Schnur*[1], *Florian Kaisen*[1], *Sandra von Hardenberg*[1]

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Background: Rapid whole exome and genome sequencing (rWES/rWGS) studies in critically ill children have reliably demonstrated diagnostic yields above 30% and clinical utility, but implementation in routine diagnostics and in non-university hospitals remains a challenge. Here we present preliminary results of the first German multicenter rWGS study (DRKS00025163), which commenced in May 2022.

Methods: Critically ill children in neonatal or pediatric intensive care units (NICUs/PICUs) across 12 German hospitals are eligible for study inclusion, if a genetic etiology is suspected and non-genetic causes are considered unlikely. Pediatric patients (0-14 years) and preferably both parents are included based on consultations at interdisciplinary video conferences, where results are also discussed. Rapid WGS is performed at Hannover Medical School. Clinical utility is measured using the C-GUIDE survey and parental perception is monitored using a second survey.

Results: In the first 40 weeks, 45 children were included, 19 from NICU and 26 from PICU. 43 trio and 2 duo rWGS were performed. Mean turnaround time from sample reception to disclosure of preliminary results was 2,9 days. A causative variant was reported in 20 patients (44%), highly likely causative variants of unknown significance were reported in two patients (4,4%).

Conclusion: rWGS in critically ill children in a multicentered patient recruitment setting has demonstrated high diagnostic yield and very fast turnaround time. The implementation of online multidisciplinary meetings allowed hospitals without a human genetics department to recruit eligible patients, resulting in a similar diagnostic yield as in tertiary centers.

Grant References: flow cells supplied by Illumina

Conflict of Interest: Bernd Auber Illumina supplies reagents for this study, Gunnar Schmidt: None declared, Chen Du: None declared, Alexander von Gise: None declared, Bettina Bohnhorst: None declared, Michael Sasse: None declared, Harald Köditz: None declared, Michaela Losch: None declared, Bernd Haermeyer: None declared, Benedikt Schnur: None declared, Florian Kaisen: None declared, Sandra von Hardenberg: None declared

P16.055.C Analysis of mitochondrial genome and detection of heteroplasmy using Oxford Nanopore sequencing

Barbara Slapnik[1], *Robert Sket*[1], *Barbara Cugalj Kern*[1], *Barbara Jenko Bizjan*[1], *Tine Tesovnik*[1], *Blaž Vrhovšek*[1], *Tadej Battelino*[2], *Jernej Kovač*[1]

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Background: The mitochondrial genome is an important source of genetic variations that cause mitochondrial diseases. These are heterogeneous disorders characterized by a shortage of cellular energy. Heteroplasmy is a state where we find two or more (geno) types of mitochondrial DNA (mtDNA) in a single organism. The clinical phenotype can be significantly affected by the level of

heteroplasmy therefore its accurate quantification plays a vital role in diagnostics.

Methods: We used targeted nanopore sequencing with Cas9 technology, which produces long reads and enables amplification-free sequence enrichment of mitochondrial DNA. Long reads were aligned on reference with Minimap2, followed by an assessment of heteroplasmy and variant calling. Then we performed de-novo assembly with Flye and hybrid de-novo assembly with Unicycler. For both assemblies, we identified variants and compared detected variants for all the above strategies.

Results: We demonstrated that technology enriches the mitochondrial genome and generates full-length nanopore reads of mtDNA. We then verified the ability of nanopore sequencing for the detection of low-level DNA heteroplasmy and determined that nanopore sequencing in combination with Cas9 technology can detect up to 10 % heteroplasmy. The number of variants detected on hybrid de-novo assembly was lower than on de-novo assembly with long reads and long reads aligned on reference.

Conclusion: Long read sequencing generates full-length mtDNA and produces uniform coverage of the mitochondrial genome, which is especially important in detecting heteroplasmic variations. Moreover, long reads improve de-novo assembly and detection of large sporadic deletions.

Grant References: Young Research Fellowship SRA#56916; UMC-tertiary grants: TP20210119, TP20210130

Conflict of Interest: None declared

P16.056.D Accessible fragment analysis instrumentation allows resolution of challenging genotypes associated with pathogenic repeats, structural variants, SNVs and INDELs

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Capillary electrophoresis (CE) instruments provide a robust platform for sequencing and fragment analysis. Advances have improved access to these instruments, reducing complexity and price. Here, we challenge the Spectrum Compact CE System (Promega) using difficult gene targets (*FMR1, C9orf72*, *CFTR*, and *SMN1/2)*, demonstrating accurate detection of structural variation and GC-rich repeat sizing for clinically relevant genes.

We tested 30 DNA samples isolated from human cell lines or whole blood using AmplideX PCR/CE *FMR1*, *C9orf72*, *CFTR*, and *SMN1/2* Plus kits (RUO, Asuragen) with the Applied Biosystems™ 3500 Genetic Analyzer (ThermoFisher) and Spectrum Compact CE System (Promega). Samples covered relevant genotypes for each gene, including all *FMR1* CGG and *C9orf72* G_4C_2 repeat categories, >20 *CFTR* variants, and 0-4 copies of *SMN1/SMN2*. All data were processed using AmplideX Reporter Software. We compared Spectrum Compact results to the 3500.

The overall percent agreement between the genotype calls was >95% for *FMR1, C9orf72*, *CFTR*, and *SMN1/2* across all relevant genotype categories. Bias observed between instruments was minimal, with no impact on sizing precision, genotype category, variant detection, or copy number calls. The peak signal intensities were within performance specifications and similar between instruments.

The PCR products of *FMR1, C9orf72*, *CFTR*, and *SMN1/2* genes, representing diverse and complex variant classes, can be resolved on the Spectrum Compact using automated software interpretation. The instrument performs consistently with other supported platforms, with benefits that include simplified capillary maintenance, reduced footprint, and accessible price. This excellent performance demonstrates versatility, as these genes challenge the technological limits of CE applications.

Conflict of Interest: Sarah Edelmon Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Steven Partin Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Adrian Lara Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Jackie Peda Promega, John Hedges Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, John Milligan Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand

P16.057.A De novo genome assemblies from Congolese participants show placement of novel sequence and structure relative to the reference genome

Jonathan LoTempio[1]*, Kizito Mosema[2], D'Andre Spencer[3], Kevin Karume[2], Miguel Almalvez[1], Celeste Musasa[3], Johanna Nsibu[2], Matthew Bramble[3], Désiré Tshala Katumbay[2], Dieudonné Mumba[2], Eric Vilain[1]*

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Background: Sequencing technology has advanced to the point where reads longer than, and as accurate as, those made for the Human Genome Project can be produced at a fraction of the cost. Software improvement puts de novo assembly within reach of laboratories with smaller compute resources. This has led to the democratization of reference genomics, which shows great scientific and social utility.

Objective: We use 30x-HiFi genomes assembled from trios recruited from Kinshasa to show the critical need for engaging more people from more places in genomics

Methods: Our ethics proposal was based on the 1000 Genomes protocol. Trios were recruited in Kinshasa. All self-reported geographic and ethnic backgrounds. Peripheral blood mononuclear cells were isolated from whole blood. Each member of the trio was sequenced with Illumina to a depth of 30x. Probands were sequenced on PacBio HiFi flowcells to ~30x depth, and Bionano optical maps were generated to 300x coverage. Assemblies were trio binned with yak, assembled with HiFiasm, and scaffolded with optical maps.

Results: We chose four trios for sequencing by selecting those thought to be historically distant from one another. Even with this perceived distance, mitochondrial groups did not follow geographic lines. We highlight novel large, and consistent structural variation that does not present deleterious phenotype. We further show that African Pangenomic Contigs can be placed on these genomes, resolving outstanding issues in missing data.

Conclusion: HiFiasm assemblies, scaffolded with Bionano, show advancement in placement and arrangement of known, unmapped data, representing a step forward in genomics.

Conflict of Interest: Jonathan LoTempio: None declared, Kizito Mosema: None declared, D'Andre Spencer: None declared, Kevin Karume: None declared, Miguel Almalvez: None declared, Celeste Musasa: None declared, Johanna Nsibu: None declared, Matthew Bramble: None declared, Désiré Tshala Katumbay: None declared, Dieudonné Mumba: None declared, Eric Vilain Owns stock in Bionano Genomics

P16.058.B High diagnostic potential of short and long read genome sequencing with transcriptome analysis in exome-negative developmental disorders

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dana dabbagh[3], isabelle gilles[4], Claire GAYET[5], Pascale Saugier-Veber[1], Alice Goldenberg[1], Anne-Marie Guerrot[1], Gaël Nicolas[1]

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Introduction: Exome sequencing (ES) has become the method of choice for diagnosing rare diseases, while the availability of short-read genome sequencing (SR-GS) in a medical setting is increasing. In addition, new sequencing technologies, such as long-read genome sequencing (LR-GS) and transcriptome sequencing, are being increasingly used. However, the contribution of these techniques compared to widely-used ES is not well established, particularly in regards to the analysis of non-coding regions.

Methods: In a pilot study of 5 probands affected by an undiagnosed neurodevelopmental disorder after ES, we performed trio-based short-read GS and long-read GS as well as case-only peripheral blood transcriptome sequencing.

Results: We identified 3 new genetic diagnoses (60%), none of which affected the coding regions. More specifically, LR-GS identified a balanced inversion in *NSD1*, highlighting a rare mechanism of Sotos syndrome. SR-GS identified a homozygous deep intronic variant of *KLHL7* resulting in a pseudo exon inclusion, and a de novo mosaic intronic indel in *KMT2D*, leading to the diagnosis of Perching and Kabuki syndromes, respectively. All three variants had a significant effect on the transciptome, which showed decreased gene expression, mono-allelic expression and splicing defects, respectively, further validating the effect of these variants.

Discussion: Overall, in undiagnosed patients, the combination of SR and LR-GS allowed the detection of cryptic variations not or barely detectable by ES, making it a highly sensitive method at the cost of complex bioinformatics approaches. Transcriptome sequencing is a valuable complement for the functional validation of variants, particularly in the non-coding genome.

Conflict of Interest: None declared

P16.059.C Capture RNA-seq as supplement to DNA germline testing to increase diagnostic yield

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Introduction: Hereditary tumor syndromes are responsible for 5-10% of cancers. Accurate identification of causal genetic variants in affected patients is highly important in patient management. The diagnostic rate of whole-exome sequencing (WES), which is the current state-of-the-art molecular diagnostics approach, is between 30-50% across studies. Whole-genome sequencing (WGS) is estimated to increase the diagnostic rate by 5%. To further uplift diagnostic rates, high-throughput functional studies like RNA-seq are highly desired that reclassify variants of uncertain significance (VUS).

Methods: We developed a cost-efficient high-throughput capture RNA-seq approach for the analysis of RNA phenotypes in 49 cancer-associated genes from PAXgene RNA samples.

Results: We achieved ultra-high coverage sequencing data with of ~20,000 mean target coverage and on average 88% of exons covered with >50 read depth. As proof-of principle, we could verify aberrant phenotypes in 84% (22/26) of tumor syndrome patients with known pathogenic variants, as we could successfully identify aberrant splicing and allelic loss. Further, we were able to reclassify 16% (5/31) VUS cases as pathogenic.

Conclusion: Our workflow provides high quality RNA-Seq data, which allow the assessment of splicing events and allelic imbalance, and can be automated for high sample throughput. This work represents the basis for future implementation of targeted RNA-Seq in cancer diagnostics.

Conflict of Interest: None declared

P16.060.D Development of a universal assay for multiple targeted NGS tests supported by a novel sample data management tool

Skevi Kyriakou[1], *Michaella Georgiadou[1], Achilleas Achilleos[1], Christos Lemesios[1], Christodoulos Savva[1], Chrystalla Havadjia[1], Kyriakos Tsangaras[1], Gaetan Billioud[1], Chrysovalando Sotiriou[1], Louisa Constantinou[1], Haris Kkoufou[1], Lygia Ioannou[1], Michalis Spyrou[1], Stelia Pissaridou[1], Antonia Matsentidou[1], Christos Prokopi[1], Melina Vaki[1], Styliana Georgiou[1], Elena Kypri[1], Marios Ioannides[1], George Koumbaris[1], Philippos Patsalis[1,2]*

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Background/Objectives: Advances in genetic testing led to improvements in rare diseases and oncology, including early detection, management, and treatment. Currently, NGS workflow solutions can be laborious and non-scalable when manually applied. Our aim was to develop a single workflow solution to be used for identification of genetic variants using our proprietary technology platform in the fields of postnatal, neonatal, reproductive and oncology to accommodate the needs of any lab size.

Methods: The assay consists of a novel universal, single-tube library preparation and in-solution hybridization protocol supported by a data management tool. Probes are designed and synthesized to cover all coding regions of interest and grouped accordingly to form cardiac, neonatal, carrier, metabolic, infertility, PGS and oncology panels. The sensitivity and specificity of the assay was assessed followed a blind validation study performed on samples with known variant status and samples found to be carriers of mutations previously identified by an independent laboratory.

Results: SNVs and INDELS were detected at sensitivity of 100% (CI: 98-100%) and specificity of 100% (CI: 99.9-100%). The algorithm was designed to detect CNVs at a few exon resolution with high sensitivity and specificity. All variants were confirmed by an orthogonal method.

Conclusion: We have developed and validated a universal workflow including a novel NGS library hybridization pipeline and data management software that can multiplex different tests in a single sequencing run. Our end-to-end CE-IVD solution accommodates the needs of laboratories by enabling the consolidation of the analysis of many different tests using manual or automated protocols.

Conflict of Interest: Skevi Kyriakou full time employment at Medicover Genetics, Michaella Georgiadou full time employment at Medicover Genetics, Achilleas Achilleos full time employment at Medicover Genetics, Christos Lemesios full time employment at Medicover Genetics, Christodoulos Savva full time employment at

Medicover Genetics, Chrystalla Havadjia full time employment at Medicover Genetics, Kyriakos Tsangaras full time employment at Medicover Genetics, Gaetan Billioud full time employment at Medicover Genetics, Chrysovalando Sotiriou full time employment at Medicover Genetics, Louisa Constantinou full time employment at Medicover Genetics, Haris Kkoufou full time employment at Medicover Genetics, Lygia Ioannou full time employment at Medicover Genetics, Michalis Spyrou full time employment at Medicover Genetics, Stelia Pissaridou full time employment at Medicover Genetics, Antonia Matsentidou full time employment at Medicover Genetics, Christos Prokopi full time employment at Medicover Genetics, Melina Vaki full time employment at Medicover Genetics, Styliana Georgiou full time employment at Medicover Genetics, Elena Kypri full time employment at Medicover Genetics, Marios Ioannides full time employment at Medicover Genetics, George Koumbaris full time employment at Medicover Genetics, Philippos Patsalis full time employment at Medicover Genetics

P16.061.A Accurate inference of parent-of-origin of pathogenic variants in SDHD without parental data

Kasmintan Schrader[1;2;3], *Vahid Akbari*[4], *Vincent Hanlon*[5], *Katherine Dixon*[4], *Kieran O'Neill*[4], *Yaoqing Shen*[4], *Alshanee Sharma*[6], *janine senz*[3], *Yanni Wang*[5], *Chan Daniel*[5], *Alexandra Fok*[6], *Jennifer Nuk*[1], *Quan Hong*[7], *Dean Regier*[7;8], *Alice Virani*[2;9], *Louis Lefebvre*[2], *Fabio Feldman*[6], *Marco Marra*[2;4], *Sophie Sun*[1], *Stephen Yip*[6;10], *Peter Lansdorp*[2;5], *Steven Jones*[2;4]

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Background: Parent-of-origin-aware genomic analysis (POAga) using Oxford Nanopore Technologies long-read sequencing and single-cell DNA template strand sequencing (Strand-seq) can infer parent-of-origin for all autosomal chromosomes with an average mismatch error rate of 0.31% for SNVs and 1.89% for indels, using only the sample from the child. We examined whether POAga could accurately predict variant parent-of-origin in real-world samples from *SDHD* and *SDHAF2* pathogenic variant carriers with known parental segregation. *SDHD* and *SDHAF2*, demonstrate parent-of-origin effects, with high lifetime risks for paragangliomas and pheochromocytomas only when pathogenic variants are transmitted through the male gamete.

Methods: Blood samples from *SDHD* and *SDHAF2* pathogenic variant carriers were used for Oxford Nanopore Technologies long-read genome sequencing and Strand-seq under an approved research ethics board protocol, with parent-of-origin analysis performed in a blinded manner. Prior clinical testing and patient and family phenotype data was assessed to determine the rate of correct assignment.

Results: With a combination of automated calling and manual review, long-read sequencing detected all known *SDHD* pathogenic variants (n = 18) in 18 individuals. In all 18 cases, the pathogenic *SDHD* variant could be correctly assigned to the known parent-of-origin using the POAga method.

Conclusion: Preliminary findings suggest POAga is accurate and feasible from whole blood samples. For carriers, knowledge of parent-of-origin for *SDHD* pathogenic variants is essential when being advised on lifelong surveillance. When segregation is unknown, POAga could dramatically improve the clinical care of this group of patients.

Conflict of Interest: None declared

P16.062.B Galeas™ Pan-Cancer: a novel comprehensive solution to clinical genetic tumour profiling

Agata Stodolna[1], *Robert Hastings*[1], *Laura Delfino*[1], *Karen Cook*[1], *Samuel Clokie*[1], *Andrew Feber*[1], *Michael Parks*[1]

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Introduction: Next generation sequencing (NGS) has been an invaluable tool in the translation of genomic sequencing into clinical practice. Cost effective solutions allowing the targeted assessment of hundreds of cancer-related genes simultaneously has become the mainstay of clinical genomics. The clinical utility of gene panel testing is well established, not only allowing the genetic basis of an individual's tumour to be defined, but also allowing insight into potential treatment options.

Materials and methods: Using the Nonacus proprietary design algorithm, we have generated a comprehensive pan-cancer panel for SNV, MSI, TMB and comprehensive genome wide CNV profiling, which covers all clinically relevant alterations across solid tumour cancers.

Results: Galeas™ Pan-Cancer represents an automated sample-to-report workflow platform, that leverages our purpose-built bioinformatics pipelines to allow assessment of important cancer related biomarkers, including SNV, CNV, SV, MSI and TMB. The Galeas™ Pan-Cancer panel was tested across multiple reference materials to prove detection of low copy variants in FFPE derived DNA down to 1% VAF, and from ctDNA derived DNA to a VAF of 0.1%. Clinical validation of SNV and MSI calls was performed on 54 colorectal cancer derived DNA samples.

Conclusions: We have developed a NGS Pan-Cancer assay, to support comprehensive genomic profiling in routine clinical genomics. The clinically informed design and bespoke informatics workflows of the Galeas™ Pan-Cancer solution allows the sensitive and specific identification of clinically relevant somatic variants, along with comprehensive copy number profiles, microsatellite instability and tumour mutational burden from solid tumour FFPE and ctDNA.

Conflict of Interest: Agata Stodolna Nonacus Ltd, Robert Hastings Nonacus Ltd, Laura Delfino Nonacus Ltd, Karen Cook Nonacus Ltd, Samuel Clokie Nonacus Ltd, Andrew Feber Nonacus Ltd, Michael Parks Nonacus Ltd

P16.063.C Deciphering the role of non-coding variants in the etiology of neurodegenerative diseases (NDDs) by massively parallel reporter assay (MPRA)

Lucia Corrado[1], ***Beatrice Piola***[1], *fjorilda caushi*[1], *Endri Visha*[1], *Nadia Barizzone*[1], *Erica Melone*[1], *Diego Cotella*[1], *Laura Follia*[1], *Martina Tosi*[1], *Fabiola De Marchi*[2], *Luca Magistrelli*[3], *Letizia Mazzini*[2], *Alfredo Brusco*[4], *sandra d'alfonso*[1]

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Background/Objectives: In neurodegenerative disorders few patients showed a disease family history indicating that genetics

play a role in disease etiology. Missing heritability is still present and could be explained by variants in the non-coding regions of the genome. This study aimed to perform a high-throughput analysis using MPRA, to analyze several putative regulatory variants simultaneously from WGS data.

Methods: 41 rare non-coding VUS located in regulatory regions were selected. A library of 2460 probes (identified by different barcodes) was designed and cloned in pMPRA-vectors upstream of an ORF-sequence and transfected into SHSY5Y cells. After RNA isolation and sequencing, bioinformatics analysis was performed using R version 4.2.0 and mpralm function.

Results: Five variants mapping in the upstream region of *ELOVL5, GIGYF2, OMA1, CWF19L1* and *NEK1* genes, were found to decrease (*OMA1, CWF19L1, NEK1*) or increase (*ELOVL5, GIGYF2)* gene expression (Pvalue ≤ 0,01). The variants in *GIGYF2* and *NEK1*, genes known to be associated with PD and ALS, respectively and involved highly conserved nucleotides, were found each in one patient with consistent clinical phenotype. Variant *in OMA1* (a mitochondrial stress response gene) w*as* found in an ALS patient while the variants in *CWF19L1* and *ELOVL5 (*known ataxic genes*)*, in two ataxic patients. All these variants localized in sequences annotated as promoter by UCSC-GRCh38/hg38-ENCODE.

Conclusion: Even though the regulatory activity of these variants needs to be confirmed with other functional assays, MPRA was confirmed to be a good tool to screen simultaneously hundreds of non-coding putative pathogenetic variants.

Grants: PRIN 2017(2017SNW5MB_005), PRIN 2020(20203 P8C3X).

Conflict of Interest: None declared

P16.064.D Efficient and specific depletion of abundant and uninformative transcripts using a novel, algorithmic probe design tool to improve meaningful transcript sensitivity in RNA-seq

Rajat Roy[1], *Travis Sanders*[1], *David Gelagay*[1], *Kailee Reed*[1], *Jennifer Pavlica*[1], *Philip Benson*[1], *Giulia Corbet*[1], *Thomas Harrison*[1], *Brian Kudlow*[1]

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Background/Objectives: Whole transcriptome analysis is a powerful clinical tool to assess mRNA expression as well as biologically relevant long noncoding RNAs that serve as disease biomarkers and therapeutic targets. Sensitive and cost-effective sequencing of these RNAs requires upstream depletion of abundant ribosomal RNAs (rRNAs) and/or hemoglobin transcripts from total RNA. While technologies to deplete these RNAs are widely used, minimal efforts have been made to remove other abundant and uninformative transcripts, which reduce assay sensitivity. Thus, we developed a novel probe design algorithm which generates specific custom-designed probes capable of integrating into existing rRNA and hemoglobin depletion chemistry. Here we target, and specifically deplete, a broad group of abundant transcripts of disinterest to improve gene detection sensitivity and sequencing economy.

Methods: Considering ACMG SF 3.1 guidelines, we selected abundant and uninformative transcripts to target using empirical sequencing data from human, blood-derived RNA. Transcripts were selected to assess probe design and depletion efficiency across a wide range of GC-contents. We generated a custom probe pool using our algorithm and evaluated its performance with total RNA inputs ranging from 10 to 1000 ng.

Results: We observed excellent depletion efficiency of custom-targeted, as well as rRNA and hemoglobin, transcripts across all GC-contents evaluated with no measurable off-target effects. Ultimately, sequencing economy was improved with more reads supporting features of interest.

Conclusion: Our custom-designed probes efficiently and specifically deplete transcripts of disinterest to maximize sequencing economy. Beyond human-specific targets, the wide-GC viability makes this technology relevant for metatranscriptomic samples, as well.

Grant References: N/A

Conflict of Interest: Rajat Roy: None declared, Travis Sanders Employed full-time at Watchmaker Genomics, David Gelagay Employed full-time at Watchmaker Genomics, Kailee Reed Employed full-time at Watchmaker Genomics, Jennifer Pavlica Employed full-time at Watchmaker Genomics, Philip Benson Employed full-time at Watchmaker Genomics, Giulia Corbet Employed full-time at Watchmaker Genomics, Thomas Harrison Employed full-time at Watchmaker Genomics, Brian Kudlow Employed full-time at Watchmaker Genomics

P16.065.A Optical genome mapping as an innovative method for detecting repeat expansions and contractions

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Fully repetitive reads are difficult to accurately align to the reference sequence, thus short-read sequencing based methods have limitations to detect expansions and contractions which underly many disorders including facioscapulohumeral muscular dystrophy (FSHD), cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS), and Fragile X Syndrome (FXS). Usually phenotype severity of listed disorders correlates with number of repeats.

Bionano Optical Genome Mapping (OGM) is recently introduced innovative method allowing to detect structural variants. High resolution OGM provides more detailed analysis than traditional cytogenetics methods and apart from short-read NGS has power of detecting the repeats instability. We used OGM technology to study FSHD, CANVAS and FXS patients. We examined four patients with suspected FSHD, one with FXS and three with CANVAS. One of the tested patient had externally confirmed FSHD1 with Southern Blot. In all FSHD patients we detected D4Z4 repeats region contractions to less than 10 repeats on a permissive haplotype 4qA which indicates FSHD1. No copy number gains and losses were identified in the *SMCHD1* gene. In a group of CANVAS suspected patients one exhibited homozygous repeat expansion in the *RFC1* gene (AAGGG tandem repeats exceed 1000) which was further confirmed by TP-PCR method. Furthermore, we confirmed the power of OGM in detecting of FXS. We identified over than 800 CGG pathogenic repeats in the *FMR1* gene which is considered a full mutation.

In conclusion, OGM is a valuable next generation tool for detecting SVs which can be a potential supplement to the available clinical genetics diagnostics methods.

Conflict of Interest: None declared

P16.066.B HybrAmp approach for CYP21A2 pathogenic allele screening and diagnosis

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Objectives: Our reproduction clinic specializes in detection of pathogenic alleles in unaffected carriers of several recessive diseases. Genetic screening of congenital adrenal hyperplasia (CAH) is rather challenging due to the close proximity of duplicated region with the presence of highly homologous pseudogene (*CYP21A1P*) and high occurrence of several rearrangements leading to complete gene loss or formation of chimeras between gene and its pseudogene. For a long time we were confident in only dedicated number of pathogenic alleles in *CYP21A2* gene.

Methods: As a starting point we work with WES data (see our other contribution) where all samples undergo special data evaluation to elucidate presence of pathogenic allele in *CYP21A2* gene. Here we present a new approach which is added ad hoc. Locus specific long-range PCR amplicons are the starting material for NGS library preparation by a HybrAmp approach (PMID: 35697147). Amplicons are enzymatically fragmented and technical sequences are added, libraries are pooled and mixed with routine hybridization library prior to Illumina sequencing. *CYP21A2* gene is scanned for variants from the promotor down to the 3´UTR including introns. If all called variants show homozygous status, we apply second round of HybrAmp with long-range PCR where forward primer is *CYP21A1P*-specific and reverse primer is *CYP21A2*-specific.

Results: By HybrAmp approach we were able to assign pathogenic alleles and define presence of chimera and its description.

Conclusion: Under reduced cost this approach provides highly confident diagnosis of *CYP21A2* gene, but could be easily adjusted for other genes with pseudogenes or other challenging DNA diagnostics.

Conflict of Interest: None declared

P16.067.C Clinical validation of the Galeas™ Hereditary Cancer NGS panel, a comprehensive sample-to-report platform for clinical cancer risk profiling

*Panagiota Paganopoulou[1], Geoff Woodward[2], Laura Delfino[1], Karen Cook[1], Yvonne Wallis[3], Samantha Butler[3], **Samuel Clokie**[1], Michael Parks[1], Andrew Feber[1]*

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Introduction: Understanding the genetic basis of cancer risk is essential for the identification of patients with hereditary cancer syndromes (HCS) and Pis important to detect families who can benefit from preventive surgeries and surveillance due to increased risk of developing cancer. Hereditary cancer accounts for approximately 5-10% of all cancers. The use of targeted NGS-based multigene panels to provide the comprehensive analysis of cancer susceptible genes has proven to be a viable option, providing the accurate and robust detection of a wide range of clinically relevant HCS associated variants.

Materials and methods: A clinically informed hereditary cancer NGS panel (HCP) was designed using the Nonacus proprietary design algorithm. When combined with a novel analysis pipeline, incorporating a reference panel of 50 samples with no reported familial conditions, the Galeas™ HCP allows the sensitive and specific identification of germline SNV, INDELs and CNVs.

Results: The Geleas™ HCP was validated on a panel of reference standards and patient samples with orthogonal NGS and MLPA data. SNV recall on clinical samples was 100%, across a wide range of alteration types, including small and large (>10bp) indels. When assessing the detection of clinically relevant CNVs, the Geleas™ HCP demonstrated a sensitivity and specificity of 96.9% and 99.6% respectively.

Conclusions: The Galeas™ HCP provides a novel, automated, sample-to-report NGS workflow, optimised for detection of SNV, indel and CNV events associated with hereditary cancer syndromes. With a simple workflow this panel provides a comprehensive, easy and economical tool for clinical cancer risk profiling.

Conflict of Interest: Panagiota Paganopoulou Nonacus Ltd, Geoff Woodward: None declared, Laura Delfino Nonacus Ltd, Karen Cook Nonacus Ltd, Yvonne Wallis: None declared, Samantha Butler: None declared, Samuel Clokie Nonacus Ltd, Michael Parks Nonacus Ltd, Andrew Feber Nonacus Ltd

P16.068.D Bridging the gap: experiences of the Unsolved Cases Unit Groningen

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After solving 30-40% of all patients submitted for genetic testing, the possible avenues for follow-up testing are diagnostically limited. Experimental diagnostics can help to bridge the gap between the clinic and research.

At the UMCG, we have implemented a team of clinicians, researchers and laboratory geneticists focused on solving well-phenotyped unsolved cases. To date, forty-four patients have been registered. The phenotypic spectrum includes developmental delay, congenital anomalies and skin ailments. Re-analysis of NGS data, RT-PCR, qPCR and Western blot techniques have been applied. Analyses are ongoing, four cases have been solved so far.

Using RT-PCR, a homozygous missense variant in *PLAA* was found to initiate alternative splicing, resulting in truncation of the gene. With this result the patients' brain anomalies, hypotonia, epilepsy and edema of the hands are explained. A likely pathogenic in-frame deletion in the *CHD3* gene was found by re-analysis of NGS data and explains the phenotype of a patient with hypertelorism, macrocephaly and global developmental delay. A VUS missense variant in *EDA* in a family with ectodermal dysplasia could be re-classified by comparing healthy *vs*. affected family members *EDA* function using qPCR and Western blot techniques. A tandem duplication of exon 5 in the *BRWD3* gene in a patient with overgrowth, macrocephaly and axial hypotonia was confirmed using RT-PCR.

Our results demonstrate the added value of incorporating an experimental diagnostic approach in genome diagnostics to assist in solving the unsolved cases. Our experience shows that this approach is supported by patients and clinicians.

Conflict of Interest: None declared

P16.069.A Minimizing artifacts in NGS libraries from FFPE with an innovative enzymatic library preparation workflow

Ann-Cathrin Lindner[1], *Giulia Corbet[1], Philip Benson[1], Kailee Reed[1], Skyler Mishkin[1], Thomas Harrison[1], Kristin Scott[2], Zane Jaafar[1], Travis Sanders[1], Kristina Giorda[1], Ross Wadsworth[1], Amy Liu[1], Josh Haimes[1], Martin Ranik[1], Brian Kudlow[1]*

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Clinical oncology heavily relies on FFPE tissue samples for histology and molecular characterization. These samples harbor

nucleic acid modifications which negatively impact molecular profiling and vary between samples. Conventional sonication and ligation-based library preparation is considered the gold-standard approach for FFPE samples, but it is time-consuming, expensive, and introduces artifacts that impact downstream analysis and interpretation (Haile et al., 2019).

In this study, we have developed a novel fragmentation chemistry that is highly scalable and exhibits minimal sequence bias, reducing cost and workflow inefficiencies associated with sonication. Our unified library preparation method produces similar insert sizes across variable input mass and FFPE sample quality. We evaluated the performance of this method relative to a sonication-based approach with 50-200 ng of FFPE DNA. Targeted sequencing was performed using a custom oncology panel to investigate molecular complexity.

Our workflow virtually eliminated hairpin artifacts that were present in up to 4.5% of reads in sonication-based libraries. Soft-clipping was also 3- to 7-fold lower relative to sonicated DNA libraries, improving overall sequencing economy. The mean target coverage achieved with the Watchmaker kit was comparable to or higher than sonication libraries using the same input mass. Compared to sonication, which typically results in 20-40% sample loss, our approach yields significantly higher coverage when normalizing to pre-sonication input..

Watchmaker DNA Library Preparation with Fragmentation enables high-quality library preparation from FFPE samples, producing high target coverage, uniform insert size, and minimizing sequencing artifacts. This approach is highly scalable and automatable, enabling various oncology applications.

Conflict of Interest: Ann-Cathrin Lindner: None declared, Giulia Corbet Watchmaker Genomics, Watchmaker Genomics, Philip Benson Watchmaker Genomics, Watchmaker Genomics, Kailee Reed Watchmaker Genomics, Watchmaker Genomics, Skyler Mishkin Watchmaker Genomics, Watchmaker Genomics, Thomas Harrison Watchmaker Genomics, Watchmaker Genomics, Kristin Scott: None declared, Zane Jaafar Watchmaker Genomics, Watchmaker Genomics, Travis Sanders Watchmaker Genomics, Watchmaker Genomics, Kristina Giorda Watchmaker Genomics, Watchmaker Genomics, Ross Wadsworth Watchmaker Genomics, Watchmaker Genomics, Amy Liu Watchmaker Genomics, Watchmaker Genomics, Josh Haimes Watchmaker Genomics, Watchmaker Genomics, Martin Ranik Watchmaker Genomics, Watchmaker Genomics, Brian Kudlow Watchmaker Genomics, Watchmaker Genomics

P16.070.B Clinical validation of RNA-seq to complement whole-genome sequencing

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In the past decade, clinical exome and genome sequencing have resulted in increasingly higher diagnostic yields across a broad spectrum of rare diseases and unknown syndromes. However, variants of unknown significance remain challenging with the lack of functional data provided by DNA sequencing making it difficult to confirm pathogenicity in many patients.

Here, we have paired genome sequencing with RNA-sequencing (PAXgene n = 126, blood-EDTA n = 99) in 225 whole-blood samples from patients. Potential splice variants called in the genome data was correlated to the RNA-sequencing data to evaluate the functional effect on splicing of those variants. Furthermore, we also investigated mono allelic expression and allelic drop out.

The results showed that 54% of morbid OMIM genes were expressed at >1 TPM in blood with some variability for different disease panels with the highest expression observed for genes in the neurodegenerative diseases panel (65%). Furthermore, the use of PAXgene tubes increased the number of expressed genes with 9% compared to EDTA tubes. Finally, our initial analysis confirm that non-coding variants in several patient are indeed functional. For instance RNA-sequencing could confirm intron retention in a patient with a splice variant in the SCLT1 gene.

In addition our study has shown that the choice of sampling (PAXgene, blood-EDTA) and sampling preparation (whole blood, white blood cells) is important and might be complementary for the clinical work-flow. In conclusion, we have demonstrated the RNA-sequencing is an important next step to further improve clinical diagnostics of rare diseases.

Conflict of Interest: None declared

P16.071.C Design and set up of NGS-based newborn screening approach from dried blood spots

Alessia Mauri[1;2], *Sara Benedetti*[2], *Andrea Meta*[2], *Clarissa Berardo*[1;2], *Stephana Carelli*[1;2], *Luisella Alberti*[2], *Cristina Cereda*[2]

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Background/Objectives: Newborn Screening (NBS) is a neonatal-based program for early detection of congenital disorders by biochemical methods based on dried blood spots (DBS) collected 48-72 hours of life. Notably, the number of diseases screened hasn't kept pace with genomic innovation and technical limitations related to tandem mass spectrometry may occur. Next-generation sequencing (NGS) has the potential to overcome many NBS drawbacks and provide large amounts of molecular data, broadening the conditions investigated. To evaluate the technical feasibility of NGS from DBS and the potential of genomic-NBS (gNBS) as first-tier test, we set up an NGS-based method starting from 30 newborn DBS.

Methods: gDNA was extracted from DBS samples using Chemagic™360 (PerkinElmer), its quantity and integrity was estimated using Qubit and Agilent 4200TapeStation. Whole-exome sequencing was performed using three target enrichment kits from Twist, Agilent and Illumina companies, and sequenced on Illumina NS500. Data were analyzed on enGenome's eVai, Alissa (Agilent) and Dragen™ (Illumina) platforms to identify SNVs, indels and CNVs. Genetic interpretations were performed focusing on virtual gene panels related to disorders with high medical actionability in neonatal/pediatric age.

Results/Conclusion: Preliminary results suggested that amount and quality of DBS-extracted gDNA were adequate to perform high-throughput sequencing. A high read depth (80-100X) with 95% coverage uniformity was achieved for most samples, comparable among the workflows tested. The variants identified from DBS were compared to those previously detected on blood samples, confirming that DBS may be a suitable material for future gNBS programs and thus allowing to widen the diseases actually screened.

Conflict of Interest: None declared

P16.072.D Evaluation of library amplification systems for high stringency applications

Josh Haimes[1], *Philip Benson*[1], *Giulia Corbet*[1], *Skyler Mishkin*[1], *Thomas Harrison*[1], *Martin Ranik*[1], *Kristina Giorda*[1], *Ross Wadsworth*[1], *Amy Liu*[1], *Brian Kudlow*[1]

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Many challenging sample types and NGS applications, such as FFPE, liquid biopsy, single cell, and epigenetics, require library construction from ultra-low inputs, making low-bias library amplification critical for accuracy. The field demands faithful representation of the input sample in order to produce accurate conclusions for high-stringency assays. Additionally, hybridization capture methods require high pre-capture library yield which places importance on high-efficiency amplification to minimize PCR cycles. Together these constraints place great importance on the performance of the library amplification system.

We developed a family of novel ultra-high-fidelity DNA polymerases in optimized hot start master mix formats. Performance metrics characteristic of high-quality, low-bias library amplification were evaluated and compared between several commercially available PCR systems. High-efficiency amplification affords the least number of PCR cycles to be applied, minimizing bias and artifacts, and produces uniform UMI family depths which supports efficient error correction. Hence, we studied PCR efficiency across a range of GC content, as well as uniformity analysis of UMI family depth distributions. The ability to generate high yield for hybrid capture was also evaluated. The ability to faithfully amplify longer library inserts also supports robust library yields and was examined. Furthermore, efficient amplification should perform robustly in the presence of purification and capture beads to support these workflows. Base incorporation fidelity was assessed using a UMI consensus based NGS assay. Finally, an optimized antibody-based hot start formulation was shown to inhibit both exonuclease and polymerase activities superiorly, enabling automation processes.

Conflict of Interest: Josh Haimes Watchmaker Genomics, Watchmaker Genomics, Philip Benson Watchmaker Genomics, Watchmaker Genomics, Giulia Corbet Watchmaker Genomics, Watchmaker Genomics, Skyler Mishkin Watchmaker Genomics, Watchmaker Genomics, Thomas Harrison Watchmaker Genomics, Watchmaker Genomics, Martin Ranik Watchmaker Genomics, Watchmaker Genomics, Kristina Giorda Watchmaker Genomics, Watchmaker Genomics, Ross Wadsworth Watchmaker Genomics, Watchmaker Genomics, Amy Liu Watchmaker Genomics, Watchmaker Genomics, Brian Kudlow Watchmaker Genomics, Watchmaker Genomics

P16.073.A T-cell receptor repertoire analysis reveals age and disease severity-related changes in the T-cell population of COVID-19 patients

Fernando Marín-Benesiu[1;2], Silvia Martínez-Diz[3], Esperanza de Santiago[1], Juan Miguel Guerrero-González[1], Carmen María Morales-Álvarez[1;2], Maria Jesus Alvarez Cubero[1;2], ***Luis Javier Martínez-González***[4]

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Background/Objectives: The architecture and dynamics of T-cell populations are closely related to the cellular immune response to SARS-Cov2. In this study we characterized T-cell receptor repertoire via sequencing and bioinformatic analysis (TCRseq) to identify key changes associated with COVID-19 severity.

Methods: This study analyzed blood samples from 175 COVID-19 patients with confirmed post-infection, consisting of 98 mild and 75 severe cases with a median age of 53 years. The TCRβ chain complementarity determining region (CDR3) was amplified via RT-PCR and sequenced. Bioinformatic analysis was applied to measure repertoire richness, diversity, clonality, and allelic usage metrics.

Results: The results indicated a reduction in richness ($p = 0.007$), α diversity ($p = 0.0006$) and an increase in clonal expansion ($p = 0.0009$) of TCR repertoires in severe COVID-19 patients younger than 55 years old. These values were similar to those observed in patients over 55 years. Moreover, a decrease in the use of certain V alleles, such as TRBV14, was observed in relation to both severity ($p = 0.041$) and age ($p = 0.029$), as severe patients younger than 55 years old showed an allelic usage pattern comparable to those above 55 years.

Conclusion: These findings suggest that severe patients under 55 years old may have a more deteriorated TCR repertoire, leading to a worse disease outcome, with similar dynamics to severe and mild patients under 55 years old.

Grant references: Present study has specific funding and it is part of the PECOVID-0006-2020 project of the Ministry of Health of the Andalusian Regional Government and co-financed with FEDER funds.

Conflict of Interest: None declared

P16.074.B Beyond the Exome: Setting up a genomics-based undiagnosed genetic disease research program

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Background: Just over half of 9,000+ rare genetic disorders have a known cause and most patients who undergo clinical WES fail to obtain a diagnosis. To address these issues we created the University of Wisconsin Undiagnosed Disease Program (UW UDP), which takes a "beyond the exome" approach to evaluating genetics patients.

Objectives: 1) discover new disease genes; 2) improve our understanding of genetic disorders; 3) provide patients with actionable diagnoses; and 4) evaluate novel technologies. Our workflow begins with clinical WES reanalysis, followed by trio short read genome sequencing. Long read sequencing, RNA-Seq, and epigenomic profiling are utilized ad hoc.

Results: To date, the UW UDP has enrolled 53 probands and 108 relatives. >90% of probands had prior clinical WES. We identified candidate causal variants for 5 of the first 10 patients. Short and long read WGS, WES reanalysis, and RNA-Seq each played a role in finding a deletion and an instance of chromoplexy missed by clinical testing; three new candidate disease genes; and a patient whose novel phenotype is the likely result of synergy between two rare disorders. Additional analyses are on-going.

Conclusion: Our initial results suggest that a significant fraction of clinical WES-negative patients can be diagnosed using combinations of WGS, long-read WGS, and RNA-Seq. An undiagnosed genetic disease program can serve as an important component of a comprehensive center for rare diseases, as it offers patients access to emerging technologies and facilitate the discovery of new disease genes while advancing our understanding of rare genetic disorders.

Conflict of Interest: Stephen Meyn minor stock ownership in Gene42/PhenoTips, Bryn Webb: None declared, Derek Pavelec: None declared, Heather Heilmann: None declared, Heather Motiff:

None declared, Xiang Qiang Shao: None declared, Vanessa Horner: None declared, April Hall: None declared

P16.075.C High throughput multiomic analysis for human genomics on PacBio Revio system

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Improved throughput and cost of long-read sequencing, driven by recent technological advances of the PacBio Revio system, enables investigation of whole human genomes across larger populations. To support growing capabilities of long-read sequencing, high throughput sample and library preparation solutions are necessary. While short-read sequencing workflows are well established, long-read workflows handling high molecular weight (HMW) DNA are not widely available. We present automated 96 well plate-based high throughput HMW DNA extraction, shearing, and library preparation workflow for human whole blood samples for PacBio HiFi sequencing.

First, HMW DNA extraction is performed utilizing Nanobind magnetic disk technology on automated Hamilton NIMBUS Presto. Nanobind disks feature micro-and-nanostructured silica wrinkles to shield bound DNA from damage during extraction. We obtained ~6 µg of DNA per 200 µL blood sample (96 well plate) and ~30 µg of DNA per 1 mL blood sample (24 well plate) in 2.5 hours. Alternatively, Thermo Fisher KingFisher instruments provide a semi-automated option with comparable metrics.

HMW DNA is then sheared to 15-20 kb using robotic pipette shearing. Automated PacBio library and loading preparation is then performed on the fully automated Hamilton NGS STAR.

The methods presented utilize standard configurations of Hamilton instruments and can easily be incorporated into existing workflows. Data is presented using human whole blood for a workflow which can prepare 96 samples from blood to library ready for loading in ~10 hours. A Revio SMRT Cell typically generates ~30X coverage of high-quality sequence data sufficient for analysis including phasing, 5mC, and variant calling.

Conflict of Interest: Jeffrey Burke PacBio, stock, Julian Rocha PacBio, stock, Renee Fedak PacBio, stock, Duncan Kilburn PacBio, Stock, Deborah Moine PacBio, Stock, Enrique Bayo Iglesias Hamilton, Dominik Laubscher Hamilton, Birgit Ottenwaelder Hamilton, Suzanne Dee PacBio, stock, Heather Ferrao PacBio, Stock, Kelvin Liu PacBio, Stock

P16.076.D Long-read capture with Twist target enrichment system

Tina Han[1], *Holly Corbitt[1], Leonardo Arbiza[1], Esteban Toro[1], Chad Locklear[1]*

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Background/Objectives: Targeted resequencing allows for high-resolution characterization of gene regions at a scale and cost that is more accessible than WGS. While long-read PacBio HiFi sequencing has been shown to interrogate complex clinically actionable loci accurately and comprehensively, studies have been primarily focused on single genes using PCR amplicon-based methods. Here we describe a method to leverage Twist Bioscience target enrichment workflow for gene panels sequenced with HiFi reads.

Methods: The content of 2 Alliance Panels – a 50-gene Pharmacogenomics (PGx) panel and a nearly 400-gene panel of challenging, medically relevant "Dark Genes" – was developed in collaboration with leading institutions. Our protocol starts with 200ng of fragmented gDNA. After end-repair and dA-tailing, truncated Y-shaped adapters were ligated to adapted gDNA. A pair of 10-bp UDIs for sample barcoding were added during PCR. Multiple samples can be pooled for an overnight hybridization. The post-capture libraries then undergo SMRTbell library preparation and sequencing on Sequel II or Revio. 24 PGx or 4 Dark Genes samples may be multiplexed and sequenced in one SMRT Cell 8M with HiFi read length of 5-10 kb.

Results: We demonstrate that this method efficiently enables comprehensive coverage of gene targets, including complex regions like CYP2D6, HLA, SMN1/SMN2, and GBA.

Conclusion: The demonstrated method allows for scalable and cost-efficient hybrid capture with long read lengths, minimizing coverage bias, and maximizing accuracy to fully capture all variant types. This includes structural variation and haplotype phasing information which are inaccessible to short-read and Sanger sequencing.

Conflict of Interest: Tina Han Full-time employee at Twist Bioscience, Owner of stock options at Twist Bioscience, Holly Corbitt Full-time employee at Twist Bioscience, Owner of stock options at Twist Bioscience, Leonardo Arbiza Full-time employee at Twist Bioscience, Owner of stock options at Twist Bioscience, Esteban Toro Full-time employee at Twist Bioscience, Owner of stock options at Twist Bioscience, Chad Locklear Full-time employee at Twist Bioscience, Owner of stock options at Twist Bioscience

P16.077.A INFRAFRONTIER: World-class in vivo models to understand human gene function

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Background: INFRAFRONTIER is the European Research Infrastructure for modelling human diseases focused on the development, archiving, phenotyping, and distribution of mouse models. The INFRAFRONTIER network consists of 22 partners from 15 countries and is continuously improving and expanding the services and resources offered to the research community on a non-profit basis.

Results: The main services of INFRAFRONTIER include disease model generation and systemic phenotyping of genetically modified mice. Moreover, the European Mouse Mutant Archive (EMMA) - the principal resource of the infrastructure - provides the archiving and distribution of mouse-mutant strains for research. The incorporation of axenic services and a new enriched INFRAFRONTIER bibliography supported by a tailor-made "Publications Curation Tool" are some examples of the effort that INFRAFRONTIER makes to showcase the translatability of mouse genomics into human disease research. One of the novelties is the enrichment done by expert curators on the existing resources by clustering them under disease areas, such as cancer, rare diseases, and COVID-19. Furthermore, INFRAFRONTIER offers trans-national access calls for projects that are accessible to the research community and provides funding based on the quality of submitted proposals.

Conclusion: We want to introduce our specialised resources for specific research areas. INFRAFRONTIER offers tools, such as a revamped website for easy access to strains archived in the EMMA repository related to specific disease areas, such as cancer, rare

diseases or COVID-19. Overall, INFRAFRONTIER provides world-class resources for biomedical research and contributes to the understanding of gene function in human health and disease using mouse models.

Conflict of Interest: None declared

P17 Diagnostic Improvements and Quality Control

P17.001.D Genetic diagnosis by rapid exome sequencing in acutely unwell children has immediate and long term management implications not only for the patients but also for their families including extended family members

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Background/Objectives: National rapid exome sequencing (R14) was launched in England on 1st October 2019 for acutely unwell children with a likely monogenic disorder, predominantly in the neonatal (NICU) or paediatric intensive care (PICU) setting. We present a 3 year patient cohort from the West Midlands Clinical Genetics centre.

Methods: Data was collected retrospectively for a 36 months period (01.10.2019 – 30.09.2022) from the clinical genetics database using standardised proforma.

Results: R14 was performed on 297 patients from the region. 47% of requests were from NICU/PICU, 35% from other specialties and 27% from clinical genetics colleagues. 95% had trio analysis, 3% duo and 2% had singleton analysis. The median turnaround time, after the DNA samples received by the testing lab was 12 days. The diagnostic rate was 38%, providing accurate recurrence risk counselling for these the families. Diagnostic rate was highest in neuroregression, skeletal dysplasias, neuromuscular and neurometabolic disorders. In 5%, diagnosis was made with concurrent testing such as microarray and 10% had a reported variant of uncertain significance. In 70% cases diagnosis infuenced acute care including pharmacological treatment, orientation of care and referral to specialist services. In recessive conditions, cascade screening was offered where appropriate.

Conclusion: Rapid exome sequencing is a useful tool for rapid genetic diagnosis in acute paediatric settings, with some diagnoses which were not possible by clinical evaluation alone.The rapid genetic diagnosis not only influences the acute management, but also helps with the longer term management for patients and their families.

Conflict of Interest: None declared

P17.002.B Lessons from the 2022 GenQA 'variant validation' external quality assessment for combined reporting of single nucleotide and copy number variants

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Background/Objectives: Diagnostic genomics laboratories routinely confirm the results of research testing and whole exome / genome analysis. Genomics Quality Assessment (GenQA) has therefore provided an external quality assessment (EQA) for 'variant validation' since 2019 to assess the quality of testing, interpretation and clinical reporting. In previous years a single nucleotide variant (SNV) had been provided, however in 2022 a copy number variant (CNV) was also included.

Methods: DNA samples from a trio were provided along with research results describing a heterozygous splice site SNV in the MFSD8 gene and a heterozygous CNV encompassing the entire gene. Participants were asked to test the DNA samples for the SNV and incorporate with the results of confirmatory CNV testing which had already been carried out. The genotyping results, clinical interpretation and clerical accuracy of reports was assessed by a panel of assessors against peer-reviewed marking criteria.

Results: All 27 participating laboratories correctly genotyped the SNV and provided the correct clinical conclusion. Most participants merely stated that the CNV encompassed the entire MFSD8 gene with only 11 specifically classifying the variant as pathogenic. Reporting of the parental samples was of a lower quality than the index case with significant omissions particularly relating to the CNV.

Conclusion: Expansion of the 'variant validation' EQA to include both SNV and CNV results has highlighted that some laboratories are struggling to combine these results in their reports. This EQA will promote improvements to reporting as the capabilities of genomic testing expand in the clinical setting.

Conflict of Interest: None declared

P17.003.C Performance of whole-genome sequencing for rare disease diagnosis in ancestrally diverse populations: the UK 100,000 Genomes Project

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Background/Objectives: Whole-genome sequencing (WGS) has become a routine tool for rare disease diagnosis within the UK National Health Service (NHS). However, questions remain regarding the efficacy of current WGS-based diagnostic approaches in ancestrally diverse populations. Here, we explore these questions in a cohort of 72,947 participants in 34,101 families recruited through the NHS as part of the 100,000 Genomes Project rare diseases programme.

Methods: We use PCA to characterise the population structure of the cohort according to each participant's genetic similarity to a selection of globally diverse reference genomes in the UK Biobank. We then use multivariate generalised linear regression to model the effect of genetic similarity on candidate pathogenic variant prioritisation and diagnosis rates alongside additional demographic and study related factors.

Results: Compared to participants genetically similar to Europeans, we observe more reported candidate variants in all other groups ($p < 10^{-5}$). For example, candidate variants were 2.9 times more likely to be reported in participants genetically similar to East Africans (SE 2.4-3.3, $p < 10^{-36}$). However, we find genetic similarity appears to have had little independent effect on a participant's likelihood of receiving a diagnosis ($p > 0.05$).

Conclusions: In conclusion, our results show clear disparities in the numbers of variants of uncertain significance across ancestrally diverse groups, likely reflecting imbalanced representation among control cohorts used to prioritise variants on basis of their allele frequency. This highlights a need to increase the genetic

diversity of WGS datasets to improve the precision of pathogenic variant discovery.

Conflict of Interest: Samuel Tallman Genomics England, Thuy Nguyen Genomics England, Yoonsu Cho Genomics England, Maxine Mackintosh Genomics England, Loukas Moutsianis Genomics England, Karoline Kuchenbaecker Genomics England University College London

P17.004.D Getting the most from your sample: Inter-laboratory variability in DNA extracted from blood and formalin-fixed paraffin-embedded (FFPE) tissue

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With a growing number of molecular genetic technologies requiring high quality and quantity of DNA, ensuring that laboratories are getting the most from the source tissue provided is more important than ever.

To determine the efficiency of laboratories extraction methods, laboratory participants were provided with blood and tissue samples from the same source and required to extract DNA using their routine extraction methodology and return to GenQA for analysis.

The quantity of DNA extracted was determined using digital PCR and weight of DNA. For DNA extracted from blood, the quality was assessed using DNA integrity numbers (DIN). For DNA extracted from FFPE, quality was determined by library amplification which were then quality scored.

The resulting yield and integrity of DNA was variable within and across laboratories, and extraction methodologies for both DNA extracted from blood and FFPE tissue. The mass of DNA extracted from a 1ml blood sample ranged from 0.95µg to 31.75µg, 3ml sample ranged from 5.85µg to 100.27µg and 4.5ml sample ranged from 6.63µg to 145.23µg. The DIN values ranged from 5.8 to 8.9.

For DNA extracted from FFPE tissue, there was variability across samples, with a lower mean yield being extracted from lung tissue of 0.15µg, compared to GIST (3.57µg) and colorectal tissue (2.11µg).

The variability of mass and quality of DNA extracted demonstrates the need for improvement and standardisation of extraction protocols to aid the roll out of more advanced technologies such as long read sequencing into routine practice.

Conflict of Interest: None declared

P17.005.A Investigating the variable expression of housekeeping genes in normal human keratinocytes

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Background/Objectives: Real-time PCR (RT-PCR or qPCR) is a useful technique to assess and compare levels of mRNA expression. Many stably expressed endogenous genes (also known as housekeeping genes) are used to normalise expression of genes of interest during this process. Common housekeeping genes used for qPCR, such as *GAPDH*, have been shown to have variable expression in normal human keratinocytes (NHEKs) when comparing basal NHEKs to differentiated NHEKs, which can skew gene expression results. In order to yield meaningful results, it is vital to choose a housekeeping gene that is consistent throughout the differentiation process of NHEKs.

Methods: Basal NHEKs isolated from human tissue were grown until 70% confluent. Differentiated NHEKs were produced by treating basal NHEKs with 1.15mM $CaCl_2$ at 3 different time points (24, 48, and 72 hours). RNA was extracted from basal NHEKs and the differentiated NHEKs and converted to cDNA. Expression for 7 housekeeping genes (*18S, CLTC, GAPDH, GUSB, HPRT1, PPIB, and YWHAZ*) were compared at different differentiation time points using probe-based qPCR and analysed using the delta-delta Ct method.

Results: *CLTC* and *18S* showed the most stability in Ct values between samples, suggesting consistent gene expression, while *GAPDH*, *HPRT1*, and *PPIB* showed the least stability.

Conclusion: The data highlights the importance of characterising housekeeping gene expression in cell types to ensure meaningful results and suggests that *CLTC* and *18S* are good housekeeping genes for keratinocytes, especially when both are used to normalise expression data. Further research on normalising gene expression in 3D models is needed.

Conflict of Interest: None declared

P17.006.B Analysis of factors determining success in FFPE based NGS panel testing for lung and ovarian cancer in the NHS

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Background/Objectives: The identification of oncogenic driver mutations in both lung and ovarian cancer is now central to personalised oncological treatment. Next generation sequencing (NGS) approaches are routinely implemented for mutation detection but optimisation of pre-analytic factors is crucial for success.

Methods: A large multi-cohort retrospective audit assessing pre-analytic factors related to in-house designed custom NGS panel testing for solid tumours, consisting of lung ($n = 801$) and ovarian ($n = 882$) FFPE cancer samples, alongside a further targeted analysis of a lung sample cohort ($n = 461$) submitted from a single high-volume referral centre, was undertaken.

Results: Overall NGS cohort success ranged from 74-85% with large regional variation amongst referring laboratories. Multivariate logistic regression analysis revealed DNA yield and quality to be significant predictors of NGS success ($p < 0.001$) alongside sample type for lung ($p = 0.035$) and use of macrodissection for ovarian ($p = 0.025$). Univariate analysis revealed poor performance within lung biopsy samples and number and length of core biopsy samples alongside fixation time for lung cytology and ovarian samples was associated with NGS failure ($p < 0.05$). Only 50% of Lung EBUS samples met existing local recommendations for optimal fixation times of <24hours with 62% of prolonged fixation samples arriving in the pathology laboratory for processing >24 hours later.

Conclusion: Specific updated recommendations for Lung EBUS samples regarding fixative agent, fixation time and quality of core biopsies, alongside fixation time for ovarian samples, may ensure improved NGS success. Better collaboration between NHS genomic hubs and referring pathology laboratories is crucial to improve future NGS testing.

Conflict of Interest: None declared

P17.007.C Considerations for the use of microsatellite instability analysis by PCR on endometrial cancer samples

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Microsatellite instability (MSI) is a biomarker with a long history of use to identify patients for Lynch syndrome (LS) testing. Along with mismatch repair (MMR) protein expression by immunohistochemistry (IHC), MSI analysis by PCR has routinely been used when testing colorectal cancers (CRC) for potential LS. MSI status is now also recognized as an important predictor of response to immune check point inhibitors. As a result, MSI testing is being utilized more broadly, including in additional tumour types. Among these tumour types, endometrial cancers represent a particular interpretive challenge for MSI analysis by PCR due to their more subtle phenotype (shift-size), relative to CRC samples. Here we share MSI results by PCR from a cohort of 60 endometrial cancers previously characterized for MMR status by IHC (39 deficient and 21 proficient in expression of MMR proteins). We catalogue concordance between IHC results and MSI data as well as the shift-sizes observed based on overall MMR status, individual MMR proteins, and neoplastic cell content. We share considerations for performing MSI analysis by PCR testing in non-colorectal cancer samples and strategies for the detection of subtle shifts in MSI results and the impact of sample-related factors including neoplastic cell content. Ultimately, we demonstrate the utility of MSI analysis by PCR in endometrial cancer specimens and its correlation with MMR status.

Conflict of Interest: Manisha Maurya: None declared, Marty Ensenberger Promega Corporation, Kirsty Trewellard: None declared, Samantha Lewis Promega Corporation, Glenn McCluggage: None declared, Manuel Salto-Tellez: None declared, David Gonzalez de Castro: None declared

P17.008.D Assessment of variants of unknown significance using RNA sequencing to improve diagnostic yield

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Introduction: RNA-sequencing is now being used as a complementary tool to DNA sequencing in diagnostics where DNA has been uninformative. RNA-sequencing allows us to identify alternative splicing and aberrant gene expression allowing for improved interpretation of variants of unknown significance (VUS). Our aim is to improve diagnostic yield of rare diseases by assessing VUSs using blood-based RNA-sequencing.

Methods: RNA was extracted from blood in 87 patients with likely genetic disorders of which 49 had a VUS encompassing 38 genes. Samples were sequenced in four batches and aligned to the reference genome using STAR. MAJIQ, rMATS-turbo, and LeafCutterMD were used to detect alternative splicing. For cases with unknown causes of disease, two novel approaches were assessed to identify potential diagnostic candidates.

Results: Mean number of reads was 76.6 million/per-sample and on average 80% of reads were uniquely mapping. Visual inspection of BAM files allowed identification of alternative splicing in 14 cases. For cases where alternative splicing events were not detected 12/49 variants could not be assessed due to insufficient gene coverage while the rest showed no detectable splicing abnormalities. In cases with no VUSs, we identified at least three new candidates using outputs from the splicing tools.

Conclusion: While identification of splicing abnormalities was limited by gene expression in blood for some genes; this easily accessible tissue allowed us to increase the molecular diagnostic yield by validating splicing abnormalities in 14 patients with a VUS and identified at least three new alternative splicing events leading to new diagnoses.

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Conflict of Interest: None declared

P17.009.A Visualizing DNA for long read sequencing by moles, not mass

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Traditional measurements during NGS library preparation include the determination of the sample mass within specific size ranges. This analysis is easily performed with the Agilent automated electrophoresis instruments, which provide visual results in the form of a digital gel and electropherogram. The electropherogram displays the fluorescent signal as a graphical representation, with the size on the X-axis and relative fluorescence units (RFUs) on the Y-axis. The height of the fluorescent signal is therefore directly proportional to the mass of sample at a given size. While this representation has been widely used for quality control of sheared gDNA and the final NGS library, examining the molarity of a sample may provide a better visual representation of the number of sequencing reads that can be produced by a sample, especially for long read sequencing. High molecular weight samples were analyzed using the Agilent Femto Pulse system and the accompanying ProSize data analysis software. ProSize allows the user to visualize the electropherogram image as a product of either mass or molarity by switching the Y-axis. By visualizing the data in moles and utilizing a smear analysis, the Femto Pulse can be used to determine the number of moles of sample found within different sizing brackets and providing a prediction of long read sequencing read length. This data can be used to make informed size selection experiments prior to sequencing, allowing customers to make informed long read sequencing decisions.

Conflict of Interest: None declared

P17.010.B An R package (vaRHC) to assist variant classification for hereditary cancer genes following gene-specific ClinGen and the updated ACMG/AMP guidelines

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Background/Objectives: Variant classification is a major challenge. Only an accurate classification allows proper genetic counseling, personalized risk estimation and subsequent clinical management. Classifying a variant is a manual, time-consuming

process that combines information of distinct nature and must follow published updated guidelines. The automation of this task can be very helpful to speed up the classification, search comprehensively through available databases and avoid manual errors. We present vaRHC, an R package to assist in variant classification process.

Methods: From the input of a single variant, a dataframe or a vcf file vaRHC automates criteria related to mutation type, population frequencies and in silico predictors, and provides information from clinical databases. The novelty of this tool is that it implements gene-specific guidelines for *ATM, CDH1, CHEK2, MLH1, MSH2, MSH6, PMS2, PTEN* and *TP53* and updated general ACMG/AMP guidelines for the remaining cancer susceptibility genes. vaRHC assigns criteria, generates a classification and provides a user-friendly report to examine and store results.

Results: A performance assessment conducted with 659 manually classified variants has demonstrated its robustness and accuracy, and has allowed us to identify 47 manual errors. Additionally, Cohen's Kappa test revealed that vaRHC compares favourably to cancer SIGVAR (PMID:33565189), the only other hereditary cancer variant classification tool.

Conclusions: vaRHC will facilitate the task of variant curators in clinical settings by assigning classification criteria, reducing time for variant classification and limiting manual errors.

Grant References

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P17.011.C Hidden hereditability in a rare disease: Hereditary Hemorrhagic Telangiectasia

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Background/Objectives: Hereditary Haemorrhagic Telangiectasia (HHT) is a rare autosomal dominant vascular dysplasia. Causative genes are *ACVRL1*, *ENG*, *SMAD4*, *GDF2*. Clinical diagnosis is based on Curaçao criteria: epistaxis, telangiectases, arteriovenous malformations, family history. According to literature and our HHT reference centre experience, in 10% of HHT-clinically diagnosed patients, a pathogenic variant cannot be identified by standard diagnostic analyses. For these "Not Found" patients we aim to identify novel pathogenetic mechanisms by WGS (Whole Genome Sequencing).

Methods: 47 Not Found patients previously underwent standard analyses (WES, NGS-custom panel, Sanger, MLPA). 17/47 were selected for WGS NovaSeq6000. Candidate variants were filtered by location in HHT genes and in silico prediction. Sanger sequencing was performed for confirmation and segregation studies. When possible, RNA was extracted from peripheral blood, retrotranscribed and analyzed by PCR and qPCR (ΔΔCq method).

Results: Genetic data revision led to find 4/30 definite pathogenic variants, including the first HHT branch point alteration; other 2/30 were splicing and transcription-altering intronic variants, respectively. The latter shows a 40% *ENG* expression reduction. From WGS analysis we found 11/17 candidate variants. Of these, the first large inversion involving *ENG*, one wide *ACVRL1* intron deletion, one intronic transcription-altering, four exonic and several deep-intronic splicing variants.

Conclusion: Data revision allow to find 6/30 variants, while WGS data 11/17 (59%) possible causative variants, significantly increasing the diagnostic rate. Therefore, WGS could be an optimal solution in Not Found HHT cases and may contribute to the pathogenicity determination, elucidating novel HHT mechanisms.

Grant References: Fondo Beneficienza Intesa San Paolo.

Conflict of Interest: None declared

P17.013.A Exploring the knowledge of genetics laboratories on CFTR modulator therapy eligibility: findings and reflections during external quality assessment

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Cystic fibrosis (CF) is caused by variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene inherited by both parents. Revolutionary CFTR modulator therapy has improved the quality of life of patients living with CF in recent years. Drug eligibility is mainly based on the patient's genotype, stressing the importance of genetic analysis. During this retrospective study on CF external quality assessment (EQA) data over three scheme years, we analyzed reporting approaches by genetics laboratories regarding modulator eligibility. We looked at 496 laboratory reports in total (181 reports in 2018; 158 in

2020;157 in 2022). The majority of laboratories correctly addressed genotype's eligibility. Nonetheless, we observed a lot of variation between laboratories in the way how they reported on this issue. There was no statistically significant effect of sample volume, previous EQA experience, accreditation status, laboratory setting nor if the laboratory was familiar with testing of patients' samples on performance. Some laboratories refused to comment on therapy options since it was not considered the responsibility of the laboratory. Nevertheless, since the genotype plays a key role in addressing CFTR modulator eligibility, early reporting on drug eligibility by the laboratory could potentially decrease time-to-treatment.

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P17.014.B Critical Assessment of Genome Interpretation (CAGI) community challenge improves rare disease diagnosis

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Background/Objectives: CAGI (Critical Assessment of Genome Interpretation) organizes challenges by providing unpublished datasets for analysis. CAGI provides a forum to evaluate the effectiveness of different methods by an independent assessor, and to learn as a community.

Methods: In CAGI6, there were >500 submissions from 51 teams participating from 17 countries in 13 challenges that ranged from predicting measured functional impact of variation in a gene to improving polygenic risk scores to improving genetic diagnosis. In the Rare Genomes Project (RGP) Challenge, 16 teams submitted 52 models analyzing genome sequence data and phenotypes from 30 families consisting of "solved" and "unsolved" cases. Models were assessed, including a weighted metric based on rank position of the causal variant(s).

Results: Performance ranged widely with the top performing teams identifying the causal variants in up to 13 of the 14 solved cases; four teams (Invitae, Lichtarge lab, enGenome, Exomizer) performed particularly well. After review of prioritized candidates, two additional results were returned (after confirmatory RNA-seq) and two candidates were submitted to Matchmaker Exchange.

Conclusion: The RGP CAGI6 challenge increased rare disease diagnosis by at least 12.5% (2/16). In future CAGI challenges, we will distinguish methods providing fully-automated prioritization from those with human input, and increase the number and variety of unsolved cases. Careful design of a diverse set of challenges and high levels of participation from researchers and industry can help to drive innovation and inform selection and application of the most effective methods for interpreting human genome variation.

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Conflict of Interest: None declared

P17.015.C Increasing the diagnostic yield of Whole Exome Sequencing (WES) through CNV detection

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Background/Objectives: Whole-Exome Sequencing (WES) has proven valuable in the identification of the genetic etiology of most rare diseases (RDs). An important approach to extend the diagnostic yield of WES involves the detection of Copy Number Variants (CNVs), which explain ~10-15% of all inherited RDs. Recent improvements in NGS technologies and bioinformatics have contributed to the development of accurate and highly sensitive tools, capable of calling CNVs from WES data.

Methods: Of 920 patients referred for WES in our lab, 454 unresolved cases were further analyzed for CNVs using the ExomeDepth WES-based CNV-calling algorithm. CNVs called, were evaluated and categorized according to ACMG and ClinGen recommendations.

Results: In 39 patients pathogenic CNVs were identified, increasing the diagnostic yield of WES from 50.7% (466/920) to 54.9% (505/920). Out of the 39 CNVs, 21 were available for validation and all were confirmed, and 7 were novel. Furthermore, 20 of the 39 CNVs were characterized as AD, 13 as AR and 6 as X-linked. Regarding the 13 AR-inherited CNVs, 2 were homozygous deletions and 11 were found in compound heterozygosity with pathogenic/likely pathogenic SNVs. Finally, the complex phenotype described in one patient was attributed to two distinct genetic diseases caused by a CNV and a SNV respectively.

Conclusion: The use of a specific algorithm for calling CNV from WES data enables ancillary detection of different types of causative genetic variants, making WES a critical first-tier diagnostic test for patients with RDs.

Conflict of Interest: None declared

P17.016.D Diagnostic performance of exome sequencing on 18,995 patients with suspected rare genetic disorders

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We report on 18,995 independent patients (19,764 individuals) referred for exome sequencing (ES) with a wide range of clinical indications. Patient samples came from 50 countries and ~half were from East Asia.

ES data were analyzed using an AI-based system and reviewed by medical geneticists and physicians. The overall diagnostic rate (DR) was 30.4% (5,775/18,995). Majority (93%) were diagnosed with SNVs, yet a subset of the patients was diagnosed with CNVs (2%, $n = 393$), repeat expansions ($n = 3$) and LINE-1 insertions

(n = 3). 110 patients were given dual diagnoses and 61 were given potential diagnosis with genes that are yet to be associated with a disease in OMIM. Earlier onset (~childhood) disorders were correlated with higher DR of 36.1%, while later onset (adolescence ~ adulthood) disorders had 20.9% DR. In 92% of the families, the disease-causing variant was within the top-5 variants proposed by 3billion's AI-based variant prediction system. Inconclusive results were given to 2,413 families (12.7%) with either VUS in AD disease genes or heterozygous P/LP variants in AR disease genes.

In addition to providing diagnosis to individual patients, we were able to use this large genomic dataset as a population data. For example, we reclassified 558 P/LP/VUS ClinVar variants to likely benign as they were found to be too common in our samples, mostly in Asian ethnicity underrepresented in other major population databases. As we accumulate more data, we expect to continue improving the diagnostic performance and contribute to the rare disorder community by providing valuable genomic data.

Conflict of Interest: None declared

P17.017.A DNA extraction kits and WGS PCR free library preparation kits have an impact on whole-genome data quality from FFPE samples

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Background/Objectives: The France Genomic Medicine Plan introduces WGS into healthcare pathway for rare diseases and cancers. The Reference Center for Technology, Innovation and Transfer (CReflX) investigated the impact of preparation kits for WGS PCR free protocols on sequencing data accuracy and mutations analysis from FFPE samples.

Methods: Extraction kits (Qiagen, Covaris, Promega), WGS PCR Free library preparation kits (Illumina, NEB) with different DNA inputs (1000 - 100 ng) and a DNA repair kit (NEB) were tested on standard FFPE samples and lung cancer tissues. Matched FF and FFPE samples were sequenced on NovaSeq 6000. Somatic mutational profiles (SNVs, CNVs, SVs, SBS signature …) were performed. The F1-score was also reported, FF samples used as gold standard.

Results: FFPE extraction kits affected DNA quantities and quality. One extraction kit created more low frequency artefacts than the ones caused by FFPE. For one WGS PCR Free library preparation kit, insufficient library quantities to sequence were obtained, when artefacts specific to another kit were observed. The FFPE DNA repair kit tested had no effect on pre and post-sequencing results.

Conclusion: Introducing FFPE samples into clinical pathway still requires protocols adjustments, specifically for low input FFPE samples. The choice of kits combination is crucial and every kit needs testing as some increase the number of artefacts already high in FFPE samples. With the right kits combination, FFPE samples could be used with caution for clinical interpretation, although it will never reach the same quality as FF.

Grant References: ANR-18-INBS-0001 (French National Research Agency).

Conflict of Interest: None declared

P17.018.B Streamlining cytogenetics analysis of genome sequencing data: a comprehensive guide for Balanced Structural Variants

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The identification of Balanced Structural Variations (BSVs) through short-read genome sequencing (GS) has become increasingly feasible. However, there are currently no established guidelines for variant filtering and pathogenicity classification. Herein, we present a strategy for managing BSVs in a diagnostic setting, based on the analysis of >2,000 trios for rare diseases. After variant detection using Manta and a 1% population frequency filter (1kb window considered for breakpoints'similarity), we typically retain an average of 5-15 variants consistent with Mendelian inheritance for analysis. Among these, it is crucial to distinguish classical cytogenetics rearrangements from "sequence structural variants" (e.g. mobile elements), as their genomic consequences significantly differ. To streamline the" manual curation process, we recommend considering only (i) de novo variants, (ii) gene-disrupting variants affecting a predefined gene list based on the phenotype, and (iii) variants in trans with another prioritized variant (SNV, CNV, etc.). This approach minimizes the workload for trained cytogenomics specialists to a maximum of 2 variants (zero for more than 90% of cases) that require read alignment inspection and formal interpretation. We will illustrate our decision algorithm for pathogenicity classification of curated variants with the 12 (likely) pathogenic (0.6%) and 6 balanced structural variants of unknow significance (0.3%) we reported. This work clearly underscores the need for more comprehensive guidelines for BSV analysis in the diagnostic setting and provides a starting point for future research.

Conflict of Interest: None declared

P17.019.C Pharmacogenomics in the Canary Islands population: a comparison of assessment by different technologies

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Background/Objectives: Pharmacogenomics facilitates the implementation of Personalized Medicine Programs, providing individual genetic considerations for drug dosage, efficacy, and adverse reactions, among others. Here we assessed the utility of different genomic approaches for providing germline pharmacogenetic information.

Methods: We used the available and diverse genomic dataset from the Canary Islands database (CIRdb) which includes samples assayed for two SNP arrays (Axiom Genome-Wide Human CEU 1 Array [CEU1] or Axiom Spain Biobank Array [SBA]), and next-generation sequencing approaches (whole-exome [WES] or whole-genome sequencing [WGS]). Variants with clinical annotation from the PharmGKB database (3,054 curated variants in 2,761 positions) were used as the target for comparing the number of called variants or positions by the different approaches. These comparisons were conducted at different levels: raw, filtered, and TOPMed-imputed data.

Results: For the arrays, the best scenario was obtained with TOPMed-imputed data, recovering 79.1% and 80.1% of the target variants for CEU1 and SBA, respectively. WES only covered 37.7% of the target variants, although it covered almost all of those residing in exons (96.9%). As expected, WGS provided calls for nearly all (97.6%) target variants.

Conclusions: While WGS allows to capture most of the target variants, imputing of array data on TOPMed allows to efficiently retain nearly 80% of them, thus constituting a cost-efficient alternative for pharmacogenetic studies.

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Conflict of Interest: None declared

P17.020.D The retrocopy challenge: unmasking real genetic variants

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Background: Retrocopies are gene copies generated by reverse transcription and genomic integration of transcribed mRNAs, some of which have been fixed and acquired a biological role becoming retropseudogenes. This is a widely known mechanism of evolutionary genomic variability, rarely taken into account, that has a direct effect on the analysis and genetic diagnosis by next-generation-sequencing (NGS).

Methods: The high amount of NGS data generated in our laboratory has allowed us to detect some frequent retrocopies (like *SMAD4* or *MTMR2*). When a variant in a potential retrocopy with relevant diagnostic involvement was detected, its confirmation was performed using bioinformatics analysis, such as structural variant or RNA enrichment tools, or molecular assays.

Results: We identified SNVs within genetic regions affected by retrocopies presenting an anomalous allele frequency and coverage pattern that, in many cases, may hinder its genomic localization and their possible involvement of the parental gene. In these cases, confirmation by another approach was performed. However, to be certain of the existence of a potential CNV involving these genes is currently a limitation.

Conclusions: As it has already stated, retrocopy existence hinds genetic diagnostic. Its suspicion requires additional analysis performance to confirm SNV localization. Nevertheless, CNVs confirmation is a challenge that will be minimized with the technology improvement through long-read sequencing or the increase of whole-genome performance. Although further research is still needed, it is necessary to be aware of the existence of this mechanism to prevent both false positive and false negative results in order to achieve accurate genetic diagnosis.

Conflict of Interest: None declared

P17.021.A How accurate is haematological neoplasms chromosome microarray testing and interpretation – the EQA experience

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Objectives: The identification of chromosome abnormalities plays a pivotal role in the management of patients with haematological neoplasms enabling an accurate diagnosis, risk assessment, and informing therapeutic choice.

Cytogenomic testing is mandatory at diagnosis for several disease entities and the method(s) used depends on the disease testing pathway and laboratory preference. These include chromosome microarray analysis (CMA), to identify small copy number aberrations (CNA), and large imbalances.

GenQA has provided external quality assessments (EQAs) for CMA testing in haematological neoplasms since 2014, measuring the accuracy of results and interpretation.

Methods: DNA samples were provided for testing and additional samples/test results were provided where appropriate for full interpretation.

Laboratories were expected to analyse and interpret the clinical significance of the results in the context of the disease referral based on current guidelines. The results were submitted as a standard laboratory report that was assessed by a panel of assessors.

Results: There was a high level of accuracy in the identification of the recommended CNA present, according to recommendations.

However, variation was noted in the number of additional 'optional' abnormalities reported and how CNA were counted, resulting in 1-15 abnormalities being reported for one case. In addition, there was also variability in the genes of interest included in the reports and the interpretation of chromosomal regions with multiple aberrations.

Conclusion: The EQA results demonstrated an overall high accuracy of CMA testing but revealed a difference in the way recommendations were applied and interpreted by participants, highlighting areas where further guidance is required.

Conflict of Interest: Katrina Rack part time, Fiona Morgan full time, Ros Hastings part time, Zandra Deans full time

P17.022.B A cloud-based bioinformatic tool to enable automated diagnostic analysis of raw genomic sequence data from people with rare monogenic diseases

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Introduction: In recent decades, whole genome and whole exome sequencing have been increasingly used both for clinical diagnostic purposes and for research. However, one of the biggest barriers to realising the potential of these methods is the need for expertise and experience in bioinformatics and computer science in order to process the raw genomic sequence and make it accessible and ready for analysis for researchers and clinicians. In order to deal with this problem, we present a cloud-based bioinformatic tool for raw genomic sequence analysis. This tool simplifies the existing complexity of raw genomic germline sequence processing and enables fast and easy automated analysis of a large number of samples for identifying potentially pathogenic variants.

Methods: This tool was built based on GATK4 algorithms and deployed using Cromwell Azure technology. An R script was added to create a unique feature that allows dynamic variant filtering using gene panels to obtain a shortlist of variants for American College of Medical Genetics (ACMG) classification in accordance with the patient's phenotype.

Results: A proof of concept of this tool will be presented, performed using patient-derived whole genome and whole exome sequences of epilepsy and renal disease patients.

Conclusion: This tool can support faster and more effective clinical decision-making and can be integrated into a variety of clinical and research settings.

Conflict of Interest: None declared

P17.023.C Detecting copy number variations in routine diagnostic samples using next generation sequencing data

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Background/Objectives: Copy number variation (CNV) refers to the genomic alterations that involve variations in the copy number of specific DNA segments, which are important determinants of genomic diversity and can have significant clinical implications. Accurate detection of CNVs is crucial for understanding genetic disease mechanisms, diagnosis, and treatment. However, conventional methods for CNV detection in routine diagnostic practices are known to have limitations. Next-generation sequencing (NGS) technology offers a robust solution for detecting CNVs with enhanced sensitivity and accuracy.

Methods: We at the Department of Medical Genetics, St. Olavs Hospital, aimed to detect CNVs in routine diagnostic samples utilizing NGS data. For that purpose, we developed a robust bioinformatics pipeline, which incorporates NGS data obtained from targeted gene panel and whole genome sequencing to identify CNVs. This pipeline integrates multiple detection methods based on diverse approaches including read coverage-depth, paired-end, and split-read alignments. This comprehensive approach not only enables accurate and sensitive CNV detection, but also helps to minimize false positives and increase the robustness of the results.

Results: The pipeline was evaluated against control samples with known CNVs and have demonstrated 100 % sensitivity by detecting all the CNVs in these control samples, consistent with existing diagnostic standards. It demonstrates the potential of this pipeline for accurate CNV detection in routine diagnostics.

Conclusion: This pipeline is now implemented as part of our routine diagnostic practice, which provides us the opportunity to find CNVs in a faster and more cost-effective manner and helps in improving disease diagnosis and management.

Conflict of Interest: None declared

P17.024.D Clinical databases and extended family pedigrees: the missing tool for intrafamilial risk estimation analysis

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Background/Objectives: The very rare *CDH1* c.1901C>T is a founder variant in the Northern Portuguese population, shared by hereditary diffuse gastric cancer (HDGC) families. Pedigree analysis revealed an apparent lower penetrance than expected for this gene, but extremely young age of cancer onset. Our aim was to design and create a clinical database that could be used to generate accurate lifetime-risk estimation in variant carriers and identify sub-cohorts for identification of disease modifiers.

Methods: We collected extended family pedigrees and compiled clinical and demographic data of individuals from 11 families carrying the c.1901C>T variant, through appointments and clinical registries' mining at associate hospital. SPSS was used to create a reference database.

Results: 173 individuals were tested for the c.1901C>T variant, from which 74 were positive. Pedigrees were expanded with information on a total of 1000 relatives through testing in several hospitals and/or patient reports at genetic counseling appointment. 18% (13/74) of all carriers developed HDGC related cancers with clinical manifestation (8 DGC, 5 LBC), with a mean age at diagnostic of 37.6±13.7 years, with a mortality of 62%. 21 carriers were found to have early gastric cancer confirmed at risk-reducing gastrectomy. Seven carriers without gastrectomy remained HDGC-free after 65 years of age. The database is currently ready for lifetime-risk estimation of a single *CDH1* variant.

Conclusion: This work highlights the path towards data collection for intrafamilial lifetime-risk estimations. Further analysis on this clinical database will guide current and future studies on disease modifiers and contribute for guidelines' development.

Grant References: PTDC/BTM-TEC/6706/2020

Conflict of Interest: None declared

P17.025.A One sample, multiple results

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Background/Objectives: Diagnostic genomic testing should be accurate and reproducible, however, when different laboratories report the same result in multiple ways, this raises concerns about reliability which may have implications for wider family testing. Four Genomics Quality Assessment (GenQA) 2022 external quality assessments (EQA) included cases where single or multiple exons were deleted/duplicated which highlighted reporting inconsistency.

Methods: DNA samples and case scenarios were sent to participants to analyse using their routine testing protocol. Participants were required to upload clinical reports which were assessed by a panel of expert advisors against peer-reviewed marking criteria.

Results: In all four EQAs, participants attempted to describe variants using HGVS/ISCN nomenclature and/or exon numbering, with varying degrees of accuracy; in the familial colorectal cancer EQA a single exon deletion was described differently based on the numbering system used; for the familial hypercholesterolaemia EQA the extent of a deletion was not reported consistently; HGVS descriptions of multi-exon deletions for the cystic fibrosis and epilepsy disorders EQAs varied considerably - with up to 8 different results reported for cystic fibrosis. The significant variation in reporting practice will be presented along with recommendations on how to minimise misinterpretation.

Conclusion: HGVS and ISCN should be a universal language yet review of the 2022 EQAs showed significant variation in the results reported following analysis of the same sample. It is recommended that a user-friendly description of the variant (e.g., deletion including exon 'a' to 'b' of gene name) be used instead of, or alongside the HGVS/ISCN.

Conflict of Interest: None declared

P17.026.B Rapid whole exome sequencing (rWES) in neonatal care in an Italian maternal-children hospital

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Background/Objectives: Genetic disorders represent the main cause of infant morbidity and mortality and rWES proved to be an accurate approach to provide a precise molecular diagnosis. We here report on rWES performed in a selected cohort of Italian neonates for a faster diagnosis.

Methods: Eight critically ill neonates (aged <1 month) with suspected monogenic diseases underwent *in trio* rWES (data analyses included both SNVs/CNVs).

Results: Mean turnaround time of rWES was 20 days; pathogenetic or likely pathogenetic variants were identified in 5/8 patients (63%). In particular, two neonates (1,2) resulted positive to a metabolic screening. As regards 1), compound heterozygous mutations within *ACADVL* gene were detected, leading to the diagnosis of long chain Acyl-CoA deficit and allowing to change patient's diet. For 2) no mutations were detected, thus excluding the presence of a metabolic disease. Another patient (3) was tested for suspected *ATP6V1B1*-related disorder: instead, compound heterozygous mutations within *GJB2* were identified, confirming a diagnosis of hearing loss and thus excluding renal genetic disease; behind-the-ear hearing aid was implemented. For patient (4), born with hyperinsulinism, one heterozygous variant in *ABCC8* gene was detected, indicating focal familial hyperinsulinism and guiding the correct surgical management. Finally, a de novo variant in *TGFBR2* was detected in patient (5), leading to the correct diagnosis (Loeys-Dietz syndrome) and the resulting patient's management.

Conclusion: This is the first Italian rWES study carried out so far suggesting its importance to: a) confirm a diagnosis, b) guide therapy and specific follow-up, c) be used in routine clinical diagnosis.

Conflict of Interest: None declared

P17.027.C A validated PCR-free clinical whole genome sequencing system for the detection of germline variants

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Background/Objectives: Clinical whole genome sequencing (WGS) increases diagnostic yield and decreases time to diagnosis over usual care in patients with undiagnosed genetic disease. Despite cost reductions and expanding reimbursements, the complexity of analysis and burden of analytical validation impedes the wide-spread adoption of clinical WGS as a first line test for the detection of germline disease. Here, analytical validation is presented for a PCR-free clinical WGS system, with automated and manual library preparation, and sequencing and analysis for the detection of single-nucleotide variants (SNVs), insertions and deletions (Indels), copy number variants (CNVs), runs of homozygosity (ROH), short tandem repeat (STR) expansions, mitochondrial SNVs (MitoSNVs), and SMN-associated variants.

Methods: Modeling of empirical data was used to set reportable ranges and stratify variant types into genomic context categories (high, intermediate, and low confidence). System quality control metrics were selected, and performance was assessed by analytical validation.

Results: Validation with >450 clinical samples demonstrated the system is compatible with blood collected in EDTA tubes, commercially available extraction kits, 280ng DNA input, and robust to expected sources of variability while supporting ≥35.0x average autosomal coverage with <5% sample failures. Additionally, the LoD for MitoSNVs is 4.75% and the system provides accurate and reproducible variant calls for all the supported variant types reported with high and intermediate confidence.

Conclusion: The validated WGS system starts from genomic DNA extracted from peripheral whole blood with output including sample QC reports and genome VCF files that can be used for various clinical applications, including genetic disease testing.

Conflict of Interest: Christine Glidewell-Kenney Illumina, Inc., Illumina, Inc. stock/stock options, Vitor Onuchic Illumina, Inc., Illumina, Inc. stock/stock options, Konstantin Sabourov Illumina, Inc., Illumina, Inc. stock/stock options, Noah Dukler Illumina, Inc., Illumina, Inc. stock/stock options, Mike Mehan Illumina, Inc., Illumina, Inc. stock/stock options, Joyce Lee Illumina, Inc., Illumina, Inc. stock/stock options, Francesca Fiocco Illumina, Inc., Illumina, Inc. stock/stock options, Nafeesa Khan Illumina, Inc., Illumina, Inc. stock/stock options, Jing Su Illumina, Inc., Illumina, Inc. stock/stock

options, Ying Liu Illumina, Inc., Illumina, Inc. stock/stock options, Jairus Kleinert Illumina, Inc., Illumina, Inc. stock/stock options, Ramya Akula Suresh Babu Illumina, Inc., Illumina, Inc. stock/stock options, Adrian Leelin Illumina, Inc., Illumina, Inc. stock/stock options, Laura Rivas Yepes Illumina, Inc., Illumina, Inc. stock/stock options, Krizelle Minde Illumina, Inc., Illumina, Inc. stock/stock options, Christian Abaya Illumina, Inc., Illumina, Inc. stocks/stock options, Sruja Iyer Illumina, Inc., Illumina, Inc. stocks/stock options, Devina Naidu Illumina, Inc., Illumina, Inc. stocks/stock options, Carrie Ludman Illumina, Inc., Illumina, Inc. stocks/stock options, Tram Nguyen Illumina, Inc., Illumina, Inc. stocks/stock options, Mitch Bekritsky Illumina, Inc., Illumina, Inc. stocks/stock options, patent, Victoria Corey Illumina, Inc., Illumina, Inc. stocks/stock options, Daniel Andrews Illumina, Inc., Illumina, Inc. stocks/stock options, Derek Blythe Illumina, Inc., Illumina, Inc. stocks/stock options, Paul Wenz Illumina, Inc., Illumina, Inc. stocks/stock options, Kelechi Eluwa Illumina, Inc., Illumina, Inc. stocks/stock options, Ali Crawford Illumina, Inc., Illumina, Inc. stocks/stock options, Ryan Taft Illumina, Inc., Illumina, Inc. stocks/stock options

P17.028.D Whole genome sequencing as the sole method to characterize genetically pediatric B-cell precursor acute lymphoblastic leukemia in a diagnostic setting

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Background/Objectives: Genetic characterization of pediatric acute lymphoblastic leukemia (ALL) is used to tailor individually the treatment intensity, which significantly contributed to improve the outcome. Today, diagnostics in ALL patients demands a multimodal and cumbersome analysis, hence, we have investigated the suitability of whole genome sequencing (WGS) as the sole method to detect all clinically relevant genomic aberrations in ALL.

Methods: A total of 87 ALL patients were selected to represent genetic subgroups mandatory to detect in the treatment protocol ALLtogether and cases lacking recognized stratifying aberrations, so-called B-other. We compared diagnostic yield of paired-end WGS by analyzing paired leukemia/normal and leukemia-only samples. Following downstream processing, the WGS data were analyzed regarding copy number aberrations (CNAs) and structural variations (SVs). Furthermore, capability of detecting variants at 30x coverage was tested in only leukemia samples by in-silico down-sampling of ten 90x cases and sequencing of 20 cases.

Results: We were able to detect all mandatory aberrations in the treatment protocol as well as to allocate most of the B-other cases to one of the emerging genetic subgroups, both through the analysis of paired leukemia/normal as well as of leukemia-only. Low coverage analysis also successfully identified all class-defining events. Due to the short-read sequencing limitations, rearrangements in known repetitive regions, e.g. D4Z4 containing DUX4, were not identified.

Conclusion: As standalone method, WGS allows detection of clinically relevant genomic events. This represents a promising approach as sole method in the diagnostic setting of ALL.

Grant References: Swedish Childhood Cancer Fund PR2017-0063, PR2019-0072 and Tj2015-0047

Conflict of Interest: None declared

P17.029.A Comparison of short-tandem repeat detection from whole genome sequencing to standard of care clinical testing

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Background/Objective: Short tandem repeats (STRs) are small repetitive sequences (2-6bp) varying in length between individuals and where expansions can cause disease. WGS distinguishes pathogenic expansions from non-expanded alleles. However, for clinical use, it is important to understand the uncertainty of the allele size call.

We evaluated STR lengths detected using clinical WGS, comparing these to standard of care (SOC) locus specific tests.

Methods: We compared 12 STR loci (ATXN7_CAG, ATXN2_CAG, ATXN1_CAG, ATXN3_CAG, CACNA1A_CAG, FXN_GAA, PPP2R2B_CAG, TBP_CAG, ATN1_CAG, DMPK_CTG, AR_CAG, C9orf72_GGGGCC) and 1167 alleles. SOC testing used PCR. STR detection from WGS (150bp paired-end sequencing) used Expansion Hunter v2.5.6 as part of DRAGEN v3.2.

Results: We see very similar repeat lengths from WGS and SOC methods (98% within ±2 repeats) with 85% within the ±1 reported with SOC tests. Lengths were mostly within the WSG read length (99th percentile = 38 repeats = 114bp).

Some loci (ATXN7_CAG, ATXN2_CAG, CACNA1A_CAG, DMPK_CTG, AR_CAG) have very high correspondence (490/502 identical).

For others (FXN_GAA, ATN1_CAG, ATXN_CAG) differences are explained by locus complexity. Some variation (TBP_CAG, ATXN3_CAG, PPP2R2B_CAG) is explained by PCR calibration in SOC tests, with consistent differences only affecting some labs.

Conclusion: Our data supports using WGS as a diagnostic test for STRs. For allele sizes shorter than read lengths, WGS size estimation is at least as accurate as current SOC tests and can be used help to calibrate PCR tests. Although orthogonal testing by PCR is required for confirmation of expansions, WGS could replace PCR in excluding expansions for clinically relevant loci.

Conflict of Interest: None declared

P17.030.B Whole genome sequencing for unsolved exomes – a case from 30 years ago

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Background: In patients with neurodevelopmental disorders (NDD), exome sequencing (ES), the diagnostic gold standard, reveals an underlying monogenic condition in only ~40% of cases, suggesting a considerable diagnostic gap. Here, we report the case of a female patient with profound NDD who died 30 years ago at the age of 3 years and for whom genome sequencing (GS) now identified a one exon deletion in TBCK previously missed by ES.

Methods: DNA was extracted from frozen muscle tissue of the index patient and the parents' blood. Genome data were analysed using a phenotype-based filter and a filter for structural variants.

Results: Biallelic variants in TBCK, which are linked to the autosomal recessive disorder "Hypotonia, infantile, with psychomotor retardation and characteristic facies 3" (OMIM #616900), were detected in the affected individual: a maternally inherited frameshift variant (NM_033115.5:c.1392dup) and a paternally inherited deletion of exon 21. While common calling algorithms for variant calling were able to identify the frameshift variant in the previous exome analysis, they failed to find the intragenic deletion. The patient's phenotype (profound NDD, muscular hypotonia, areflexia, dysmorphic facial features) highly resembles those of previously described patients with biallelic TBCK variants.

Conclusion: Our case illustrates the added value of GS for the detection of structural variants previously missed by ES. Furthermore, it shows the importance of "molecular or genetic autopsy" allowing genetic risk counselling for other family members as well as the end of a diagnostic odyssey after 30 years.

Conflict of Interest: None declared

P17.031.C Diagnostic yield of next generation sequencing-based copy number variation analysis in Mendelian Disorders

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Background/Objectives: Copy number variations (CNVs), play an important role in the emergence of Mendelian disorders. It has been possible to detect CNVs with the help of next generation sequencing (NGS) data and bioinformatic tools. Herein, we present 12 patients diagnosed by NGS-based CNV analysis and aim to evaluate the diagnostic yield of this recently used method and expand the clinical spectrum of intragenic CNVs.

Method: Twelve patients followed with various preliminary diagnoses at Ege University Pediatric Genetics Department and diagnosed by NGS-based CNV analysis (SEQ Platform's CNV analysis, GATK gCNV pipeline) were evaluated. Clinical exome sequencing (Roche HyperCap DS CES kit) was performed for 5 patients, whole exome sequencing (WES) for 4 patients and trio-WES for 3 patients.

Results: Indications for genetic testing were heterogenous including cardiomyopathy, lactic asidosis, spastic paraplegia, neuronal ceroid lipofuscinosis, epidermolysis bullosa, unidentified vision loss with deafness and preliminary diagnoses of Kabuki, Bardet Biedl, Cornelia de Lange, Ehler Danlos syndromes. NGS-based CNV analysis revealed 12 different CNVs consistent with patients clinical findings; monoallelic deletion in *KMT2D, EIF5A, LAMP2* genes; biallelic deletion in *ZFYVE26, AP4S1, PPT1, PDHX, BBS9, COL7A1, RPGRIP1* genes; monoallelic duplication in the *HDAC8* gene and biallelic duplication in the *PLOD1* gene. Dual molecular diagnosis, one as a result of a single nucleotide variant and the other as a result of CNV, was established in three patients.

Conclusion: Our study highlights the role of CNVs in the etiology of Mendelian disorders and the importance of using NGS based CNV analysis in routine diagnostic process.

Conflict of Interest: None declared

P17.032.D Methylation-specific droplet digital PCR is a suitable method for molecular testing for 11p15 associated Imprinting Disorders

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Background/Objectives: Beckwith-Wiedemann and Silver-Russell syndromes (BWS, SRS) are imprinting disorders (ImpDis) caused by opposite molecular alterations of imprinting centers IC1 and IC2 in 11p15.5. For both syndromes, identification of the precise molecular disturbance is required in respect to specific monitoring (BWS) and treatment (SRS). The majority of patients exhibit IC1 or IC2 imprinting defects, and paternal uniparental disomy 11 (upd(11)pat) in BWS. However, these molecular changes might escape detection due to mosaic occurrence and the limitations of the currently applied methods. To improve diagnostic testing, we developed a droplet digital PCR approach (ddPCR) for the IC1 and IC2.

Methods: Two methylation-specific ddPCR approaches targeting the IC1 and IC2 were developed, addressing the same CpGs as MS MLPA assays. ddPCRs were validated by analyzing samples from BWS and SRS patients with different (epi)genotypes. As upd(11)pat mosaicism is particularly challenging to diagnose, a cohort of 15 patients was screened by ddPCR. Data were compared with results from MS-MLPA, MS pyrosequencing, SNP array and WES.

Results: The ddPCR tests for both IC1 and IC2 confirmed the different types of molecular disturbances in all samples. The mosaic levels were comparable with those obtained from other assays, with a clear-cut discrimination between aberrant and normal ranges.

Conclusion: We show for the first time that ddPCR is a sensitive method to identify aberrant imprinting in ImpDis as it accurately discriminates between aberrant and normal (epi)genotypes. Though methylation-specific ddPCR does not allow to differentiate molecular subtypes, its sensitivity will further improve the identification of low-level mosaicism.

Conflict of Interest: None declared

P17.033.A Genetic Testing for Mitochondrial Disease: The United Kingdom Best Practice Guidelines

Eleni Mavraki[1;2], *Robyn Labrum*[3], *Kate Sergeant*[4], *Charlotte Alston*[1;2], *Cathy Woodward*[3], *Conrad Smith*[4], *Charlotte Knowles*[1;2], *Yogen Patel*[3], *Philip Hodsdon*[4], *Jack Baines*[1;2], *Emma Blakely*[1;2], *James Polke*[3], *Robert Taylor*[1;2], *Carl Fratter*[4]

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Background: Primary mitochondrial disease describes a diverse group of neuro-metabolic disorders characterised by impaired oxidative phosphorylation. Diagnosis is challenging; pathogenic variants in >350 genes, both nuclear and mitochondrial DNA (mtDNA) encoded, are known to cause mitochondrial disease, leading to all possible inheritance patterns and further complicated by heteroplasmy of the multicopy mitochondrial genome. Technological advances, particularly next-generation sequencing, have driven a shift in diagnostic practice from 'biopsy first' to genome-wide analyses of blood and/or urine DNA. This has led to the need for a reference framework for laboratories involved in mitochondrial genetic testing to facilitate a consistent high-quality service.

Methods: In the United Kingdom, consensus guidelines have been prepared by a working group of Clinical Scientists from the NHS Highly Specialised Service followed by national laboratory consultation and ratification by our professional body, the Association for Clinical Genomic Science (https://www.acgs.uk.com/quality/best-practice-guidelines/).

Results and Conclusions: Here we outline the genetic testing strategies for diagnosis, family testing and reproductive options including prenatal diagnosis. Importantly, recommendations for the minimum levels of mtDNA testing for the most common referral reasons are included, as well as guidance on appropriate referrals and information on the minimal appropriate gene content of panels when analysing nuclear mitochondrial genes. Finally, we discuss variant interpretation and recommendations for reporting of results, focussing particularly on the unique challenges of interpreting and reporting mtDNA variants.

Conflict of Interest: Eleni Mavraki NHS full time, Robyn Labrum NHS, Kate Sergeant NHS, Charlotte Alston NHS, Cathy Woodward NHS, Conrad Smith NHS, Charlotte Knowles NHS, Yogen Patel NHS, Philip Hodsdon NHS, Jack Baines NHS, Emma Blakely NHS, James Polke NHS, Robert Taylor NHS, Carl Fratter NHS

P17.034.B Blood transcriptome database to detect aberrant splicing in rare disease diagnostics

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Genome sequencing (GS) is the first line diagnostic tool for the diagnosis of rare disease patients at our center. However, GS typically achieves a diagnostic rate of ~40%, partially due to poor detection of non-coding variants. Such variants can result in aberrant splicing or aberrant expression. Transcriptomics can help to close this diagnostic gap as it directly captures information related to splicing and expression levels. In this project we aimed to create a RNA transcript database to which individuals can be normalized in order to find rare transcripts.

RNA-seq data from 126 patients was used to generate a non-redundant set of transcripts that together with their frequencies were collected in a database. A custom pipeline was built that allows input of a patient transcript file and a target gene list to find rare splicing events in genes of interest.

We then applied the pipeline to a cohort of 126 patients of which 22 had a confirmed splicing defect. Each patient had on average 29000 deviating transcripts. Using a list of known disease causing genes and the database described herein we filtered out common transcripts and technical artefacts and reduced the list of candidate disease causing transcripts by a factor 100, while detecting 15 of the confirmed aberrant transcripts.

In aggregate, our data shows that transcriptomics can complement GS in rare disease diagnostics and increase the diagnostic rate by ~15% when only looking at splice variation. As such, transcriptomics is a promising new avenue to explore to improve rare disease diagnostics.

Conflict of Interest: None declared

P17.035.C Towards cross-border access to human genomes and clinical information at scale for research and healthcare

Juan Arenas[1], ***Serena Scollen***[1], *Nikki Coutts*[1], *Ruben Kok*[2], *Toni Andreu*[3], *Jan Korbel*[4], *Denis Horgan*[5], *Regina Becker*[6], *Jasper Bovenberg*[7], *Ivo Gut*[8], *Jeroen Beliën*[9], *Tommi Nyrönen*[10], *Ilkka Lappalainen*[10], *Dylan Spalding*[10], *Bengt Persson*[11], *Sergi Beltran*[8], *Astrid Vicente*[12], *Ilse Custers*[13], *Elena Doménech*[14], *Fernando Martin-Sanchez*[14], *Ángela Ponce*[14], *Giselle Kerry*[1], *Marco Tartaglia*[15], *Giovanni Tonon*[16], *Andres Metspalu*[17], *Andreas Scherer*[18]

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Background/objectives: Genomics data will soon be routinely generated and integrated into national healthcare systems. The 1 + MG Initiative, an initiative of 24 EU countries, the UK, and Norway, aims to enable secure access to genomics and the corresponding clinical data across Europe for better research, personalised healthcare and health policy making. The Beyond 1 Million Genome project (B1MG) supports the 1 + MG initiative in its design and testing phase to produce recommendations and guidelines based on stakeholder feedback.

Methods: The project, working with Member States' national experts deployed in 12 1 + MG Working Groups (Governance, ELSI, Data Quality, Data Standard, Infrastructure, Health economics, Industry, Rare Diseases, Cancer, Common and Complex Diseases, Infectious Disease and Populations Genomics/Genome of Europe), has developed guidance and a set of recommendations to advance towards the deployment of personalised medicine at the European scale.

Results: Recommendations are captured in the 1 + MG Trust Framework that, once adopted by 1 + MG member states representatives, would be implemented at the national level. This includes guidance on data governance, standards, quality and infrastructure, and recommendations on how to approach citizen engagement, as well as a tool for countries to self-assess implementation into healthcare.

Conclusion: Recommendations are being used to promote governance and technical interoperability of genomic and clinical data across European initiatives including the HealthData@EU and the European Cancer Image Initiative.

Supporting this ambition, the European Genomic Data Infrastructure (GDI) project will deploy an infrastructure across 20 countries.

Grant references: Beyond 1 Million Genomes EC H2020 Research and Innovation programme #951724

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P17.036.D The detection of a two-exons-deletion in the ATL3 gene in a patient with sensory polyneuropathy

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Introduction:Hereditary sensory neuropathies (HSN) are a group of neuropathies that impact the peripheral sensory nerves. Patient's symptoms vary in terms of severity and onset. Few cases of HSN1F, a subtype of HSN, have been reported transmitted in an autosomal dominant manner, caused by heterozygous mutations in the *ATL3*. However, structural variants (SVs) have never been reported in *ATL3*.

Materials and Methods:In our study, we employed targeted NGS (Next Generation Sequencing) and the CovCopCan bioinformatics tool to analyze the sequencing data of a patient presenting with symptoms of sensory polyneuropathy.

Results: We reported a deletion of approximately 3kb in *ATL3* gene, that included exons 11 and 12. Further analysis of the sequences, using bioinformatic tools, revealed the presence of transposable elements at the breakpoints' area. Fact that underlined a possible implication of the Non-Allelic-Homologous-Recombination mechanism that could result to the appearance of this large deletion. Examination of patient's nerve biopsy through electronic microscopy (EM) revealed severe rarefaction of the myelinated fibers and the induction of demyelinating-remyelinating processes. In addition, EM pointed out an abnormal aspect of patient's endoplasmic reticulum.

Conclusions: This study is the first to report a large SV in *ATL3* in a patient presented with sensory polyneuropathy. Hence, we highlight the importance to search also for SVs in addition to point-mutations, to optimize the diagnosis of patients. If SVs research is incorporated in routine diagnosis procedure, patients' diagnosis could be improved, not only for patients suffering from HSN but also for other inherited diseases.

Conflict of Interest: None declared

P17.037.A Long read sequencing technology to overcome the challenge of regions with high sequence homology

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The detection of genetic variations in regions with high sequence homology has always been a challenging task for molecular biologists. Paralogous genes and pseudogenes are part of these complex regions, where mapping and variant calling remains difficult with short read sequencing. Orthogonal confirmation of these low-confident variants most likely requires nested-PCR but this approach has disadvantages: improper alignment, contamination, challenging primer design…. Longer reads, spanning specific regions, allow for a more accurate mapping and then can improve variant calling. We validated this technology on three variations (p.(Ala248Val), p.(Ala302Thr), p.(Arg380Cys)) identified through genome sequencing in *TUBB2A*. The last exon of this gene is known to have a great sequence homology with 4 paralogous genes and 1 pseudogene so that variant calling is known to be challenging (Ragoussis *et al*, 2022). These three variations, called by different bioinformatics pipelines, had different VAF (73, 50, 44% respectively) and different kind of caveat aspects on the read alignment visualization. To enrich specifically this exon, we long-range PCR amplified a 3,5 kb region exceeding the homology region. We then prepared libraries with Oxford Nanopore Technologies (ONT) protocol and sequenced them on R9.4.1 flongles. Data were basecalled with Guppy and Minimap2 was used for alignment. Presence of the variants was unequivocally confirmed. This straightforward strategy advantageously replaces the previously used nested-PCR approach for Sanger sequencing. With its ability to generate long reads and distinguish between closely related sequences, long read sequencing is poised to become an essential tool for geneticists studying such challenging regions.

Conflict of Interest: None declared

P17.038.B A systematic review of the diagnostic yield of whole genome sequencing in patients with rare disorders

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Objectives: This review aims to report on the diagnostic yield of whole-genome sequencing (WGS) in patients with suspected genetic disorders as well as compare it with the current standard-of-care genetic testing practices.

Methods: We conducted a comprehensive literature search of the PubMed database followed by screening and selection of the search results based on the inclusion and exclusion criteria and identified studies reporting on the diagnostic yield of WGS in patients with suspected genetic disorders. We extracted and analyzed the data including the diagnostic yield, reported variants, sequencing and analysis approach, and outcome by disease type.

Results: Our review identified 54 studies reporting on 23,888 index patients with the molecular diagnosis reported in 5,305 patients. Exome sequencing (ES) would fail to discover the

diagnostic variant in 10.9% of cases in which small and complex structural variants, tandem repeat expansions and non-coding variants were most commonly reported. The highest percentage of such variants was in patients with neurogenetic disorders.

Conclusion: Our review suggests that the diagnostic yield of WGS is superior to individual other methods including WES, however, the increase in some disease groups is minimal. WGS may be valuable in the diagnostic workup of patients with a complex clinical presentation, in disease types with a wide spectrum of expected pathogenic variant types and in selected patients with undiagnosed conditions following a negative WES report. Additional studies are required to determine the impact of WGS in individual disease types.

Grant references: None

Conflict of Interest: None declared

P17.039.C Harnessing the power of social media to rapidly translate novel discoveries into new diagnoses

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Next generation sequencing has accelerated rare disease gene discovery. However, ~50% of individuals affected by rare genetic conditions remain undiagnosed. Historically, increase in diagnostic yield has mostly been related to improvements in gene panel content, through curation of new disease-gene associations by ClinGen, Genomics England PanelApp, PanelApp Australia and laboratories. This is particularly important when a phenotype-driven virtual gene panel approach to data analysis is taken. However, there is often >1 year delay between the initial report of a disease-gene associations and the inclusion in the relevant gene panel. We set-out to increase diagnostic yield by reducing this delay.

Our methods involved refining traditional search methodologies alongside widely disseminating findings and expert crowdsourcing using a Twitter bot (@DiseaseGenes). We then used custom scripts to identify undiagnosed individuals within the Genomics England research environment with de novo or biallelic likely or pathogenic variants in the newly identified genes.

The bot has identified and shared >120 disease-gene associations in a seven-month period. The majority were associated with autosomal (55 dominant vs 60 recessive) neurodevelopmental phenotypes (59.5%). Within the Genomics England research environment, we identified and fed back 40 previously unrecognised diagnoses of 24 newly described conditions. These included individuals with ATP6V0C- DOHH- and KCNK3- related disorders expanding the genotypic and phenotypic spectrum associated with these conditions. @DiseaseGenes is now also utilised by Australia Genomics as a tool for updating PanelApp panels.

Our approach accelerates translation of disease-gene discoveries into new diagnoses for patients and, with utility to automate reanalysis of genome data.

Conflict of Interest: None declared

P17.040.D Use of long-read sequencing to improve identification and characterisation of complex structural variants in NF2-schwannomatosis

Cristina Perez-Becerril[1;2], *George Burghel*[2], *Claire Hartley*[2], *Charles Rowlands*[1;2], *D Gareth Evans*[1;2], *Miriam Smith*[1;2]

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Background: *NF2*-schwannomatosis is a tumour predisposition disorder resulting from pathogenic variants (PVs) in the *NF2* gene (chr22q12.2). Structural variants are important contributors to *NF2*-schwannomatosis pathogenesis with well-defined genotype-phenotype correlations. We used long-read sequencing to characterise a complex rearrangement disrupting *NF2*, originally identified in a *NF2*-schwannomatosis family, as part of a study aimed at increasing the rate of detection of PVs in non-mosaic cases.

Methods: Initial screening of 168 *NF2*-schwannomatosis families was carried out by targeted next generation sequencing (NGS) and multiplex ligation-dependent probe amplification (MLPA). For refractory cases, linkage and karyotype analyses were performed. One family was referred to Genomics England for whole genome sequencing (WGS). For this family, we also performed WG Long-read sequencing using a HiFi approach (PacBio), along with a customised bioinformatics pipeline.

Results: Extended genetic screening increased the rate of detection of PVs from 90% (NGS/MLPA only) to 96%, enabling identification of PVs in non-coding regions of *NF2* as well as structural variants including a large (~15 Mb) complex rearrangement. Long-read sequencing analysis and assembly of this complex variant revealed several structural variants within the affected region.

Conclusion: PVs located in non-coding regions, and structural variants are an important component of the genetic landscape of schwannomatosis. Long-read sequencing technologies are particularly well suited for identification and characterisation of these variants and a powerful tool for improving accuracy of diagnosis of schwannomatosis and similar disorders.

Grant references: USAMRAA CDMRP Neurofibromatosis Research Program, Investigator-Initiated Research Award (W81 XWH1910334).

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Conflict of Interest: Cristina Perez-Becerril University of Manchester, Pending: MRC Career Development Award, George Burghel Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre (MAHSC), Manchester, UK., Claire Hartley 1Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre (MAHSC), Manchester, UK., Charles Rowlands The Institute of Cancer Research, D Gareth Evans The University of Manchester, Manchester National Institute for Health Research (NIHR) Biomedical Research Centre (IS-BRC-1215-20007) Smith MJ (PI), Evans DG. Detecting missing heritability for risk stratification and clinical management of the neurofibromatoses. US Dept of Defense NFRP. $524,983.80USD. 2019-2023., Miriam Smith The University of Manchester, NIHR Biomedical Research Centre, CoI in Cancer Prevention and Early Detection Theme and Hearing Health Theme. IS-BRC1215-20007. £4,000,000. 2017-2022. Smith MJ (PI). Improving genetic diagnosis for patients with schwannoma and meningioma tumours. Francis Barbara Thornley Trust. £14,000, 2018-2023. Smith MJ (PI), Evans DG. Detecting missing heritability for risk stratification and clinical management of the neurofibromatoses. US Dept of Defense NFRP. $524,983.80USD. 2019-2023.

P17.041.A Impact of reference materials on limit of detection in analytical performance evaluation of liquid biopsy NGS assays

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Background: Liquid biopsy (LB) for non-invasive diagnostics to support precision medicine was recently introduced into clinical practice. In order to evaluate the performance of LB NGS assays in accordance with the In Vitro Diagnostic Regulation (IVDR), appropriate reference materials are crucial. They enable the accurate determination of the limit of blank (LoB) and detection (LoD), which are essential estimating the assay's sensitivity and specificity.

Methods: To assess the suitability of NA24385 based cfDNA reference materials (SensID, Coriell, SeraCare) for analytical performance evaluation, we analyzed three wild-type (WT), five ctDNA positive reference materials (0.1% to 5% variant allele frequency, VAF) and 15 patient samples using our Duplex Sequencing-based LB NGS assay.

Results: Comparison of three WT reference materials showed <0.1 variants per kilobase with VAFs <0.1% in the SensID and Coriell materials, whereas 16.9 of such variants were detected per kilobase in the SeraCare material. This direct comparison of reference materials in combination with <0.1 variants per kilobase with VAFs <0.1% observed in 15 patient samples support our findings that these variants are truly present. In contrast, SeraCare cfDNA reference materials closely resembles native cfDNA, providing a meaningful source for determining the detection rate of known spike-in variants.

Conclusion: In summary, careful selection of reference materials is required to achieve the full potential of NGS assays down to a LoB of 0.1%. While reference materials with well-defined variants are preferable for determining the LoB, reference materials with a broad range of spike-in variants should be considered for determining the LoD.

Conflict of Interest: None declared

P17.042.B The Occurrence of Variants in Difficult-to-Sequence Genes, Noncoding Variants and Copy Number Variants in Whole-Exome Sequencing: The Blueprint Experience

*Kimberly Gall[1], Milja Kaare[1], Kirsty Wells[1], Maria Calvo del Castillo[1], Inka Saarinen[1], **Mari-Liis Lukke**[1], Mikko Muona[1], Tuuli Pietilä[1], Matias Rantanen[1], Massimiliano Gentile[1], Sari Tuupanen[1], Lotta Koskinen[1], Tiia Kangas-Kontio[1], Pertteli Salmenperä[1], Jussi Paananen[1], Samuel Myllykangas[1], Juha Koskenvuo[1]*

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Introduction: It is widely reported that whole-exome sequencing (WES) provides a lower diagnostic yield than whole-genome sequencing (WGS), often attributed to missed variants in difficult-to-sequence (DTS) genes, copy number variants (CNVs), and variants in noncoding regions. The occurrence of such variants in an unselected population undergoing WES is not well understood. We assessed the diagnostic yield of our WES assay with attention to pathogenic (P) and likely pathogenic (LP) variants identified in DTS genes, CNVs, and disease-causing noncoding variants.

Methods: We examined results from 10,435 patients tested with a WES assay at Blueprint Genetics. The target region included all coding exons (including +/-20 bp from intron/exon boundaries) of protein coding genes and 1,501 noncoding variants. CNV analysis was performed bioinformatically from next generation sequencing data.

A positive result was defined as a P or LP variant(s) consistent with the patient's reported phenotype and expected inheritance.

Results: A positive result was reported for 27.3% patients. Ten genes accounted for >22% of the positive results. Three of these genes (*ANKRD11*, *TTN*, and *PTEN*) were DTS genes. CNVs accounted for 13.9% of all positive results with 12.2% <1000 bp in size. Variants in DTS genes and in disease-causing noncoding regions accounted for 7.5% and 1.1% of positive results respectively.

Conclusions: Variants in DTS genes, CNVs and disease-causing noncoding variants comprised 22.5% of positive results in an unselected population undergoing WES. WES assays optimized for detection of these variants may provide benefits similar to those of WGS.

Conflict of Interest: Kimberly Gall full, Milja Kaare full, Kirsty Wells full, Maria Calvo del Castillo full, Inka Saarinen full, Mari-Liis Lukke full time, Mikko Muona full, Tuuli Pietilä full, Matias Rantanen full, Massimiliano Gentile full, Sari Tuupanen full, Lotta Koskinen full, Tiia Kangas-Kontio full, Pertteli Salmenperä full, Jussi Paananen full, Samuel Myllykangas full, Juha Koskenvuo full

P17.043.C CYP21A2 genetic diagnostics by NGS data evaluation in a clinical laboratory setting

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Our clinic specializes in detection of pathogenic alleles in unaffected carriers of several recessive diseases. Genetic screening of congenital adrenal hyperplasia (CAH) is rather challenging due to the close proximity of duplicated region with the presence of highly homologous pseudogene (CYP21A1P) and high occurrence of several rearrangements leading to complete gene loss or formation of chimeras between gene and its pseudogene. For a long time we were confident in only dedicated number of pathogenic alleles in CYP21A2 gene.

To improve this, with the advent of NGS and whole exome sequencing we focused on data analysis. By incorporation of CYP21A1P masking we aligned all reads to CYP21A2 region and evaluated the number of achieved coverage in a ratio with a selected reference gene FGFR3 (duplication and deletion events in this region are scarse). By this approach we were able to evaluate the number of alleles CYP21A2 + CYP21A1P. We analyzed frequencies of all SNPs and further, we focused on the most frequently mutated positions within the CYP21A2 that have originated from microconversions with CYP21A1P pseudogene. However, microconversion processes also create reverse variants where pseudogene carries a nucleotide that originally was present in a gene. To overcome this, we applied special haplotype bioinformatic pipeline which showed to be successful in determination if selected SNP is present in gene or pseudogene.

We present our newly set approach for CYP21A2 genetic diagnostics from NGS data. Preliminary results with reference CYP21A2 samples and detailed description will be provided. All suspicious findings are further analyzed by HybrAmp approach.

Conflict of Interest: None declared

P17.044.D Exome sequencing for patients with rare genetic diseases in an Indian start up genomics laboratory

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Background/Objectives: Clinical Genetics is rapidly making strides into practice of medicine in India with several clinical laboratories offering a diverse range of services. We share the early experiences of a new clinical genomics laboratory.

Methods: Exome sequencing (ES) was performed in patients with referral diagnosis of a rare genetic disease (Solo- 517; Duo- 3; Trio- 9) by using commercially available exome capturing kits (TWIST- 425; CREV2- 58; CREV3- 56). The variants of clinical interest found were validated by Sanger sequencing and segregation was performed in affected and unaffected family members to determine the clinically relevant variants. Copy number variant analysis was employed in all the ES data and relevant CNVs were confirmed by MLPA.

Results: We evaluated 539 patients (including 300 males, 229 females with ages ranging from birth to 64 years and 10 foetuses with unknown gender). Overall, our diagnostic yield was 50.65% (273/539 patients). We identified copy number variants (CNVs) in ten patients. A total of 296 variants (P- 157, LP- 74, VUS- 65) were reported in 230 genes. Bi-allelic variants (recessive disease) were seen in 139 patients (homozygous- 110, compound heterozygous- 29). 120 patients had monoallelic variants (dominant disease). 12 patients had hemizygous variants (X-linked). We noted multilocus disease-causing genetic variations as blended/ distinct phenotypes in nine patients. We also share the challenges faced by the start-up.

Conclusion: Exome sequencing is a useful tool for diagnosis of rare disease if good prescription of tests, segregating candidate variants, interpretation by clinical geneticists are implemented.

Conflict of Interest: Chakshu Chaudhry Suma Genomics Private Limited, Sandesh Salvankar Suma Genomics Private Limited, Katta Girisha Suma Genomics Private Limited

P17.045.A Beyond genomics: Using RNA-seq in filter cards to unlock the clinical relevance of non-coding variation in splicing

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Background: Many patients remain undiagnosed, with variants of unknown significance (VUS) or no relevant variants detected even after genome sequencing. We aim to assess the impact of splicing variants reported in our laboratory to gain insight into their clinical relevance.

Methods: We selected 103 consecutive patients with known/ suspected splicing variants for RNA-seq. A protocol was developed to perform RNAseq from dried blood spots (DBS). Splicing in genes of interest was inspected using IGV with at least three unaffected individuals as controls. Relative gene expression was calculated with *GAPDH* and *ACTB* housekeeping genes and compared to average of 20 unaffected individuals.

Results: 87 variants were single base substitutions and 16 were small indels. 78 variants were heterozygous (72%), 26 were homozygous (24%) and 4 hemizygous variants (4%). Abnormal splicing was clearly detected in 40 samples (37%). Abnormal splicing was likely in 27 additional variants, but detected in a low number of reads, warranting an independent confirmation. No evidence of abnormal splicing was detected in 14 samples. Inconclusive results were noticed in 27 cases.

Additional evidence of pathogenicity by an independent method was available in three individuals with variants in the *HEXA, GAA* and *MANBA* genes.

Conclusion: We propose a method for systematic experimental evaluation of the splicing impact of intronic variants through RNAseq from DBS, integrated in diagnostic exome/genome sequencing, which impact the assessment of their clinical relevance. The approach can be implemented in the routine workflow by diagnostic laboratories, adding an additional -omics layer to the diagnosis of rare disorders.

Conflict of Interest: Aida Bertoli-Avella Centogene GMBH, Ruslan Al-Ali Centogene GMBH, Mandy Radefeldt Centogene GMBH, Jorge Pinto Basto Centogene GMBH, Peter Bauer Centogene GMBH

P17.046.B The added diagnostic yield of copy number variant (CNV) analysis in gene panels from whole exome sequencing (WES) data: a two year experience

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Background/Objectives: In clinical diagnostics whole exome sequencing (WES) data is being increasingly used for copy number variant (CNV) detection. The aim of this study was to uncover the added diagnostic yield of CNV analysis in gene panels from WES data.

Methods: We evaluated the CNV's detected in clinical diagnostic gene panel analysis (34 indications) from WES data between January 2021 and December 2022. CNV analysis was performed by read-depth method using dynamic bins (BAM Multiscale Reference Method by BioDiscovery). Detected CNV's were confirmed by an orthogonal method. Classification and reporting were according to local and international guidelines.

Results: A total of 3650 gene panel analysis were performed. A CNV was reported in 127 (3.5%) analysis, of which 61 (1.7%) (likely) pathogenic variants yielded the molecular diagnosis, 30 (0.8%) variants of uncertain significance and 18 (0.5%) pathogenic variants in autosomal recessive disorders (potentially) fitting the phenotype but without a second variant detected. The remaining 18 (0.5%) were pathogenic variants not fitting the phenotype (incidental findings), of which 14 were already known by other genetic tests performed.

Conclusion: The added diagnostic yield by CNV analysis in gene panels from WES data is between 1.7-3.0%.

Grant References: not applicable

Conflict of Interest: None declared

P17.047.C Clinical and analytic workflow considerations for re-analysis of genomic data

Adrienne Elbert[1;2], *Indhu Rajan Babu*[2], *Laura Li*[3], *Stephanie Huynh*[1], *Hyun Lee*[1], *Cuong Bui*[3], *Nan Zhang*[3], *Karar Al-Mamaar*[4], *Haidar Al-Khafaji*[5], *SHELIN ADAM*[2], *Jan Friedman*[2], *Cornelius Boerkoel*[1;2]

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BACKGROUND/ OBJECTIVES: Despite advances in the clinical application of genomic sequencing for diagnosis of rare disease, there is a paucity of refereed information on the clinical process spanning bioinformatics-based variant prioritization through to diagnosis. Herein we address many outstanding questions regarding how elements within the process contribute to

successful diagnosis, and how re-analysis in undiagnosed cases can be efficiently implemented in the clinical workflow.

METHODS: We used a partially retrospective approach focused on undiagnosed cases. Raw genomic data was obtained and re-aligned for two clinical populations 1) 532 diagnosed and undiagnosed research patients (2015-2018), and 2) 72 undiagnosed patients ascertained after uninformative clinical exome sequencing (2017-2021). Clinical features were captured as HPO terms. Genomic variants were prioritized in exomiser, LIRICAL and Virtual Geneticist. Patient-relevant variants were identified by the clinical geneticist.

RESULTS: Implementation of clinical re-analysis by the geneticist revealed candidate diagnoses not reported by private laboratories, due to variability in the threshold for reporting uncertain variants.

Variability in phenotypic terms influences the ranking of genomic variants in certain cases.

Parallel analysis through several variant prioritization tools leads to efficient diagnosis by the clinician.

Patients with dual molecular diagnoses may be enriched among unsolved cases and benefit from separate analytic approaches.

CONCLUSION: We provide implementable process strategies to optimize diagnostic yield on genomic testing. We propose workflow that improves diagnosis of the subset of patients in whom multiple genetic diagnoses together explain the phenotype. Further work will establish whether these approaches have benefit in a prospective manner.

Conflict of Interest: None declared

P17.048.D Clinical impact of RNA-sequencing in diagnostics

Laurenz De Cock[1;2], *Erika D'haenens*[1;2], *Sarah Vergult*[1;2], *Lies Vantomme*[1;2], *Annelies Dheedene*[1;2], *Robin de Putter*[2], *Tim Van Damme*[2], *Jo Sourbron*[2], *Bert Callewaert*[1;2], *Olivier Vanakker*[1;2], *Björn Menten*[1;2]

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Background: Several studies have shown that transcriptomics (RNA-seq) nicely complements whole exome and whole genome sequencing (i.e., WES and WGS) in variant interpretation and leads to a 7-36% increase in diagnostic yield. Therefore, we have set up a novel minimally-invasive ready-to-use RNA-seq protocol based on short-cultured peripheral blood monocytes (PBMCs).

Methods: In short, PBMCs are isolated from blood and cultured for 2-3 days. After which the culture is split in two, one of which is treated with cycloheximide to allow detection of nonsense mediated decay sensitive transcripts. Subsequently RNA is extracted and subjected to polyA-sequencing. The RNA-seq data is then processed with OUTRider and/or FRASER or subjected to manual inspection via IGV.

Results: Isolation and culture of PBMCs is faster and less labour intensive in comparison with fibroblasts. Moreover, we show that 63.5% and 62.8% of genes of the Mendeliome (4542 genes) are respectively expressed in PBMCs and fibroblasts, highlighting the potential of PBMCs.

Our workflow revealed an aberrant splicing event in 6/9 individuals with a suspicious splice variant of unknown significance, detected via WES. As these aberrant splicing events were more complicated than anticipated, targeted cDNA sequencing failed to detect the event in 4/6. Based on the RNA-seq results, the six variants could be reclassified and thus lead to a correct clinical diagnosis for these individuals, highlighting the added value of RNA-seq.

Conclusion: We present an optimized RNA sequencing protocol and analysis workflow and show its added value for interpretation and classification of putative splice site variants.

Conflict of Interest: None declared

P17.049.A nf-core/raredisease: a community driven open-source pipeline for raredisease diagnostics

Ramprasad Neethiraj[1], *Anders Jemt*[2;3], *Lucia Pena-Perez*[2;4;5], *Mei Wu*[6], *Annick Renevey*[2], *Gwenna Breton*[7], *Sima Rahimi*[7], *Emma Andersson*[7], *Lauri Mesilaakso*[8], *Subazini Thankaswamy Kosalai*[9], *Emil Bertilsson*[3], *Ryan Kennedy*[10], *Viktor Henmyr*[10], *Henrik Stranneheim*[2;3;4], *Valtteri Wirta*[1;2;3]

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Background: Several open-source computational pipelines are available to analyse whole genome sequencing (WGS), and whole exome sequencing (WES) data for rare disease (RD) diagnostics. Despite using similar tools, these pipelines are often not portable across different IT infrastructures, and they usually require a commercial software to facilitate variant interpretation. Herein we describe the development of a rare disease pipeline that addresses these issues.

Methods: The pipeline can call and annotate SNV/indels, SVs and CNVs. Furthermore, it runs a specialized analysis workflow for mitochondrial variants. Variants are ranked by genmod (https://github.com/Clinical-Genomics/genmod), an open-source tool, according to their predicted pathogenicity. The pipeline is developed in nextflow, and its modular architecture enables switching between different bioinformatic programs to fit local needs and specialties.

Results: The pipeline is an extension of the workflow used in the Stockholm healthcare region (https://github.com/Clinical-Genomics/MIP) to analyze nearly 3,000 WGS and 1000 WES yearly, with a diagnostic yield of 40% in a first cohort of 3219 patients. The development is a part of the nf-core initiative (https://nf-co.re/), and is a major collaborative effort within Genomic Medicine Sweden, to establish a national bioinformatic pipeline for RD diagnostics.

Conclusion: Our rare disease pipeline adheres to nf-core community's standards ensuring that it will install, run and perform on most computing infrastructure, including cloud. The nf-core/raredisease pipeline's portability and modular architecture opens up the opportunities for broad collaboration, and utilization across the diagnostic RD community.

Grant references

Conflict of Interest: None declared

P17.050.B A case with a DNM1L associated acute onset encephalopathy highlights the urge for rapid genome sequencing

Matias Wagner[1;2], *Theresa Brunet*[1;2], *Elisabeth Graf*[1], *Riccardo Berutti*[1], *Juliane Winkelmann*[1;3], *Thomas Meitinger*[1], *Katharina Vill*[1;2], *Ingo Borggräfe*[2], *Florian Heinen*[2], *Florian Hoffmann*[4]

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Oberschleißheim, Germany; [4]Dr. v. Hauner Children's Hospital, Munich, Germany

Background: About 4 million people in Germany suffer from a rare disease reflecting the clinical heterogeneity, which requires rapid and comprehensive diagnostics. Rare diseases that become symptomatic in childhood often have very severe manifestations and are initially being treated in pediatric and neonatal intensive care units. In most cases, this is a purely symptomatic therapy until the underlying disease is correctly diagnosed.

Methods: A 6-year-old female patient was admitted to the pediatric intensive care with status epilepticus. Parent-child trio genome sequencing was performed within the project "Bavariant Genomes".

Results: Extensive diagnostic workup included multiple CSF samplings, four MRI examinations under anesthesia, two computer tomographies, 13 EEGs, a muscle biopsy, 13 X-rays, extensive laboratory testing for infectious, immunological and metabolic diseases as well as a brain biopsy. Rapid genetic testing by panel sequencing of >100 genes was negative. Therapeutic approaches included 18 different antiseizure drugs, high-dose cortisone therapy, plasmapheresis and administration of monoclonal antibodies. Diagnostic and therapy costs were over 100,000 € within 60 days of treatment. Trio genome sequencing identified a pathogenic heterozygous variant in *DNM1L* (NM_001330380.1: c.1246C>T, p.Arg416Cys) which was inherited from the father where it was present in mosaicism on day 62 after admission.

Conclusions: Patients with rare diseases account for a relevant percentage of pediatric intensive care bed occupancy and are therefore of great importance in terms of health economics. Rapid diagnosis is important for better therapy, but also for more targeted diagnostics to improve patient outcome and reduce costs urging for rapid genome sequencing in Europe.

Conflict of Interest: None declared

P17.051.C A false-positive diagnostic GBA1 finding for an allele that harbors a benign conversion of GBA1 gene sequence into GBAP1 pseudogene sequence

Mandy Radefeldt[1], *Tama Dinur*[2;3], *Ruslan Al-Ali*[1], *Ari Zimran*[2;3], *Shoshana Revel-Vilk*[2;3], *Peter Bauer*[1], *Christian Beetz*[1]

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Background/Objectives: Variants in *GBA1* are associated with Gaucher Disease and Parkinson's Disease. The *GBA1* mutational spectrum contains variants that result from recombination between *GBA1* and its nearby pseudogene *GBAP1*. Some genetic diagnostic approaches fail to reliably identify corresponding alleles. False-negative findings are a well-recognized and well-understood consequence. False-positive findings are rare, and no explanation has been provided. We came across a case in which our next generation sequencing (NGS) approach did not confirm presence of the pseudogene-derived *GBA1* variant p.L483P as reported by another lab.

Methods: The case was re-tested using a primary *GBA1*-specific long-range PCR followed by secondary exon-wise PCRs and Sanger sequencing. The Integrative Genomics Viewer (IGV) was used for visual exploration of NGS-derived sequencing reads mapping to the relevant *GBA1*/*GBAP1* regions in the case vs. in controls.

Results: Sanger sequencing confirmed absence of the p.L483P variant. IGV exploration suggested that the relative number of NGS reads mapping to the *GBA1*/*GBAP1* is significantly deviating from what is observed in controls. Closer examination revealed that this imbalance is caused by a subset of reads which span a certain region that contains three single nucleotide gene-pseudogene differences.

Conclusions: The number and identity of NGS reads in the case is consistent with a (benign!) conversion of *GBA1* sequence into *GBAP1* sequence. *GBA1* variant p.L483P is a false positive call, which can be avoided by (i) usage of an appropriate BED file, (ii) consideration of read number imbalances, and (iii) setting of stringent variant frequency thresholds.

Conflict of Interest: Mandy Radefeldt Centogene GmbH, Rostock, Germany, Tama Dinur: None declared, Ruslan Al-Ali Centogene GmbH, Rostock, Germany, Ari Zimran: None declared, Shoshana Revel-Vilk: None declared, Peter Bauer Centogene GmbH, Rostock, Germany, Centogene GmbH, Rostock, Germany, Christian Beetz Centogene GmbH, Rostock, Germany

P18 Bioinformatics, Machine Learning and Statistical Methods

P18.001.A scQCEA a framework for annotation and quality control report of single-cell RNA-sequencing data

Isar Nassiri[1], *Benjamin Fairfax*[2], *Angela Lee*[1], *Yanxia Wu*[1], *David Buck*[1], *Paolo Piazza*[1]

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Background/Objectives: Systematic description of library quality and sequencing performance of single-cell RNA sequencing (scRNA-seq) data is imperative for subsequent downstream modules, including re-pooling libraries. We present scQCEA (acronym of the single-cell RNA sequencing Quality Control and Enrichment Analysis), an R package to generate interactive reports of process optimization metrics for comparing sets of samples and visual evaluation of quality scores. In addition, scQCEA provides automated cell type annotation on scRNA-seq data using differential patterns in gene expression for expression-based quality control.

Methods: scQCEA is written in R, combining Shiny and Markdown. scQCEA is available at https://isarnassiri.github.io/scQCEA/ as an R package. Full documentation including an example is provided on the package website.

Results: To demonstrate the utility of scQCEA, we apply the workflow to the sixteen gene expression profiles of eight patients with metastatic Melanoma, and 286 single cell gene expression profiles from humans and mice. The interactive report of quality control metrics and image QC of profiles allowed visual evaluation and comparison of comprehensive QC metrics. The results suggest that the cell type enrichment analysis captures the main clusters across cells, and samples share similar cellular compositions in agreement with existing labels. In addition, we provide example of application to discriminate between true variation and background noise for samples which the knee plot works poorly due to a wetting failure.

Conclusions: In summary, the scQCEA package with two functions for cell-type enrichment analysis and generating an interactive QC provides infrastructure for different applications, including delivery of high-quality genomic services.

Conflict of Interest: None declared

P18.003.C Automated identification of manuscripts describing genetically-determined developmental disorders using machine learning

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Introduction: Disease-phenotype data is crucial in interpretation of genome-wide sequencing results, particularly for genetically-determined developmental disorders (GDD). There is a need for automated search of the peer-reviewed literature to facilitate diagnostic phenotypic data extraction at scale.

Methods: PubMed search strategies were evaluated for all diseases in Developmental Disorders Gene2Phenotype (DDG2P). A corpus of abstracts from this search for 125 exemplar GDD in DDG2P was used to evaluate 10 supervised machine learning classifiers, for the purpose of identifying GDD case reports/case series. 33 literature derived features associated with the title, abstract, PubMed metadata, or MeSH terms were used.

Results: For 2164 GDD, a PubMed {gene symbol}[title] search was chosen as the best balance between manageable number of results (411,783) and genes with zero results (30). This search was then used for 125 exemplar GDD, generating 6578 abstracts: 2008/6578 (31%) manuscripts described the relevant GDD. The top performing classifiers on this set were Gaussian Process and Random Forest, with precision/recall of 0.76/0.79 and 0.74/0.79. The least performant classifiers, Naïve Bayes and Quadratic Discriminant Analysis, had the useful property of high recall (0.99, 1.00). The top five features were 'case report' in metadata, fuzzy match to disease name in title and 'mutation', 'infant' and 'intellectual disability' in the MeSH terms.

Conclusions: We demonstrate effective classification of GDD-relevant papers using a supervised machine learning model. Integration of this system with named entity recognition should enable extraction of phenotypic data at scale to enhance GDD diagnostics.

Grant reference: Wellcome Strategic Award 200990/Z/16/Z

Conflict of Interest: None declared

P18.004.D Leveraging healthy population data to assess the pathogenicity of rare variants in WGS using an extension of the PSAP method

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Background/Objectives: High genetic heterogeneity in rare diseases makes it extremely difficult to identify a patient's causal variant with sequencing data and traditional analysis methods. The PSAP (Population Sampling Probability) method uses gene-specific null distributions of CADD pathogenicity scores to assess the probability of observing a given genotype in a healthy population. Here, we propose an extension of the PSAP method to the non-coding genome using predefined functional regions as testing units. Our method broadens the spectrum of variants detectable by PSAP and also improves the performance of PSAP in coding regions by reflecting functional constraint.

Methods: We simulated disease exomes by inserting 315 best-reviewed ClinVar pathogenic variants in healthy exomes. Inserted variants were evaluated using either genes or functional regions as testing units, by ranking them based on their PSAP p-value in the context of the exomes. The percentage of variants ranked at top positions was compared between the different strategies and against a simple ranking based on CADD scores only.

Results: When using PSAP on functional regions, 91% of the ClinVar variants were ranked first compared to 87% when using genes, and 0.3% when using CADD scores only. On real data from 6 patients with Cerebral Small Vessel Disease (CSVD), the prioritization of the causal variants was improved by using PSAP on functional regions.

Conclusion: The PSAP method extended to functional regions is an efficient priorization tool, which offers promising results for the diagnosis of unresolved cases of rare diseases.

Grant: French-Priority Research Program on Rare Diseases, ARED-Britanny region

Conflict of Interest: None declared

P18.006.B PheWAS-based clustering of Mendelian Randomisation instruments reveals distinct mechanism-specific causal effects between obesity and educational attainment

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Mendelian Randomisation (MR) is a statistical method that estimates causal effects between risk factors and common complex diseases using genetic instruments. Heritable confounders, pleiotropy and heterogeneous causal effects violate MR assumptions and can lead to biases. To tackle these, we propose an approach employing a PheWAS-based clustering of the MR instruments (PWC-MR). We apply this method to revisit the surprisingly large causal effect of body mass index (BMI) on educational attainment (EDU): $\hat{\alpha} = -0.19$ [-0.22, -0.16].

As a first step of PWC-MR, we clustered 324 BMI-associated genetic instruments based on their association profile across 407 traits in the UK Biobank, which yielded six distinct groups. The subsequent cluster-specific MR revealed heterogeneous causal effect estimates on EDU. A cluster strongly enriched for traits related to socio-economic position yielded the largest BMI-on-EDU causal effect estimate ($\hat{\alpha} = -0.49$ [-0.56, -0.42]) whereas a cluster enriched for primary impact on body-mass had the smallest estimate ($\hat{\alpha} = -0.09$ [-0.13, -0.05]). Several follow-up analyses confirmed these findings: (i) within-sibling MR results ($\hat{\alpha} = -0.05$ [-0.09, -0.01]); (ii) MR for childhood BMI on EDU ($\hat{\alpha} = -0.03$ [-0.06, -0.002]); (iii) step-wise multivariable MR (MVMR) ($\hat{\alpha} = -0.06$ [-0.09, -0.04]) where time spent watching television and past tobacco smoking (two proxies for potential confounders) were jointly modelled.

Through a detailed examination of the BMI-EDU causal relationship we demonstrated the utility of our PWC-MR approach in revealing distinct pleiotropic pathways and confounder mechanisms.

Conflict of Interest: None declared

P18.007.C Innovative applications of the Human Phenotype Ontology to achieve diagnoses in rare disease cohorts

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Background: The genome sequence of patients with rare monogenic diseases often lacks a clearly identifiable disease-causing genetic variant or mutation. Such patients often remain undiagnosed despite the application of numerous computational variant prioritization methods, necessitating the development of novel tools to increase diagnostic rates. We have developed an approach that matches undiagnosed patients to phenotypically similar diagnosed patients, then utilizes the known diagnostic genes to prioritize variants in the undiagnosed case.

Methods: The hierarchically organized Human Phenotype Ontology (HPO) terms used to describe disease phenotypes enable the phenotypic similarity of pairs of patients to be calculated. These patient-to-patient similarity scores are highest for the most clinically similar patients, without requiring that they share exact phenotypes. We then use these scores to identify the most similar diagnosed patients to an undiagnosed patient, build a list of diagnostic genes, and then search for plausible diagnostic variants within these genes for the undiagnosed case.

Results: We have applied our methods to the difficult-to-diagnose patient cohort within NIH's Undiagnosed Diseases Network (UDN). We have shown that patients with matching clinical or genetic diagnoses show significantly higher phenotypic similarity scores than patients with different clinical or genetic diagnoses. We have identified multiple undiagnosed cases for whom our method identifies compelling candidate variants based on their similarity to a diagnosed patient.

Conclusion: Our tool prioritizes candidate variant lists of undiagnosed patients based on their phenotypic similarity to previously diagnosed cases. Initial successes suggest that this will be an exciting and valuable tool for diagnostic variant prioritization.

Conflict of Interest: Isabelle Cooperstein: None declared, Alistair Ward Part-time, Co-I on 1U01HL153007

Co-I / Site lead on 1R01HG012286

PI on 1R41HG012536, Stock in Frameshift Genomics, Gabor Marth Co-PI on 1U01HL153007

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P18.008.D Multivariable MR can mitigate bias in two-sample MR using covariable-adjusted summary associations

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Background: Genome-Wide Association studies (GWAS) are hypothesis free studies that survey the whole genome for polymorphisms associated with a trait of interest. To increase power and to estimate the direct effects of these variants on a trait GWAS are often conditioned on a covariate (such as body mass index). This adjustment can introduce bias in the estimated effect of the SNP on the trait. Two-sample Mendelian randomisation studies use summary statistics from GWAS estimate the causal effect of an exposure on an outcome. Covariate adjustment in GWAS can bias the effect estimates obtained from MR studies.

Methods: Multivariable MR (MVMR) is an extension of MR that includes multiple traits as exposures. Using simulations we show that MVMR can recover unbiased estimates of the direct effect of the exposure of interest by including the covariate used to adjust the GWAS within the analysis. We apply this method to estimate the effect of systolic blood pressure on type-2 diabetes and waist circumference on systolic blood pressure both adjusted and unadjusted for BMI.

Results: Our simulation results show that this method provides consistent effect estimates when summary stats have been adjusted for a covariate. The results from the applied analysis mirror these results, with equivalent results seen in the MVMR with and without adjusted GWAS.

Conclusion: When GWAS results have been adjusted for a covariate, unbiased direct effect estimates of an exposure on an outcome can be obtained by including that covariate as an additional exposure in a MVMR estimation.

Conflict of Interest: Joseph Gilbody PhD student at University of Bristol, MRC studentship grant, George Davey Smith University of Bristol, Work in a unit funded by the MRC (MC_UU_00011), Carolina Borges University of Bristol, Supported by a Vice-Chancellors Fellowship from the University of Bristol and the Bristol BHF Accelerator Award (AA/18/7/34219), Eleanor Sanderson University of Bristol, Work in a unit funded by the MRC (MC_UU_00011)

P18.010.B ExDepth: a large deletions analysis algorithm resolved 3% of exome negative cases from a cohort of 1000 neurodevelopmental samples

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Objectives: Exome sequencing has been widely used to diagnose rare genetic disorders, especially for neurodevelopmental disorders. Despite advances in sequence data analysis and finding novel disease genes, there is still a large proportion of neurodevelopmental cases remaining undiagnosed. Here we hypothesised that this is not solely because of missing non-exonic variants or variants of unknown human disease genes, but this could be due to our analysis approach.

Methods: An algorithm, named ExDepth, was developed to detect large deletions from exome sequence data. This gets a cohort of samples that are sequenced using the same platform and creates a reference read coverage from control exomes. Then compares patients' read coverages with the reference to find large deletions.

Results: We applied ExDepth on a cohort of 1000 exomes, 700 of which are affected neurodevelopmental patients. About 500 of these cases are genetically undiagnosed. We detected 14 homozygous deletions in previously unresolved cases, some of them were circulating for several years to get a diagnosis. These deletions are validated using SNP-array or PCR, and confirmed by matching the defective genes with patients' phenotypic features.

Conclusion: ExDepth could detect exonic deletions ranging from less than 100 base pairs to several hundred kilobases long. Regarding the limitation of array techniques in detecting deletions smaller than several kilobases long, ExDepth can fill the gap between short variant calling and large copy number variant detection. In addition, compared to similar algorithms, ExDepth has a low false positive rate, making the variant interpretation process much easier.

Conflict of Interest: Shahryar Alavi Palindrome, Rauan Kaiyrzhanov UCL Queen Square Institute of Neurology, Ellie Self UCL Queen Square Institute of Neurology, David Murphy UCL Queen Square Institute of Neurology, Kristina Zhelcheska UCL Queen Square Institute of Neurology, Reza Maroofian UCL Queen Square Institute of Neurology, Henry Houlden UCL Queen Square Institute of Neurology

P18.012.D Statistical Analysis of Genomic in-silico Pathogenicity Predictors

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Genomic variant interpretation is a critical step in diagnostic short-read sequencing. A "supporting" evidence for pathogenicity (PP3) in the ACMG/AMP guideline is defined by the agreement of multiple lines of in-silico evidence assessments. Many in-silico predictors have been developed in this context. However, as data accumulate in databases such as ClinVar, it has been observed that these predictors do not always provide accurate predictions for each gene. Therefore, choosing the predictors would create a bias, and there should be a standard on which tool to use for diagnostic variant interpretation. Here we present a tool that statistically compares the efficiency of 45 different in-silico predictors in the dbNSFP (v4.3a) dataset for missense variants by using variants reported to ClinVar to ensure correct gene-specific predictor matching.

Only two gold star ClinVar variants (criteria provided, multiple submitters, no conflicts) are kept for reliable pathogenicity assessment when assigning the variants to 3 main groups; Benign, Pathogenic, and Unknown. Only dbNSFP (v4.3a) ranked scores (0-1) of in-silico predictors were used for the comparability via variance-based multiple comparisons. Then, in-silico predictors were listed according to their gene-based statistical significance in predicting a variant's pathogenicity.

The tool was able to upgrade the ACMG/AMP PP3 criterion's strength level to moderate, leading to potential re-classification, a possible diagnosis, or vice versa downgrading when promoting a variant's benignity. The tool will be available in April 2023 as an open-access web platform that enables gene-specific in-silico predictor analysis for users.

Conflict of Interest: None declared

P18.013.A Using machine learning to find brain correlates of genetic risk for mental health conditions

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Mental health conditions such as schizophrenia (SCZ), bipolar disorder (BD), as well as Alzheimer's disease (AD) have a strong genetic component and are associated with changes in brain structure and function. Little is known on how the genetic risk for SCZ, BD and AD might mediate brain changes.

Magnetic Resonance Imaging (MRI) is a promising route to understand how genetics influence the neurobiology of mental health conditions. However, this highly promising research area has been limited by; the heterogenous nature of the data, small sample sizes of datasets (typically 100-200 cases), use of imaging derived phenotypes (IDPs) that lose information embedded in raw images, overlooking rare variants typically associated with large effects by only using polygenic score (PGS).

To overcome these limitations, we used IDPs, and raw MRI data derived from $n = 40{,}000$ individuals of the UK Biobank. A logistic regression model using the IDPs acts as a baseline for comparison with a more complex convolutional neural net (CNN) model using the images. Participants were split into high and low-genetic risk groups in two separate ways; using the top and bottom 10% PGS of each condition, as carriers (or not) of rare variants. These models can then pinpoint differences in brain structure and function that are relevant to diseases. This knowledge will advance our understanding of the underlying neurobiology and might inform diagnostic strategies. We will present preliminary results of the two approaches using both common and rare variants, including known pathogenic copy number variants and single nucleotide variants.

Conflict of Interest: None declared

P18.014.B Integrating online variant interpretation resources and automating NHS variant data sharing to improve a clinical bioinformatics decision support system

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Background/Objectives: East Genomic Laboratory Hub (East GLH) is part of the NHS Genomic Medicine Service. East GLH is co-developing bioinformatics tools for clinical variant interpretation with Zetta Genomics, including the OpenCGA framework, which stores patient cases and associated data. This project aims to integrate OpenCGA with web tools to provide useful information during variant interpretation and to establish a method to automate variant sharing across the NHS, as there is currently no routine data sharing system.

Methods: The DECIPHER rare disease variant database was used as the exemplar. An app ("Pandora") was developed to automate submission of cases from OpenCGA to DECIPHER, leveraging their respective APIs.

Results: Pandora consists of two parts; it retrieves cases from OpenCGA, including HPO terms and causative variants interpreted by clinical scientists. It then creates a corresponding DECIPHER patient record and populates HPO terms and variants. Pandora can also update existing patient records. The app is not yet in routine service, but has been successfully tested with a number of test variants; preliminary work has designed Pandora so that a

future release could take data from other sources and submit cases elsewhere, such as to ClinVar.

Conclusion: Increased NHS variant data sharing is a goal supported by the ACGS, BSGM and ESHG; this project enables this to be automated. Sharing data means that clinical scientists can access more evidence to conclusively classify variants and provide patients with a diagnosis.

Conflict of Interest: None declared

P18.015.C Genetic and brain imaging phenotype joint prediction of longitudinal Parkinson's Disease subtypes

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Background/Objective: The characterization of Parkinson's Disease (PD) progression involves several heterogeneous clinical attributes, such as motor function or cognitive decline, to define disease stage. PD subtyping to identify fast or slow disease progressors remains especially challenging. We utilize -OMICs data (genomic and brain imaging) along with HPO (Human Phenotype Ontology)/GO (gene ontology) term embedding (node2vec), CSF biomarkers, and longitudinal clinical measurements to predict PD progression subtypes.

Methods: A Neural Net based Long Short-Term Memory (LSTM) model was employed on participants of the Parkinson's Progression Marker Initiative (PPMI, N = 600) with clinical features at 2 time points (baseline/2 years) capturing motor changes, cognitive impairment and memory scores. Whole genome sequenced data filtered on 90 known PD associated genetic variants along with HPO/GO terms and imaging data (T1-weighted neuroimaging for volume and thickness) were used to train the LSTM model into a 2-D embedded vector.

Results: We identified specific genes (*CTNNB1*, *SHH*, and *GLI2*), HPO embeddings/biologic pathways via GO terms combined with brain regions characterizing fast/slow PD progressors independent of Hoehn and Yahr disease stage. Utilizing embedding algorithms along with HPO/GO terms increased the Area Under the Curve (AUC) by ~5% compared to using genetic variants directly. Clinical +imaging inputs provided the highest AUC for two (92.2%) or three (77.4%) subtype models.

Conclusions: Our findings provide mapping between genetic risk variants, symptom progression along with brain network health useful for clinical trial inclusion. Future personalized medicine applications include specific drug targets by brain region and PD-related biologic mechanism.

Conflict of Interest: Linda Polfus Ambry Genetics and REALM IDx, Hamed Masnadi Shirazi Ambry Genetics and REALM IDx, Alexandra Reardon Invicro, Matthew Varga Ambry Genetics, Alexander Tchourbanov Ambry Genetics, Taylor Gosselin Invicro, Anant Dadu Data Tecnica International, Participation in this project was part of a competitive contract awarded to Data Tecnica International LLC by the National Institutes of Health, National Institutes on Aging to support open science research. Participation in this project is part of an open science collaboration between Center for Alzheimer's and Related Dementias (CARD) and Invicro., Faraz Faghri Data tecnica International, Participation in this project was part of a competitive contract awarded to Data Tecnica International LLC by the National Institutes of Health, National Institutes on Aging to support open science research. Participation in this project is part of an open science collaboration between Center for Alzheimer's and Related Dementias (CARD) and Invicro., Mike Nalls Data Tecnica International, Participation in this project was part of a competitive contract awarded to Data Tecnica International LLC by the National Institutes of Health, National Institutes on Aging to support open science research. Participation in this project is part of an open science collaboration between Center for Alzheimer's and Related Dementias (CARD) and Invicro., Advisor for Clover Therapeutics and Neuron23 Inc, Jacob Hesterman Invicro, Roger Gunn Invicro UK and REALM IDx, Adam Chamberlin Ambry Genetics, Brian Avants Invicro and REALM IDx

P18.016.D Multi-layered genetic approaches to identify approved drug targets

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Background/Objectives: Drugs whose targets have genetic support were found to be more likely to succeed in trials. Various approaches have been proposed to identify likely causal genes for complex diseases, including gene-based genome-wide association studies (GWAS), rare variant burden tests in whole exome sequencing studies (Exome) or integration of GWAS with expression/protein quantitative trait loci (eQTL-GWAS/pQTL-GWAS).

Methods: Here, we compare gene-prioritization approaches on 30 common clinical traits and benchmarked their ability to recover drug target genes defined using a combination of five drug databases.

Results: Across all traits, the top prioritized genes were enriched for drug targets with odds ratios (ORs) of 2.17, 2.04, 1.81 and 1.31 for the GWAS, eQTL-GWAS, Exome and pQTL-GWAS methods, respectively. We quantified the performance of these methods using the area under the receiver operating characteristic curve and adjusted for differences in testable genes and data origins. GWAS performed significantly better (54.3%) than eQTL (52.8%) and pQTL-GWAS (51.3%), but not significantly so against the Exome approach (51.7% vs 52.8% for GWAS restricted to UK Biobank data). Furthermore, our analysis showed increased performance when diffusing gene scores on gene networks. However, substantial improvements in the protein-protein interaction network may be due to circularity in the data generation process, leading to the node (gene) degree being the best predictor for drug target genes (OR = 8.7, 95% CI = 7.3-10.4).

Conclusion: We systematically assessed strategies to prioritize drug target genes highlighting promises and potential pitfalls of current approaches.

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Conflict of Interest: None declared

P18.017.A SMA Finder detects previously undiagnosed spinal muscular atrophy cases from exome and targeted sequencing data

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Background: Spinal muscular atrophy (SMA) is a relatively common autosomal recessive progressive neuromuscular disorder which is primarily caused by homozygous deletion of the *SMN1* gene, while severity is modified by the copy number of the *SMN2* gene. Due to high homology between *SMN1* and *SMN2* genes, identifying *SMN1* deletion from short-read sequencing data has been challenging. SMA Finder (https://github.com/broadinstitute/sma-finder) is a novel tool for detecting SMN1 homozygous deletion from exome and genome sequencing data. It has been proposed for use on other short-read data such as targeted gene panels, however this has not been assessed.

Methods: SMA Finder detects the count of *SMN1*-unique C nucleotides in the positions c.840 of the reads mapped to both *SMN1* and *SMN2* loci, indicating the SMA diagnosis if the patient is likely to have zero copies of *SMN1*. We re-analysed 9923 samples (1157 exome and 8766 targeted sequencing samples) sent for molecular diagnostics for various suspected disorders to Tartu University Hospital.

Results: SMA Finder flagged eleven samples for SMA equally in exome and targeted data, corresponding to six unique patients: three previously known and three novel cases. The novel SMA cases were confirmed using multiplex ligation-dependent probe amplification (MLPA). Two patients had 4 *SMN2* copies (clinically SMA type III), and one patient had 5 *SMN2* copies (asymptomatic at the time of testing).

Conclusion: SMA Finder can facilitate SMA diagnostics in targeted sequencing analysis, helping to diagnose SMA in a clinical setting, which can be elusive for SMA types III-IV.

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Conflict of Interest: None declared

P18.018.B Improved detection of functionally relevant aberrant splicing using the Intron Jaccard Index

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Background: Detection of aberrantly spliced genes from RNA-seq data is an important step in RNA-seq based rare disease diagnostics. We recently developed FRASER, a denoising autoencoder-based method for aberrant splicing detection that outperformed alternative approaches. However, when systematically investigating FRASER results on 303 rare disease samples and 16,213 GTEx samples, we noticed that >20% of the detected introns did not result in substantial major isoform change, mainly due to the intron-centric splicing metrics used by FRASER.

Methods: We introduce the Intron Jaccard Index, a new intron excision metric that combines alternative donor, alternative acceptor, and intron retention signal into a single value. As with FRASER, we model this metric using a beta-binomial based denoising autoencoder, thereby controlling for potential confounding sources of variation. We refer to this new approach as FRASER2.

Results: On GTEx, FRASER2 called 10 times fewer splicing outliers and increased the recall of splice-disrupting variants 8.4 times. On a rare disease dataset, FRASER2 also reduced the number of splicing outliers (by two-thirds) with a slight loss of sensitivity (only 2 out of 22 pathogenic splicing cases not recovered with default cutoffs). Finally, we introduce an option to select the genes to be tested on each sample instead of a transcriptome-wide approach, which we demonstrate to be particularly useful in the rare disease field.

Conclusion: By leveraging a new, more robust, splicing metric, the Intron Jaccard index, FRASER2 is able to maintain the sensitivity of FRASER while focusing on more functionally relevant outlier calls.

Conflict of Interest: None declared

P18.019.C MR-AHC: a fast, efficient and robust method for two-sample summary data Mendelian randomization based on agglomerative hierarchical clustering

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Background: Mendelian randomization (MR) uses genetic variants as instrumental variables for estimating the causal effect of a risk factor on an outcome. Standard MR usually assumes a homogeneous causal effect from the exposure to outcome. We allow violation of this assumption such that the variants can be divided into clusters identifying distinct causal effects driven by different biological mechanisms. Variants are classified into the same cluster only if their variant-specific causal estimates are similar.

Methods: We adapt the general method of agglomerative hierarchical clustering (AHC) to the two-sample summary-data MR setting, enabling the detection of variant clusters driving distinct causal mechanisms between exposure and outcome. We also significantly extend the MR-AHC method to handle two outcomes and a common exposure to aid the deeper investigation of a shared but possibly heterogenous causal pathway between multimorbid conditions.

Results: In simulations MR-AHC correctly detects variant clusters and is much faster than the existing method MR-Clust. In an applied example studying the causal effect of body fat percentage (BFP) on type-2 diabetes (T2D), MR-AHC detects three SNP-clusters identifying causal effects of different signs, further supporting the finding of heterogenous metabolic effects from adiposity to T2D. We also apply MR-AHC to identify BFP SNP-clusters implicated in simultaneously affecting T2D and osteoarthritis, a disease pair known to have a large genetic correlation. We identify a novel 124 SNPs cluster associated with increasing risks of both conditions and present a detailed enrichment analysis of downstream traits using PhenoScanner.

Grant References: UK Medical Research Council grant (MR/W014548/1)

Conflict of Interest: Xiaoran Liang: None declared, Ninon Mounier: None declared, Nicolas Apfel: None declared, Timothy M. Frayling Tim Frayling has received funding from GSK and consulted for Sanofi and Boehringer Ingelheim., Jack Bowden Jack Bowden is a part time employee of Novo Nordisk, engaged in work unrelated to this project.

P18.020.D A whole genome sequencing tool 'WGSPrisma' to support variant filtering and prioritization

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Background/Objectives: As the implementation of whole genome sequencing (WGS) is increasing, it is desired to standardize and simplify data analysis pipelines. We have developed an application, coined WGSPrisma, for automated variant filtering and prioritization in inherited retinal dystrophies (IRD).

Methods: This study comprised of 111 IRD cases that remained genetically unsolved after pre-screening using targeted sequencing of IRD-associated genes. Four pathogenicity prediction tools (CADDv1.6, Grantham, PhyloP and Revel) were compared using 1,810 curated variants (333 likely pathogenic and 1,477 likely benign) from the VKGL-datashare-database. We developed an Rstudio shiny application that colors WGS variants based on variant type and in silico prediction tools. This workflow was validated using WGS data from 42 solved IRD individuals (validation cohort). Finally, WGS-data from 69 genetically unexplained IRD-individuals (discovery cohort) were analyzed using WGSprisma.

Results: Development of WGSprima showed that CADDv1.6 (>15) and Revel (>0.3) provided the optimal combination to detect missense variants. WGSprisma also identified 100% of causative variants in our validation cohort. Moreover, we determined the genetic cause in 24.6% (17/69) of the individuals in the discovery cohort. The use of this automatized process reduced filtering and prioritization time by 70%. Furthermore, it provided a user-friendly interface, producing a visual prioritization of variants.

Conclusion: WGSprisma provides an efficient strategy for WGS-data variant filtering and prioritization, which aids in determining genetic diagnosis in unsolved cases.

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Conflict of Interest: None declared

P18.021.A MR-link-2: a pleiotropy robust cis Mendelian randomization method for -omics exposures

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Background: Mendelian randomization (MR) is a causal inference approach for heritable traits. MR may have high false positive rates (FPR) when limited genetic markers are available, which is typical for omics exposures where a single *cis* region is investigated.

Methods: We present a *cis* MR method, MR-link-2, requiring GWAS summary statistics and a linkage disequilibrium (LD) reference. MR-link-2 models all SNPs in a region, estimating a causal relationship. We compare MR-link-2 to four *cis* MR methods and two colocalization approaches in 9'800 simulation scenarios and in real-data using 259 reactions from metabolite networks as true positives.

Results: Simulating 100 causal SNPs in LD, explaining 3% of the exposure variance and 1% pleiotropic variance, MR-link-2 has well-calibrated FPR with power of 0.758 when simulating a 0.2 causal effect. Here, the area under the receiver operator characteristic curve (AUC) is 0.95, (best competing AUC: 0.72, MR-PCA-IVW).

Across scenarios, power increases by 1.4% per percent of exposure *cis* heritability. Our simulations show that violating the MR-link-2 assumptions increases FPR: noisy LD (max FPR: 0.175), limited number of causal SNPs (max FPR: 0.08) and strong LD between causal SNPs (max FPR: 0.318). Despite this, AUC for MR-link-2 is higher than competing methods in 75% of simulated scenarios.

Using mQTLs (249 metabolites) combined with causal relationships from metabolite networks, we find an AUC of 0.77 for MR-link-2, outperforming tested methods (max AUC: 0.72).

Conclusions: MR-link-2 is a MR method for *cis* causal inference. In simulations and real data, MR-link-2 compares favorably to competing approaches.

Conflict of Interest: None declared

P18.022.B Rare variant burden tests (RVBTs) – A feasibility study on inherited retinal diseases (IRDs)

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Background/objectives: IRDs are a group of conditions causing progressive degeneration of the retina and resulting in vision loss and blindness. Their missing heritability is ~30-50%. Traditional statistical tests have limitations in identifying the genetic factors contributing to IRDs. RVBTs measure the cumulative effect of rare variants that are not detectable with standard methods. Here, a burden test was performed on patients with Stargardt disease and variants in *ABCA4*, as well as on controls, to show its applicability to the identification of novel disease genes.

Methods: Whole exome sequencing (WES) was performed on 18 Swiss Stargardt patients and 193 controls of European ethnicity. After variant calling and annotation, variants were selected according to their population allele frequency (AF), type of alteration and predicted damaging scores. Selected variants in each gene were collapsed into single scores. Odds ratios (OR) in all genes were calculated using two-sided Fisher's exact test with Bonferroni correction.

Results: *ABCA4* was the most enriched gene across different AFs and variant groups. It was observed that significance increased whenever AF decreased, and damaging scores of the variants increased. The highest burden was observed in the group of loss of function and very damaging missense variants, at the lowest AF threshold ($OR = Inf$, $p = 3.01E\text{-}12$).

Conclusion: This feasibility test shows an enrichment in rare damaging variants in *ABCA4* for Stargardt patients. The effect of variant filtering on allele frequency and identical damaging properties was also clearly observed. In conclusion, this study demonstrates that RVBTs can provide information on missing heritability in IRDs.

Conflict of Interest: None declared

P18.023.C Interaction analysis in mendelian randomization

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Mendelian Randomization (MR) is a widely used tool to scrutinize causal associations. Yet, only little research has been conducted on the elucidation of environment specific causal effects, which may be subject to biases when performed in a naïve way. Such bias can result from non-linear effects of the exposure on the outcome if the exposure is not independent of interacting environment.

To control for bias resulting from quadratic effects in MR interaction analysis, we extended two-stage-least-squares MR (2SLS-I). We first tested 2SLS-I in a wide range of realistic simulation settings including quadratic environment-dependent causal effects. Next, we applied 2SLS-I to estimate age- and sex-specific non-linear causal effects of body mass index (BMI) on 16 cardiometabolic outcomes in the UK Biobank (n ~300'000).

In simulations, 2SLS-I successfully identified the X*E-interaction effects with <6% relative bias, while the naïve MR approach showed up to 97% bias. Despite 2SLS-I having much better controlled type I error rate, it still maintained ~80% power compared to the (upward) biased naïve approach. Applied to real data, 2SLS-I led to significant changes in several interaction estimates, e.g. sex-dependent BMI causal effect on alanine aminotransferase (ß $(SE)_{naive} = 0.036$ (0.0035), ß $(SE)_{2SLS-I} = 0.041$ (0.0047), $p_{diff} < 0.0001$).

We present 2SLS-I, a method that simultaneously models environment-specific non-linear causal effects and substantially reduces bias resulting from quadratic effects in MR interaction analysis. Our results highlight the importance of considering this source of bias when estimating interactions using MR.

Conflict of Interest: None declared

P18.024.D Cell type deconvolution of methylated cell-free DNA at the resolution of individual reads

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Background: Cell-free DNA (cfDNA) are DNA fragments originating from dying cells that are detectable in bodily fluids, such as the plasma. Accelerated cell death, for example caused by disease, induces an elevated concentration of cfDNA and a change of its cell type composition. As a result, determining the cell type origins of cfDNA molecules can provide information about an individual's health. In this work, we aim to increase the sensitivity of methylation-based cell type deconvolution by adapting an existing method, CelFiE (Caggiano *et al*., Nat Commun 2021), which uses the methylation beta values of individual CpG sites to estimate cell type proportions.

Methods: Our new method, CelFEER, differentiates cell-types by the average methylation values within individual reads rather than using CpG site averages. We additionally improved the originally reported performance of CelFiE by using a new approach for finding marker regions that are differentially methylated between cell types. This approach compares the methylation values over 500 bp regions and solely takes hypomethylated regions into account.

Results: We show that CelFEER estimates cell type proportions with a higher correlation than CelFiE on simulated mixtures of cell types. Moreover, using our new approach, we uncovered significant differences between skeletal muscle cfDNA fractions in four ALS patients and four healthy controls.

Conclusion: We developed a new method to estimate cell type proportions from methylated cfDNA. Our tool is available on GitHub at https://github.com/pi-zz-a/CelFEER and uses read averages instead of beta values of CpG sites, thereby resulting in a more accurate cell type deconvolution.

Conflict of Interest: None declared

P18.025.A Complete resolution of gene/paralog pairs with PacBio HiFi sequencing

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Many medically relevant genes fall in regions – including segmental duplications - where variant calling is limited due to highly similar paralogs and pseudogenes. High sequence homology promotes unequal crossing over and gene conversion, resulting in frequent structural variants that are difficult to assess for short read sequencing methods. PacBio HiFi long-read sequencing can resolve many regions with high sequence homology, but informatics methods are still lacking for segmental duplications.

We developed a software tool, Paraphase, that assembles long sequence reads into haplotypes of known gene/paralog pairs and performs per-haplotype variant calling. Hereby, copies of the gene family, including genes and their paralogs or pseudogenes, can be annotated with functional status, enabling accurate determination of disease or carrier status.

We applied Paraphase to resolve ten clinically relevant genes with highly similar paralogs or pseudogenes: *SMN1*, *CYP21A2*, *TNXB*, *STRC*, *IKBKG*, *F8*, *PMS2*, *NCF1*, *NEB* and *CFC1*. We characterized the frequencies of pathogenic small and structural variants in these genes across 254 samples from five populations. This analysis identified major population haplotypes for each gene, found genetic markers for complex multi-copy alleles, identified duplications in cis with pathogenic variants that are difficult to genotype by other technologies, and catalogued signatures of frequent gene conversion.

Paraphase, provides a single framework for resolving a class of the most difficult, clinically important genes using accurate long reads. Resolving these highly homologous regions enables accurate detection of pathogenic variants for better clinical testing, as well as enable research to discover novel gene-disease associations in previously inaccessible genes.

Conflict of Interest: Xiao Chen PacBio, PacBio, Emily Farrow: None declared, Isabelle Thiffault: None declared, Dalia Kasperaviciute: None declared, Christian Gilissen: None declared, Alexander Hoischen: None declared, Tomi Pastinen: None declared, Michael Eberle PacBio, PacBio

P18.026.B Dissecting the interplay between ageing, sex and body mass index on a molecular level

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Mark McCarthy[3], Ewan Pearson[9], Jochen Schwenk[10], Mark Walker[1], Andrew Brown[9], Ana Viñuela[1]

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Ageing is a complex process, entangled with a variety of age-related traits. Here, we aim to separate age-related molecular changes from the influence of sex and BMI.

Utilizing data from the DIRECT consortium, whole blood and plasma transcriptomic, targeted proteomic and metabolomic data from 3,027 participants, we identified molecular phenotypes associated with age, sex and BMI and their interplay to modulate the abundance of these molecules.

Using linear models, we identified 10,643 (66%), 10,163 (63%) and 8,256 (51%) genes differentially expressed with age, sex and BMI respectively (FDR<0.05). Protein associations were 316 (85%), 260 (70%) and 271 (73%). More than 20% of genes and 45% of measured proteins were independently associated with all three factors such as ITGB1, and EGFR. When studying the overlap of associations between biological factors, for instance, exclusively sex-related proteins were mostly involved in T-cell activation responses. Moreover, exclusively BMI-related proteins included CDH1, where mutations in the corresponding gene have been correlated with multiple types of cancer. Using interaction models, we identified 850 genes, 103 proteins and 48 metabolites with significant sex-dependent changes with age. For example, abundance of Sclerostin increased with age in men, but decreased in women, while levels of L-carnitine showed the inverse effect. BMI-dependent changes with age were detected for GAS6 levels that decreased with age in individuals with high BMI.

A majority of tested molecular phenotypes were independently associated with age, sex and BMI. However, we identified how the abundance of several molecules changed with age, depending on these factors.

Conflict of Interest: None declared

P18.028.D Polygenicity of Alzheimer's disease beyond the effect of APOE differentially impacts joint modulation of brain structure in preclinical stages of the disease

***Patricia Genius**[1;2], Malu Calle[3], Raffaele Cacciaglia[1;2;4], Tavia Evans[5;6], Carles Falcon[1;2;7], Carolina Minguillón[1;2;4], Hieab Adams[5;6;8], Manel Esteller[9;10;11;12], Arcadi Navarro[1;10;13;14;15], Juand D. Gispert[1;2;7;16], Natalia Vilor-Tejedor[1;2;5;14;15]*

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Background: Individual genetic variation may influence the structural heterogeneity of the brain. We aim to explore the joint brain structural variation depending on the genetic predisposition to Alzheimer's disease (AD) in a sample of cognitively unimpaired (CU) participants at risk of AD.

Methods: A total of 351 participants classified as CU amyloid-beta positives (Aβ+) and CU Aβ- from the ALFA cohort were included. Genetic predisposition to AD (PRS-AD) was calculated through polygenic risk scoring including genetic variants at the genome-wide suggestive level ($p < 5 \times 10\text{-}6$). The PRS-AD was also calculated excluding the APOE region (PRS-ADnoAPOE), as well as uniquely including the APOE region (PRS-ADAPOE). Individuals were classified into high/low genetic risk groups (cut-off quantile 0.8). Compositional data analysis was used to compute the optimal brain structural variation signature (elastic net selection). Association with the genetic risk groups of AD was then computed using logistic regressions. Models were stratified by Aβ status and adjusted for age and sex.

Results: Despite slight differences, we did not observe Aβ status-specific effect of the overall polygenicity of AD (PRS-AD) on the joint structural variation, which was mainly defined by regions of the temporal lobe and visual areas in both groups. Differences between and within groups were found when excluding APOE (PRS-ADnoAPOE) and when its specific effect (PRS-ADAPOE) on brain structure was considered.

Conclusions: Results showed Aβ status-specific volumetric variations associated with an increased genetic predisposition to AD beyond the effect of APOE. These findings may have implications for brain targets and prevention.

Conflict of Interest: Patricia Genius: None declared, Malu Calle: None declared, Raffaele Cacciaglia: None declared, Tavia Evans: None declared, Carles Falcon: None declared, Carolina Minguillón: None declared, Hieab Adams: None declared, Manel Esteller: None declared, Arcadi Navarro: None declared, Juand D. Gispert JDG has received research support from GE Healthcare, Roche Diagnostics and Hoffmann-La Roche, JDG has received speaker's fees from Philips and Biogen, Natalia Vilor-Tejedor: None declared

P18.029.A Clinical validation of GATK-gCNV for germline CNV calling using a targeted NGS capture

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Background/Objectives: Analysis of copy number variants (CNVs) associated with disease is a requirement of the Rare Disease NHS National Genomic Test Directory. Here we present the clinical implementation of GATK-gCNV into routine germline variant analysis performed in East Genomics Laboratory Hub.

Method: The validation sample set included 22 unique CNVs previously identified using MLPA (17 deletions, 5 duplications). Sequencing was performed within 3 runs of 48 samples on NovaSeq sequencers using a custom 162-gene targeted Twist

Biosciences capture. Each run was assessed for quality and analysed using GATK-gCNV in 'COHORT' mode. Variants were annotated using Ensembl-VEP and compared to the MLPA results for concordance and sensitivity calculations.

To assess repeatability, replicates of 4 unique CNV control samples were analysed within the same sequencing run. To assess reproducibility, replicates of the same 4 samples were analysed within different sequencing runs. Appropriate thresholds for minimum read count per sample and minimum sample count per run were established via downsampling and subsampling of the validation dataset respectively. To calculate specificity, 7 normal controls containing 426 unique intervals with normal copy number (established via MLPA) were assessed for concordance.

Results: Overall sensitivity was calculated at 100% (95% CI 85-100%) with overall specificity of 100% (95% CI 99-100%). Robustness experiments indicated a minimum threshold 20 million reads per sample and a minimum cohort size of 32 samples required for CNV calling.

Conclusion: We identified that GATK-gCNV accurately called all known CNVs and has been implemented in routine clinical practice to increase diagnostic yield in patients.

Conflict of Interest: None declared

P18.030.B Evaluation of pathogenic repeat expansion detection from exome sequencing data

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Background/Objectives: Repeat expansions (RE) are a well-known disease mechanism of various genetic disorders. RE are mostly detected using targeted approaches and are usually missed in exome sequencing (ES). The aim of the study was to evaluate the clinical utility of pathogenic RE detection from available ES.

Methods: For RE detection, we have developed a bioinformatic workflow consisting of ExpansionHunter with an extended RE catalogue from Stranger, and visualization using REViewer. Only REs covered in our ES were included (n = 31/52). For the validation, we sequenced and analyzed positive controls (n = 7) with known pathogenic REs in *ATXN8OS*, *AR*, *DMPK* and *HTT* genes. To investigate the clinical utility, we have analyzed archival (n = 314) and prospective (n = 158) ES samples. To reduce the number of false positives, REs were visualized and only good quality REs fitting patient's phenotype were further confirmed with a targeted assay.

Results: In positive controls, we were able to identify all known REs (n = 7/7) using our pipeline but underestimated the precise repeat number for large REs (for *DMPK* and *ATXN8OS*). Analysis of the archival data revealed pathogenic RE in 6/314 (2%) cases: in *DMPK* (n = 4), *HTT* (n = 1) and *ATXN2* (n = 1) genes. All 6 REs were confirmed with RP-PCR. In prospective cohort, we identified 1/158 RE that is currently being validated.

Conclusion: We demonstrate that REs can be detected from ES, if covered in ES, with high sensitivity, but underestimate the size of large REs and require visualization to reduce the number of false positives, as well as confirmation with PCR.

Conflict of Interest: None declared

P18.031.C Splicing analysis of RNAseq data using IntEREst R/Bioconductor package (V1.24.0)

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Background/Objectives: Alternative splicing process regulates the choice of individual splice sites to generate different mRNA isoforms from an individual gene. It is a central post-transcriptional process regulating gene expression and includes: cassette exons, alternative 3′ or 5′ splice site activation, mutually exclusive exons, and intron retention (IR). Intron retention is, however, not easily detectable from RNAseq data, given the relatively large length of introns, their frequent overlapping with ncRNAs and their enrichment with repetitive sequence elements.

Methods: We originally developed Intron Exon Retention Estimator (IntEREst) R/Bioconductor package to reliably detect IR events from RNAseq data. Recently, however, we have upgraded IntEREst to improve the accuracy of its IR findings and detect alternative splicing events other than IR, e.g., exon skipping. Now IntEREst features:

1. Quantifying the reads that skip exons, as well as those that span introns, map to introns and map to intron-exon junctions.
2. Measuring Percentage Spliced In (i.e. PSI) for exon inclusion as well as IR.
3. Differential exon inclusion and IR statistical analysis.
4. strand-awareness: For stranded RNAseq data, the accurate estimation of IR or exon-inclusion levels in genes from opposite DNA strands that overlap.

Results: We applied the latest IntEREst on muscle RNAseq data from patients with neuromuscular diseases (NMD). Here, we shows how we apply IntEREst to detect splicing abnormalities in TTN and OBSCN, in patient samples.

Conclusion: IntEREst can detect splicing abnormalities in NMD patients. The latest IntEREst updates are available at https://www.bioconductor.org/packages/devel/bioc/html/IntEREst.html.

Grant References: This work is supported by Magnus Ehrnrooth foundation.

Conflict of Interest: None declared

P18.032.D Inferring genetic ancestry subgroups in 50,000 whole genomes from the Genome Aggregation Database (gnomAD) v3

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Introduction: The Genome Aggregation Database (gnomAD) provides a useful resource of population allele frequencies that assists researchers and clinicians with variant interpretation. One feature that aids with this interpretation is the breakdown of allele frequencies for large genetic ancestry groups as well as for subgroups within those ancestry groups. However, gnomAD v2 only provides variant information for a limited number of ancestry subgroups. The addition of 76,156 genomes in gnomAD v3, including many samples with known subgroup labels not present in gnomAD v2, provided the opportunity to expand on the number and diversity of available subgroup annotations.

Materials and Methods: After applying both sample and variant-level filters, we utilized known subgroup labels from the Human Genome Diversity Project (HGDP), the 1000 Genomes Project (1KG), and select v3 cohorts to train a random forest model and infer subgroup labels for unlabeled genomes in gnomAD v3.

Results: Our pipeline for subgroup inference allowed us to classify over 50,000 samples in gnomAD v3 into subgroups, such as Costa Rican, Han Chinese, Iberian, Pakistani, etc., with 70-80% confidence. This analysis increased the count of unique subgroups from seven in gnomAD v2 to over 60 in gnomAD v3.

Conclusion: The inclusion of more diverse subgroups provides an additional tool for analyzing the frequency and pathogenicity of variants.

Conflict of Interest: Kristen M. Laricchia: None declared, Julia Goodrich: None declared, Michael Wilson: None declared, Katherine Chao: None declared, Grace Tiao: None declared, Konrad Karczewski K.J.K. is a consultant for Vor Biopharma and Tome Biosciences, and is on the Scientific Advisory Board of Nurture Genomics, Heidi Rehm: None declared

P18.033.A BayesRB: a Markov Chain Monte Carlo-based polygenic genetic risk score algorithm for dichotomous traits

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Identifying high-risk individuals using polygenic risk score (PRS) algorithms can guide screening and preventive treatment. Among the many PRS algorithms developed so far, BayesR shows good characteristics of unbiasedness, accuracy, sparseness, and robustness. It detects the associated SNPs, estimates the SNP effects, and predicts disease risk using all SNPs simultaneously. However, it assumes that the phenotype follows a Gaussian distribution, which cannot be met in case-control studies. Here, we create an extended method called BayesRB by adding auxiliary variables to the BayesR model. We examined the characteristics, efficacy, and accuracy of BayesRB when estimating SNP effects and predicting disease risks compared with three traditional algorithms under different conditions using both simulated data and real data from the Welcome Trust Case Control Consortium (WTCCC). For SNP effect estimation, BayesRB shows unbiasedness and sparseness for large and small effect SNPs, respectively. For disease risk prediction, BayesRB had the best performance among the methods. This study provides a theoretical basis for complex disease risk prediction and disease prevention.

BayesRB links: Preprint: https://doi.org/10.1101/2022.02.27.482193

GitHub: https://github.com/sylviashanboo/bayesRB

This study makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk. Funding for the project was provided by the Wellcome Trust under award 076113, 085475 and 090355, and National Natural Science Foundation of China 82204148.

Conflict of Interest: Ying Shan Full time, National Natural Science Foundation of China (82204148) and Scientific Research Foundation of Peking University Shenzhen Hospital (KYQD2022203)., Daniel Weeks Full time, Award 076113, 085475 and 090355

P18.034.B Machine learning identifies novel exosomal RNAs for differentiating hepatocellular carcinoma patients from healthy individuals

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Background/Objectives: Current surveillance of hepatocellular carcinoma (HCC) relies on detection of alpha fetoprotein (AFP) level. However, AFP has limited sensitivity and specificity in detection for early stage of HCC. As RNA sequencing in exosomes has emerged to be a promising tool for diagnosing and characterizing various cancers, its potential in HCC detection has not been thoroughly investigated. With the advancement of high-throughput sequencing and machine learning techniques, this study explores if machine learning can be used to identify biologically significant markers using exosomal RNAs to predict HCC.

Methods: The exosomal RNA expression data of HCC patients and healthy individuals were downloaded from exoRBase 2.0 (http://www.exorbase.org/). The data was first split into train and unseen test set, followed by selection of predictive features using permutation importance. The train set with selected features was then trained using a support vector machine (SVM) classifier. The predictive features were finally validated across six different models in an unseen test and their biological significance was evaluated by pathway analysis.

Results: A total of 9 exosomal RNAs were selected to be potential predictors of HCC. These potential predictors achieved good predictive performance with accuracies between 0.70-0.83 in the unseen test set. Pathway analysis showed that 7 out of the 9 RNAs are involved in immune pathways.

Conclusion: 9 exosomal RNAs were shown to be potential predictors of HCC in machine learning models with biological significance. These exosomal RNAs may facilitate the design of potential clinically useful biomarkers or therapeutic targets.

Conflict of Interest: Josephine Yu Yan Yao: None declared, Laura Shih Hui Goh: None declared, Ashley Jun Wei Lim: None declared, Samuel Chong National University of Singapore, Lee Jin Lim National University of Singapore, Caroline Lee Full Time at the National University of Singapore

P18.035.C WiNGS SV: A platform for analysis and federated sharing of structural variants

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Neurodevelopmental disorders are etiologically extremely heterogeneous and individually ultra-rare genetic disorders. Currently, about 40% of patients remain without a genetic diagnosis. We hypothesize that structural variants (SVs) cause many of these unsolved cases. Because the complexity of SVs is much higher as compared to SNVs, data sharing will be paramount to correlate phenotypes. Hence we present WiNGS SV, a federated platform developed to simplify the identification of candidate SVs and enable genomic data sharing amongst institutions in a privacy preserving way.

WiNGS SV enables users to upload Variant Call Format files into a docker container retained within the institute of origin. Files are parsed into a database making data accessible through the platform's web-based interface. Each file is linked to a specific individual. The primary investigator (PI) is proprietor. PI's associates are granted access and allowed to input data to the PI's database.

WiNGS SV allows users to visualize data inside a table and to filter the SVs based on type, length, chromosome, and genotype. Additionally, data can be visualized using circus and ideogram plots. Trio analysis to detect de novo SVs is enabled. Data can be organized into datasets and shared among researchers engaged in a joint project.

Our federated platform facilitates collaboration among researchers and enhances the process of SV identification. The future integration of annotation and population-wide frequency analysis within the platform is expected to boost SV related gene discovery and increase accurate patient diagnoses.

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Conflict of Interest: None declared

P18.036.D GiRAFR improves gRNA detection and annotation in single cell CRISPR screens

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Novel methods that combine single cell RNA-seq with CRISPR screens enable high-throughput characterization of transcriptional changes caused by genetic perturbations. Dedicated software is however lacking to annotate CRISPR guide RNA (gRNA) libraries and associate them with single cell transcriptomes. Here, we generated a CRISPR droplet sequencing (CROP-seq) dataset. During analysis, we observed that the currently available tool fails to detect mutant gRNAs. We therefore developed GiRAFR, a python tool to characterize intact and mutant gRNAs. We show that mutant gRNAs are dysfunctional, and failure to detect and annotate them leads to an inflated estimate of the number of untransformed cells as well as an underestimated multiplet frequency. These findings are mirrored in publicly available datasets, where we find that up to 34 % of cells are transduced with a mutant gRNA. Applying GiRAFR hence stands to improve the annotation and quality of single cell CRISPR screens.

Conflict of Interest: Qian Yu Stichting tegen Kanker (F/2020/1544), Paulien Van Minsel The Research Foundation – Flanders (FWO), Eva Galle KU Leuven, Bernard Thienpont Stichting tegen Kanker (F/2020/1544)

P18.037.A Semi-automated genotype-phenotype matching within a database identifies candidate disease genes and resolves variants of uncertain significance

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Objective: Widespread application of next-generation sequencing has provided diagnoses for countless families with genetic disorders. For unresolved cases, Matchmaker Exchange platforms have facilitated gene discovery. In order to exploit the power of Hadassome, an in-house database, in reclassifying variants of uncertain significance (VUS) shared by similarly affected patients, and to identify new disease-causing genes, we implemented an unbiased semi-automated genematching algorithm based on genotype and phenotype matching. We focused on homozygous variants, leveraging the uniqueness of the consanguineous cohorts that represent a major proportion of the referral population.

Methods: Rare homozygous variants identified in two or more affected individuals, and absent from reportedly healthy individuals, were extracted from Hadassome which currently consists of ~14,000 exomes. Phenotype-similarity scores (PSS) based on human phenotype ontology (HPO) terms were assigned to each pair of individuals using HPOsim.

Results: 33,792 genotype-matched pairs were discovered, reflecting variants in approximately 7,500 unique genes. There was an enrichment of PSS ≥0.1 amongst pathogenic/likely pathogenic (P/LP) variant-level pairs (94.3% in P/LP variant-level matches vs. 34.75% in all matches). Most genotype and phenotype-matched cases reflected known founder or region-specific variants. Other genotype-matched cases were helpful in resolving VUS. Most notably, the process enabled the identification or reinforcement of candidate disease genes. Variant-level matches were particularly helpful in highlighting in-frame indels and splice region variants beyond the canonical splice sites, which may otherwise have been overlooked.

Conclusions: Semi-automated genotype matching combined with PSS is a powerful tool to identify candidate disease genes, and to resolve variants of uncertain significance.

Conflict of Interest: None declared

P18.039.C Inferring genetic architecture and predictive ability with LDpred2-auto

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Many common problems in statistical genetics can be boiled down to properly modeling the genetic architecture of the phenotype in question. These include (1) estimating heritability and polygenicity, where the infinitesimal model is commonly assumed although non-infinitesimal priors can provide more accurate heritability estimates and also infer polygenicity; (2) deriving polygenic scores, where both non-infinitesimal and infinitesimal priors have been used; (3) fine-mapping causal variants, where non-infinitesimal models are (per definition) required. However, there is currently no single method that provides solutions to all of these problems simultaneously.

Here we show that LDpred2-auto can solve all of these problems in a single unified statistical framework. We evaluate how accurate it is at inferring heritability, polygenicity, strength of selection, and fine-mapping using both simulations and a diverse set of 248 outcomes in the UK Biobank. Furthermore, we propose a new equation to simultaneously estimate out-of-sample prediction accuracy (without any validation or test data). Last but not least, we propose to extend the widely-used set of HapMap3 variants to improve genome coverage and show how this new set increases heritability estimates by 12%, and prediction accuracy by 6%, on average for the 248 UKB phenotypes.

Therefore LDpred2-auto can be used not only for deriving polygenic scores, but also to infer the genetic architecture of traits.

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Conflict of Interest: Florian Privé: None declared, Clara Albiñana: None declared, Bogdan Pasaniuc: None declared, Bjarni Vilhjalmsson B.J.V. is on Allelica's international advisory board.

P18.040.D GestaltMatcher Database - a FAIR database for medical imaging data of rare disorders

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Introduction: Next-generation phenotyping (NGP) technology is increasingly used in the diagnostic workup of patients with facial dysmorphism. The performance of these tools increases with the training set's size and diversity, but properly labelled training data is currently the biggest bottleneck. Therefore, we developed GestaltMatcher Database (GMDB) - a database for medical image data that complies with the FAIR-principles.

Methods: An entry in GMDB consists of a medical image such as a portrait, X-ray or fundoscopy, and machine-readable meta information such as clinical features or a disease-causing mutation. Starting by collecting imaging data from the literature, the GMDB now serves as a new publication medium, allowing to share previously unpublished cases and updating them dynamically after further consultations. Patients can easily provide data due to a patient-centred digital consent system. To enable inter-cohort comparisons, a research platform can compute the pairwise syndromic similarity between hand-picked cases.

Results: At the time of writing GMDB consisted of 8316 cases with 781 different disorders. We collected data from 2038 case reports and 574 individuals that are not published elsewhere. The web interface enables gene- and phenotype-centred queries. GMDB also serves as a repository for medical images that cannot be included in medRxiv. The research app within GMDB was used to generate syndromic similarity matrices to characterise novel phenotypes (e.g. CSNK2B, PSMC3).

Conclusion: GMDB is a database for NGP where data are findable, accessible, interoperable, and reusable. GMDB connects clinicians with a shared interest in particular phenotypes and simultaneously improves the performance of AI.

Conflict of Interest: Hellen Lesmann: None declared, Shahida Moosa: None declared, Jean Tori Pantel: None declared, Alexander Hustinx: None declared, Tom Kamphans GeneTalk, Wolfgang Meiswinkel GeneTalk, Jing-Mei Li: None declared, Hannah Klinkhammer: None declared, Behnam Javanmardi: None declared, Alexej Knaus: None declared, Stanislav Rosnev: None declared, Merle ten Hagen: None declared, Pilar Caro: None declared, Ibrahim Abdelrazek: None declared, Clara Velmans: None declared, Frédéric Ebstein: None declared, Sebastien Küry: None declared, Ebtesam Abdalla: None declared, Matthias Höller: None declared, Kimberly Christine Coetzer: None declared, Miriam Elbracht: None declared, Felix Marbach: None declared, Cordula Knopp: None declared, Claudio Graziano: None declared, Annabelle Arlt: None declared, Artem Borovikov: None declared, Christian Netzer: None declared, Annette Uwineza: None declared, Rami Abou Jamra: None declared, Markus Nöthen: None declared, Gholson Lyon: None declared, Peter Krawitz: None declared, Tzung-Chien Hsieh: None declared

P18.041.A Leveraging polygenic enrichments for risk gene prioritisation from GWAS summary statistics

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Despite GWAS's success in linking genetic regions with disease risk, finding the genes mediating that risk remains challenging. Other gene properties can be used to prioritise causal candidates, such as expression in relevant cells or tissues, presence in pathways and protein-protein interaction with other disease-related genes. We developed PERiGene (Polygenic Enrichments for Risk Gene Prioritization), which combines these properties with gene-level genetic association statistics, such as MAGMA z-scores, to calculate gene prioritisation scores. Training PERiGene independently on two GWAS of Alzheimer's disease (AD), we found that its predictions replicated better than scores based only on the underlying genetic signal ($rho_{PERiGene}$~0.58, $p = 0$; rho_{MAGMA}~0.33, $p = 0$), showing the benefits of exploiting multiple sources of information. The top genes identified by PERiGene were also enriched in known AD pathways, such as in microglial phagocytosis (OR = 45; adjusted $p = 5.8e\text{-}21$). Applied to AD, Parkinson's disease, schizophrenia and height, we compared PERiGene's predictions against genes identified in familial forms of the diseases, in OMIM, or with independent methods, such as WES. We observed significant GSEA enrichments (NES: 1.7-4.7 depending on the disease; 10k permutations), consistently as large as or greater than the enrichments obtained with MAGMA on the same sets (NES: 1.4-2.7). Where the causal gene is known and near a GWAS locus, PERiGene identified it in 39% of cases, compared to 27% when using only genetic information. Our results demonstrate the value of PERiGene for post-GWAS analyses, providing a more comprehensive picture of disease risk and leading to more accurate identification of causal genes in complex traits.

Conflict of Interest: Théo Dupuis Roche, Will Macnair Roche, Andrew Brown: None declared, Martin Ebeling Roche, Julien Bryois Roche

P18.042.B BDNF gene implication in primary ovarian insufficiency through fragile X syndrome pathway

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Background/Objectives: Primary ovarian insufficiency (POI), a clinically and genetic heterogeneous condition, is a major cause of female infertility due to early loss of ovarian function. Premutation in the *FMR1* – a gene implicated in fragile X syndrome (FXS), is the most frequent monogenic cause of POI. About 20% of the carriers of premutation alleles (55-200 CGGs) will develop POI. To shed light on the mechanisms underlying POI, molecular pathways were investigated.

Methods: PathVisio software was used to create the POI pathway. Comparison between the FXS and POI pathways was performed using Cytoscape software. Additional pathways for investigating overlapping molecular networks were provided by WikiPathways database.

Results: A novel pathway containing all genes involved in POI was developed and is available on WikiPathways (WP5316). Investigating the overlap with the FXS pathway (WP4549) showed *AKT1*, *BDNF*, *FMR1* and *PTEN* genes as well as the mTOR complex were common to both pathways. This observation, along with that of other studies that provide evidence to individually relate each of these genes to POI, support the hypothesis of their interaction in infertility pathophysiology.

Conclusion: Overall results prompt us to speculate *BDNF* implication in the regulation of the PTEN-Mediated PI3K/Akt/mTOR Signaling pathway in POI through a decrease in *FMR1* coded protein levels. Interestingly, overlapping genes have also been implicated in longevity decrease and cardiovascular diseases. By providing the newly designed POI pathway, we warrant further research towards understanding the synergy between those pathways, and a centralized resource for the patients with POI.

Grant References: UIDB/00215/2020; UIDP/00215/2020; LA/P/0064/2020

Conflict of Interest: None declared

P18.043.C Exomiser is an efficient tool to prioritize candidate genes in unsolved myopathy singletons

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Objectives: Exome sequencing provides a molecular diagnosis only for 40-60% of patients. Sometimes the variant is not covered by the exome, but often the issue is the lack of evidence for pathogenicity of the identified variant or the relevance of the gene to the disease. Clinical interpretation is particularly challenging for sporadic cases where only the patient DNA is tested (singleton).

Here we introduce our approach to utilise phenotype-based prioritization on exome data to recognise possible novel disease genes in our cohort of unsolved singleton myopathy cases.

Methods: We used Exomiser which prioritizes genes and variants by combining pathogenicity scores with a phenotypic relevance score. We benchmarked our approach by introducing two heterozygous truncating variants to 45 previously identified candidate muscle disease genes in model VCFs, creating mock data. We defined the best analysis settings to prioritize our candidate genes and applied these settings to a real cohort of over 323 unsolved myopathy cases.

By combining the mean Exomiser score for genes observed in our cohort with the reoccurrence of the gene in our cohort we generated a list of genes with the highest probability to be novel disease genes.

Results: We demonstrated that our method is an efficient strategy to prioritize candidate genes responsible for muscle diseases even on singletons.

Grant References: Academy of Finland, Samfundet Folkhälsan, University of Helsinki

Conflict of Interest: None declared

P18.044.D DeNovoCNN: A deep learning approach to de novo variant calling in next generation sequencing data

Gelana Khazeeva[1], *Karolis Sablauskas*[2], *Bart van der Sanden*[1], *Wouter Steyaert*[1], *Michael Kwint*[1], *Dmitrijs Rots*[1], *Max Hinne*[3], *Marcel van Gerven*[3], *Helger Yntema*[1], *Lisenka Vissers*[1], *Christian Gilissen*[1]

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Background/Objectives: De novo mutations (DNMs) are a well-established cause of genetic disorders. However, the accurate identification of DNMs remains a major challenge, due to sequencing errors, uneven coverage, and mapping artifacts. Recent developments in deep learning have shown the power and applicability of neural networks to a wide range of topics, including biology.

Methods: We used convolutional neural networks to develop a novel DNM detection algorithm (DeNovoCNN), that represents the alignment of sequence reads for a trio as 160×164 resolution images. DeNovoCNN was trained on DNMs from 5,616 whole exome sequencing (WES) trios. We compared the performance of DeNovoCNN with other approaches such as GATK, DeNovoGear, DeepTrio and Samtools using various validation datasets.

Results: DeNovoCNN achieved a total of 96.74% recall and 96.55% precision on the test dataset. Using a set of 1,323 DNMs from the Genome in a Bottle reference dataset we show that DeNovoCNN outperforms other approaches with 97.16% precision and 90.55% recall. DeNovoCNN was able to identify all 24 true DNMs on an independent set of 20 WES trios, confirmed by Sanger sequencing. We showed that DeNovoCNN has the highest concordance with PacBio HiFi LRS on a set of 7 WGS trios with 81.83% recall and 21.8% precision.

Conclusions: We showed that de novo mutation detection could be improved by applying convolutional neural networks. Our results suggest that DeNovoCNN is likely robust against different exome sequencing and analysis approaches, thereby allowing the application on other datasets.

Grant references: The Netherlands Organisation for Scientific Research [917-17-353 to C.G.].

Conflict of Interest: None declared

P18.045.A Clinical validation of AION, an artificial intelligence platform for automated variant interpretation, using data from the 100,000 Genomes Project

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Introduction: AION is an AI-driven platform that helps classify, prioritize and interpret genetic variants by analyzing patient clinical information with exome and genome sequencing data. This study aims to evaluate AION's clinical performance using a sample from the 100,000 Genomes Project (100kGP) cohort.

Methods: This study included 318 individuals (n = 57 singletons, n = 261 trios) with monogenic disease and validated causative variant(s), diagnosed by a Genomic Medicine Centre (GMC), with research consent and WGS data in GRCh37. Individuals with other types of causative variants (CNV, SV, STR, etc) were excluded. The study assessed AION's sensitivity (percentage of cases where the causative variant(s) was identified) and causative variant rank.

Results: Overall, the disease-causative variant(s) was detected in 91.3% of cases (95% CI = 88-94), increasing to 93.1% (n = 243/261, 95% CI = 89.4-95.6) for trios. We observed higher sensitivity in the pediatric cohort (94%, n = 172/183, 95% CI = 89.5-96.9) and in the most common disease category within this group, intellectual disability (96.8%, n = 92/95, 95%CI = 91.0-99.3). In most cases analyzed, the top-ranked variant by AION was the diagnostic variant reported by clinicians across GMCs.

Conclusions: This study demonstrates AION's power to quickly and accurately analyze WES/WGS in a clinical setting, providing a promising step towards the integration of AI in healthcare. Future work will consist of a phase 2 study applying AION v3.4 to 1718 affected individuals from the 100kGP cohort Pilot study.

Conflict of Interest: Kristina Ibáñez Garikano Nostos Genomics GmbH, Andrea Bertana Nostos Genomics GmbH, Edgard Verdura Nostos Genomics GmbH, Max Schelker Nostos Genomics GmbH, Joe Rayner Nostos Genomics GmbH, Ilona Lehtinen Nostos Genomics GmbH, Genomics England Research Consortium: None declared, David Alberto Neville Nostos Genomics GmbH, Rocio Acuna Hidalgo Nostos Genomics GmbH, Nostos Genomics GmbH

P18.046.B Statistical ratification of detected CNVs from WES-based pipelines with the sharc R package

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Background/Objectives: WES samples from the ICGNMD (530) and Newcastle (218) were analysed by a CNV detection pipeline which used three popular detection tools (clincnv, cn.mops, exomedepth) and multiple annotation steps including vep and ACMG guidelines. Filtering low confidence variants left thousands of detected CNVs, and many of these were false positives. I developed statistical handling of annova in regions with cnvs (sharc) R package as a means to statistically validate CNVs detected from such pipelines.

Methods: Input for sharc includes a bed file containing detected CNVs from the detection pipeline, and cov files showing the read depths of the samples. Mean read scores are contrasted against detected CNV values, and quantile distributions are used to ratify detected duplications and deletions. Anova and subsequent post-hoc examinations by dunnet's and bonferoni's tests provide adjusted p.values which determined the likelihood of the detected CNVs showing a significant variation in the genomic region of interest.

Results: Deployment of the downstream statistical package filtered out over 50% of CNVs picked up by detection tools and provided statistical ratification for remaining CNVs. Following visualisation and clinical correlations were less labour intensive, and lead to several novel CNVs being validated from the ICGNMD and Newcastle cohorts.The statistical package will be deposited into Bioconductor following further optimization.

Conclusion: Statistical ratification of WES based CNV detection pipelines greatly decreased labour of clinicians in our consortium, and streamlined the process of indetifying diagnostic CNVs. Many other WES based CNV detection projects may benefit from sharc.

Grant References: MR/S005021/1

Conflict of Interest: None declared

P18.047.C Uplifting the diagnostic yield in Rare Disease Patients through the AI-based hypothesis-driven "Suggested Diagnosis"

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Introduction: Despite the importance of ACMG/AMP guidelines for Variant Interpretation (VI), class-based systems may not be effective in clinical settings, when thousands of variants need to be examined to detect few causatives. eVai (www.engenome.com) integrates AI-based "Suggested Diagnosis" feature to boost VI process.

Materials and Methods: The "Suggested Diagnosis" AI-based model exploits variants pathogenicity, phenotypic similarity and family segregation to assign a clinical score to each sample's variant. The model is trained on a large in-house dataset of variants from phenotypically heterogeneous diagnosed patients.

We benchmarked this model within the NIH-funded Rare Genome Project CAGI6 challenge, assessed by the Broad Institute. Furthermore, we tested the "Suggested Diagnosis" on a dataset of 17 prenatal WES samples with heterogeneous phenotypes and 85 samples from the "Deciphering Developmental Disorder" (DDD) study for performance evaluation purposes.

Results: Our model resulted as a best performer among worldwide solutions within the CAGI challenge and it was the only solution enabling the diagnosis of 2 unsolved cases, increasing the diagnostic yield by 12.5%. In the cohort of 17 prenatal samples, the model pinpointed the causative variants in the top 20 positions in the 100% of cases (88% cases in the top 5), while on the 85 DDD probands the genetic diagnoses were ranked in the top 20 in 83% of cases (75% in the top 5).

Conclusion: Exploiting phenotypic description and family segregation information in addition to variant's pathogenicity allows eVai's Suggested Diagnosis to break down the limits of traditional VI.

Conflict of Interest: Federica De Paoli enGenome S.r.l., Patent request N. 102021000006353, Giovanna Nicora enGenome S.r.l., Ivan Limongelli enGenome S.r.l., Has shares of enGenome S.r.l

Patent request N. 102021000006353, Ettore Rizzo Has shares of enGenome S.r.l

Patent request N. 102021000006353, Susanna Zucca enGenome S.r.l., Has shares of enGenome S.r.l

Patent request N. 102021000006353

P18.048.D Enabling large-scale clinical sequencing through the automation of bioinformatic workflows and data management

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Background/Objectives: As the demand for DNA and RNA based diagnostics increases in the clinical setting, previous manual bioinformatic workflows need to be automated for scalable throughput and shorter turnaround times. We present how small databases and accompanying software, virtual machines and a high performance cluster creates an efficient and scalable compute infrastructure for clinical genomics.

Methods: Four basic ideas are used to automate bioinformatic workflows and data management. (1) Standardising the interaction with pipelines to enable a streamlined integration and usage for all analyses. (2) The usage of metadata databases to automate configuration of bioinformatic pipelines by organising sample metadata, such as sample type, requested analysis type and requested delivery of result. (3) A small database for file tracking, facilitating access, storage, clean-up and delivery of files. (4) Finally, a tool which monitors ongoing computational analyses helps the user to get an overview and handle errors. All tools are developed in-house and are publicly available.

Results: Over the past year, an average of 330 whole genome sequencing (WGS) samples, 63 whole exome sequencing (WES) samples, and 131 gene panel samples were analysed monthly. Filtered variants, ranked by predicted pathogenicity, were delivered to clinicians, with a median turnaround time of 11 days, including library preparation, sequencing and analysis. Extensive automation, with automated starts, quality controls and deliveries, provides resource efficient data management.

Conclusion: Standardising sequencing output and constructing supporting software infrastructure is a necessity for scalability and continued growth of WGS/WES based diagnostics in healthcare.

Grant References:

Conflict of Interest: None declared

P18.049.A Phenome-wide associations of multi-allelic copy number variants with health-related traits

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Background: Complex structural variants, such as multi-allelic CNVs (mCNVs), have not been widely studied for their contribution to disease risk. Though they may disrupt biological processes, mCNVs have been difficult to characterise at large-scale genome-wide and are often not strongly associated with flanking SNVs, limiting imputation approaches. Improved understanding of the role of mCNVs in disease risk may lead to novel insights into disease pathobiology.

Methods: We called mCNVs from UK Biobank whole exome sequences (n = 200453) with ClinCNV, identifying 28 mCNV loci that passed stringent quality control. Phenome-wide association studies were performed on these mCNVs using the DeepPheWAS R package. Participants of non-European ancestries and relatives closer than second degree were excluded.

Results: For 10 mCNVs, fifty-two phenotype associations were found, with a false discovery rate ≤0.01. We found well-known associations, such as *RHD* with rhesus alloimmunisation in pregnancy ($p = 1.01 \times 10\text{-}41$, OR = 0.045 [0.028, 0.069]), *CYP2D6* with adverse effects from opiate use ($p = 5.05 \times 10\text{-}7$, OR = 1.37 [1.21, 1.55]) and *CFHR1/CFHR3* with age related macular degeneration ($p = 5.41 \times 10\text{-}11$, OR = 1.26 [1.18, 1.35]). 48% of these gene-phenotype associations implicated the same genes as SNP associations from the GWAS Catalog, including for a locus near *EPO* (erythrocyte count, $p = 8.44 \times 10\text{-}45$, beta = -0.047 [-0.054, -0.041]), and *CYP2E1* with acetone levels ($p = 3.88 \times 10\text{-}13$, beta = -0.16 [-0.21, -0.12]). We also identified 2 potentially novel associations including *FCGR2A/3A* with haematuria ($p = 2.21 \times 10\text{-}8$, OR = 1.25 [1.15, 1.35]).

Conclusion: We show that accurate typing and large sample sizes can reveal association of mCNVs with clinically important traits, and our work provides several novel loci that contribute significantly to clinical traits.

Conflict of Interest: None declared

P18.050.B VIsoQLR: an interactive tool for the detection, quantification and fine-tuning of isoforms in selected genes using long-read sequencing

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DNA variants altering the pre-mRNA splicing process represent an underestimated cause of human genetic diseases. Their association with disease traits should be confirmed using functional assays from patient cell lines or alternative models to detect aberrant mRNAs. Long-read sequencing is a suitable technique to identify and quantify mRNA isoforms. Available isoform detection and/or quantification tools are generally designed for the whole transcriptome analysis. However experiments focusing on genes of interest need more precise data fine-tuning and visualization tools.

Here we describe VIsoQLR, an interactive analyzer, viewer and editor for the semi-automated identification and quantification of known and novel isoforms using long-read sequencing data. VIsoQLR is tailored to thoroughly analyze mRNA expression in splicing assays of selected genes. Our tool takes sequences aligned to a reference, and for each gene, it defines consensus splice sites and quantifies isoforms. VisoQLR introduces features to edit the splice sites through dynamic and interactive graphics and tables, allowing accurate manual curation. Known isoforms detected by other methods can also be imported as references for comparison. A benchmark against two other popular transcriptome-based tools shows VIsoQLR accurate performance on both detection and quantification of isoforms. Here, we present VIsoQLR principles and features and its applicability in a case study example using nanopore-based long-read sequencing. VIsoQLR is available at https://github.com/TBLabFJD/VIsoQLR.

GRANTS: PI20/0085, ONCE, PI22/00579, PI18/00579, IMP/00019

Conflict of Interest: None declared

P18.052.D Predictive modelling of genetic variant reclassification using variant submissions to the ClinVar database

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Background/Objectives: Genetic variant classification can be unstable and can be subject to change over time (variant reclassification) with no predictive certainty as to when this might occur or for what reasons. Although genetic variant reclassification has been examined in the literature, comprehensive prediction models using large scale databases of genetic variants have never been attempted.

We aimed to identify predictors of reclassification of variants in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Thereafter, we examined variant dependent factors that are associated with variant reclassification using a logistic regression model.

Methods: We created a dataset combining present and historical records of genetic variant submissions to ClinVar from 2014 to 2022 using the EDirect package. Using logistic regression and machine learning techniques, we analysed this large dataset to determine factors that could be combined in a model for the prediction of genetic variant reclassification.

Results: There were 2,192,693 individual variant submissions eligible for inclusion in our analysis. Variant reclassification frequency from 2014-2022 was 8.6% in ClinVar. Variant reclassification is strongly associated with Variants of Uncertain Significance and Likely Pathogenic variants, Year First Submitted, and Allele Frequency. Chromosomal location is significantly associated with reclassification in only a few chromosomes.

The accuracy of the prediction model created from our logistic regression was 0.92 [sensitivity 0.99, 95% confidence interval (0.92 – 0.9216)].

Conclusion: We identified previously unknown predictors of variant reclassification within our dataset. Machine learning techniques demonstrated usable accuracy of our test model at predicting reclassification within variant submission records that are currently publicly available from the ClinVar database.

Conflict of Interest: None declared

P18.053.A Québec population-specific reference panel helps improve genotype imputation

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We created and evaluated a Québec genotype imputation reference panel using 2,113 deeply sequenced genomes from the BIOBANQUE QUÉBÉCOISE DE LA COVID-19 to investigate its value for GWAS of COVID-19 and related diseases.

We conducted three imputations using 800K genotyping array positions in sequenced unrelated individuals of European, American, East Asian, Central South Asian, Middle Eastern, and African ancestries (20 of each), comparing the performance of the Québec panel (N = 1,993), TOPMed panel (N = 97,256), and meta-imputation.

The Québec panel covered more true alternate alleles (AA) than TOPMed, 98.83% vs 97.32%, in all ancestries, with the lowest difference in Africans (98.3% vs 97.5%). However, the percent of imputed AA concordant with true AA was higher in TOPMed in all ancestries (96.15% vs 97.80%). The Québec panel still had higher absolute numbers of concordant imputed AA in individuals across all ancestries except Africans (Europeans: 2,693,014 vs 2,662,039, Africans: 3,101,557 vs 3,246,180). The meta-imputation improved the coverage of true AA (99.59%) while maintaining high concordance (97.60%). Meta-imputation had slightly lower concordance than TOPMed due to the inclusion of panel-specific variants from the smaller panel, a known limitation of the algorithm.

The 1,577,814 variants present in 1,122 Europeans in the Québec panel but absent in TOPMed had higher AA frequencies compared to gnomAD non-Finnish Europeans ($M_{Quebec} = 0.0022$, $M_{nFE} = 0.0018$, Wilcoxon signed-rank P-value < 0.0001), and 11,287 of them showed statistically significant frequency differences (Fisher's exact P-value $< 4 \times 10^{-8}$).

Our findings demonstrate the benefits of population-specific imputation panels and the need for further improvements in meta-imputation methods.

Conflict of Interest: None declared

P18.054.B Automated syndrome classification across ethnicities

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Craniofacial dysmorphism, from a clinical perspective, reflects disturbed embryonic development. At least 40% of Mendelian disorders present with distinctive facial characteristics, and such features often contribute to initial diagnosis. Current practice is the evaluation by a clinical expert, who may be guided by recent machine learning-based approaches to automatically classify syndromes using 2D or 3D facial scans. However, descriptions of syndrome-specific facial gestalts and automated methods are often based on study populations that are predominantly white, and it is yet to be evaluated how clinical presentations translate to other ancestral populations.

3D facial images, demographics and/or genomic data were available from 4909 healthy control subjects and 5613 patients (n = 94 genetic syndromes, 76% self-reported white). Genomic ancestry axes were established in the control population and projected onto the syndromic samples. We then standardized facial shape by the obtained ancestry scores using partial least-squares regression and evaluated syndrome classification accuracy following the work of Hallgrimsson et al. (2020).

When comparing classification results of ancestry-adjusted and unadjusted faces, we observed an increase in mean sensitivity for some of the syndromic groups (e.g., Williams-Beuren). This suggests that, for these groups, syndromic effects are shared among diverse populations. In contrast, sensitivity was reduced for e.g., Down syndrome and CHARGE, potentially indicating a syndrome-by-ancestry interaction effect.

In conclusion, we studied the potential bias in syndrome classification due to ancestry by removing common ancestry effects from the face. However, future recruitment of diverse samples remains crucial to validate the current work.

Grant References: NIH-NIDCR U01DE024440

Conflict of Interest: None declared

P18.055.C A knockoff filter selection study of single nucleotide polymorphisms associated with multiple sclerosis in a highly dense region of chromosome 17

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Background: When tackling feature selection, the aim is heading toward the most important subset of variables which best explains the outcome, considering that no information should be lost. In association studies between phenotypic traits and DNA segments, scientists may incur in testing thousands of variants. Though the reduction of the number of features is a central step to be performed. Analysis must include efficient strategies to account for correlation patterns among genomic regions and methods to reach as many independent variants as possible. This problem increases in complexity when the study design deals with family-based samples because the limited sample size may lead to underpowered analyses and relatedness inevitably causes dependencies in genotypes, which must be accounted for when including resampling methods to increase accuracy estimate.

Methods: Our work aimed to find a filter selection method to study the most important genetic variants associated with Multiple Sclerosis (MS), performing a multi-steps analysis: knockoff filter was applied, limiting spurious findings, correcting for all the possible correlations among variants due to linkage disequilibrium. The genomic region 30820506:32483270 bp on chromosome 17 was investigated, since it has been strongly associated with MS from previous studies. A sample of 157 trios and 198 unrelated subjects from the isolated Sardinian population was employed.

Results: Significant associations with MS were found in variants belonging to ASIC2 and MYO1D regions, highlighting notable genomic spots involved in MS development, since it is a complex trait disease for which genetics may be determinant.

Conflict of Interest: None declared

P18.056.D High-definition mixture modeling of local genetic correlations

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The determination of genetic correlations between complex traits is a pivotal aspect in comprehending shared genetic foundation. The limitations of existing methods in estimating genetic correlations, particularly when confined to specific genomic regions or exhibiting variability in diverse directions across various loci, necessitate the development of new approaches. To address these limitations, we present a novel method called Mixture High-Definition Likelihood (HDL-Mix) which offers more precise estimates of genetic correlation and the extent of polygenic overlap between complex traits at various genomic regions. Our comprehensive simulations demonstrate that HDL-Mix surpasses state-of-the-art methods in terms of both estimation efficiency and interpretation of cross-trait genetic architecture. Applications of HDL-Mix on large-scale genome-wide association analyses provide comprehensive dissection of genetic correlations at functional genomic regions and established loci for human complex traits.

Grant references: X.S. was in receipt of Swedish Research Council (Vetenskapsrådet) grants (No. 2017-02543 & No. 2022-01309).

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P18.057.A Uncovering context-specific and dynamic genetic regulation of gene expression in immune cells at single-cell resolution

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Background: Single-cell genomics has transformed our understanding of human biology and disease by allowing the molecular profiling of their fundamental functional units: cells. Deployed across thousands of individuals with matched genotype, single-cell technologies provide an opportunity to measure the impact of genetic factors on cell types and states at unprecedented resolution. Yet, current strategies to map expression quantitative trait loci (eQTL) - where one assesses the effects of genetic variants on gene expression - rely on conventional 'bulk' transcriptome approaches, and may fail to identify genetic regulation in rare cell types or transitional cell states, which may be key in disease.

Methods: Here, we leverage a dataset of matched single-cell RNA sequencing (scRNA-seq) and genotype data from nearly 1,000 Australian individuals to map the context-specific effects of genetic variation on single-cell expression in blood. We use CellRegMap, a method we recently proposed to identify and characterise genetic effects that vary across cellular types and states estimated from scRNA-seq. Using recently-generated whole-genome sequencing data, we additionally begin to assess the effects of rare variation (population frequency <1%) on single-cell expression in blood cells.

Results/Conclusion: Together, these data and methods allow us to identify new -and better characterise existing- variants and genomic regions associated with changes in expression. Combined with disease association information, these results help improve our understanding of mechanisms underlying blood traits and immune-mediated diseases. As more cohort-scale single-cell datasets are generated, these strategies will generalise to a wide range of biological systems and diseases.

Grant reference: EMBO Fellowship 2022.

Conflict of Interest: None declared

P18.058.B PubCaseFinder CaseSharing: A management system for case information on rare diseases

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Background: The cost of whole genome sequencing (WGS) tests has been decreasing, and more providers have been offering WGS tests for rare diseases. Therefore, more genomic information will be accumulated in hospitals that treat patients with rare diseases. The case information, which is a combination of clinical and genomic information, is an essential resource for future genomic medicine and should be managed in a format suitable for international sharing.

Methods: We have developed a management system (beta version) for case information on rare diseases in PubCaseFinder (https://pubcasefinder.dbcls.jp/), a clinical decision support

system. Users can manage many items in common with the Phenopackets format, which is becoming a standard format for sharing case information on rare diseases, and store case information not in the cloud, but in their local storage in JSON format. In addition, users can output case information in Phenopackets format.

Results: There is no need to worry about case information crossing national borders since case information is managed in local storage. This means that users can protect their case information within their country without compromising the interests of their respective countries. In addition, case information can be output in Phenopackets format, so that only case information with informed consent can be shared internationally in an appropriate format.

Conclusion: For the future of genomic medicine, we developed a management system for case information on rare diseases, that allows users to protect appropriately their data without compromising the convenience of sharing.

Grant References: Challenging Exploratory Research Projects for the Future

Conflict of Interest: None declared

P18.059.C SeqOneRank+: a machine learning model to rank genetic variants based on phenotypes and ACMG criteria

Jiri Ruzicka[1], *Nicolas Duforet-Frebourg*[1], *Jerome Audoux*[1], *Sacha Beaumeunier*[1], *Denis Bertrand*[1], *Nicolas Philippe*[1], *Laure Raymond*[2], *Julien Thevenon*[3;4], *Michael Blum*[1], *Kevin Yauy*[1;3;5], *Laurent Mesnard*[6;7;8]

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Background/Objectives: Diagnostic of rare diseases relies on genetic sequencing and interpretation of genomic variants. One of the most challenging parts of interpretation relates to variant prioritization accounting for the patient's phenotype.

Methods: We developed SeqOneRank+, a multi-layered machine learning model, enabling fast and interpretable variant prioritization. In order to predict variant pathogenicity class, SeqOneRank+ is trained on ClinVar dataset using ACMG-AMP standards. To provide rankings, clinical context is exploited with Phenogenius, an AI method to rank genes based on the provided HPO (Human Phenotype Ontology) terms. We compared SeqOneRank+ to Exomiser 13 using an exome cohort of 242 phenotyped nephrology patients with known disease-causing variants.

Results: Prioritizing variants and genes from exome sequencing, SeqOneRank+ ranks the diagnostic gene in the top 10 genes for 88.9% (185/208) of the patients whereas this percentage is 79.8% (166/208) for Exomiser 13. For two thirds (159/242) of the diagnoses, SeqOneRank+ ranks the causative variant as the top candidate variant. For explainability purposes, SeqOneRank+ reports an ACMG probability score for each variant, the importance of the different ACMG-AMP criteria used for classification as well as a gene compatibility score based on the patient's phenotype.

Conclusion: SeqOneRank+ provides support for diagnosis of rare diseases by ranking genomic variants, and providing explainable results at a variant and gene level. Its ranking performance is superior to Exomiser, the current state-of-the-art prioritization tool for genomic variants.

Grant References:

Conflict of Interest: Jiri Ruzicka SeqOne Genomics, Nicolas Duforet-Frebourg SeqOne Genomics, Jerome Audoux SeqOne Genomics, Sacha Beaumeunier SeqOne Genomics, Denis Bertrand SeqOne Genomics, Nicolas Philippe SeqOne Genomics, SeqOne Genomics, Laure Raymond: None declared, Julien Thevenon: None declared, Michael Blum SeqOne Genomics, Kevin Yauy Previous employee, Laurent Mesnard: None declared

P18.060.D Combined bulk transcriptomic and machine learning analyses identify immune markers differentially regulated during various stages of COVID-19 infection

Shweta Pipaliya[1;2], *Yassine Damergi*[1;3], *Jacques Fellay*[1;2;4]

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Background/Objectives: During SARS-CoV-2 infection, the range of symptoms ranges from asymptomatic to mild, severe, or even critical COVID-19 in a small fraction of the population. We sought to determine the transcriptomic profile of these various clinical presentations in comparison to healthy donors.

Methods: We used a combination of published and in-house whole blood bulk RNA sequencing datasets from healthy donors, from individuals with asymptomatic SARS-CoV-2 infection, and from patients with mild, severe, or critical COVID-19 to identify sets of differentially expressed genes (DEGs) between these groups. Subsequently, from these DEGs, we determined a subset that are markers of critical COVID-19 through a supervised machine learning (ML) approach that consisted of recursive feature elimination with cross-validation to perform feature selection.

Results: Our differential gene expression and gene-enrichment analyses revealed an upregulation in interferon stimulated genes (ISGs) in asymptomatic and mild condition, contrasting with an upregulation in inflammatory cytokines, markers of T-lymphocytes and neutrophils, and cell proliferation signatures in severe and critical COVID-19. Additionally, our ML approach identified FcγR1 genes (-A, -BP, and -CP) as biomarkers differentiating critical COVID-19 from healthy samples.

Conclusion: Overall, our bulk transcriptomic analyses identify mRNA signatures of the various clinical presentations of SARS-CoV-2 infection. They specifically show an upregulation of ISGs in milder cases and of inflammatory genes in more severe disease. Using a machine learning approach to extract the most informative mRNA biomarkers of COVID-19 severity, we identify FcγR1 genes as important markers of COVID-19 severity.

Grant References: Swiss National Science Foundation Grant 310030L_197721

Conflict of Interest: Shweta Pipaliya: None declared, Yassine Damergi: None declared, Jacques Fellay Swiss National Science Foundation Grant 310030L_197721

P18.061.A Selphi, a BiDirection PBWT for genotype imputation, boosts GWAS power by increasing imputation accuracy of rare variants

Adriano De Marino[1], *Abdallah Mahmoud*[1], *Mykyta Matushyn*[1], *Jon Lerga-Jaso*[1], *Charlie Manson*[1], *Biljana Novković*[1], *Varuna Bamunusinghe*[1], *Sandra Bohn*[1], *Puya G. Yazdi*[1]

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Genotype imputation is a powerful tool for inferring missing genotype data in large-scale genetic studies. However, current

imputation methods are of limited accuracy, particularly for rare variants. Here we present Selphi, a novel genotype imputation algorithm based on the Bidirectional Positional Burrow Wheeler Transform (PBWT) and a new heuristic method for haplotype selection. When compared to state-of-the-art methods such as BEAGLE5.4, IMPUTE5, and MINIMAC4, Selphi showed a 10-30% higher accuracy on the 1000 Genome Project dataset. Selphi performed better than existing imputation methods across all super-populations and allele frequencies. Improvement was particularly pronounced for rare variants. Similar results were obtained on the UK Biobank dataset. Selphi's improvements in imputation accuracy, especially for low frequency variants, leads to a boost in power in GWAS discovery and the ability to test for rarer variants that carry larger effect sizes.

Conflict of Interest: Adriano De Marino Full, stock, Abdallah Mahmoud full, stock, Mykyta Matushyn full, Jon Lerga-Jaso full, stock, Charlie Manson full, stock, Biljana Novković full, stock, Varuna Bamunusinghe full, Sandra Bohn full, Puya G. Yazdi full, stock

P18.062.B Analyses of Polygenic Risk Scores that Consider Individual-Level Admixture Proportions Outperform Single Ancestry Approaches

Scott Kulm[1], *Jen Kintzle*[1], *Alessandro Bolli*[1], *Paolo Di Domenico*[1], *Giordano Bottà*[1], *George Busby*[1]

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Background: A polygenic risk score (PRS) is calculated as a weighted sum of common risk variants. The risk variants that lead to superior risk predictions for a group of individuals who share common ancestors may perform very poorly for another group of individuals. We could mitigate this issue by developing a unique panel of risk variants for each ancestry group, however it is difficult to assign some individuals to a single ancestry group.

Methods: We created a system that sums continental ancestry specific PRSs weighted by an individual's continental admixture components. As a proof of concept, we assessed the validity of this system in the context of coronary artery disease and individuals from the UK Biobank with an admixture of European and African ancestries (iAdmix African ancestry >= 80%). We primarily measured the accuracy of each PRS by its odds ratio per standard deviation (ORxSD) measured within a logistic regression model adjusted by age and sex.

Results: The ORxSD of an African ancestry specific PRS was 1.326 (95% CI 1.131 - 1.556), whereas the ORxSD of the admixture weighted score was 1.415 (95% CI 1.153 - 1.739). When considering all individuals with an African ancestry component greater than 50%, a level of admixture that most analyses avoid, the ORxSD of the admixture weighted score increased to 1.509 (95% CI 1.338 - 1.719).

Conclusion: These results suggest that weighting an individual's PRS by their admixture components is a superior strategy than simply assigning that individual to a single ancestry group.

Conflict of Interest: Scott Kulm Allelica Inc, Jen Kintzle Allelica Inc, Alessandro Bolli Allelica Inc, Paolo Di Domenico Allelica Inc, Giordano Bottà Allelica Inc, George Busby Allelica Inc

P18.063.C Genetic contribution to TCR repertoire

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Background/Objectives: The T cell receptor repertoire plays an important role in cancer, autoimmune and infectious diseases. However, little is known about the extent to which the usage of different Variable (V) genes in the TCR repertoire is shaped by host genetics, especially in the context of cancer and Immune Checkpoint Blockade (ICB). This study aims to investigate whether we can establish a genetic basis for TCR repertoire in CD8 T cells in a cohort of patients with melanoma and study interactions with treatment status.

Methods: Genotyping and TCR repertoire sequencing of CD8 T cells isolated from peripheral blood of patients with melanoma (n = 199) receiving ICB treatment. A linear regression model was fitted between V gene usage and genotyping or *HLA* allele including age, gender and CMV status as covariates.

Results: Associations were identified at GWAS significance at chromosomes 14 (*TRBV*) and 7 (*TRAV*), *cis* to the respective genes. The peak associations being rs4725599 upstream of *TRBV* for TRBV-28 usage (beta = -0.89, $P = 2.0 \times 10^{-21}$) and rs7148819 associated with TRAV26-2 usage (beta = -0.77, $P = 2.0 \times 10^{-10}$). Independent associations were identified with *HLA* alleles including HLA C 07:02 with TRBV5-6 (beta = -0.72, $P = 1.4 \times 10^{-5}$) and HLA B13 with TRAV26-1 (beta = 1.77, $P = 8.3 \times 10^{-5}$).

Conclusion: Our observations demonstrate a complex relationship between genetic variation and TCR repertoire usage in patients undergoing treatment with checkpoint immunotherapy. Given TCR usage is associated with immunotherapy responses, these observations have potentially important implications with respect to oncological outcomes.

Conflict of Interest: None declared

P18.064.D Mendelian randomization and colocalization identifies plausible causal proteins across autoimmune diseases

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Background: This study aims to use mendelian randomization and colocalization to identify the putative causal effects of proteins on autoimmune diseases in order to elucidate shared pathways and druggable targets.

Methods: We obtained summary statistics of genome-wide protein quantitative trait loci (pQTL) analysis from Pietzner et al., 2021 (N = 10,708), as well as disease outcome genome-wide association studies in ten autoimmune diseases – ulcerative colitis, Crohn's Disease, type 1 diabetes, rheumatoid arthritis, juvenile idiopathic arthritis, psoriasis, multiple sclerosis, autoimmune thyroid disease, ankylosing spondylitis and lupus. We performed phenome-wide Mendelian randomization (MR) with disease as the outcome and protein as the outcome variable.

Results: We analyzed 3,892 plasma proteins on genetic liability to autoimmunity. At FDR of 0.05, we identified 18 proteins that are associated with at least one disease. As a confirmatory example, TM11D, a transmembrane serine protease, is associated with both Crohn's Disease (beta = -0.32, P = 1.1e-14) and Ulcerative Colitis (beta = -0.27, P = 4.9e-19). The top SNP is rs3197999, which is a missense variant in the gene MST1, (and has a trans-effect on TM11D abundance). We replicated the TM11D association using pQTL data available from Sun et al., 2018 (N = 3,301): UC (beta = -0.24, P = 5.4e-11) and CD (beta = -0.27, P = 1.05e-17).

Conclusion: Our large-scale MR and coloc study suggests that certain autoimmune diseases may share a common proteomic basis. Proteomics studies within disease cohorts would help to confirm this. Functional studies are currently being designed.

Conflict of Interest: None declared

P18.065.A Investigating bias and precision in Mendelian randomisation estimation using educational attainment and smoking initiation as negative control outcomes

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Mendelian randomisation (MR) uses genetic variants (SNPs) in an instrumental variable framework to infer casual relationships. A key assumption for the instruments to be valid is that the instruments are only associated with the outcome through the exposure. The SNPs used as instruments in MR are typically obtained from a genome-wide association study (GWAS) of the exposure trait. However, as the sample size of these studies increases so does the number of variants identified. This has the potential to increase the likelihood that SNPs identified are invalid. Negative control outcomes can be used in MR to identify the presence of bias, by testing for a seemingly impossible relationship, e.g. by exploiting the temporal relationship between traits.

In this study we used two-sample MR to estimate the effect of circulating Vitamin D and C-reactive protein on outcomes that preceded their measurement; smoking initiation and educational attainment. For each exposure-outcome combination, we compared estimates using all genetic variants returned from exposure GWAS with just genetic variants for which a biological association was known to see if they increased bias in effect estimates.

None of the MR effects estimated provided evidence for a non-zero effect. Using all genetic variants identified in the GWAS did not notably increase the precision of the estimates obtained. Further investigation showed that this was due to high levels of heterogeneity and pleiotropy in the additional genetic variants.

Conclusion: Including large numbers of SNPs in MR estimation does not necessarily provide a tangible benefit over using known functional variants.

Conflict of Interest: None declared

P18.066.B Epistatic signals discovered between MAPT and WNT3 genes and within SNCA help shaping the genetic landscape of late onset Parkinson´s disease

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Epistatic interactions can be defined as the statistical departure from additive effects in SNP based models of disease. Epistasis might explain some of the genetic variability that remains unknown in disease, but it remains difficult to investigate. We therefore developed the R software called Variant-variant interaction through variable thresholds (VARI3) which performs non-exhaustive genome-wide epistasis screens by prioritising and testing promising variants (i.e., high minor allele frequency, MAF>0.05) and nominally associated with the phenotype (P < 10-5) against the rest of the variants in the genome. Using VARI3, we investigated the role of epistasis within Parkinson's Disease (PD) in the International Parkinson's Disease Genomics Consortium (IPDGC) cohort (14671 cases and 17667 controls, all of European origin). We identified 14 significant epistatic signals. Signals within the *MAPT* and *WNT3* loci (17:43992943 - 17:44865439; $P < 6.06 \times 10^{-9}$) and in the *SNCA* locus (4:90607126 - 4:90610135; $P < 3.05 \times 10^{-20}$) replicated in a Latino genetic ancestry (LARGE-PD) cohort (807 cases and 609 controls). Based on the effect of the epistatic signals on the phenotype we observed similar genotype-effect combinations in both cohorts in that, for example, the G/G-T/T genotype combination in the *SNCA* locus was associated with a higher risk for both cohorts (Odds ratio (OR) [95% CI] = 3.16 [1.35, 8.26] IPDGC and OR [95% CI] = 2.43 [1.61, 3.73] LARGE-PD). Here we demonstrate the utility of utilising VARI3 in examining epistatic interactions in the context of disease pathology and expanding on the genetic architecture of PD in diverse populations. VARI3 is accessible at https://github.com/alexcis95/VARI3.

Conflict of Interest: Alejandro Cisterna García: None declared, Bernabé Bustos: None declared, Sara Bandrés: None declared, Thiago Peixoto: None declared, Elif Irem Sarihan: None declared, Christie Jok: None declared, Cornelis Blauwendraat: None declared, Mike Nalls Founder and CEO/Consultant of Data Tecnica International LLC, and serves on the scientific advisory board for Clover Therapeutics and is an advisor to Neuron23 Inc., Dimitri Krainc Founder and Scientific Advisory Board Chair of Lysosomal Therapeutics Inc. and Vanqua Bio. D.K. serves on the scientific advisory boards of The Silverstein Foundation, Intellia Therapeutics, AcureX and Prevail Therapeutics and is a Venture Partner at OrbiMed., Andrew Singleton: None declared, Ignacio Mata: None declared, Steven Lubbe: None declared, Juan Botia: None declared

P18.067.C LoRID: a bioinformatic pipeline to discover alternative isoforms using nanopore long-read sequencing

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Background/Objectives: Resolution of mRNA transcripts is a challenge for molecular research and diagnosis. Current approaches are based on short read sequencing or RT-PCR and only identifies a partial structure of RNA isoforms. Since the development of long-read sequencing, resolution of full-length transcript is becoming a reality. We propose a bioinformatic pipeline to analyze long-reads RNA sequencing data.

Methods: We present LoRID, a pipeline based on guppy for basecalling and demultiplexing, minimap2 for alignment, StringTie for transcript assembly and expression, and homemade tools to allow transcript filtering and their interpretation. LoRID annotates transcripts based on ENSEMBL and generates de novo annotations for aberrant transcripts, providing expression level of each transcript. We tested LoRID on an innovative targeted capture of long-read RNAseq analysis using Nanopore sequencing for 28 genes on 8 samples.

Results: Using LoRID, we found that 50% of the transcripts are at least fully covered by one read. At least 44% of the transcripts found in a sample are known in ENSEMBL and this figure rises to 61% by counting all transcripts of the cohort. These metrics showed that the majority of assembled transcripts are physiologic

as expected in an human context. We detected all pathogenic isoforms in positive controls consisting of exon duplication, retrotransposon insertion and intronic genomic deletion.

Conclusion: Using Nanopore sequencing, LoRID was able to detect alternative and pathogenic isoforms in samples. LoRID allows detection of novel isoforms regardless of their structural complexity and could enhance molecular diagnosis based on a RNA first approach.

Grant references:

Conflict of Interest: Nicolas Soirat: None declared, Camille Aucouturier: None declared, Nicolas Philippe SeqOne CEO, SeqOne CEO, Dominique Vaur: None declared, Denis Bertrand SeqOne's bioinformatics manager, Leman Raphael: None declared, Sophie krieger: None declared, Laurent Castera: None declared

P18.068.D Shading light on pathogenic digenic mechanisms with Explainable AI

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Background/Objectives: Digenic inheritance is increasingly investigated to solve Rare Disease cases. Artificial Intelligence (AI) tools greatly support digenic variant interpretation (DVI), but their development depends on the availability of bona fide cases for training purposes. Additionally, most of these tools focus on pathogenicity predictions, without further exploring the different digenic pathogenic mechanisms observed: True Digenic (TD), Composite (CO) and Dual Molecular Diagnosis (DM). We have curated a dataset of pathogenic digenic combinations and we show how we expanded our AI-based approach for DVI, DIVAs, to dissect the digenic mechanisms (TD/CO versus DM).

Methods: We gathered almost 800 pathogenic combinations associated with different disorders, such as Alport Syndrome and Ciliopathies, from public databases, internal cases and manual curation. For each combination, the phenotypic profiles in the corresponding study were inferred in terms of Human Phenotype Ontology terms. We then improved our phenotype-based AI system for digenic pathogenicity prediction, named DIVAs, by computing Explainability of predictions in terms of Shapley values, and by using these explanations to automatically infer the digenic mechanism (TD/CO or DM).

Results: On a test set of about 400 combinations, our approach shows 80% sensitivity in pathogenic prediction. On the same data, the Explainability-based approach to dissect the digenic mechanism of predicted pathogenic combinations reports an accuracy of 91%, while Varcopp, a similar tool, has almost 70% accuracy.

Conclusion: We have developed and improved DIVAs by including an Explainability layer that predicts the digenic mechanism of pathogenic-predicted combinations.

References

PMID: 28977688
PMID: 35411390
PMID: 26481352

Conflict of Interest: Giovanna Nicora Full employee (enGenome), Federica De Paoli Full employee (enGenome), Patent N. 102021000006353, Ivan Limongelli Full employee (enGenome), enGenome shares

Patent N. 102021000006353, Ettore Rizzo enGenome, enGenome shares

Patent N. 102021000006353, Susanna Zucca enGenome, enGenome shares

Patent N. 102021000006353

P18.069.A Data standards and the European Genomic Data Infrastructure

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Background/Objectives: Secure cross-border access to high-quality genomic and phenotypic data for research and personalised medicine requires a supporting data infrastructure. Standards are essential to ensure data can be reused via FAIR (Findable, Accessible, Interoperable, Reusable) principles. A demonstration deployment or proof-of-concept has been developed by the Beyond 1 Million Genomes project and is the starting point for the technical development to enable access to the federated genomic and phenotypic data from the European 1+ Million Genomes initiative (via the European Genomic Data Infrastructure (GDI) project).

Methods: A software stack (starter kit) has been designed and implemented utilising existing, open source applications and components to build this infrastructure, aiming to maximise interoperability by providing a coordinated technology solution to the 1 + MG initiative signatory countries. All open-source components support standards, such as those from the Global Alliance for Genomics and Health. Each component can be updated or replaced, allowing adaptation to changing use cases and requirements.

Results: The B1MG Proof-of-Concept and GDI starter kit demonstrates data infrastructure for secure cross-border genomic data access. Global standards ensure the solution can link to European Data Spaces that manage information for health register data, cancer images and others. Other projects that aim to manage genomic information can benefit from and reuse the GDI starter kit.

Conclusion: The GDI starter kit facilitates the deployment of a federated data infrastructure throughout Europe and increased technological capacity to sustain national personalised medicine programmes across Europe.

Grant References: The European Genomic Data infrastructure European Union Digital Europe programme #101081813.

Conflict of Interest: None declared

P18.070.B Pediatric bone age assessment of patients with genetically-caused skeletal malformations by prior-free deep learning

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Background/Objectives: Assessing skeletal maturity via determining bone age (BA) is valuable for diagnosing and monitoring pediatric growth disorders. Hereby, reliable BA estimates are especially crucial for timing hormone treatment and orthopedic interventions. However, genetically-caused growth disorders can also induce skeletal malformations, which impede precise BA estimates. While manual BA assessment is time-consuming and

suffers from intra- and inter-rater variability even in healthy individuals, automatic methods frequently fail to generalize to some skeletal malformations.

Methods: We developed end-to-end deep learning models without relying on priors such as bone shapes. The models were trained on the public RSNA BA dataset and tested on a hold-out fraction of this dataset (n = 200) combined with the Digital Hand Atlas (DHA, n = 1382). To test generalization to skeletal malformations, we curated the German Dysplastic Bone Dataset (GDBD), comprising n = 568 images from 189 patients with seven genetically-confirmed disorders including Turner and Léri–Weill syndrome, and Achondroplasia.

Results: Our approach achieves state-of-the-art performance at a mean absolute difference (MAD) of 3.87 months on the RSNA (w.r.t. six reference BA ratings) and 5.88 months on the DHA (two ratings). On the GDBD, the model achieves at-least human-level performance with a MAD of 5.84 months (two ratings). Further, we estimate the test-retest reliability at 2.74 months based on the longitudinal data within the GDBD, which is at least as good as the associated clinical rating.

Conclusion: Our proposed prior-free approach suits assessing and monitoring the BA in patients with genetically-caused skeletal dysplasias.

Conflict of Interest: None declared

P18.071.C smORF-EP: predicting the effect of variants in small open reading frames

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Small open reading frames (smORFs) are small translated regions (typically <100 amino acids) that can encode functional small peptides. Recent ribosome profiling experiments have shown that tens of thousands of smORFs exist in humans, but few have been fully characterised. Hence, the contribution of variants in smORFs to human diseases remains unclear. Standard variant annotation tools such as the Ensembl variant effect predictor (VEP) only annotate variant consequence with respect to known protein-coding ORFs, limiting studies into smORF-impacting variants.

We developed smORF-EP, a tool to annotate variants in smORFs. smORF-EP takes as input any hypothetical ORF and variant within it and annotates the variant based on input transcript information (i.e., GENCODE). We tested smORF-EP on a high-confidence smORF dataset (Chothani et al. 2022). We annotated 485 ClinVar variants found within those smORFs and compared them to annotations from VEP.

We identified 82 ClinVar variants with a more severe annotation on a smORF than the annotation from VEP, 11 of them classified as (Likely) Pathogenic. Running smORF-EP for the 485 ClinVar variants takes less than 37±2 seconds on a M1 Pro processor with 16GB, with possibility for improvement through parallelisation.

smORF-EP enables investigation of smORF variant consequences. This opens the avenue to further investigate the potential role of smORFs, and variation within them, in human diseases.

Grant References: Wellcome Trust and the Royal Society (220134/Z/20/Z); Rosetrees Trust (PGL19-2/10025).

Conflict of Interest: Maria Fernandes: None declared, Elston Neil D'Souza: None declared, Alex Geary: None declared, Nicola Whiffin Receives research funding from Novo Nordisk

P18.072.D An automated pipeline for standardized biomedical knowledge graph construction

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Background/Objectives: In the last few decades, massive amounts of biological data have become universally available. Knowledge Graphs (KGs) have increasingly been used for the integration of complex and heterogeneous data. However, the lack of interoperability between databases is still a challenge. To address this issue we aim to develop software tools to automate the process of building standardized biomedical KGs, relating entities such as genes, proteins, non-coding RNAs, diseases, phenotypes and anatomical entities, among others.

Methods: Our tool, ngest, integrates data from 14 high quality curated resources: Ensembl, STRINGDB, GO, GOA, MONDO, HPO, HPOA, RNACENTRAL, UBERON, Cell Ontology, Bgee, DisGeNET, NPInter and MirTarBase. The data processing pipeline was implemented using the Snakemake workflow engine. The Biolink Model was used to standardize the categorization of different entities and relationships using, whenever possible, the KGX command line tool.

Results: We developed a scalable and flexible pipeline for automated download, data processing and production of biomedical KGs. The current version extracts information from 14 data sources to produce a KG containing around 300K entities and 20M relationships. Analysis of the KG identified several non-coding RNAs potentially implicated in Autism Spectrum Disorder.

Conclusions: We developed a software tool, ngest, for automated building of standardized Biomedical KGs. As a use-case, we explore the KG to identify non-coding RNAs potentially implicated with ASD.

Grant references: EXPL/CCI-BIO/0126/2021,UIDB/04046/2020, UIDP/04046/2020

Conflict of Interest: None declared

P18.073.A Automated orchestration of clinical NGS bioinformatics pipelines in the cloud

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Background/Objectives: The shift towards cloud-based analysis of high-throughput NGS data enables greater scalability in compute capacity compared to a traditional HPC. A key challenge in the clinical setting presented by this shift includes the timely uploading and management of data generated by the sequencing instruments. Typical HPC set-ups often have analysis automated through a local job scheduling system, which does not easily translate into the cloud environment. Triggering of bioinformatics processing pipelines involves multiple steps that may not be easily connected. Automated secondary and tertiary bioinformatics analysis presents a key area for reducing turn-around times.

Method: We implemented an automated process for data streaming from sequencers and initiation of analyses within the DNAnexus cloud platform. When launched, samples present in the data are identified to trigger appropriate workflow(s), that

transform raw BCL files to annotated VCFs and interpretation reports with no manual intervention by bioinformaticians.

Results: Automation has significantly reduced the time taken to initiate analysis and time between intermediary steps. For one such NGS assay averaging 48 samples per run, the average time to manually launch analysis was ~21.5 hours, and full pipeline runtime was ~19.4 hours, equating to 40.9 hours for post sequencing processing. Automation reduced this to a total of ~3 hours, a reduction of 37.9 hours / 93% (46 sequencing runs from 07/01/2022 - 21/12/2022).

Conclusion: Automated cloud processing substantially reduces the processing time for bioinformatics analysis, reducing test turn-around times for patients and enabling our laboratory to meet the 10-fold increase in patient referrals.

Conflict of Interest: None declared

P18.074.B KmerToCN: an alignment-free method for copy number estimation directly from next generation sequencing reads

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Background/Objectives: Copy variable genes in the human genome are linked to various traits and disease susceptibility. Estimating the exact copy number from next-generation sequencing data requires mapping reads to the reference genome, a lengthy process prone to errors in complex repetitive regions. However, *k*-mers (short DNA substrings with length *k*) have proven effective in managing vast genomic data and could be utilized for fast alignment-free copy number estimation.

Methods: *K*-mer databases are created containing *k*-mers from gene regions and single-copy flanking regions. *K*-mers are chosen based on region specificity, uniqueness in the genome, and GC content value. Frequencies of these *k*-mers can be obtained from raw sequencing data for copy number estimation. The KmerToCN toolkit includes tools for both copy number estimation and creating *k*-mer databases.

Results: *K*-mer databases were compiled for different gene regions and copy numbers were estimated from Illumina reads of 500 individuals from the Estonian Biobank. Copy number distributions resembled those previously described for Europeans. Copy numbers for three amylase genes were compared to Droplet Digital PCR results for 40 individuals, showing high correlation ($R = 0.9921$) and concordance (90%). Results were similar to those from a read-depth based approach ($R = 0.9925$ and 87.6%).

Conclusion: The *k*-mer-based alignment-free approach for gene copy number estimation has proven to be fast and reliable with highly precise results, comparable to a method that requires read alignment.

Grant References: EU ERDF grant No. 2014-2020.4.01.15-0012, the cost of the sequencing was partly covered by the Broad Institute (MA, USA)

Conflict of Interest: None declared

P18.075.C Predicting DNA structure from sequence sheds insights into multifactorial mechanisms of loop formation

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Background/Objectives: The causal link between DNA sequence and its 3D structure, although definite, remains resistant to explicit characterization. Among foundational units of 3D structure are hairpins and loops, the latter of which are often associated to CTCF-cohesin complexes through a loop extrusion mechanism. As the presence of CTCF binding sequences is not always sufficient for looping, we assessed whether other sequence specificities were important in "loopability" of a genomic stretch.

Methods: We built machine learning models to predict loop or hairpin status from local sequence alone. The inputs mix handcrafted features such as k-mer entropy, GC-content and CTCF binding sequence matrix score with features derived from a variable-length sequence embedding (dna2vec) inspired from natural language processing.

Results: We assessed our predictions using a set of 10,000 "validated" loops identified by three independent tools (HiCCUPS, cooltools, HiCExplorer) in five human cell lines. Using only naïve local sequence as an input, we correctly predicted the capacity of a sequence to loop and/or create hairpins >80% of the time. This high accuracy was independent from cell lines or detection tools. While the explicit presence of a CTCF consensus site did not improve accuracy, loop status was sensitive to broad-scale similarity of multiple sequence consensus, suggesting a cooperative binding mechanism rather than strong site recognition. Conversely, hairpins were not associated with presence of CTCF consensus sites.

Conclusion: Taken together, our results stress the importance of sequence information as a crucial contributor to DNA structure and could provide insight into its most critical building blocks.

Conflict of Interest: None declared

P18.078.B Accelerating NGS Diagnostics with GPU Optimization on HPC Systems

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Background: Diagnostic sequencing generates a large quantity of data, making the analysis of such data a time-consuming and challenging task. In clinical settings, the need for data analysis speed is paramount, and high recall is necessary for accurate diagnosis. Moreover, diagnostic sequencing involves sensitive data, which requires strict privacy and security measures.

Methods and Results: This study focuses on benchmarking the accuracy and time of the germline variant calling analysis following Global Alliance for Genomics and Health Benchmarking Team standards. We compared CPU-accelerated processes using GATK4 - Best Practices Workflows with GPU-accelerated processes using NVIDIA Clara and Parabricks. The performance of each pipeline was evaluated using wall-clock time, processor time, and memory usage. We expected significant improvements with GPU acceleration, as it is known to provide substantial speedups compared to CPU acceleration. The analysis was performed on a high-performance computing (HPC) system to leverage its computational capabilities. We estimated the cost-effectiveness of each pipeline for diagnostic sequencing. The GPU-accelerated processes showed a higher computational cost, but the time savings provided by the acceleration were substantial, making the overall cost per sample more efficient.

Conclusion: The findings suggest that optimized GPU acceleration on HPC systems can help achieve the necessary speed while maintaining high recall for clinical procedures, which is critical for timely diagnosis and treatment.

Conflict of Interest: None declared

P18.079.C Precise ancestry deconvolution reveals regional genetic history of Latin Americans and improves fine mapping in GWAS

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Even closely-related populations may display subtle genetic variations that can impact complex phenotypes. Recognizing the need for this level of precision, we developed Orchestra, a local ancestry inference method with a base layer that mimics the natural process of recombination and a deep learning smoothing module. We demonstrate that Orchestra performs better than other state-of-the-art ancestry inference algorithms such as RFmix, Gnomix or FLARE, both on non-admixed and admixed samples, and using either sequencing or array data, with an overall 15% accuracy improvement compared to the second best model. Orchestra also retains high accuracy across all tested populations, unlike other methods, providing a unique opportunity to distinguish between closely related ancestries. To demonstrate Orchestra's utility, we retraced the genetic history of Latin Americans within the 1000 Genomes Project and UK Biobank, obtaining a fine-grained regional break-down supported by well-known historical migration events. Finally, we applied Orchestra to the entire UK Biobank dataset and performed GWAS while leveraging local ancestry, showing that Orchestra discovers subcontinental ancestry-specific effect size differences that allow for fine mapping of variants. Accordingly, high-precision ancestry deconvolution algorithms like Orchestra have a great potential to improve discovery in downstream GWAS analysis.

Conflict of Interest: Jon Lerga-Jaso Full, Stock, Biljana Novković Full, Stock, Deepu Unnikrishnan Full, Varuna Bamunusinghe Full, Marcelinus Rocky Hatorangan Full, Alex Osama Full, Haley Pedersen Full, Charlie Manson Full, Stock, Adriano De Marino Full, Stock, Sandra Bohn Full, Andrew Terpolovsky Full, Stock, Abdallah Mahmoud Full, Stock, Manfred Grabherr Full, Puya G. Yazdi Full, Stock

P18.080.D Optimal statistical power for clinical subgroup analysis in COVID-19 genome wide association study

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Background/Objectives: There is evidence that critically ill COVID-19 patients exhibit a range of clinical phenotypes which can affect the patient's response to treatment. Understanding the underlying biological reasons for this variation can contribute to the development of advanced therapeutic interventions.

Methods: Using the latent component analysis method we have stratified 26,420 critically-ill COVID-19 patients into distinct subgroups using data on the presence of 25 symptoms, as reported upon a patient's hospitalisation. The dataset is derived from the International Severe Acute Respiratory Infection Consortium Clinical Characterization Protocol (ISARIC) Coronavirus Clinical Characterization Consortium (4C) study (ISARIC4C).

Results: The resulting subgroups appear to be stable, as at least 6 subgroups are consistently present even on models with a higher number of cluster solutions. Most subgroups exhibit at least two of three core symptoms (fever, cough, and shortness of breath). The subgroups distinction is based on the presence of less common symptoms, such as myalgia, loss of taste and smell, and gastrointestinal symptoms. The identified subgroups will be used for a Genome-Wide Association Study (GWAS) in a one-vs-rest design. The statistical power for such design was calculated for all the subgroups in a range of solutions from 2 to 9, with the minimum identifiable Odds Ratio per cluster being between 1.13-2.05.

Conclusions: The identifiable Odds Ratio are in line with results of recent studies about COVID-19 severity genetics. An optimal solution for one-vs-rest GWAS, lies between 5 and 7 subgroups as indicated by each subgroup's statistical power, stability and clinical relevancy.

Conflict of Interest: None declared

P18.081.A A rigorous benchmarking of methods for SARS-CoV-2 lineage abundance estimation in wastewater

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Background/Objectives: While wastewater-based genomic (WBG) surveillance is promising for viral propagation and dynamics at a population level, there is a need for comprehensive benchmarking of methods for SARS-CoV-2 lineage abundance estimation in wastewater samples. To fully unlock the potential of WBG surveillance we created extensive benchmarks to measure the accuracy of bioinformatics methods aimed to estimate the relative abundance of SARS-CoV-2 lineages in the wastewater samples.

Methods: We benchmarked 17 tools by exploring the dependence of the accuracy on several parameters, including different sequencing technology, regions for sequencing, length of sequencing fragments, read length, error rate, and sequencing coverage. In total, we have more than 100 in-silico and 12 in-vitro benchmarks that mimic different properties of waste-water samples.

Results: Our results on 42 simulated samples mixed with different abundances of lineages and sublineages show that in terms of accuracy and limits of detection, Kallisto outperforms other tools, showing 25% from total number of estimations of abundances in mixed samples with an absolute error less than 0.1 at 50X whole genome coverage. Also, the Kallisto tool can detect the lower frequency of 1% below the relative error of 0.2. In general, frequencies tend to be under-estimated and/or sub-estimated in lineages, as a consequence of closely related genomes. The more divergent lineages tend to be more precisely estimated.

Conclusion: Our research will inform the broad biomedical community about feasible bioinformatics methods for quantifying SARS-CoV-2 strains abundances in wastewater samples.

Conflict of Interest: None declared

P18.082.B Face2HPO: Simultaneous HPO Labeling and Disorder Classification of Syndromic Faces

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Background/Objectives: The exponential growth of unlabeled medical data in the last two decades presents a challenge in the accurate labeling of complex medical data such as syndromic faces. We propose a novel approach that uses a convolutional neural network (CNN) to automatically label syndromic faces with their respective Human Phenotype Ontology (HPO)-terms and predict the patients' disorders. This approach not only helps clinicians with their diagnosis and administration but also facilitates users in the GestaltMatcher DataBase (GMDB) when adding new patient photos or supplementing old ones with more information.

Methods and Results: Our CNN-based approach leverages the tree-like data structure of HPO to allow for multiple levels of abstraction, known as HPO-levels, which help us address the data scarcity of underrepresented cases in the GMDB. This approach also provides new explainable artificial intelligence (XAI) insights into the decision-making process of the black box CNN, as we condition the feature space on both HPO and disorder classification. With the HPO-classifier, we can infer the regions of interest and consider the spatial and descriptive information of the predicted HPO terms to justify the disorder classification.

Conclusion: Our proposed approach for combining HPO and disorder classification for automatic labeling and diagnosis of syndromic faces is another step forward in the field of bioinformatics in genetics. The use of HPO terms in our model inherently makes the process more explainable than most other diagnostic tools. Additionally, it will help to reduce the labor-intensive labeling process and enable clinicians to work more efficiently.

Conflict of Interest: None declared

P18.089.A Orphadata : Orphanet data and tools to power rare disease knowledge generation

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Orphadata (www.orphadata.com) is a platform dedicated to providing downloadable, computable and re-usable aggregated datasets and services derived from the Orphanet rare disease knowledge base (www.orpha.net). Orphadata services aim to help health professionals, academic researchers, industry researchers, policy makers, software editors, advocacy organisations and the biotech/pharma industry better understand rare diseases (RD) and the RD landscape.

Users can access through Orphadata.com Orphadata Science, an open data suite (CC-BY 4.0 licence) derived from the Orphanet knowledge base and recognised as an ELIXIR Core Data Resource and a Global Core Biodata Resource: this includes the Orphanet nomenclature pack for coding of RD in 9 languages, datasets from the Orphanet scientific knowledge base (terminology alignments, classifications, genes associated with rare diseases, clinical signs and symptoms in RD, epidemiology, natural history and functional consequences), and the Orphanet Ontology of Rare Diseases (ORDO) and HPO-ORDO Ontological Module, all updated twice a year. The ORPHAcodes API is also made available to aid the implementation of ORPHAcodes in health information systems and third-party tools so as to improve data interoperability. These products were downloaded over 420 000 times in 2022.

Users can also access Orphanet's textual information, orphan drug and designations, and directories of expert centres, networks of expert centres, medical laboratories and diagnostic tests, patient organisations, and research activities through Orphadata after signature of a Data Transfer Agreement or a service contract depending on their status.

Orphadata will be offering additional APIs and services in 2023 in order to help users make the most of Orphanet data.

Conflict of Interest: David Lagorce Fulltime INSERM US14 - Orphanet, collaborator OD4RD2; EJP RD, ELIXIR-FR; Solve-RD; TEHDaS JA, Valerie Serriere-Lanneau INSERM, US14-Orphanet: Full time, collaborator: EJP-RD, OD4RD2, Marc Hanauer Full-time INSERM, US14 - Orphanet, collaborator OD4RD2; EJP RD, ELIXIR-FR; Solve-RD; EHDS Pilot 2; TEHDaS JA;, advisory board: Scientific Advisory board national support group "Aide aux Jeunes Diabétiques", Support group Chairman (Association ENT'RED-paris, réseau enfance diabète), Charlotte Rodwell Part time (90% FTE) INSERM, US14 - Orphanet No other employment., Collaborator: EJP RD; Solve-RD, Advisory board: Maladies Rares Info Services, Ana Rath Full-time, PI OD4RD2; collaborator: EJP RD; Solve-RD; EHDS Pilot 2; TEHDaS JA; (tu as besin des tous les numéros de contrats? Sylvie peut te le donner, advisory board: ERN-LUNG; ERN-BOND; Share4Rare

P18.090.B The dark side of Moon and other commercial genome analysis or interpretation tools

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Background/Objectives: Genome diagnostics requires software tools that are not only accurate and fast but also available over a reasonable time. Sudden unavailability of such tools can severely impact established pipelines. Current example is the recent discontinuation of the in-2017-launched fast and reliable variant interpretation platform Moon outside of the USA. Since Moon is a cloud-based artificial intelligence engine, after its discontinuation no further use is possible, e.g. local for temporary life cycle extension. Here, we address the disappearance of innovative genome analysis tools, suggesting potential solutions.

Methods: Considering "make vs. buy" and software life cycle management in (bio-)informatics, we explored various options for replacing discontinued genome analysis tools, such as developing new software in-house or through (open-source) collaborations and acquiring (long-term) commercial solutions.

Results: Short term, "buy" an on-site tool is clearly preferred. Long term, "make" an open-source tool, like Linux/Firefox/OpenOffice, would be the solution to reduce the risk of a Moon-like complete loss of access. Software for Oxford-Nanopore-Technologies sequencers and NCBI BLAST search are well-known examples of powerful open-source solutions for genome analysis. The residual risk of open-source projects being abandoned, leading to non-update scenarios is acceptable, since in life cycle management it is still much better than the worst-case scenario of a commercial (cloud-based) tool, where the software is suddenly inaccessible.

Conclusions: To deal with the sudden disappearance of software, we propose that the genomics community support each other by redirecting resources from various in-house developments towards a few open-source genome analysis tools accessible for long-term usage.

Conflict of Interest: None declared

P18.091.C Assessment of sequencing coverage uniformity for two whole-exome enrichment capture solutions

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Background/Objectives: Whole-exome sequencing (WES) has been incorporated for routine genetic testing of disease. However, the performance of the different WES solutions is heterogeneous. Here we compared two widely used exome capture solutions using reference samples.

Methods: Four NIST-GIAB references were sequenced with Illumina DNA Prep with Enrichment and Agilent SureSelect Human V8 solutions. Sequencing was done with NextSeq550 (Illumina Inc.) and PE75. Genes and exonic annotations were obtained from RefSeq. The transcript-exon level read data was filtered keeping genes with >1 exon and a mean read depth >10X. The median and interquartile range (IQR) of the exonic coverage was calculated to assess variability. We relied on the 80-fold penalty (80FP) to measure capture efficiency.

Results: The average depth was 120-141X across samples and solutions. The captures overlapped 95% for genes and 96.5% for transcript-exons. Median coverages per gene were 107X and 125X for Illumina and Agilent, respectively. Average coverage IQR were 54X and 50X, while the dispersion of IQR was 37X and 27X, for Illumina and Agilent, respectively. 80FP was higher ($p < 10^{-4}$) in Illumina (3.0) in comparison to Agilent (2.0).

Conclusion: Agilent showed both a relatively higher mean depth and uniformity than Illumina's, despite their average dispersions were comparable.

Grant References: ACIISI, Gobierno de Canarias (ProID2021010073; ProID2021010084); Instituto de Salud Carlos III (PI20/00876), co-financed by the ERDF 'A way of making Europe' from the EU; Fundación Canaria Instituto de Investigación Sanitaria de Canarias (PIFIISC20/57); ECIT (CGIEU0000219140); ITER (OA17/008); CEUCD-ECIT 2021-2025 (CGIAC0000014697).

Conflict of Interest: None declared

P18.092.D Computational analysis of metatranscriptomic data with simultaneous detection of changes in human transcriptome, microbiome and viriome - COVID-19 pilot

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Background/Objectives: During recent pandemics, it has been shown how much potential tools of molecular biology and bioinformatics may have in coping with dangerous infection outbreaks. To understand the disease, it is important to see how is a pathogen acting and how it influences the host. Here we studied this problematic on the level of transcriptomics, but not investigating only virus and host, but also microbiome present in our samples.

Methods: We analysed RNA-seq data originating from COVID-19 positive patients classified by severeness of disease plus COVID-19 negative patients. Samples were obtained from nasopharyngeal swabs and positivity/negativity was tested by qRT-PCR. Microbial transcript content and abundance was inspected was measured by *Kraken 2* using human-unmapped reads. Expression profiles for human and SARS-CoV-2 transcripts were studied with differentially expressed genes analysis. SARS-CoV-2 variants in our samples were classified using *Galaxy* pipeline and *Nextclade*.

Results: We observed that our samples contained various microbes, mostly *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria*. Differential expression and pathway enrichment analyses showed mostly disease or immunity related and signalling pathways. There were detected Alpha, Delta and Omicron variants in our study cohort.

Conclusion: SARS-CoV-2 infection is influencing host transcription profile. We will be further inspecting what is the relationship between the local host, virus and microbial gene expression.

Grant references: This work was supported by the OPII programme as the project - Research on COVID-19 progressive diagnostic methods and biomarkers useful in early detection of individuals at increased risk of severe disease, ITMS: 313011ATA2, co-financed by the ERDF.

Conflict of Interest: Dominik Hadzega Medirex Group Academy n.p.o., Klaudia Babisova Medirex Group Academy n.p.o., Michaela Hyblova Medirex Group Academy n.p.o., Nikola Janostiakova Medirex Group Academy n.p.o., Peter Sabaka: None declared, Gabriel Minarik Medirex Group Academy n.p.o., principal investigator, APVV agency, VEGA grant agency

P18.093.A Hydra-genetics, a modular framework for bioinformatics pipeline development

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Background: Processing information from massively parallel sequencing/next-generation sequencing (NGS) data involves several steps that transform millions of rows of input data into more accessible genetic information. The combination of bioinformatics tools that extract all requested information for a particular clinical/research application, how they are tuned and the order in which they are executed constitute a bioinformatics pipeline. Software is often re-used in several pipelines and regularly updated. For clinically validated NGS pipelines it may be challenging when individual components of several pipelines needs updating or when tools are replaced with new applications.

Methods: The Hydra-genetics framework takes advantage of version controlled Snakemake modules. Pipeline steps are split into modules that can be configured and tested individually. The modules can be combined to build complete bioinformatics analyses, or be added to existing pipelines. All modules are subjected to extensive testing to ensure that new releases do not unexpectedly break existing pipelines or deviate from guidelines and best practices on how to write code.

Results: Bioinformaticians from five Genomics Medicine Sweden centers used Hydra-genetics to develop the bioinformatics pipeline for the comprehensive solid tumor panel, GMS560. The pipeline analyses tumor DNA and/or RNA data and generates information on genetic variation including complex biomarkers such as tumor mutation burden and microsatellite instability. It is validated and in clinical use.

Conclusions: The Hydra-genetics framework provides a platform for structured bioinformatics pipeline development and facilitates joint development projects involving multiple partners. It makes clinical pipeline development easier, faster and more structured.

Conflict of Interest: None declared

P18.095.C A machine learning approach for the detection of incidental findings in genetic testing

Silvia Berardelli[1], *Federica De Paoli*[2], *Giovanna Nicora*[2], *Ivan Limongelli*[2], *Ettore Rizzo*[2], *Paolo Magni*[1], *Susanna Zucca*[2]

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Introduction: Medical and ethical implications on reporting incidental findings (IF) in genetic testing results raised up a controversial worldwide debate amongst genetic community. Currently, the American College of Medical Genetics and Genomics (ACMG) recommends reviewing and reporting pathogenic variants in 78 genes as IFs (PMID:23788249, PMID:34012068). Beyond this list, causative variants prioritization among thousands could be challenging: even the most advanced Variant Interpretation systems struggle to discriminate the variants mostly related to patient's phenotypes. Machine Learning (ML) could help refining the prioritization, identifying IFs.

Materials and Methods: We developed an approach capable of tagging possible IFs in a list of prioritized candidate variants, given the patient's phenotypes. Built upon the eVai Suggested Diagnosis (www.engenome.com), this method tags variants reported in the IF ACMG genes list, and predicts additional IFs by exploiting variants pathogenicity and phenotypic similarity based on the Human Phenotype Ontology. The model was trained to distinguish IF from causative variants on in-house and public dataset of diagnosed patients and validated using independent data from the Deciphering Developmental Disorder (DDD) study (PMID:25533962).

Results: On 100 repeated hold-out, the model showed promising results (F-score = 82%). On 160 validation samples the model shows 90% accuracy in the identification of the causative variant. The number or variants prioritized by the Suggested Diagnosis is reduced by 80% through IF tagging.

Conclusion: Exploiting a ML model that combines ACMG guidelines and phenotypic information to tag IFs allows the reduction of the number of candidate variants to be reviewed, speeding up genetic diagnosis.

Conflict of Interest: Silvia Berardelli: None declared, Federica De Paoli enGenome Srl, Patent request N: 102021000006353, Giovanna Nicora enGenome Srl, Ivan Limongelli enGenome Srl, Patent request N: 102021000006353

He has shares in enGenome Srl, Ettore Rizzo Patent request N: 102021000006353

He has shares in enGenome Srl, Paolo Magni Patent request N: 102021000006353

He has shares in enGenome Srl, Susanna Zucca enGenome Srl, Patent request N: 102021000006353

She has shares in enGenome Srl

P18.096.D Rare variant gene-based burden testing aids gene discovery in rare Mendelian diseases - an Exomiser-based R pipeline

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Background/objectives: The large scale of current sequencing datasets in rare Mendelian diseases, such as the 100,000 Genomes Project (100KGP) in the UK, now allows for the application of case-control association methods that have been more typically used to identify gene susceptibility in complex disorders, e.g. rare variant gene-based burden testing for Mendelian disease gene discovery. Yet, analytical tools for such analyses at scale are not largely available.

Methods: In a recent report of the rare disease component of the 100KGP (27,591 families and 197 different rare disorders available at the time of the analysis; Smedley et al., *NEJM*, 2021), we have developed an Exomiser-based analytical framework for gene-based burden testing. Exomiser is a phenotype-aware (Human Phenotype Ontology, HPO-based) variant prioritisation software that uses semantic similarity to integrate genotype–phenotype animal model data into the search for Mendelian causative variants. This analysis led to the identification of 3 new disease-gene discoveries that have been recently independently confirmed and 19 new associations.

Results: We have now extended our pipeline for generic use beyond the application to the 100KGP data and complemented it with visualisation scripts to produce volcano, Manhattan, variant lollipop and HPO-based plots. We also repeated the analysis of the 100KGP data on a larger set of families (i.e. 35,008) and identified 259 most-probable, novel disease-gene associations for further investigation.

Conclusion: By making our analytical framework openly available and for generic use beyond the 100KGP data, we expect to aid substantially gene discovery in rare Mendelian diseases.

Grant references: NIH 1R01HD103805-01

Conflict of Interest: None declared

P18.097.A Open Targets Genetics: common and rare variant gene-based analyses increase the accuracy of our locus to gene model

Yakov Tsepilov[1;2], *Daniel Considine*[1;2], *Xiangyu Ge*[1;2], *Ellie McDonagh*[2;3], *Ian Dunham*[1;2;3], *Maya Ghoussaini*[1;2]

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Background/Objectives: We have previously developed a machine learning framework to systematically predict causal gene(s) at established human GWAS loci. A combination of genomic features comprises the locus-to-gene (L2G) predictive features for model training detailed in past publications. Here, our aim is to increase the accuracy of the model, by adding the results of gene-based analysis (GBA), gene set analysis (GSA) and tissue enrichment analysis (TEA) as novel features.

Methods: Rare variant burden test p-values were sourced from seven projects and used as features. Common variant GBA and GSA were performed using MAGMA. For pathway analysis, we used curated gene sets and GO terms from the Molecular Signatures Database. TEA was performed with the EPIMAP dataset

using the CHEERS method on all GWAS in the Open Targets Genetics (OTG) portal (https://genetics.opentargets.org/). TEA results were used to weight existing GWAS-QTL colocalisation features.

Results: Inclusion of rare variant burden tests increased the average precision of L2G by 1%, and AUROC increased by 0.5%. Similar results were observed after including common variants GBA and GSA. Together these features increase precision from 0.65 to 0.69 and AUROC from 0.93 to 0.94. TEA did not significantly change the L2G accuracy, most likely because the overlap between the cell types used for tissue enrichment analysis and those available for colocalisation was limited.

Conclusion: Integration of these novel genomic features yielded a small but significant improvement in L2G accuracy. Updated results will be made available through OTG.

Conflict of Interest: None declared

P18.098.B Exploring the use of machine learning techniques and synthetic data creation with CoCoBi dataset

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Background/Objectives: The aim of this collaboration is to explore the use of machine learning techniques and synthetic data creation. We used a population-based harmonized dataset created in the CoCoBi (Connecting Cohorts with Biobanks) infrastructure project from THL Biobank. The dataset contains disease history information provided by cohort participants via questionnaire at baseline visit, and genome-wide imputed SNP data.

Methods: The CoCoBi dataset includes 362 harmonized variables, selected to support the study of healthy aging, from approximately 40,000 sample donors. The harmonized data was complemented with polygenic risk score (PRS) data for asthma, BMI, diabetes, systolic blood pressure, diastolic blood pressure, and cognitive performance. PRSs were calculated with PRS-CS method. Synthetic data version was created from the dataset, to ensure anonymity. Machine learning classification was then used as a test case on both datasets to validate the quality of synthetic data and its creation method.

Results: Diabetes could be predicted from the CoCoBi dataset with accuracy of 94% and recall of 33%. Lesser but still effective accuracy was found for 3 other diseases out the tested 27. This method could be used to focus healthcare resources to most probable cases.

Conclusions: Using real healthcare data is complex for business partners and non-EU researchers because of strict regulation. If real datasets could be converted into synthetic datasets, it would be easier to give access to the data to researchers for different purposes, since it is not considered personal health data.

Grant References:

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Conflict of Interest: None declared

P18.099.C altAFplotter – a web app for reliable UPD detection in NGS diagnostics

***Johanna Moch**[1], Maximilian Radtke[1], Thomas Liehr[2], Thomas Eggermann[3], Christian Gilissen[4], Rolph Pfundt[5], Galuh Astuti[4], Rami Abou Jamra[1], Julia Hentschel[1], Isabell Schumann[1]*

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Background: Uniparental disomy (UPD) is the inheritance of both alleles of a chromosome from only one parent. So far, the detection of UPDs in sequencing data is not well established and a common gap in NGS diagnostics. To address this we developed a tool for UPD-detection based on sequencing data which is easy to use and publicly available.

Methods: We established a UPD classification approach, based on the detection of runs of homozygosity (ROH) and the distribution of maternal and paternal variants (inheritance ratio). This approach was validated with 30 positive controls, including all known UPD-types and then used to evaluate a cohort of >9000 samples (panel and whole-exome data) in a single, duo or trio-constellation. Novel UPDs identified were validated with a secondary method.

Results: We defined cutoff values that allow for reliable UPD detection. Suspicious runs of homozygosity and inheritance distributions are identified and affected chromosomes are flagged accordingly. A sensible selection of cutoff values allows for the differentiation between different UPD types. With these parameters, we have identified nine UPDs in our cohort of which five are potentially causative and have gone unnoticed in previous NGS diagnostics. Detection of heterodisomies is only possible in a duo- or trio-constellation whereas isodisomies can be detected in a single analysis.

Conclusion: Our new tool aims to detect changes in alternative allele frequency and SNV inheritance distribution in clinical NGS data, which will help to detect UPDs detection. It is designed for easy accessibility and offers a comprehensive UPD interpretation guide.

Conflict of Interest: None declared

P18.100.D The German Human Genome-Phenome Archive (GHGA) – A national infrastructure for secure archival and community-driven analysis of omics data

***Anna Benet Pages**[1;2], Christian Mertes[3;4;5]*

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The German Human Genome-Phenome Archive (GHGA) is building a secure national omics data infrastructure, aiming to enable the secondary use of human omics data for research purposes. It is part of the German National Research Data Infrastructure (NFDI) and will serve as the national hub of the Federated European Genome-Phenome Archive (FEGA).

GHGA strives to provide the necessary computing infrastructure, an ethical-legal framework to handle omics data in a data-protection-compliant and FAIR (Findable, Accessible, Interoperable, Reusable) manner, a harmonized metadata schema, and standardized workflows to uniformly process the incoming data. GHGA will be based on cloud computing infrastructures managed in a network of data generators. Utilizing the Global Alliance for

Genomics and Health (GA4GH) standards, researchers will have controlled access to raw and processed sequence data.

We will showcase the first set of tools GHGA has developed to support our communities: a FEGA-compliant metadata model that links omics data with experimental and phenotypic information to make data traceable, and tools to help overcome challenges such as legacy consent forms, consent to share secondary data, and GDPR compliance. Furthermore, GHGA has co-developed workflows (together with the nf-core community) for data analysis, benchmarking, statistical analysis and data visualization.

Initially focusing on stakeholders that drive the national efforts for research and clinical sequencing at scale (cancer, rare and infectious diseases), GHGA will enable cross-project analysis and hence promote new collaborations and research.

This project is funded by the *Deutsche Forschungsgemeinschaft (DFG)* - project number 441914366.

Conflict of Interest: None declared

P18.101.A ComPyTool – A Python based software hub for visualizing and comparing the quality of NGS data providing an operating system independent user interface

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Background: Quality control (QC) of NGS samples is critical to identify poor quality data. To enable early detection of quality issues, QC should be performed at multiple levels, from single samples to comparative QC across multiple sequencing runs. However, most open-source tools only provide QC of single samples or, at most, single sequencing runs and often require Linux knowledge.

Methods: ComPyTool is an open source Python code that combines 5 established open source tools such as FastQC to create a comprehensive overview of NGS data including raw data, alignments and variants. The data is stored in a SQLite3 database. ComPyTool can also be hosted as a local web service.

Results: ComPyTool quickly compares new NGS data with existing quality data stored in the database. All metrics are presented in interactive, customizable graphs that can be downloaded as a combined report or as separate files. The database allows comparison of samples from single runs, from different runs, or between different laboratories. As a web application, ComPyTool provides a user-friendly way to access data, download results, or interact with the database through a graphical user interface. It can also be used from the command line to be integrated into NGS analysis pipelines.

Conclusion: ComPyTool is a user-friendly software for assessing the quality of NGS data from single or multiple sequencing runs. The web application enables non-bioinformaticians to easily assess QC data for their NGS applications. It will be available on GitHub, the Python Package Index, Anaconda, and Docker.

Conflict of Interest: None declared

P18.104.D Comparative interpretation of possible consequences of variant classification performed on different platforms

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Background and Objectives: The ACMG/AMP guideline for sequence variant interpretation is a crucial resource for standardizing the classification of single nucleotide variants (SNVs). However, automated pipelines may produce inconsistent classifications for the same variant, even when following the same guideline. To investigate the reasons for these discrepancies, we examined variant profiles in ClinGen-curated variants (n = 3371) using a range of variant prediction tools and platforms. This analysis aimed to identify factors contributing to differences in variant interpretation and ultimately improve the accuracy and resolution of SNV classification.

Materials and Methods: ClinGen dataset, comprised of 618 P, 697 LP, 1023 VUS, 367 LB and 499 B variants, was annotated and classified using ACMG/AMP classification criteria utilizing Genomize-SEQ and a competitor platform. Differences between classifications were analyzed using Python and R environments.

Results: The major discrepancy was observed in VUS classification. The Genomize-SEQ Platform identified 88% of ClinGen-determined VUS variants as VUS and 8.3% as LP while 52.7% were categorized as VUS and 33.6% as LP by the competitor software. We also investigated additional pieces of evidence to illuminate this disparity.

Conclusion: We have identified PVS1, PM2, PP3, PP5 evidence codes as the main source in diverging variant classification. Further comparative reviews of multiple platforms will contribute prevention of potential misclassification of variants in Mendelian diseases. Analysts should take the tendency of "over-classification" of variants by automated pipelines and re-evaluate the results, especially when using software & pipelines with low agreement with the known truth-sets.

Conflict of Interest: Burak Islek Burak İşlek is employed full-time by Genomize., Tolga Aslan Tolga Aslan is employed full-time by Genomize., Erşen Kavak Erşen Kavak is the founder and CEO of Genomize., Tuncay Şeker Tuncay Şeker is employed full-time by Genomize.

P18.105.A Integrating structural variant calling, annotation and prioritization into whole genome analysis workflows: a practical application in the molecular diagnosis of neurodevelopmental disorders

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Background: WGS is increasingly used as a first-line diagnostic test for patients with rare genetic diseases such as neurodevelopmental disorders (NDD). Clinical applications require a robust infrastructure to support processing, storage and analysis of WGS data. The identification and interpretation of SVs from WGS data

also needs to be improved. Finally, there is a need for a prioritization system that enables downstream clinical analysis and facilitates data interpretation. Here, we present the results of a clinical application of WGS in a cohort of patients with NDD.

Methods: We developed highly portable workflows for processing WGS data, including alignment, quality control, and variant calling of SNVs and SVs. A benchmark analysis of state-of-the-art SV detection tools was performed to select the most accurate combination for SV calling. A gene-based prioritization system was also implemented to support variant interpretation.

Results: Using a benchmark analysis, we selected the most accurate combination of tools to improve SV detection from WGS data and build a dedicated pipeline. Our workflows were used to process WGS data from 77 NDD patient-parent families. The prioritization system supported downstream analysis and enabled molecular diagnosis in 32% of patients, 25% of which were SVs and suggested a potential diagnosis in 20% of patients, requiring further investigation to achieve diagnostic certainty.

Conclusion: Our data suggest that the integration of SNVs and SVs is a main factor that increases diagnostic yield by WGS and show that the adoption of a dedicated pipeline improves the process of variant detection and interpretation.

Conflict of Interest: None declared

P18.106.B Functional gene embeddings enable state-of-the-art performance for a wide range of predictive tasks in genetics

Felix Brechtmann[1], *Thibault Bechtler*[1], *Shubhankar Londhe*[1], *Christian Mertes*[1], *Brian Clarke*[2], *Oliver Stegle*[2], *Julien Gagneur*[1]

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The notion of gene function is central to genetics. However, functional annotations be free text or based on controlled vocabularies are complex and often partial or missing. Therefore, their integration into machine learning models is cumbersome and biased for well-studied genes.

Functional gene embeddings, numerical vectors capturing gene function, offer an exciting avenue to address this issue. Such embeddings are obtained by applying self-supervised approaches on various data types including quantitative measurements, protein interaction networks, and literature. However, their utility and relative performance for diverse prediction tasks has not been assessed so far.

Here we benchmarked functional gene embeddings obtained from multiple data sources for predicting disease gene lists, cancer drivers, phenotype-gene associations, and scores derived from large-scale genome-wide association studies.

To assess the embedding usefulness, we compared basic off-the-shelf prediction algorithms (elastic net, gradient boosted trees) trained directly on the embedding to state-of-the-art predictors specifically developed for the respective tasks.

Remarkably and despite their simplicity and low dimensionality (ca. 500), the embeddings always reached or outperformed state-of-the-art performances, demonstrating their high utility for modeling in genetics.

Importantly, while embeddings based on literature and low-throughput experiments performed best on predicting human-curated labels, those derived from genome-wide experimental data (transcriptomics, deletion screens, protein sequence) performed better in predicting genome-wide association signals and were not biased for highly-studied genes. These results indicate that literature-based embeddings suffer from ascertainment biases and should be avoided.

Altogether, our study and embedding resource will facilitate the development of machine learning models in genetics and related fields.

Conflict of Interest: None declared

P18.108.D Investigating the impact of rare pathogenic variants linked to Intellectual Disability genes on cognitive ability in adults: exome analysis of the UK Biobank cohort

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Objective: Identification of genetic variants (SNVs & CNVs) linked to intellectual disability (ID) (with prevalence 3-5%) has been extensively studied on children or young populations to address developmental delay and early onset of IDs. However, reduced penetrance of these variants has also resulted in unaffected obligate adult carriers in the population.The current work investigates the impact of rare and ultra-rare, protein-coding pathogenic SNVs in known ID-genes on the cognitive abilities of unaffected UK-Biobank (UKBB) individuals.

Methods: 50,000 UKBB jointly-genotyped (GATK) samples (non-ID) were processed for variant annotation (gnomAD, CADD, Clinvar etc.) and filtering. Subsequently, variants were filtered for frequency (rare: MAF $< = 1\%$, ultra-rare: AC$< = 5$), protein coding, impact, consequences, pathogenicity, deleteriousness and quality metrics.The filtered set of variants were regressed (generalised linear model) upon a set of 6 cognitive tests (CTs) scores available in the UKBB portal.

Results & Conclusions: 40 rare pathogenic variants ($p < = 0.02$) associated with these 6 CTs had both negative (53%;$n = 21$) or positive (47%; $n = 19$) impacts (with symbol digit substitution test mostly impacted). Similarly, 98 ultra-rare pathogenic variants ($p < = 0.001$) were identified with either negative (66%;$n = 63$) or positive (34%;$n = 25$) impacts on cognitive functioning (with pairs matching test mostly impacted). Cumulatively, enrichment of rare/ultra-rare pathogenic variants with reduced penetrance in a large non-ID cohort exemplifies their impact on cognitive functioning. Integrating pathway enrichment and gene network analysis will further characterise their distinct roles.

Grant: UK Research and Innovation; Canadian research funding agencies (Reference ES/T013435/1)

Conflict of Interest: None declared

P18.109.A DeepHRD-LR is an AI-based powerful and cost-effective method to detect GIS in ovarian cancer samples

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Background: Homologous Recombination Repair Deficiency (HRD) leads to tumor genomic instability (GIS) and sensitivity to PARP inhibitors in ovarian cancers (OC), and the identification of

HRD status is considered a major therapeutic issue. The current determination of the HRD status is the subject of much research and development: as four new methods were clinically validated in Europe in 2022. We developed and technically validated deepHRD-HR, combining high-resolution genomic backbone sequencing and an AI algorithm for GIS determination in OC. We tested a low resolution approach, deepHRD-LR, to obtain the best minimal set of data to detect GIS in an economical perspective.

Method: Based on the data obtained for deepHRD-HR, we extracted the sequencing data of the Oneseq Low Resolution (LR) backbone design of 2.7Mb. Using AI, we determined the theoretical performances of deepHRD-LR to detect GIS in the 200 tumors sequencing data, and resequenced 32 tumors with the LR design to confirm efficiency of the method. We challenged the AI tool to determine GIS status with a sample set of tumors with gradually falling parameters, cell purity from 40% to 20%, and HRD scores from 47 to 38.

Results: DeepHRD-LR demonstrated over 95% sensitivity and specificity in samples from 25% cell purity with HRD score over 42.

PERSPECTIVES

DeepHRD-LR is a powerful, robust and cost-saving AI-based method for OC HRD testing. Clinical validation is ongoing with PAOLA-1 OC samples. GIS determination in breast, prostate, and pancreatic cancer is ongoing, using scarHRD algorithm on in-house WES data as gold-standard.

Conflict of Interest: None declared

P18.110.B Integrating tumor multiomics with patient-specific drug screening for improved cancer drug response prediction

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Background: Owing to a lack of clinically actionable molecular alterations in most tumors, standard of care cytotoxic chemotherapy remains the mainstay of cancer treatment. Deep learning (DL) models incorporate high-dimensional omics features, facilitating drug response prediction in the absence of clinically actionable vulnerabilities. However, existing methods perform poorly in precision oncology-relevant settings. To address these limitations, we developed ScreenDL, a novel deep learning-based cancer drug response prediction method.

Methods: ScreenDL accepts drug chemical structures and tumor molecular profiles as input and predicts drug response. When available, ScreenDL incorporates partial drug screening data through a patient-specific finetuning strategy. We rigorously validated ScreenDL in cell lines and patient-derived models, emphasizing the ability to predict differential drug response in never-before-seen cell lines and patients.

Results: ScreenDL outperforms state of the art DL methods in precision oncology-relevant testing, achieving a Pearson correlation of 0.55 between observed and predicted response compared to 0.32 for existing methods. When possible, incorporating partial, patient-specific drug screening data further improves overall Pearson correlation to 0.70. Importantly, biomaterial from surgical tumor resection is often available for patient-specific drug screening in practical clinical scenarios, informing an approach to precision oncology wherein deep multiomic tumor characterization and patient-specific drug screening can be integrated to provide reliable precision treatment recommendations.

Conclusion: Here, we demonstrate the utility of incorporating partial, patient-specific drug screening data with deep multiomic tumor characterization for improved cancer drug response prediction. ScreenDL represents an exciting new direction, bringing deep learning-based cancer drug response prediction models closer to clinical application.

Conflict of Interest: None declared

P18.111.C A web-based bioinformatic tool LYNX for targeted LYmphoid NeXt-generation sequencing data analysis and visualization for hematooncology

Tomas Reigl[1], ***Jakub Porc***[1;2], *Veronika Navrkalová*[1;2;3], *Jakub Hynšt*[1], *Karol Pál*[1;2], *Kamila Stránská*[1;3], *Jana Kotašková*[1;2;3], *Sarka Pospisilova*[1;2;3], *Karla Plevová*[1;2;3]

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Background/Objectives: Next-generation sequencing (NGS) is one of the most rapidly expanding technologies in laboratory medicine. In routine diagnostics, a single NGS experiment can replace several laboratory tests and provide more information about the disease being tested. Commonly, the assistance of a qualified bioinformatician or a dedicated tool is necessary for the data analysis.

Methods: Our team has created an integrative, targeted NGS panel that detects genetic markers common for most abundant lymphoid malignancies (PMID: 34082072). For this panel, we have developed a bioinformatic tool, LYNX, with a user interface (UI) that allows diagnosticians to easily analyze the data and interactively visualize the results. This facilitates data interpretation and speeds up diagnostic procedures.

Results: We have built a web-based bioinformatic tool LYNX for targeted NGS data analysis. LYNX utilizes computational pipelines to provide information about single nucleotide variants in specific genes, copy number variations in the whole genome, antigen receptor rearrangements and lymphoma-specific translocations. The UI allows users to execute the analysis and presents the results in interactive tables and graphs.

Conclusion: LYNX bioinformatic tool streamlines the analysis and interpretation of data from targeted NGS panels for laboratories that lack in-house bioinformatic support. The UI allows diagnosticians to perform interactive data analysis and exploration, helping them to identify genetic markers for individual patients. In addition, the modular implementation of our tool allows adaptation to different diagnostic panels, making it transferable to other diagnostic applications.

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Conflict of Interest: None declared

P18.112.D Characterizing the inaccurate quality metric in genotype imputation using the TOPMed reference panel

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Genotype imputation is a common practice in genome-wide association studies. The imputation accuracy (squared correlation between imputed dosage and true genotype; Dosage-Rsq)

depends largely on the reference panel and target population. We imputed the East Asian (EAS) using the TOPMed panel and found that the quality metric (Rsq) was much higher than Dosage-Rsq. Our variance component analysis of Rsq showed that the specific imputed-dosage distribution (closer to 0 and 1) in the TOPMed imputation caused the upward biased Rsq, indicating some systemic bias in the imputation process. In fact, we revealed that the increased Rsq could be attributed to the decreased template switching rate (θ value) of the hidden Markov model in the imputation pipeline, whereas Dosage-Rsq was roughly maintained in that situation. In simulated multi-ancestry panels, the estimated θ value decreased with panel size and increased with ancestral diversity. For example, for imputing European samples, adding 3,760 EAS samples to a European panel of size 403 resulted in a 50.8% decrease in the θ value and 22.4% more variants passed Rsq > 0.7. For imputing EAS samples, adding the 1KG samples to a Japanese panel of size 3,256 resulted in a 29.2% increase in the θ value and 3.69% fewer variants passed Rsq > 0.7. In conclusion, we underscored the θ value's impact on Rsq. Appropriate θ value, target population, panel size and ancestral component should be considered to obtain accurate Rsq and facilitate downstream analyses.

Grant References: This study is supported by AMED Grant Number JP20km0405215.

Conflict of Interest: None declared

P18.113.C Cross-trait genetic analyses indicate pleiotropy and complex causal relationships between headache and thyroid function traits

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Epidemiological studies have reported a comorbid relationship between headache and thyroid traits; however, little is known about the shared genetics and causality that contributes to this association. We investigated the genetic overlap and associations between headache and thyroid traits using genome-wide association study (GWAS) data. We found a significant genetic correlation (r_g) with headache and hypothyroidism ($r_g = 0.09$, $p = 2.00 \times 10^{-4}$), free thyroxine (fT4) ($r_g = 0.08$, $p = 5.50 \times 10^{-3}$), and hyperthyroidism ($r_g = -0.14$, $p = 1.80 \times 10^{-3}$), a near significant r_g with secondary hypothyroidism ($r_g = 0.20$, $p = 5.24 \times 10^{-2}$), but not with thyroid stimulating hormone (TSH). Pairwise-GWAS analysis revealed six, 14, four and five shared loci with headache and hypothyroidism, hyperthyroidism, secondary hypothyroidism, and fT4, respectively. Cross-trait GWAS meta-analysis identified novel genome-wide significant loci for headache: five with hypothyroidism, three with secondary hypothyroidism, 12 with TSH, and nine with fT4. Of the genes at these loci, six (*FAF1*, *TMX2-CTNND1*, *AARSD1*, *PLCD3*, *ZNF652*, and *C20orf203*; headache-TSH) and six (*HMGB1P45*, *RPL30P1*, *ZNF462*, *TMX2-CTNND1*, *ITPK1*, *SECISBP2L*; headache-fT4) were significant in our gene-based analysis. Our causal analysis suggested a positive causal relationship between headache and secondary hypothyroidism ($p = 3.64 \times 10^{-4}$). The results also suggested a positive causal relationship between hypothyroidism and headache ($p = 2.45 \times 10^{-3}$) and a negative causal relationship between hyperthyroidism and headache ($p = 1.16 \times 10^{-13}$). These findings suggest strong evidence for genetic correlation and complex causal relationships between headache and thyroid traits.

Conflict of Interest: None declared

P19 Personalized Medicine and Pharmacogenomics

P19.001.A Blood donor biobank as a resource for personalised genetic studies and blood donation conditions

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Backround Health questionnaires and healthy donor effect, HDE, result to accumulation of individuals with better than average health status in blood donor population. To evaluate the usefulness of blood donor-based biobank in personalised disease-associated genetic studies and in creating personalised blood donation conditions, we investigated how well a blood donor-based biobank material can provide for these purposes.

Methods Frequencies of 53 rare disease-associated and two iron overload related variants, C282Y and H63D, in *HFE* gene and one bleeding disorder mutation, rs771048666, in *GP1BA* gene were analysed among the blood donors, N = 35,239. Results of genome level PCA and donor's postcode were used to localize the carriers of the rare variants in Finland.

Results 80.7 % of blood donors carried at least one of the rare variants. Enrichment of donors carrying multiple rare variants was seen in the Kainuu region in Finland and overall enrichment of rare variants in the East of Finland. Amount of donors homozygous for C282Y mutation was 40.7 % higher than expected. C282Y/H63D compound heterozygotes and rs771048666 as heterozygous were found as expected.

Conclusion We demonstrate that despite of the commonly known HDE of blood donors, blood donor-based biobank is a useful resource in personalised genetic studies. Geographical genetic substructure of Finland should be taken into account. Furthermore, we show that blood donor biobank material can be utilized for personalized blood donation conditions; 1) taking donors genetic iron overload risk into account when providing post-donation iron supplementation 2) taking rs771048666 genotype into consideration when recruiting for thrombocyte donation.

Conflict of Interest: None declared

P19.002.B Implementing a long-read sequencing panel for pharmacogenomics: using the CYP3A locus as an example

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Background/Objectives: Pharmacogenomics aims to unravel the genetic contribution to drug response. Interestingly, many important pharmacogenes are located in complex regions of the genome, including the CYP3A locus containing CYP3A4, CYP3A5 and CYP3A7. Long-read sequencing is required to resolve and phase these complex genomics regions into individual haplotypes.

Methods: We developed a protocol for long-read targeted sequencing using capture probes from Twist Bioscience and applied this workflow to sequence 21 pharmacogenes from 41 samples with PacBio HiFi technology.

Results: In total, 41 samples had an average on target phasing of 62% (47%-73%) and the average haploblock size was 7,509bp demonstrating the large number of nucleotides in the target region that were phased. In the CYP3A locus, 1,088 unique variants were detected, of which 570 variants were located in the core regions of CYP3A4, CYP3A5 and CYP3A7. Only 27 of these variants (2%) are included in the clinically used *-allele nomenclature. Notably, 1 frameshift-, 5 missense- and 8 splice site variants which are not included in clinical nomenclature were detected. Per individual, an average of 155 unique variants were detected and 34% (5% - 86%) of nucleotides were phased in the CYP3A locus.

Conclusions: Our results indicate that a panel-based long-read sequencing approach can phase the majority of variants in complex genomic regions, revealing a high abundance of unknown but potentially impactful variants in the CYP3A locus.

Grant Reference: Not applicable

Conflict of Interest: Qinglian Zhai Full, maaike van der lee Full, Roberta Menafra: None declared, Loes Busscher: None declared, Redmar R. van den Berg: None declared, Susan Kloet: None declared, Jesse Swen Full

P19.003.C Implementation of polygenic risk scores from sequencing data towards practice by utilizing large publicly available datasets

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Introduction: Polygenic risk scores (PRSs) are a potential tool for assessing an individual's genetic predisposition for diseases or traits. Current methodologies use genome-wide summary statistics from SNP-array data. Yet, the applications might be limited due to ascertainment biases of the SNP array. Next-generation sequencing technologies such as whole-exome sequencing (WES) have higher resolution of genetic variants and reduced batch effects, allowing higher interoperability with other population cohorts. We aimed to test the applicability of WES data from large-population datasets for calculating PRSs to analyze smaller sample sizes.

Methods: We developed a workflow that processes WES data from large-reference datasets to estimate PRSs in small datasets with similar population characteristics. It generates a base exome-wide summary statistics and target dataset to increase the sample size of the small cohort. We applied the workflow using the 200,643 exomes from the UK Biobank and 30 exomes from our in-house data, IAM Frontier. We calculated PRSs for blood pressure, body mass index, LDL cholesterol, and vitamin D.

Results: The workflow generated scores with significant performance (P-value $< 2 \times 10^{-6}$) for the selected traits. For the IAM Frontier cohort, the scores had a predictive performance (R^2) of up to 11.54%. The generated scores had similar performance; compared to the reported ones in the Polygenic Score (PGS) Catalog.

Conclusions: We successfully estimated PRSs using the UK Biobank WES data for the IAM Frontier cohort. To our knowledge, this is the first study that reports PRSs purely from sequencing data.

Grant references: Flemish Special Research Fund (BOF) [BOF21DOC23].

Conflict of Interest: Alejandro Correa Rojo Full time, Flemish Special Research Fund (Bijzonder Onderzoeksfonds - BOF). Grant Project Number: BOF21DOC23, Dirk Valkenborg Full time, Flemish Special Research Fund (Bijzonder Onderzoeksfonds - BOF). Grant Project Number: BOF21DOC23, GOKHAN ERTAYLAN Full time

P19.004.D A polygenic model for Japanese height implicates the importance of localization

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Background/Objectives: Height is implicated in skeletal growth disorders and used as a model trait for studying polygenic inheritance. In a previous GWAS involving over 5 million individuals of diverse ancestry, 12,111 independent variants were identified as being significantly associated with height (Yengo et al. Nature, 2022). The cross-ancestry polygenic score (PGS) calculated based on this GWAS showed prediction accuracy (R^2) of up to 0.447 for the actual height of Europeans adjusted for age, sex, and 10 genetic principal components (PCs). However, the prediction accuracy of the PGS was significantly attenuated for the height of other populations, such as Africans (~0.154) and East Asians (~0.205). In this study, we aimed to improve the prediction accuracy of PGS for the height of East Asians.

Methods: We constructed models using PRS-CS (Ge et al. Nat. Commun., 2019) based on a previously reported GWAS for a height of 165,056 Japanese subjects (Sakaue et al. Nat. Genet., 2021). The best model was selected based on its correlation with the height of 9,811 Japanese individuals and included 1,031,950 variants from the 1000 Genomes Phase 3 dataset.

Results: The prediction accuracy of the PGS for the adjusted height of 53,305 Japanese individuals was 0.66, which is similar to heritability estimates of a height based on whole-genome sequence data (0.68) and pedigree estimates (0.7-0.8) (Wainschtein et al. Nat. Genet., 2022).

Conclusion: This finding may suggest the potential for optimizing a polygenic model for a specific population to achieve practically sufficient accuracy.

Grant References: 21tm0124006j0001 (AMED, Japan)

Conflict of Interest: None declared

P19.005.A Common risk factors enhance the predictive ability of polygenic scores for type 2 diabetes

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We aimed to examine associations between common variants in genes implicated in the development of type 2 diabetes (T2D) and evaluate the predictive value of polygenic scores calculated from these variants alone and in combination with other risk factors, including age and sex.

We tested associations between established genetic variants and T2D in 1371 participants from the Volga-Ural region of the Eurasian continent (496 people with T2D and 875 control participants). Weighted and unweighted polygenic scores were calculated from the genetic variants significantly associated with T2D in the study group according to the results of a logistic regression analysis.

We found associations with T2D for the *CCL20* rs6749704 ($OR = 1.68$, $P_{FDR} = 3.40 \times 10^{-5}$), *CCR5* rs333 ($OR = 1.99$, $P_{FDR} = 0.033$), *ADIPOQ* rs17366743 ($OR = 3.17$, $P_{FDR} = 2.64 \times 10^{-4}$), *TCF7L2* rs114758349 ($OR = 1.77$, $P_{FDR} = 9.37 \times 10^{-5}$), and *CCL2* rs1024611 ($OR = 1.38$, $P_{FDR} = 0.033$) polymorphisms. The most informative prognostic model included weighted polygenic scores for these five loci, and non-genetic factors such as age and sex (AUC 85.8%, 95%CI 83.7%-87.8%).

The polygenic approach has shown its efficacy in predicting susceptibility to T2D, especially in combination with other, non-genetic risk factors, such as age and sex. The five variants associated with T2D in people from the Volga-Ural region are linked to inflammation (*CCR5*, *CCL2*, *CCL20*) and glucose metabolism regulation (*TCF7L*, *ADIPOQ*). Further studies in independent groups of people with T2D should validate the prognostic value of the model and elucidate the molecular mechanisms of the disease.

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Conflict of Interest: None declared

P19.006.B EJHG series: Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction between CYP2C9 and HLA and anti-epileptic drugs

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Introduction: The Dutch Pharmacogenetics Working Group (DPWG) aims to facilitate PGx implementation by developing evidence-based pharmacogenetics guidelines to optimize pharmacotherapy. These guidelines are integrated in the Dutch Clinical Decision Support Systems and are used during drug prescribing and dispensing. To disseminate these pharmacogenetic guidelines, a series of articles are being published in the European Journal of Human Genetics. Here we report the guideline on *CYP2C9*, *HLA-A* and *HLA-B* and carbamazepine, lamotrigine, oxcarbazepine, and phenytoin.

Methods: A systematic review of the literature was performed, relevant articles were summarized and therapeutic recommendations were proposed by a scientist of the Royal Dutch Pharmacists Association (KNMP). All included articles were scored for level of evidence and clinical relevance. All summaries and scores were checked and discussed in the multidisciplinary DPWG. If scores differed, consensus on a score was reached within this meeting.

Results: For *CYP2C9* intermediate and poor metabolizers the DPWG recommends lowering the daily phenytoin dose by 25-60% depending on the genotype. *For HLA-B*15:02*, *HLA-B*15:11* and *HLA-A*31:01* positive patients starting on carbamazepine, lamotrigine oxcarbazepine or phenytoin, the DPWG recommends an alternative anti-epileptic drug due to increased risk of severe cutaneous adverse reactions. If an alternative is not possible, it is recommended to advise the patient to report side effects including any rash as soon as possible.

Conclusion: To date, 6 DPWG guidelines have been published in EJHG, concerning fluoropyrimidines, opioid analgesics, SSRI's, gout and arthritis medication, ADHD medication and irinotecan. Another 15 additional guidelines are expected to be published in 2023-2025.

Conflict of Interest: None declared

P19.007.C Risk assessment for hypercholesterolemia using LDL and LPA genetic scores

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Objective: Identification of disease etiology in Hypercholesterolemia by an efficient screening approach. Genetic testing is a routine procedure in patients with suspected familial hypercholesterolemia. However, causative variants in familial hypercholesterolemia genes are identified in less than 50% of cases. Most prominently elevated low-density-lipoprotein cholesterol (LDL-C) levels are causative of hypercholesterolemia but also elevated lipoprotein(a) [Lp(a)], both resulting in a 3-10-fold increase in coronary artery disease risk. To enhance the diagnostic output we established an array screening approach to analyse genetic scores associated with LDL-C and Lp(a).

Methods: 1.020 individuals including 252 clinically diagnosed hypercholesterolemia patients from the Austria FH Register and 768 population controls were analyzed. The Illumina Global Screening Array was used as the primary source of genetic data complemented by NGS and Sanger sequencing. For each individual, validated genetic scores associated with LDL-C and Lp(a) values were calculated based on imputed genotypes (Michigan Imputation Server).

Result: Elevated LDL-C in patients with high LPA score is confirmed to be partly due to cholesterol in Lp(a)-particles. The PPV of the applied genetic score is 90% and can be used to estimate biochemical levels. By introducing genetic scores for LDL-C and Lp(a) the number of individuals with a defined disease aetiology was increased from 46.6% to 68.8%.

Conclusion: The proposed screening approach resulted in a more precise diagnosis of the underlying cause of hypercholesterolemia. Analysing monogenic cause and genetic scores for LDL-C and Lp(a) will allow the application of optimal treatment strategies in the future.

Acknowledgments: Austrian FH-Register, Austrian Heart-Foundation.

Conflict of Interest: None declared

P19.008.D Blockchain and Artificial Intelligence-Enabled Stratified Trial System (BESTS) - A patient driven platform that leverages clinical and genomic data to accelerate clinical trial recruitment for precision therapies

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Background: Personalised medicine aims to provide individual treatment strategies for patients, often on the basis of an underlying monogenic cause. With accelerating genomic discovery, the number of potential targets that might enable a precision approach is rising. Additionally, clinical trials will become more personalised and stratified. However, recruitment of patients to studies is a significant barrier. We present BESTS, a cloud-based platform developed for collaborative use by patients, healthcare providers (HCPs) and clinical research organisations. It allows patients to be matched to trials via their clinical and genomic information, while retaining control and ownership of that data.

Methods: A process of requirements engineering and prototyping informed layout design and platform development. Twenty-five participants representing patients and HCPs engaged in a series of qualitative research methods to identify value proposition and user requirements across four core modules: dynamic consent enabled by blockchain; a genomics profile whereby patients can upload pre-existing data or access sequencing; a clinical profile with condition-specific information and an AI-Trial matcher to interrogate clinical and genetic data against eligibility criteria on clinical trial databases.

Results: BESTS value propositions for patients include becoming more empowered around use of their genetic data, greater knowledge of ongoing research and personalised trial-matching. Value propositions for HCPs includes the ability to quickly identify patients and demonstrate suitability as trial site.

Conclusion: User-centred design in BESTS development enables real-world effectiveness. Access to genomic and phenome data through BESTS provides for high-resolution recruitment to trials, facilitating faster introduction of treatments into care pathways.

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Conflict of Interest: None declared

P19.009.A Copy number variation in GSTM1 is associated with poor prognosis in an African population

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Background/Objectives: Genetics contribute significantly (80%) to inter-individual variations in treatment response, but there are still limited pharmacogenomic studies on cervical cancer among Africans. This study describes the role of host genetic polymorphisms on cervical cancer treatment response in an African population.

Methods: A prospective, case-only study recruited 201 women with newly diagnosed histologically confirmed cervical cancer prescribed to receive cisplatin. Pre-treatment blood was drawn to characterize genetic polymorphisms in cisplatin transporter, *ABCC2* rs717620 A>G; and metabolizers, *GSTP* rs1695A>G; *GSTM1* del/del; *GSTT1* del/del and *NQO1* rs1800566 C>T. Participants were also followed up over 12 months for cisplatin-induced toxicities, survival and disease status.

Results: *GSTP* rs16895 G was associated with protection against toxicities (OR = 0.1; 95% CI = 0.1-0.4; p = 0.012). *GSTM1 del/del* (OR = 3.5; 95% CI = 1.3-9.8; p = 0.016) was associated with relapse.

Conclusion: We report for the first time in an African population that *GSTM1* del/del is associated with poor prognosis after cisplatin therapy, confirming data from the Clinical Pharmacogenetics Implementation Consortium data that earmarks *GSTM1* deletion as a promising cisplatin pharmacogenetic marker. Therefore, *GSTM1* del/del carriers may not benefit from cisplatin and may need alternative therapies towards personalizing medicine in Africa where the cervical cancer burden is disproportionately high.

Grant references: Organisation for Women in Science for the Developing World

Conflict of Interest: None declared

P19.010.B Pharmacogenetic effect of cytochrome P450 3A4 and CALCR genes polymorphism on treatment response in patients with hip fracture and osteoporosis

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Background/Objectives: Gene polymorphisms research is important in personalized therapy. Cytochrome P4503A4 plays a key role in xenobiotic and endogenous substrates metabolism, calcitonin receptors - in bone development, remodeling and disease, calcium stone urolithiasis. The CYP3A4 and CALCR genes are located on the same arm of chromosome 7, belong to the osteoporosis gene network. The aim of the work was to assess the effects of CYP3A4 and CALCR genes polymorphisms on patients with hip fracture and osteoporosis treated by the standard protocols.

Methods: Surgical and conservative treatment of patients with fracture of the proximal femur (31 B1-B3 AO/ASIF), coxarthrosis (1-2 st. by Kelgren) was carried out. The genotyping was carried for CYP3A4 A290G, CALCR C1340T (n = 22). The linkage disequilibrium (LD) was estimated by D', r2.

Results: D'(r2) for SNPs analyzed were: 7q22.17/7q21.37 - 0,027 (0,0020). Genotypes distribution was 86.4%:9.1%:4.5% for AA:AG:GG and 19.4%:75.0%:5.6%, for TT:TC:CC. The frequency of genotypes AG + GG was 13.6%. In patients A290A of CYP3A4 gene, associated with drugs metabolism, including enalapril

maleate, metamizole sodium, omeprazole, fluconazole, diphenhydramine, ondansetron, rates of BMD (4081.7±37.9), HHS (85.0±1.6) were were higher than in patients with allele G (3836.7±32,8; 58,7±4,4), whereas functional disorders of the excretory system were two times less. Patients T1340T of CALCR gene had higher rates of BMD (4110.0±70.0), calcitonin (5.2±3.2), and HHS (91±1.0). The lowest values of HHS were determined in patients AG + GG/TC (58,7±4,4).

Conclusions: Reduced adaptability of patients G290G + A290G/T1340C, including the combined use of drugs in surgical and conservative treatment, was noted.

Conflict of Interest: None declared

P19.011.C Repurposing of HLA genotyping results to prevent drug hypersensitivity reactions

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Introduction: From pharmacogenomic studies it is known that the risk alleles HLA-A*31:01, HLA-B*15:02, HLA-B*15:11, HLA-B*57:01 and HLA-B*58:01 are associated with an increased risk of developing drug hypersensitivity reactions induced by abacavir, allopurinol, carbamazepine, oxcarbazepine, phenytoin, lamotrigine or flucloxacillin. Pre-emptive genotyping for these alleles is only introduced for abacavir-HLA-B*57:01. Since transplant patients are routinely genotyped for HLA genes to assess whether donor and recipient can be matched, we aimed to investigate the feasibility of repurposing HLA genotyping results for the prevention of drug hypersensitivity reactions.

Methods: HLA genotyping by Next Generation Sequencing (NGS) is routinely performed in the Leiden University Medical Center (LUMC) for all transplant recipients and donors. We collected the HLA genotypes of 1345 transplant recipients who were HLA typed using NGS within the LUMC since 2005.

Results: 13.3% of our transplant cohort patients carried at least one of the five HLA risk alleles and are therefore at risk for drug induced hypersensitivity. The most prevalent risk alleles were HLA-A*31:01, HLA-B*57:01 and HLA-B*58:01 with a carrier frequency of respectively 4.7%, 4.5% and 3.5%. HLA-B*15:11 was not found in our cohort.

Conclusions: Repurposing HLA NGS genotyping results for the prevention of drug hypersensitivity reactions is feasible and leads to the detection of patients at risk for drug induced hypersensitivity. Although the positive predictive value of the HLA tests is low, due to the severity of the associated drug hypersensitivity reactions, documenting these risk alleles as a contraindication in the electronic health record, may prevent drug hypersensitivity reactions of transplant recipients.

Conflict of Interest: None declared

P19.012.D Assessing medication-associated risk of Type-2 Diabetes using eQTL and pQTL-based Mendelian randomization: statins and PCSK9 inhibitors

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Background/ Objective: Cardiovascular disease is the largest cause of global mortality and morbidity. One of the main factors is high levels of LDL-cholesterol; hence, lipid-lowering medication forms an essential part of prevention and treatment of cardiovascular disease. However, there is evidence that some lipid-lowering medications (e.g. statins) increase the risk of type-2-diabetes (T2D), despite their beneficial effects on lipids. A relatively new lipid-lowering drug is PCSK9-inhibitors (PCSK9i). The impact of PCSK9i on T2D risk is currently unclear, and we aimed to assess the association of PCSK9i with the risk of T2D.

Methods: We used genetically-reduced *PCSK9* gene-expression (eQTLs) in multiple tissues from GTEx.v8, eQTLGen, and STARNET consortiums, and PCSK9 protein levels (pQTLs) in European-ancestry individuals as a proxy for PCSK9i to assess the impact on T2D using Mendelian randomization. We also assessed effects on BMI and LDL-cholesterol levels. Reduced *HMGCR* gene-expression levels (proxying statins) was used as a comparator for the relationship with T2D.

Results: We found that neither genetically-reduced *PCSK9* gene-expression levels nor circulating-PCSK9 levels were associated with BMI($p = 0.2$-0.7) the risk of T2D($p = 0.09$-0.2). However, reduced *HMGCR* gene-expression levels were significantly associated with increased BMI($p = 1.4 \times 10^{-7}$) and increased risk of T2D($p = 4.4 \times 10^{-3}$), in line with existing evidence.

Conclusion and Relevance: *PCSK9* gene-expression levels and protein levels, used to proxy PCSK9i, were not associated with risk of T2D, but *HMGCR* gene-expression levels (proxying statins) were associated with increased risk of T2D, possibly through increased BMI. This study informs the ongoing debate as to the impact of PCSK9i on BMI and T2D risk.

Conflict of Interest: None declared

P19.013.A Prevalence of rare deleterious variants in idiopathic pulmonary fibrosis patients in a Spanish cohort

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Background: Idiopathic pulmonary fibrosis (IPF) is a chronic, rare progressive lung disease characterized by lung scarring. Genetic studies have confirmed the role of common and rare variants in its etiology. Here we relied on whole exome sequencing (WES) to assess the contribution of rare deleterious variants (RDVs) in sporadic forms of IPF in a Spanish cohort.

Methods: We obtained samples from 100 unrelated patients with sporadic IPF. WES was performed using a HiSeq 4000 Illumina system. Small germline variant identification based on BWA-GATK v3.8 and the GRCh37/hg19 as reference. An in-house bioinformatic

pipeline was developed to identify RDVs (frequency < 0.01% and CADD > 15) in telomere and non-telomere related-genes previously linked to IPF.

Results: We found 28 RDVs in 21 individuals, most commonly affecting *TERT*, *RTEL1* and *KIF15*. Of these variants, nine were classified as pathogenic or likely pathogenic, the majority affecting *TERT* and *RTEL1*, which translates into a diagnostic yield of WES of 9% (95% CI: 4.8-16.2) in the cohort.

Conclusion: Together, our results indicate that up to 21% (95% CI: 14.2-29.9) of individuals of the cohort have RDVs in IPF genes, which may be causal. Subsequent polygenic risk scores studies will be conducted to assess differences in RDVs between carriers and non-carriers.

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P19.015.C Precision Drug Repurposing and Precision Risk Prediction for Metabolic Syndrome Endophenotypes in UK and Taiwan Biobanks

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Background: Metabolic syndrome (MetS) is highly heterogenous with detrimental clinical outcomes and high worldwide prevalence.

Methods: Clinically-relevant endophenotypes were identified through cluster analysis in UK Biobank MetS individuals. GWAS were performed to identify genotypic variants associated with each endophenotypes. Potential drug repurposing targets were established from GWAS results and unbiased identification of drugs targeting associated genes. Heritability and PRS models for each endophenotypes were calculated. MetS endophenotypes were further explored in Taiwan Biobank.

Results: Five MetS endophenotypes were identified; Cluster 1 (C1): non-descriptive (n = 33,707), Cluster 2 (C2): hypertensive (n = 23,215), Cluster 3 (C3): obese (n = 30,089), Cluster 4 (C4): lipodystrophy-like (n = 13,116) and Cluster 5 (C5): hyperglycaemic (n = 3,869). MetS endophenotypes showed distinct phenotypic traits and differing clinical outcomes. MetS endophenotypes were associated with partially dissimilar genotypic traits: C1 had 156 cluster-unique genes while C2 had 16, C3 98, C4 133 and C5 8. C1 associated genes were enriched in gene-set targeted by cardiovascular system drugs such as diuretics targeting *SLC12A3* and *SLC12A4*; C3 by anti-obesity drugs targeting *SLC6A2* and antithrombotic drugs targeting *F2*, *TFPI* and *VEGFA*; C4 by lipid-modifying drugs targeting *APOB* and *LPL*; C5 by drugs of alimentary tract targeting *CES1*. Using 3,800 cases subsets, PRS for endophenotypes performed better (C1 $R^2 = 0.0114$, C3 $R^2 = 0.0094$, C4 $R^2 = 0.0572$, C5 $R^2 = 0.0175$) than the heterogenous all MetS category ($R^2 = 0.0052$), with exception of C2 ($R^2 = 0.0018$) which had lowest heritability of $h^2 = 0.2198$.

Conclusion: The novel combination of unsupervised learning and genetic epidemiology methodologies allowed for potential precision drug repurposing targets and precision risk prediction specific for MetS endophenotypes.

Conflict of Interest: Aylwin Ming Wee Lim Taiwan International Graduate Program in Molecular Medicine, National Yang Ming Chiao Tung University and Academia Sinica & ASUS Intelligent Cloud Services (AICS) PhD Fellowship, Evan Unit Lim Research assistant at Institute of Biomedical Sciences, Academia Sinica, Pei-Lung Chen Physician at Department of Medical Genetics, National Taiwan University Hospital, Cathy SJ Fann Research fellow / Professor at Institute of Biomedical Sciences, Academia Sinica, Principal investigator

P19.016.D Medically important parental genomic findings unrelated to the cause of referral for proband's exome sequencing: yield and dilemmas

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Objective: Exome sequencing (ES) might detect disease-causing variants unrelated to the test indication. These include reproduction-related findings (RFs), deliberately searched secondary findings (SFs) and incidental findings (IFs). We aimed to examine the detection rate of medically important findings and to present counseling dilemmas in 840 parents of probands undergoing clinical trio ES testing.

Methods: RFs/IFs/SFs were actively searched for in the parents as part of ES data analysis. Variants were filtered by frequency, mode of inheritance, ClinVar classification, presence in local disease-causing variant databases, and protein-truncating effect.

Results: In 15/420 families (3.6%) 16 RFs were detected. Mutual carrier status for autosomal recessive disorders was identified in 23.3% of consanguineous and 2.1% of nonconsanguineous couples. SFs were found in 22/840 (2.6%), including 15 variants (7 founder) in cancer-predisposing genes and 4 in cardiac disease-related genes. IFs were found in 3 individuals without reported symptoms. Overall, variants of potential medical importance were detected in 9.5%% of families. Challenges related to the decision whether to report variants included missed parental phenotype, presymptomatic testing, difficulty in reaching a consensus on disease severity, potential medical implications for children already born, medico-legal aspects, and stigmatization.

Conclusion: Active search for RFs, IFs and SFs yields a high rate of findings that may contribute to individual medical care in parents of probands undergoing ES. A structured approach to overcome the challenges associated with reporting these findings should be considered before such active search can be broadly adopted in clinical genomic data analysis.

Conflict of Interest: None declared

P19.017.A Combining pharmacogenetics and patient characteristic polygenic scores to improve outcome prediction for calcium channel blocker treatment

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Background: Pharmacogenetic variants show modest effects on antihypertensives clinical outcomes, while patient characteristics may also predict outcomes. We aimed to test associations between genetically predicted patient characteristics plus pharmacogenetic variants with calcium channel blockers (CCB) outcomes in a large community cohort.

Methods: Extending our analysis of 32,000 UKBiobank dihydropiridine CCBs treated participants testing 23 variants, where *NUMA1* rs10898815 and *RYR3* rs877087 showed robust associations, we calculated polygenic scores for systolic and diastolic blood pressures (SBP and DBP), body fat mass, lean mass, lipoprotein A and others. Primary outcomes were CCB discontinuation and heart failure (HF).

Results: For HF, the highest risk 20% of polygenic scores for fat mass, lean mass and lipoprotein A were associated with increased risks (e.g. Hazard-Ratio $(HR)_{fat\text{-}mass}$ 1.46, 95% CI 1.25-1.70), versus the lowest of each score. *RYR3* T-allele modestly increased HF risks (HR 1.13: 1.02-1.25) versus non-carriers, but in subsets with high fat mass, lean mass, and lipoprotein A scores, estimates were substantially larger. For CCB discontinuation, high polygenic scores for fat mass and lean mass increased risks versus low, whereas high SBP and DBP scores decreased discontinuation risks. HRs for discontinuation with the pharmacogenetic *NUMA1* rs10898815 A-allele (overall HR 1.07: 1.02-1.12) were higher (HR 1.17: 1.05-1.29) in those with high scores for fat and lean mass.

Conclusion: Polygenic scores affecting adiposity and lipoprotein A levels add to pharmacogenetic variants in predicting key clinical outcomes in CCB treatment. Combining pharmacogenetic variants and relevant individual characteristic polygenic scores may help for personalizing prescribing.

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P19.018.B Linguo Franca: an automated framework to anonymize, translate and summarize clinical reports in HPO format

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Background/Objectives : Precision medicine (PM) for rare diseases requires both precision phenotyping and data sharing. However, the majority of digital phenotyping tools only deal with the English language.

Methods : Using French as a proof of concept, we have developed Linguo Franca, an automated framework to anonymize, translate and summarize clinical reports using Human Phenotype Ontology (HPO) terms compliant with medical data privacy standards. The output consists of a de-identified translated clinical report and a summary report in HPO format.

Results : We identified country-specific translation errors, pointing out 211 errors from 4639 French-to-English translations of HPO terms and institutional acronyms. In order to avoid incorrect de-identification resulting from the presence of proper noun medical terminology, we compiled a dictionary of 2994 medical exceptions from OMIM and HPO. We then conducted preliminary studies on 30 clinical reports where a summary report in HPO was accessible. The translated clinical reports were de-identified for 115 of the 117 protected health information items. Full anonymization in HPO format was found to be equivalent to the physician-generated summary, with a median difference of +0.3 terms per patient and an average of 4 terms per patient, without generating false positives.

Conclusion : By facilitating the translation and anonymization of clinical reports, Linguo Franca has the potential to facilitate inter-hospital data sharing, accelerate medical discoveries and open up the possibility of an international patient file without limitations due to non-English speakers. The framework is accessible open-source at https://github.com/kyauy/Linguo-Franca.

Conflict of Interest: None declared

P19.019.C An European pharmacogenomic study of response to opioids in advanced cancer patients identified variants associated with efficacy and toxicity

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Background/Objectives: Cancer patients usually receive opioids to control pain but unfortunately, 10-20% of patients do not benefit from treatment and experience side effects. Genetics might explain this interindividual variability in the response to opioids. The aim of this genome-wide association study (GWAS) is to identify new genetic markers of opioid efficacy and toxicity.

Methods: European cancer patients receiving morphine, oxycodone, buprenorphine, fentanyl were recruited (n = 2060). Data about efficacy (pain intensity, PI) and toxicity (nausea-vomiting score, NVS) were collected; DNA samples were genotyped using Axiom PMRA arrays. Linear regression between genotypes and NVS or PI were performed, using PLINK software. We also used the REGENIE pipeline, based on a machine learning algorithm, as an alternative method. Sex, age, study, country and opioid were included in the models as covariates.

Results: GWAS identified 4 and 7 variants associated with PI and NVS, respectively (P-value $< 1.0 \times 10^{-6}$). REGENIE found 5 variants associated with PI and 33 variants associated with NVS (P-value $< 1.0 \times 10^{-7}$), including rs111539671, intronic variant of *S100Z* gene, above the genome-wide significance threshold (P-value $< 5.0 \times 10^{-8}$).

Conclusions: This is the first GWAS for response to opioids performed in more than 2000 patients, individually genotyped. We did not detect any associations reaching the genome-wide significance threshold for the PI phenotype while, for the toxicity phenotype, we obtained a significant genome-wide association. These preliminary results, requiring further validation, highlight the need of analyzing larger cohorts, with homogeneous efficacy and toxicity data.

Grant References: AIRC MFAG 2019-ID.22950 project.

Conflict of Interest: None declared

P19.020.D Unique patient stratification method identified susceptibility and protective factors for the severe COVID-19 disease in the Hungarian population

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Background: In early 2020 it became apparent that genetic factors affect the outcome of the SARS-CoV-2 infection. In the past years, numerous - sometimes global – research projects concluded that our susceptibility to severe COVID-19 outcome, including the activation of the immune system and antiviral pathways, are genetically predisposed. Our main goal was to identify genes that play a crucial role in determining the severity of the COVID-19 disease.

Methods: We used age, severity, and clinical background-based patient stratification method to select focus groups whose disease severity is likely most influenced by their genetic background. Mutation burden analysis was performed on 180 selected patients' whole exome sequencing (WES) data. The resulting data were analyzed using a machine-learning random forest algorithm to help identify susceptibility and aggravating genes.

Results: We identified a gene set of 933 genes that allowed us to distinguish between patient groups with severe and mild disease outcomes, and to distinguish the genetically defined patient groups from the control groups. 599 genes have a higher SNP count in the severe patient group (susceptibility genes), whereas 424 genes have a higher SNP count in the patients with mild outcome (protective genes).

Conclusion: Gene set enrichment analysis on the 933 genes revealed which biological pathways were mostly affected by the susceptibility and protective genes.

Conflict of Interest: None declared

P19.021.A The Gene Discovery clinic: a personalised medicine approach to diagnose conditions not identified by WGS

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In the 100,000 Genomes project, a significant proportion (>75%) of patients remained without a genomic diagnosis. There are several possible explanations for this: (1) the patient does not have a monogenetic disorder; (2) incorrect application of virtual gene panels; (3) incorrect or incomplete phenotyping; (4) no trio analysis done; (5) gene not discovered at time of genomic analysis; (6) DNA variant not identified by the laboratory technique for other reasons e.g. intronic or structural variant(s).

In North West Thames, a monthly deep phenotyping Gene Discovery Clinic was established in 2020 for patients without primary findings from the 100,000 Genomes project. The clinic involves both a forward and reverse phenotyping approach, and has developed links with genomic research laboratories, with patient tissue samples routinely biobanked.

In >2 years' experience of diagnosing unsolved cases from the 100k Genome project, several common themes have emerged: the initial phenotype was incorrect or had not yet evolved; an incorrect inheritance pattern was assumed; a gene DNA variant was listed in Exomiser software but sufficient medical literature wasn't available; or the clinical diagnosis was that of a non-genetic condition. Other diagnoses resulted from the identification of novel genes, and routine trio analysis.

Our data suggest, that further "big data" laboratory or biostatistical analysis of a cohort of unsolved cases such as within the 100k Genome Project, may have limited success only. For a much larger yield and greater efficiency of trying to solve cases undiagnosed by WGS, we recommend a re-deep phenotyping approach.

Conflict of Interest: Virginia Clowes Consultant Clinical Geneticist

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P19.022.B Protein function informs drug usage patterns: A population-scale study of gene-drug interactions

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Background / objectives: Across populations more than 98% of individuals carry functionally relevant mutations in drug-metabolizing genes which can lead to adverse outcomes. With more people receiving more drugs it is increasingly relevant to ask what benefit each drug is providing the patient and whether current practices of drug administration align with our level of genetic insight. We sought to examine whether persons with genetically-determined higher/lower protein function display separate patterns of prescription drug usage.

Methods: We used imputed genotype data from 188,000 individuals from the Copenhagen Hospital Biobank to predict the function of 19 proteins involved in drug metabolism. These data were mapped to temporal drug prescription records spanning the years 1994 to 2022 to identify associations between predicted protein function and drug use characteristics such as drug discontinuation and dosage.

Results: The distribution of protein function phenotypes was similar to that of other European populations with more than 98% of our cohort carrying at least one actionable phenotype. Furthermore, 40% of our cohort have been prescribed drugs for which there exist actionable genotype-based recommendations. Among the drugs studied, we identified cases where genetically-determined protein function alters the daily drug dose.

Conclusion: Mapping associations between protein function and drug use patterns deepens our knowledge of how functional genetics influences drug response. This knowledge helps us understand the complex interactions between genetics and patient outcomes and sheds light on the potential advantages of genetics-informed drug administration.

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P19.023.C Mining the pharmacogenetic treasure in exome sequencing data

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A recent study from a large cohort published by the Ubiquitous Pharmacogenomic Consortium demonstrates the interest of pre-emptive pharmacogenetic panel testing, with a 30% reduction rate of clinically detectable side-effects. Our laboratory routinely performs diagnostic exome sequencing (ES), more than 7000 patients have been analysed to date. All patients could benefit from pharmacogenetics information: efficacy or safety of many medications are known to be influenced by the genetic background. We studied if meaningful pharmacogenetics information could be delivered from ES data provided for any patient, either retrospectively or prospectively.

We selected 211 variants of pharmacogenetics relevance, within 23 genes, based on evidence and frequency criteria. We evaluated their depth of coverage on 300 patients randomly selected in our cohort. Eight of those variants were not covered by ES, falling in *CYP2C19*, *CYP3A5*, *CYP3A4*, *CYP2D6*, *HLAB*(B15:02), *HLAA*(A31:01), *IFNL3*, *VKORC1* genes. We completed ES data by targeted sanger sequencing for these variants.

One challenging region was *CYP2D6* given high sequence similarity with *CYP2D7* and *CYP2D8*. To assess if we could reliably call variants in this region, we used 15 samples characterized with an orthogonal technique. We were able to properly detect variants in these complex regions in all cases, including hybrid genes.

Our preliminary data show that ES, initially ordered for suspicion of monogenic disease, could also be used to deliver pharmacogenetic meaningful information, by adding a few targeted genotyping assays. We currently evaluate the clinical utility and impact of pharmacogenetics on our cohort, applied to chronic kidney diseases and psychiatric disorders.

Conflict of Interest: Fanny PONCE Eurofins Biomnis (full time), Tanja Gatard Sonogen, Nicolas Jauniaux Sonogen, Xavier Vanhoye Eurofins Biomnis (full time), Boris Chaumette: None declared, Laurent Mesnard: None declared, Karl-Dietrich Hatz Sonogen, Laure Raymond Eurofins Biomnis (full time)

P19.024.A A single centre experience of personalised functional studies in rare diseases

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Rare disorders are collectively common but individually rare. Novel genomic technologies have been a great success in both research and diagnostics. However, even after extensive investigations with the latest methods many patients remain undiagnosed. One major diagnosis bottleneck is the presence of "variants of uncertain significance" (VUS). To be able to show causality of such variants it is necessary to either find more patients, challenging when the prevalence is low, or to perform functional studies.

At our centre, we have built a pilot setup to investigate patients with VUS findings, using a clinical and functional validation pipeline. The phenotypes of patients are varied and include intellectual disability/neurodevelopmental delay, epilepsy, neuromuscular disorders, amongst others.

The model systems established in house are patient-derived induced pluripotent stem cells (iPSCs) and the zebrafish, which are chosen depending on the needs of the individual case. While using the iPSC model we can study relevant cell types and how mutations affect global gene expression, using a humanized zebrafish model we can investigate the impact of the mutations in tissue formation and function within the organism, often the developing embryo. In specific cases, collaborations with other groups with relevant models have also been used. We will present a few success cases from our laboratory's pipeline and how to best scale up this type of approach. To date, VUS findings in 17 different genes have resulted in a diagnosis for 39 individuals, for all changing their clinical care and genetic counselling, personalised for both patients and family members.

Conflict of Interest: Raquel Vaz: None declared, Anna Lindstrand Oxford Nanopore **PacBio Illumina**

P19.026.B Genotype-first approach to identify associations between CDH1 germline variants and cancer phenotypes: a multicentre study by the European Reference Network on Genetic Tumour Risk Syndromes

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Background/Objectives: Hereditary Diffuse Gastric Cancer (HDGC) syndrome is caused by germline *CDH1* Pathogenic/Likely Pathogenic variants (PV/LPV). Rare *CDH1* missense variants are frequently classified as variants of unknown significance (VUS). Surveillance/prophylactic surgery is life-saving in asymptomatic PV/LPV carriers, but clinical management remains challenging in carriers lacking clinical criteria. We present the largest genotype-phenotype analysis in *CDH1* rare-variant carriers and their relatives to study *CDH1*-associated spectrum and optimize clinical management.

Methods: 1971 phenotypes from 854 carriers of 398 *CDH1* rare-variants and 1021 relatives from 29 institutions and 10 ERN-GENTURIS countries were analyzed. Variants were classified with *CDH1* ACMG-AMP guidelines. Genotype–phenotype associations were analyzed by Student's t-test, Kruskal-Wallis, $\chi 2$ and multivariable logistic regression models. Equivalence test, Youden

index, ROC and Z-test were used to assess performance of HDGC-clinical criteria sets.

Results: Lobular Breast cancer-LBC and Diffuse Gastric cancer-DGC had the greatest positive association with the presence of truncating-PV/LPVs (OR = 12.39[95% CI 2.66–57.74],p = 0.0014; OR = 8.00[2.18–29.39],p = 0.0017, respectively), as opposed to missense-VUS. *CDH1*-PV/LPVs occurred in 136/182 (75%) families fulfilling 2015 HDGC-clinical criteria, and in 40/672 (6%) families lacking criteria. Amongst the latter 40, 18 presented LBC but did not fulfill recent 2020 criteria. Three new LBC-centred criteria improved testing sensitivity while maintaining high specificity. Probability to find a *CDH1*-PV/LPV in patients fulfilling the LBC-expanded criteria, compared with the 2020 criteria, increased significantly (AUC 0.92 *vs* 0.88;p = 0.0004).

Conclusion: This study supports association of *CDH1* truncating-PV/LPVs, but not missense-VUS, with HDGC-specific phenotypes and supports widening HDGC-clinical criteria through the expansion of LBC-centred criteria.

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Colorectal Cancer/Polyposis Variant Curation Expert Panel; unpaid member of the German Gene Diagnostics Commission and speaker of the Centre for Hereditary Tumour Syndromes of the University of Bonn, Svetlana Bajlica Lagercrantz: None declared, JUDITH BALMAÑA Fees from AstraZeneca, Lilly, and Pfizer., Ana Blatnik: None declared, Patrick Benusiglio Fees from AstraZeneca, MSD, and Bristol Myers Squibb;

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P19.027.C Distribution of polygenic risk variants predisposing to cardiovascular disorders in Bulgarian population

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Background/Objectives: The genetic variations associated with an increased risk of cardiovascular disease are typically small and have a relatively modest effect on their own. However, when these variants are combined, they can have a significant impact on the risk of developing the disease.The aim of this study is to identify the frequency of the genetic variants predisposing to cardiovascular disorders - hypertension and dyslipidemia.

Methods: Sixty-seven subjects from Bulgarian origin were tested for ten common genetic variants: *LPL*(1595C>G), *CETP*(279G>A), *APOC3*(3175C>G), *APOE*(E2/E3/E4), *PON1*(A>G), *MTHFR*(677 C>T, 1298A>C), *FADS1*(rs174537G>T), *ACE*(I/D) and *AGT*(T>C). All of the patients filled in a questionnaire for assessment of dietary, epidemiological and occupational exposure risk factors that can interact with the genome and predispose to disease development and progression.

Results: The results demonstrate that 12% from the studied cohort are carriers of APOE4 risk variant; 7,4% are double homozygous (TT/CC) and 16,4% are double heterozygous (CT/CA) for risk variants in *MTHFR* gene. 4% are double homozygous (II/CC) and 21% are double heterozygous (ID/CT) for the *ACE* and *AGT* genes. 4,5% are carriers of four and 7,46% - of three out of ten high risk polymorphisms. 25% are carriers of more than four moderate risk variants with cumulative effect related to dyslipidemia and hypertension.

Conclusion: Among the Bulgarian population, specific combinations of multiple genetic variants with high and medium cardiovascular risk are common. Genetic screening which takes

into account the cumulative effect of multiple genetic variants can provide a more comprehensive assessment of an individual's risk of developing cardiovascular disease.

Conflict of Interest: Sofia Todorova: None declared, Boryana Gerasimova ReGena, LTD, Victoria Spasova Medical University-Sofia, Sena Karacanak-Yankova Medical University-Sofia, Savina Hadjidekova Medical University-Sofia, Darina Naydenova: None declared, Olga Antonova Medical University-Sofia

P19.028.D Severity and poor response to medical treatment in asthma patients (Canary Islands, Spain)

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Background/Objectives: The response to drug treatments is different among individuals. It is estimated that up to 70% of the therapeutic response in asthma patients is determined by genetic variation. Here, we evaluated the genotype-to-phenotype relationships from a set of asthma-related pharmacogenetic variants in asthma patients.

Methods: We assessed 79 pharmacogenetic variants associated with asthma treatments from the PharmGKB database on 216 patients who suffer from asthma at different severity levels. Variants were assayed by Axiom Genome-Wide Human CEU 1 Array and imputed using the Michigan Imputation Server. Multivariate logistic regression was performed to identify association between the variants and asthma severity, adjusting for relevant covariates. Significance was established at $p < 6.33 \times 10^{-4}$.

Results: A high percentage of individuals (82.4%) carried variant alleles associated with poor response to drug treatments or an increased risk of asthma exacerbations. In addition, the medical records showed a bad progression of the disease in many of these patients. Association was found for two frequent variants (>1%) and asthma severity (lowest $p = 5.12 \times 10^{-4}$; Odds Ratio = 0.19; 95%CI = 0.08-0.45).

Conclusions: Including information from pharmacogenetic variants in asthma treatment and prognosis could be informative for the management of these patients.

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Conflict of Interest: None declared

P19.029.A Towards an evidence-based neonatal genomic screening panel

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Background/Objectives: While novel technologies provide the means to enhance neonatal screening programs, selection of candidate diseases remains a moving target. The objective of our study was to perform a comprehensive assessment of diseases for inclusion in neonatal genomic screening panels and develop an up-to-date resource facilitating the rapid translation of novel findings into public health practice.

Methods: We have collected and analysed data from extensive genomic resources, including Orphadata, HPO, OMIM, Rx-Genes, and NHGRI CGD and developed an algorithm for severity classification. Monogenic diseases of interest were assessed based on disease severity, age of onset, and actionability.

Results: We identified 2713 childhood-onset monogenic genetic diseases with a unique Orphacode identifier, of which 2646 (97.5%) were moderate to profound, and 841 (31.0%) diseases (associated with 1587 genes) had mechanism-based interventions. Among selected diseases, 58 were classified as profound, 271 as severe and 512 as moderate. In addition, while mechanism-based interventions are in use for all included diseases, orphan drugs, including gene therapy, are currently available for 184 (21.9%) disorders. Finally, to determine the burden of rare disorders captured with this panel, the analysis of cumulative prevalence based on Orphadata was estimated at 229.3/100 000 – 1152.9/100 000, which accounts for 17.9 – 89.9 million people affected with these disorders worldwide.

Conclusions: We developed an evidence-based resource for neonatal genomic screening and identified a panel of 841 childhood-onset disorders with mechanism-based interventions. Our tool can be readily updated with novel information from existing genomic resources.

Conflict of Interest: None declared

P19.030.B Characterisation of CYP2D6, CYP2B6 and CYP2A6 haplotype variation in African populations and development of the StellarPGx diplotype calling algorithm

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Background/Objectives: Genetic variation is in part responsible for the variability in drug response across populations. However, the full catalogue of pharmacogenetic variants and their distribution are yet to be established, in particular for African populations. This study aimed to characterise variation in three core pharmacogenes (*CYP2D6*, *CYP2B6* and *CYP2A6*) across diverse African populations.

Methods: Given the challenges of genotyping these hypervariable genes, we developed a novel bioinformatics pipeline (StellarPGx) to facilitate star allele (haplotype) detection from high-depth short-read whole genome sequence (WGS) data. We used StellarPGx and other existing tools to call star alleles from 961 African genomes and over 2000 genomes from global populations (for comparison).

Results: We present frequencies across sub-Saharan Africa (SSA) for star alleles in *CYP2D6* (e.g. **17* and **5* [gene deletion]; frequency of 20% and 8%, respectively), *CYP2B6* (e.g. **6* and **18*; frequency of 33% and 10%, respectively) and *CYP2A6*, compared to European populations. StellarPGx identified novel African-

ancestry star alleles in *CYP2D6* (n = 27), *CYP2B6* (n = 18) and *CYP2A6* (n = 31); seven of these alleles were validated via targeted Single-Molecule Real-Time (SMRT) resequencing. In addition, collaboration with experts from the Pharmacogene Variation Consortium has enabled validation of a further 12 novel *CYP2D6* star alleles. Based on diplotype-phenotype translation, we found the metaboliser phenotype distributions for CYP2D6 and CYP2B6 to be non-uniform across SSA, and different compared to other global populations.

Conclusion: These findings underscore the need for investigating pharmacogene variation in the African context to reliably inform clinical pharmacogenomics implementation in Africa and across the African diaspora.

Conflict of Interest: None declared

P19.031.C Additional Findings from the 100,000 Genomes Project: a qualitative study of impacts on recipients

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Background: 100,000 Genomes Project (100K GP[1]) was a pioneering clinical/research hybrid initiative offering genome sequencing to patients with rare disease or cancer and some healthy relatives. Particpants were also offered screening for "additional findings" (AF), pathogenic variants in genes associated with inherited cancer predisposition syndromes and familial hypercholesterolaemia, and most AF have now been returned through the NHS.

The SAFE study aims to understand a range of outcomes of AF disclosure, including experiences and perspectives of people who received an AF. These data are required to inform development of future policy related to secondary genomic findings.

Methods: We used semi-structured interviews with adult recipients of an AF in Oxford, West Midlands and Wessex. Interviews covered conceptual domains: participant understanding, psychological and behavioural responses, healthcare utilisation, decision satisfaction and regret[2]. Transcribed interview data were analysed using deductive and inductive thematic analysis, informed by the expanded Health Belief Model[3].

Results: 33 adults (16 female, age range 24-75; 13 with an AF in a gene associated with familial hypercholesterolemia, 20 with an AF in a cancer predisposition gene) were interviewed. Many participants did not recall their decision about AF. Most interviewees do not regret their decision, have attended recommended clinical screening appointments and informed family members. However some participants experienced shock at receiving an AF, significant impacts on life planning and/or worry about at-risk relatives.

Conclusion: Results inform benefits and harms of secondary findings and whether, when and how to offer them.

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Conflict of Interest: Elizabeth Ormondroyd Full, Principal Investigator, Joshua Nolan Full, Jamie Forrest Full

P19.032.D Recalibrating polygenic risk scores to country-specific incidence

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Background: To translate genetic effects estimated from polygenic risk scores (PRS) to new populations, approaches are required that can integrate epidemiological data with genetic data to ensure accurate assessments of risk. This can be achieved through recalibration of PRS to disease-specific population incidence and mortality data.

Methods: We built a general recalibration solution that uses the Global Burden of Disease dataset and demonstrate the method using effect sizes from internally developed PRSs for coronary artery disease, Type 2 Diabetes, breast and prostate cancer. We recalibrated observed incidence in the UK, USA, Colombia, and Vietnam controlling for overall and disease-specific mortality by age group.

Results: To recalibrate lifetime risk, we set a baseline incidence and survival for age 0 and then apply a recursive pattern to extrapolate the incidence and survival rates for each age; the underlying data are the ancestry-based age dependent risk distribution for the disease along with historical incidence and mortality data from the country of residence. Absolute risk is then calculated using the probability of not dying, survival and incidence rates per age and risk percentile. Time range specific (e.g. 10-year) risk is computed from lifetime risk using the probability of an individual developing the disease in question by any age t_2, given that they are alive and free of that disease at age t_1 with the difference between the two ages being the time range of interest.

Conclusion: Recalibrating ancestry-specific PRSs with epidemiological data leads to their accurate translation to country-specific absolute risk estimates.

Conflict of Interest: Jen Kintzle Allelica Inc, Scott Kulm Allelica Inc, Alessandro Bolli Allelica Inc, Paolo Di Domenico Allelica Inc, Allelica Inc, Giordano Bottà Allelica Inc, Allelica Inc, George Busby Allelica Inc, Allelica Inc

P19.033.A Identifying genetic predictors of severe cutaneous adverse reactions attributable to anti-seizure medications using large-scale meta-analysis

Isabelle Boothman[1;2;3], *Wan-Chun Chang*[4;5], *Samreen Zafer*[6], *Takeshi Ozeki*[7], *Chun-Bing Chen*[8], *Mark McCormack*[1], *Ursula Amstutz*[9], *Neil Shear*[10], *Patrick Moloney*[1], *Antonio Gambardella*[11], *Graeme Sills*[12], *Zvonka Rener Primec*[13], *Holger Lerche*[14], *Wolfram Kunz*[15], *Chantal Depondt*[16], *Federico Zara*[17;18], *Pasquale Striano*[17;18], *Norman Delanty*[1], *Sarah Diehl*[19], *Roland Krause*[19], *Sanjay Sisodiya*[20], *Andrea Jorgensen*[21], *Chuang-Wei Wang*[8], *Yusuke Nakamura*[22], *Taisei Mushiroda*[7], *Colin Ross*[5;23], *Paola Nicoletti*[6], *Wen-Hung Chung*[8], *Munir Pirmohamed*[24], *Gianpiero Cavalleri*[1;2;3], *Bruce Carleton*[4;5]

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Background: Severe cutaneous adverse drug reactions (SCAR) are a group of extremely rare but life-threatening adverse reactions including Hypersensitivity Syndrome (HSS), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Aromatic anti-seizure medications (ASMs), carbamazepine, phenytoin and lamotrigine are the most common culprit agents. Genetic biomarkers, *HLA-A*31:01*, *HLA-B*15:02* and *CYP2C9*3*, are known predictors of ASM-induced SCAR. However, these markers have varying prognostic sensitivity and specificity, therefore we hypothesize that additional pharmacogenomic biomarkers for ASM-induced SCAR exist, and can be detected through meta-analysis facilitated by large consortia.

Methods: ASMs-induced SCAR cases (n = 391) and controls (n = 10,896) were obtained from a combination of published and unpublished datasets from centres in Canada, Europe, Japan, Taiwan and the U.K. Sites conducted imputation, quality control and GWAS following standardised criteria. We compared all ASMs vs. controls and then stratified by drug and clinical phenotypes. Results from each site were meta-analyzed using the software METAL.

Results: Preliminary analyses show replication of existing signals such as *HLA-A*31:01* ($p = 7.225 \times 10^{-18}$, OR = 4.7, CI = 4.32 to 5.02) in the SCAR induced by all ASMs analyses, and when stratified by carbamazepine ($p = 1.628 \times 10^{-29}$, OR = 14.14, CI = 13.68 to 14.6) and HSS ($p = 5.11 \times 10^{-19}$, OR = 7.03, CI = 6.6 to 7.5), 57% of HSS cases were induced by CBZ. Identified new signals on chromosome six, that are being validated.

Conclusion: We present one of the largest meta-analyses of ASM-attributed SCAR. We observe strong and consistent evidence for previously reported genetic predictors of SCAR, and novel genetic predictors.

Conflict of Interest: None declared

P19.034.D Successful pharmacological activation of the mild hypothermic response at 37°C

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Background/Objective: Targeted temperature management (TTM), the lowering of internal core body temperature into the mild hypothermic range (32-36°C), is frequently used in a neuroprotective manner in humans after catastrophic incidences such as hypoxic injury in neonates. This temperature activates an endogenous cytoprotective response, the mild hypothermia response (MHR), which has been shown to be neuroprotective and involve three key factors (SP1, CIRBP, RBM3).

Methods: To identify drugs that are able to activate the endogenous MHR without hypothermia, we created and validated multiple fluorescent mild hypothermia indicators (MHI) which translate transcriptional activity of the three key genes (*SP1*, *CIRBP*, *RBM3*) into fluorescence. These MHIs consistently show the strongest activation at 32°C in cell lines of diverse origins. Next, we conducted a high-throughput drug library screen (1953 FDA-approved drugs) using our SP1 + MCRE-MHI in HEK293WT cell line at 37°C.

Results: The drug screening yielded two compounds (Poziotinib and Entacapone) that can activate two of the MHIs at 37°C. We further show activation of the endogenous loci at 37°C at either expression or protein levels of the key factors of the MHR.

Conclusion: Our study reveals that: 1) Drugs exist that activate the MHR. 2) These drugs or their derivatives could be used to test the hypothesis that activation of the MHR is critical for the neuroprotective effect of TTM. 3) These drugs can be used for preclinical testing in animal models of asphyxia.

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Conflict of Interest: Salvör Rafnsdóttir: None declared, Arnhildur Tómasdóttir: None declared, Kijin Jang: None declared, Li Zhang: None declared, Kimberley J. Anderson: None declared, Hans Tómas Björnsson Dr. Bjornsson is a consultant for Mahzi therapeutics.

P19.035.C DrugOrder: an automated method for ranking variant/drug associations using large panel of cancer genes

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Background/Objectives: The sequencing of a large panel of genes to identify actionable alterations is becoming a common practice in oncology. A current limitation is the lack of a standardized and up-to-date hierarchization of targetable variants.

Methods: We developed DrugOrder that prioritizes actionable variants and their associated drug in the context of a patient tumor type. This tool is based on 32 rules for prioritizing variant-drug associations, and on 38 variant features related to the impact of the variant in the gene and its role in the patient's tumor. DrugOrder allowed the analysis of DNA and RNA sequencing panels of 639 cancer genes and 57 gene fusion transcripts of 63 tumor samples. 12 DNA/RNA tumor samples of the 63 samples were already characterized by an external provider, which were also sequenced with our laboratory diagnostic test. We generated simulated data representing 599 tumors built to contain an actionable variant.

Results: On simulated data, DrugOrder correctly classified the targetable mutation in its top predictions. The average rank of the targetable variant is 2. DrugOrder correctly identified all variant-drug associations previously detected by the external provider.

DrugOrder showed a good concordance with a manual biologist analysis of 63 patients sequenced in our laboratory.

Conclusion: DrugOrder showed good performance for prioritizing variant-drug associations. DrugOrder can provide support to biologists in order to streamline annotation of variants for large gene panels.

Conflict of Interest: Nicolas Soirat SeqOne, Mélanie Broutin SeqOne, SeqOne, Nicolas Hamadouche: None declared, Nicolas goardon: None declared, raphael lanos SeqOne, SeqOne, Jiri Ruzicka SeqOne, SeqOne, Sacha Beaumenier SeqOne, SeqOne, Anne-Laure Bougé SeqOne, Nicolas Philippe SeqOne, SeqOne, Michael Blum SeqOne, Dominique Vaur: None declared, Sophie krieger: None declared, Denis Bertrand SeqOne, SeqOne, Laurent Castera: None declared

P19.036.B A Maturity Level Model for the self-assessment of genomic medicine practices in healthcare systems

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Background/objectives: To fully exploit the potential of genomic information to benefit citizens health, it is crucial to understand how effectively healthcare systems implement genomic medicine. In the context of the 1+Million Genomes Initiative (1 + MG), we developed a Maturity Level Model (MLM) for health systems to self-evaluate the maturity of their genomic medicine practices, and define a path to optimization. A MLM pilot in eight European countries provided important information regarding common strengths, weaknesses and asymmetries across Europe.

Methods: The MLM development was based on literature reviews and input from 1 + MG experts. For MLM validation, a Delphi survey sought consensus from an expert panel of leaders of major national and international genomic initiatives. The MLM ToolKit was piloted in Belgium, Denmark, Finland, Ireland, Italy, Lithuania, Portugal and Spain.

Results: The MLM targets eight key domains (Governance, Investment, ELSI, Public awareness, Workforce, Clinical organization, Clinical infrastructure and Data management), including indicators with five maturity levels from inexistent to optimized. The ELSI and Clinical infrastructure domains showed highest maturity in all countries, while Public awareness and Data management had lower maturity. The widest maturity asymmetries across countries occurred in domains Investment and Workforce. This study evidenced domains where national efforts are needed and areas requiring cross-Europe investments to close development gaps.

Conclusion: The MLM is a valuable tool to assess genomic practices in healthcare systems, identifying and prioritizing areas that need further investment nationally or at European level.

Grant references: B1MG project funded by EU Horizon 2020 Research and Innovation Programme GA 951724.

Conflict of Interest: None declared

P19.037.A Transcriptomics analysis of intestinal biopsies for prediction of therapy response in patients with inflammatory bowel disease (IBD)

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Inflammatory Bowel Disease (IBD) is a chronic inflammation of the gastrointestinal tract and is a term that mainly describes two conditions: ulcerative colitis and Crohn's disease. The burden of IBD is rising globally with more than 6-8 million people living with the disease. When the standard treatment with immunosuppressant/steroids fails, the patient is treated with biologic therapies like tumour necrosis factor inhibitors (anti-TNF). However, this treatment is only effective in about 30-40% of cases. Therefore, identifying patients who will benefit from the treatment is essential for optimizing the current treatment strategy.

In this study, we applied total RNA sequencing on intestinal biopsies collected from IBD patients, treated with anti-TNF. We collected samples from blood, urine, faeces and tissue from

intestine pre- and 14-weeks post-treatment to identify predictive biomarkers of treatment response. When comparing differences in gene expression profiles from responding versus non-responding cohorts, preliminary data shows several important up- and downregulated genes. Interestingly the gene coding for the drug-metabolizing enzyme CYP1A1 was shown to be significantly upregulated in IBD patients not responding to treatment at baseline compared to patients responding to treatment.

Our data show promising results in identifying predictive biomarkers for IBD treatment response, which might have a great potential to optimize treatment selection and monitoring.

Grants for this study: Independent Research Fund Denmark, Knud and Edith Eriksens Mindesfond, University hospital of Southern Denmark, A.P Møller Foundation for the Advancement of Medical Science, Aage and Johanne Louis-Hansen Fond, and The Region of Southern Denmark Independent and Strategic Research Fund.

Conflict of Interest: Zainab Hikmat Knud og Edith Eriksens Mindesfond, University hospital of Southern Denmark, A.P Møller Fonden til Lægevidenskabens Fremme, Aage og Johanne Louis-Hansens Fond, and The Region of Southern Denmark Independent and Strategic Research Fund., Mads Thomassen: None declared, Trine Andresen: None declared, Vibeke Andersen Independent Research Fund Denmark and The Region of Southern Denmark Independent and Strategic Research Fund

P19.038.B Genetic variability in CTLA4 contributes to the development of asbestos related diseases

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Background: Occupational or environmental exposure to asbestos is a risk factor for various asbestos-related diseases, including pleural plaques (PP), asbestosis and malignant mesothelioma (MM). Inflammatory processes and the immune response play an important role in the development of these diseases. The immune response of peripheral T lymphocytes is controlled by multiple checkpoints, one of them being cytotoxic T-lymphocyte-associated protein 4 (CTLA4; CD125), which binds to antigens expressed on tumor cells and consequently inhibits *the immune response*. In this study, we aimed to investigate the associations between *CTLA4* polymorphisms and asbestos related diseases.

Methods: Our retrospective association study included 824 Slovenian patients with either PP (N = 376), asbestosis (N = 151) or MM (N = 297), and 78 healthy occupationally asbestos exposed controls genotyped for common *CTLA4* polymorphisms rs4553808, rs5742909, and rs231775. Logistic regression was used in statistical analysis.

Results: Carriers of two polymorphic rs4553808 G-alleles had lower risk for developing PP, asbestosis or MM (OR = 0.32, 95% CI = 0.15-0.72, P = 0.006) compared to carriers of two normal A-alleles. Specifically, these subjects had a lower risk for developing PP in dominant and additive genetic models (OR = 0.61, 95% CI = 0.37-0.995, P = 0.048 and OR = 0.28, 95% CI = 0.11-0.67, P = 0.005, respectively) and for MM in the dominant genetic model (OR = 0.56, 95% CI = 0.32-0.98, P = 0.044). Additionally, carriers of two polymorphic rs231775 G-alleles had higher risk for MM (OR = 1.96, 95% CI = 1.21-3.15, P = 0.006) compared to all other subjects. *CTLA4* rs5742909 was not associated with risk for developing asbestos related diseases.

Conclusion: *CTLA4* polymorphisms may play a role in the development of asbestos-related diseases.

Grants: ARRS L3-2622, P1-0170.

Conflict of Interest: None declared

P19.039.C Actionability and Familial Uptake after Genomic Screening in a Pediatric Cancer Cohort

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Background/Objectives: The care for patients with serious conditions is increasingly guided by genomic medicine. Future health care for the healthy part of the population may be equally transformed if genomic population screening is implemented. However, substantial knowledge gaps need to be filled before such an initiative can be realized in an ethically and clinically sound manner. This study focuses on one key issue quantifying the actionability and familial uptake, when secondary findings from opportunistic genomic screening (OGS) are disclosed.

Methods: Patients <18 years diagnosed with pediatric cancer underwent whole genome sequencing and OGS with ACMG version 2.0 genes not associated with cancer predisposition (36/59 genes). Patients and relatives were referred to genetic counseling and/or follow-up with a relevant clinical specialist, and clinical cascade testing undertaken as appropriate.

Results: In a cohort of 595 patients, 26 patients (4.4%) harbored a secondary finding (manuscript in preparation). Only 12 of these (46.2%) were found to be actionable in the patient or family and relevant for cascade testing after clinical workup. After an average of 1.6 years of follow-up 2.25 relatives pr. family had been tested. Additional surveillance or treatment was initiated in 16 relatives. Statins for familial hypercholesterolemia and betablocker treatments for Long QT syndrome were the most common interventions.

Conclusion: This real-world experience grants new insight into the potentials, pitfalls and derived health care demands of genotype-first screening. The study underscores the need for more research and construction of robust frameworks for disclosure of findings and handling of relatives for successful implementation.

Conflict of Interest: None declared

P19.040.D Additional findings in the 100,000 Genomes Project: disease manifestation and healthcare utilisation (SAFE Study)

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Introduction: Participants in 100,000 Genomes Project could choose to receive 'additional findings' (AF), pathogenic variants unrelated to their presenting condition in pre-specified genes associated with inherited cancer predisposition or familial hypercholesterolemia (FH). These serious health conditions have a long asymptomatic phase and are medically actionable. Genetic screening irrespective of personal or family history is new activity for the NHS, and little is known about genotype correlation of AF with disease, how clinical services might assess and manage risk, costs of disclosure or the behavioural and psychosocial impacts for recipients.

Methods: The SAFE study involves adult AF recipients in one geographical area of England. Objectives are to understand:

disease expression related to the AF

AF-indicated clinical referrals made at disclosure

Patient responses to AF

economic costs of genomic analysis and disclosure of AF.

Results: We will present data from 89 adults with an AF(2 *APC*, 5 *APOB*, 1 *APOE*, 12 *BRCA1*, 26 *BRCA2*, 26, *LDLR*, 1 *MSH2*, 9 *MSH6*, 1 *MUTYH* homozygote, 5 *RET*, and 1 *VHL*), from 85 families. Patients were 44% female, 83% White British and aged 21 to 92. Nineteen FH AF recipients (59%) were previously known to have FH or hyperlipidaemia. A single cancer AF recipient had a personal history for an associated cancer (male *BRCA2* AF, pancreatic cancer).

Conclusions: 100,000 Genomes Project AF programme identified manifest but genetically undiagnosed disease in patients with an FH AF.

We acknowledge support from Oxford Wellcome ISSF and Oxford Biomedical Research Centre (reference 0009729).

Conflict of Interest: None declared

P19.042.B Potential impact of pharmacogenomic variants for discrete populations within India

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Background/Objectives: Genomic variants are used to screen populations for disorders. While the ideal is individual screening, this is not always feasible. However, a relative risk for carriage of specific variants within a given population may still inform treatment e.g. pharmacogenomic (PGx) screening for drugs; especially applicable for populations with less efficacy and safety data, such as many populations from India. We investigated whether these groups would (1) benefit from PGx screening and (2) need to be considered at subpopulation, rather than national level by studying the polymorphic PGx gene, *CYP2C19*.

Methods: Indian participants were recruited from Gujarat in the North and Telangana in the south. Blood was exome sequenced. Actionable variants in *CYP2C19* were screened for frequency within the populations.

Results: The commonest alleles in both groups are CYP2C19*1 (normal function), CYP2C19*2 (decreased function) and CYP2C19*17 (increased function).

Table showing incidence of common actionable CYP2C19 variants

CYP2C19 allele	*1	*2	*17	Total no. of participants
Telangana	565 (38%)	601(41%)	289(20%)	1478
Gujarat	759 (48%)	530(33%)	268 (17%)	1585

Conclusion: In the absence of population based clinical studies demonstrating efficacy and side effect profiles, treated is based on largely western data. Not unexpectedly, the frequency of actionable variants frequently differs. Here, 61% of individuals from Telangana carried actionable variants, versus 47% from Gujarat, indicating a higher likelihood of adverse effects. This suggests that PGx screening may offer an opportunity, at the population level, to target prescriptions for maximal benefit while ensuring resources are optimized at the same time.

Conflict of Interest: Jonathan Picker Anuva (full time), Anuva, Affiliate Physician Boston Childrens Hospital, ASmi Shah Anuva (full time), Anuva, Omkar Babu Anuva (full time), Anuva

P19.043.C The impact of genetic variability in inflammatory pathways on COVID-19 severity and short-term outcomes

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Background: The exaggerated immune response in COVID-19 patients, known as "cytokine storm", leads to production of excessive amount of produce pro-inflammatory cytokines (IL-6, IL-12, IFN-γ) and chemokines (CXCL10, CCL2). This is associated with a more severe disease course and higher risk for fatal outcome. Genetic markers could be used for rapid identification of COVID-19 patients with a higher risk of a more severe course and a worse outcome of the disease. For that purpose, our aim was to analyse the associations of *IL-1β* and *IL-6* polymorphisms with disease severity, duration of hospitalization, oxygen therapy or requirement for treatment at the intensive care unit (ICU).

Methods: Our study included 175 hospitalized COVID-19 patients. We isolated DNA from patient's peripheral blood. All patients were genotyped for *IL-1β* rs1143623, *IL-1β* rs16944, *IL-1β* rs1071676 and *IL-6* rs1800795 polymorphisms. We used χ^2/Fischer test, Mann-Whitney test and logistic regression in statistical analysis.

Results: Among all patients, 66.3% were male and 33.7% female; their median (range) age was 56.8 (41–67) years. The majority of patients (66.1%) had severe illness, 19.9% had critical, 11.7% moderate and 2.3% had mild COVID-19. None of the investigated polymorphisms was associated with disease severity,

duration of hospitalization, mode and duration of oxygen therapy or requirement for ICU treatment.

Conclusion: The results of our pilot study do not suggest significant association of *IL-1β* and *IL-6* polymorphisms with COVID-19 severity and short-term outcomes. Further studies should take into account a larger number of genes involved in inflammatory pathways.

Grants: ARRS P1-0170 and P3-0296.

Conflict of Interest: None declared

P19.044.D Functional studies drive treatment of phenylketonuria to become personalized

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Background: Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism. PKU is due to defects in enzyme phenylalanine hydroxylase (PAH) that converts phenylalanine (Phe) to tyrosine. When given as a treatment, PAH chaperone tetrahydrobiopterin (BH4) corrects the biochemical phenotype in patients with milder forms of the disease also known as BH4-responsive PAH-deficiency. A significant gap of knowledge concerns the enzymatic function associated with *PAH* genotypes, which are often found in compound heterozygous state.

Methods: Improved activity landscapes method was used to determine PAH function in a cell model as a result of 100 most common *PAH* genotypes.

Results: Depicting enzyme activity of variant PAH proteins as activity landscapes allows for the determination of the optimal working range of the protein in the functional metabolic and therapeutic context. The binding of the pharmacological chaperone BH4 depends on the patient's Phe concentration. Using improved PAH activity landscapes method, the most frequent *PAH* genotypes were performed as 3D images that visualize behavior of misfolded protein in a gradient of BH4 and Phe concentrations.

Discussion: Using only the standard protocol for BH4-loading test disregarding the patient's *PAH* genotype may lead to false-negative testing results. Therefore, there is a need for employing a holistic approach that would guide pediatricians to personalize BH4-loading tests and dietary treatment schemes. The information resulting from PAH activity landscapes will allow to avoid false-negative test results and create individualized treatment regimens.

Grant References: Alexander vonHumboldt research fellowship, BioMarin research grant.

Conflict of Interest: Polina Gundorova Alexander von Humboldt research fellowship, Marta Danecka: None declared, Mathias Woidy: None declared, Viviane Kasten: None declared, Ania Carolina Muntau Biomarin, PTC Therapeutics, Soeren Gersting Biomarin research grant, PTC Therapeutics

P19.045.A competition – best drug prioritization algorithm

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Background/Objectives: A promising approach shown to improve cancer care is to utilize drug prioritization algorithms. Such algorithms utilize advancements in large scale profiling technology of various biological molecules (OMICS) to analyse the difference between tumours and predict the response of an individual to a specific therapy. Multiple such algorithms have been proposed, but their relative clinical applicability have not been reported. We suggest a competition to identify the best algorithm using an ex-vivo system.

Methods: PDX mice will be used to generate transcriptomics profiles, which will be used to rank drugs by their impact on the tumour by various predictors. The impact of drugs selected by a Trial Optimizer, built for this purpose, will be tested using the Tissue Ex-Vivo Analysis (TEVA) method. Predictors will be ranked by their distance to experimental results as determined by a specially designed Trial Ranker.

Data: PDX transcriptomics data for predictor development will be gleaned from MMHCdb, with drug IC50 gleaned from CCLA and GDSC. In addition, the transcriptomics of the PDX tumours will be made available.

Results & Conclusion: For proof of concept, the process was simulated by adding different levels of noise to prefect data to create a range of predictor accuracies. Our method successfully ranked predictors with a small, optimized set of drugs. Here we present a workflow and announce a competition to find the best algorithm for OMICs based precision oncology.

Conflict of Interest: None declared

P19.046.B Pharmacogenomic panel and interpretive software to help guide personalized pain and mental health management decisions in two different healthcare settings

Ben Pinder[1], ***Stephen Abbs***[2], *Jessica Woodley*[3], *Ania Skowronska*[3], *Abhishek Gupta*[1], *Ashwin Juneja*[1], *Kathy Siminovitch*[1]

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Background: Pain and mental health conditions are priorities for healthcare systems worldwide. Pharmacogenomics has the potential to reduce adverse drug reactions by 30%, reduce healthcare costs, and improve treatment outcomes for patients with these and other conditions(1).

Methods: We have developed a pain and mental health pharmacogenomic test and interpretive software to address the needs of individuals suffering from chronic pain and mental health. We are implementing the assay in two different settings: direct to consumers in Canada, and in a pilot study in NHS clinical pathways for mental health in the UK.

Results:The assay interrogates 121 variants in 37 genes, and includes the detection of CYP2D6 copy number and hybrid alleles. A minimum 100ng DNA from blood or cheek-swabs is added into a multiplex PCR, and products are assayed on an Agena MassArray platform. Results are uploaded into our interpretive software, which correlates genotypes with pharmacogenomic consortium guidelines from CPIC, PharmGKB, DPWG, and FDA, for over 175 medicines used to treat a range of clinical conditions. The software generates a Personalized Insights clinical report, indicating which medicines are predicted to be most effective and well tolerated for the patient, for clinicians to make informed decisions on which medicines to prescribe.

Conclusions: Several changes have been introduced to the approach and clinical report to implement this test in the UK NHS. We will discuss the different requirements of pharmacogenomics that we have encountered in the different settings of Canada and UK.

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Conflict of Interest: Ben Pinder Inagene Diagnostics Inc, Stephen Abbs Inagene Diagnostics UK Ltd, Jessica Woodley: None

declared, Ania Skowronska: None declared, Abhishek Gupta Inagene Diagnostics Inc, Ashwin Juneja Inagene Diagnostics Inc, Kathy Siminovitch Inagene Diagnostics Inc

P19.047.C Simultaneous profiling of SNP genotyping and copy number variation using 96 assays in a nanofluidic platform for pharmacogenomics studies

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The traditional qPCR-based workflow for pharmacogenomics tests uses two separate cumbersome workflows for SNP genotyping and CNV determination. In this work, we report a unified automated workflow to simultaneously profile SNPs and CNVs in buccal swabs without extraction. The PGx panel targets 69 SNPs and three CNV targets in a single workflow using a microfluidic chip with the Biomark™ X system. This enables high-throughput sample testing against 96 assays in nanoliter volumes, which lowers the cost of reagents and reduces sample input. This panel comprises 75 TaqMan® qPCR assays, including 71 genotyping assays, 3 CNV assays and 1 *RPPH1* assay to serve as internal control for CNV. The 71 genotyping assays cover the most common PharmGKB Tier 1 and Tier 2 SNPs located in 17 drug response-associated genes *CYP2D6, CYP1A2, CYP2B6, CYP2C19, CYP2C9, CYP3A4, CYP3A5, ABCB1, APOE, COMT, DRD2, F2, F5, MTHFR, OPRM1, SLCO1B1, and VKORC1*. The 3 CNV assays in the panel cover the exon 9, intron 6, and 5′ regions of the *CYP2D6* gene. Analysis and interpretation of SNP genotyping and CNV determination is performed using a single data processing package that supports the calling of star alleles. We have assessed this panel with a total of 173 Coriell DNA samples with either known genotypes or known copy numbers. The average call rates for the SNP and CNV assays were 99.88% and 100%, and average call accuracy was 99.94% and 95.80%, respectively, for extracted genomic DNA.

Conflict of Interest: Hui Ren Standard BioTools, Roberto Spada Standard BioTools, Standard BioTools, Stephen Knight Standard BioTools, Naveen Ramalingam Standard BioTools

P19.048.D Personalized treatment in a boy with NF1 and inoperable plexiform orbital neurofibroma

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Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic condition caused by dysfunction of neurofibromin protein leading to overactivation of the Ras signaling pathway. One of the most challenging aspects to manage are plexiform neurofibromas (PNs), histologically benign peripheral-nerve sheath tumours, sometimes leading to disfigurements. Selumetinib has been recently approved by FDA and EMA after evidence of durable tumour shrinkage and clinical benefit in NF1 children with inoperable PNs (NCT01362803). Here we present a 3 year old boy, with a pathogenic variant in NF1 c.1105C>T p.Arg369Ter, who came to our attention after partial surgical resection of a left orbital PN at 1 year of age. Clinically he showed severe left eyelid ptosis and low vision due to progressive growth of PN, left wing sphenoid dysplasia, spinal neurofibromas (C2-D2-L2-S1), diffused cafè au lait macules, psychomotor and language delay.

Given the deep tissue infiltration preventing a radical debulking surgery, the patient was candidate for Selumetinib treatment (Koselugo) provided by Astrazeneca under compassionate use. The therapy was approved by the local Ethic committee at 25mg/msq dosage.

Besides paronychia treated with topical therapy and one episode of diarrhea, no further adverse events were recorded during therapy. Pre-treatment versus 10 months-MRI study performed after therapy initiation showed a progressive reduction in the skin and subcutaneous tissues of the peri-orbital region, the left infratemporal fossa and the upper and lower eyelids. Clinically he showed edema resolution and improvement in eye movements.

This report stress how Selumetinib represents a valid therapeutic strategy to treat inoperable PNs.

Conflict of Interest: None declared

P19.050.B integration of socio-sanitary variables from electronic health records and genomic data of 380 patients in a novel platform called medigenomics

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Electronic Health Records are sometimes not well-designed or standardized, and it generates a loss of high quality and important patient information. This data should be ready and available for analysis using all patients' records, including genomic and socio-

sanitary information (phenotypes, treatments, diseases, gene variants, procedures, among others). The aim of this project is to generate a Phenopacket, the standard of Global Alliance for Genomics and Health to share clinical and genomic information in a standardized format.

For that purpose, we have analyzed 380 patients, including geriatrics and newborns patients from the Toledo Cohort, who were clinically followed for more than 30 years. At genomic level we have performed primary, secondary and tertiary analysis and we had collected a complete database of clinical information of each patient from different resources including doctor visit recordings, paper and electronic reports. Security methods and informed consents will be implemented to ensure data privacy and management.

We have got a fully integrated platform called MEDIGENOMICS with which experts are able to deeply analyze the genomic and clinical information of patients, obtain a genetic case report, send notifications to a specific patient when new important information about his/her condition is found according scientific literature, and patients can modify the informed consent that he/she signed before.

This project improves our ability to understand, diagnose and treat human diseases diseases, since it was designed to encourage synergy between the people, organizations and systems that comprise the joint effort to address human disease and biological understanding.

Grants: MEDIGENOMICS-52/2021(A/SER-032253/2021), FEDER

Conflict of Interest: None declared

P19.051.C Prevalence of actionable pharmacogenetic variants in a Swiss hospital cohort

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Background: Drug type and dosing recommendation have classically been designed and optimized based on average response in the general population. Yet, there is significant interindividual variability in drug response, which results in treatment inefficacy or adverse drug reactions (ADRs) in a subset of patients. This is partly due to genetic factors that typically affect drug metabolism or clearance. To verify the relevance and applicability of international pharmacogenetic guidelines in our local population, we here estimate the prevalence of clinically actionable variants in a Swiss hospital cohort.

Methods: We used samples and data from the genomic biobank and the clinical data warehouse of Lausanne University Hospital. Using blood extracted DNA and Illumina's Global Screening Array, we genotyped 1'533 patients who received at least 30 different drugs.

Results: We compared the allele frequencies of genetic variants in 12 known "high-risk" pharmacogenes to ethnically matched international databases and did not find any significant difference. Almost all study participants (97%) carried at least one actionable variant. Importantly, 46% of them were exposed to at least one drug for which they carried a specific high-risk variant.

Conclusion: Our analysis confirms the very high prevalence of actionable pharmacogenetic variants in the Swiss population. It also shows that a non-negligible fraction of multi-treated patients are exposed to drugs for which they carry potentially problematic variants. Implementing a genetically informed approach to drug prescribing could have a global positive impact on the quality of health care delivery.

Grant references: Fondation Leenaards

Conflict of Interest: None declared

P19.052.D Societal impact of genetic science (SENSE)

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Introduction: Genetic profiles hold great promise to personalize medicine. However, much is unclear about the way genetics could or should contribute societally. Although genetic profiles have been included in comprehensive risk prediction models (CRP), aspects related to ELSI, education, communication, health-economics, data governance and other social aspcts are often overlooked.

Methods: Within the SENSE proposal, we have established a consortium of experts in the Netherland in all social aspects mentioned.Together with clinical and genetic scientists, we have established four genetic applications with the highest technology readiness levels. These are breast cancer, cardiovascular disease, age-related macular degeneration and pharmacogenetics.These were selected as they might induce differing social considerations, such as involving differing healthcare contexts and professionals, or subjected to differing ELSI frameworks.

Results: Within the consortium, we have mapped social needs and considerations in each social domain and for each of these applications, We identified shared and unique considerations across these applications. Using this mapping, we will develop SENSE-ible workflows and strategies for responsible implementation of genetic risk profiles.

Discussion: The SENSE effort is in a proposal phase, and not yet funded. However, within the consortium we have already achieved relevant insights. These include a first mapping of various needs, expectations and responsibilities between the involved stakeholder, which must be refined. As well as the need for shared language and definitions, and developing consensus on the road to responsible utilization of the promise genetic profiles hold for personalized medicine. SENSE is specifically a Dutch consortium.

Conflict of Interest: None declared

P19.053.A The majority of pharmacogenomic variants associated with adverse drug events when taking endocrine therapies for breast cancer can not be replicated in UK Biobank participants

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Background/Aim: Two thirds of breast cancers are hormone receptor-positive for which endocrine therapy is the standard of care. The occurrence and severity of adverse drug events (ADEs) are a driver for suboptimal adherence, compromising BC survival. Pharmacogenomic predictors of endocrine therapy ADEs have the potential to help avoid these and improve adherence. We sought to assess previously reported associations between genomic variants and medically important side effects in UK Biobank participants on endocrine therapies.

Materials and methods: Using data from UK Biobank, we assessed previously published associations between ($n = 41$) single-nucleotide variants (SNVs) and various medically important ADE outcomes as per The European Medicines Agency (EMA).

Results: No significant ADE associations were found at baseline. For incident ADE outcomes, rs6025 was significantly associated with both venous thromboembolism and thromboembolic events in tamoxifen-treated patients (OR 1.40 95% CI 1.18-1.66, $p = 9.1E-05$) and OR 1.62 95% CI 1.43-1.83, $p = 5.60E-14$), respectively. However, only the association for venous thromboembolism

persisted in the interaction model (OR 3.02 95% CI 1.09-8.33, $p = 0.033$) and remained significant even after adjusting for relevant patient-related risk factors (OR 3.25 95%CI 1.16-9.12, $p = 0.025$).

Conclusion: Aside from rs6025 and venous thromboembolism in tamoxifen-treated patients, we are unable to replicate the previously reported associations. This study underscores the necessity for further investigations using prospective randomised controlled trials and the low level of evidence in many pharmacogenomic discovery studies. Pharmacogenomic tests for this indication should not be considered or implemented for personalised care without more robust data.

Conflict of Interest: None declared

P19.054.B First Experience with the Implementation of Pharmacogenetics (TPMT, DPYD, and UGT1A1)

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Purpose: The aim of pharmacogenetics is the application of personalized therapy based on genetic variations in the genes involved in the processes of absorption, distribution, metabolism, and elimination of drugs. More than 30 genetic variants decrease enzyme activity of thiopurine metabolizing enzyme thiopurine methyltransferase (TPMT). Activity of dihydropyrimidine dehydrogenase (DPD), involved in pyrimidines degradation, is also affected by several genetic variants, as well as activity of UDP-glucuronosyltransferases (UGT), responsible for glucuronidation of irinotecan.

Methods: Analysis of *TPMT* variants c.238G>C, c.460G>A, and c.719A>G in acute lymphoblastic leukemia patients before thiopurines treatment, *DPYD* variants c.1236G>A, c.1679T>G, c.1905+1G>A, and c.2846A>T in colorectal cancer before use of 5-fluorouracil and genotyping of the promoter for *UGT1A1* (TA)n, in the use of irinotecan therapy in colorectal cancer and solid tumors have been performed by real-time polymerase chain reaction (PCR) and PCR and reverse hybridization.

Results: Several months after the introduction of the methods, TPMT testing in 19 samples showed *TPMT**1/*1 genotype. Among seven samples for DPD analysis one had heterozygous c.1236G>A (HapB3). *UGT1A1* $(TA)_n$ promoter genotyping was conducted in 5 samples. Two samples had genotype $(TA)_6/(TA)_6$, another 2 $(TA)_6/(TA)_7$, and a single one $(TA)_7/(TA)_7$.

Conclusion: An individual approach is made possible by determining the optimal drug and its dose based on the individual's genetic predispositions, in order to achieve the maximal therapeutic response for each patient, by reducing the side effects of therapy, as well as treatment costs, to a minimum.

Conflict of Interest: None declared

P19.055.C NGS-based newborn screening - results of sequencing 7000 healthy newborns

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Background/Objectives: The next-generation sequencing (NGS) cost reduction led to the idea of sequencing all newborns to look for monogenic diseases (MDs) that haven't shown up yet.

Methods: In this project we studied 2350 genes by whole-exome sequencing (WES) in 7000 phenotypically normal infants. All parents who signed the informed consent received information about the treatment/prevention MDs. If desired, they could receive information about no cure, incomplete penetrance, or late onset MDs.

Results: Treatment/prevention MDs were found for 109 (1.54%) phenotypically normal newborns. The most common variants leading to the development of hearing loss – 12, familial Mediterranean fever – 6, neurofibromatosis – 6, MODY – 4. Variants requiring additional consent were found in 169 (2.4%) newborns, of which 54 with hereditary tumor syndromes and 28 with heart diseases and 87 monogenic syndromes with incomplete penetrance or adult debut. In addition to MDs WES reveals aneuploidy. Sex chromosome aneuploidies such as trisomy X and 47XXY are usually not detected at birth. We found 6 girls with triple X syndrome and 6 boys with Klaienfelter syndrome.

Conclusion: Clinically significant variants were found in 1 of 25 children without clinical features. Most of them cannot be detected by other methods. NGS-bases NBS is a promising method that allows, together with TMS screening, to detect a large number of monogenic diseases and can serve as the basis for preconception screening in the future

Grant References: The study was carried out within the framework of State Assignment 121092400060-5

Conflict of Interest: None declared

P19.056.D From sample to star alleles: a long-read pharmacogenomics pipeline powered by Twist target enrichment and PacBio HiFi sequencing

Sam Holt[1], *John Harting*[1], *Tina Han*[2], *Leonardo Arbiza*[2], *Sarah Kingan*[1], *Aurelie Souppe*[1], *Siyuan Zhang*[1], *primo baybayan*[1], *christine lambert*[1], *Heather Ferrao*[1], *Binglan Li*[3], *Katrin Sangkuhl*[3], *Mark Woon*[3], *Ryan Whaley*[3], *Michelle Whirl-Carrillo*[3], *Yao Yang*[3], *Teri Klein*[3], *Stuart Scott*[3], *Nina Gonzaludo*[1]

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Introduction: The development of scalable and cost-effective pharmacogenomic (PGx) assays can increase medication safety and efficacy. In particular, long-read PacBio HiFi sequencing enables highly accurate, ancestry-agnostic, comprehensive variant calling with direct phasing (for unambiguous star (*) allele assignment). However, manual bioinformatics expertise is often needed to integrate tools and workflows, impacting the scalability and cost-effectiveness of large precision medicine programs.

Methods: A 50-gene Twist Alliance Long Read PGx Panel for HiFi sequencing was designed to capture major PGx genes, including all 20 current genes with Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines. Samples were prepared following the Twist Long Read Library Preparation and Standard Hyb v2 Enrichment Protocol optimized for HiFi sequencing, then sequenced on a PacBio Sequel IIe system and processed using a novel bioinformatics workflow for targeted HiFi sequencing datasets. This workflow includes demultiplexing, marking PCR duplicates, GRCh38 alignment, and variant calling and phasing. While this workflow is generalizable to any HiFi target enrichment

panel, we describe an analysis extension with star allele calling for CYP2D6 using Pangu and other PGx genes using PharmCAT.

Results: We describe a scalable analysis workflow for targeted sequencing panels on PacBio HiFi sequencing systems. We demonstrate this workflow for pharmacogenomics, going from DNA to star allele calling for 10 GeT-RM reference samples, with a public dataset included.

Conclusion: This demonstrative workflow supports the use of HiFi sequencing for the development of scalable pharmacogenomics and its implementation, streamlining translation of sequencing data into phenotype that can help drive clinical decision support for PGx.

Conflict of Interest: Sam Holt PacBio, PacBio, John Harting PacBio, PacBio, Tina Han Twist Bioscience, Twist Bioscience, Leonardo Arbiza Twist Bioscience, Twist Bioscience, Sarah Kingan PacBio, PacBio, Aurelie Souppe PacBio, PacBio, Siyuan Zhang PacBio, PacBio, primo baybayan PacBio, PacBio, christine lambert PacBio, PacBio, Heather Ferrao PacBio, PacBio, Binglan Li: None declared, Katrin Sangkuhl: None declared, Mark Woon: None declared, Ryan Whaley: None declared, Michelle Whirl-Carrillo: None declared, Yao Yang: None declared, Teri Klein PharmGKB, Stuart Scott In-kind sequencing consumables from PacBio, Nina Gonzaludo PacBio, PacBio

P19.057.A Two in one: Pharmacogenetic profiling from whole genomes

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Backgroud: Drugs are usually prescribed using a one-size-fits-all approach, although several frequent pharmacogenetic predispositions affecting drug efficacy and safety are well established. Recently, a large randomised study of the U-PGx project has confirmed the benefit of preemptive pharmacogenetic testing. Furthermore, the costs of whole genome sequencing (WGS) are decreasing, enabling and accelerating genome-scale genetic testing, including pharmacogenetic predispositions.

Methods: Using a bioinformatic pipeline, we extract pharmacogenetic relevant single nucleotide variants and structural variations of whole genomes (60× WGS, PCR-free, PE150) of our patients with rare (aortic) disorders. The results of every patient are interpreted according to the guidelines of the Dutch Pharmacogenetics Working Group (DPWG) used in the U-PGx Project.

Results: The results are presented on the Medication Safety Card (MSC), a personalized pharmacogenetic profile in credit card format. The MSC displays individual medication and dosing recommendations and may be presented to the treating physician or at a pharmacy. As novel findings are expected, the MSC contains a QR-code, providing online access to an up-to-date version of the report.

Conclusions: We developed a WGS-based approach for the implementation of pharmacogenetics by leveraging WGS data instead of requiring a dedicated test. The MSC allows accurate medication selection and dosing, enabling precision medicine. Our approach not only benefits the patients but also saves time and costs, showing novel possibilities for the future of healthcare and personalized medicine.

Conflict of Interest: None declared

P19.059.C Introducing breast cancer genetic risk-based stratified screening service. BRIGHT clinical study. Estonian experience

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Introduction: n Europe, mammographic screening of women aged 50-74 addresses approximately 58% of all breast cancer cases. It is cost-effective and reduces the breast cancer mortality by 20-40%. We introduce BRIGHT genetic risk-based service for addressing the higher risk population below 50 years that is commonly not screened. The risk model comprises of questionnaire for assessing the hereditary cancer risk and a polygenic risk score (PRS) testing.

Materials and Methods: the BRIGHT study will address 2250 women in three clinical centers in Estonia, Sweden and Portugal. In Estonia, the clinical study has been largely completed. In Estonia, several recruitment paths were tested - web-based, pharmacy, breast clinic, primary care.

A simple web-based self-reporting questionnaire was introduced to assess the eligibility for monogenic pathogenic variant testing.

AnteBC polygenic risk score test (Antegenes OÜ, Estonia) was used to assess the breast cancer polygenic risk for all participants.

Results: 12% of women recruited in Estonia were eligible for genetic counselling. 84% of those genetically counselled were referred for MPV testing. In a few cases, the family member was already tested for MPV.

16% of participants had 1.5-fold or a higher genetic risk. In 6% of participants, the risk was increased 2- to 3-fold.

Next steps: clinical follow-up and feedback data will be collected to assess the feasibility of stratified cancer screening.

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Conflict of Interest: Madli Tamm: None declared, Laura Roht: None declared, Siim Sõber: None declared, Kristiina Ojamaa: None declared, Krista Kruuv-Käo: None declared, Peeter Padrik: None declared, Sander Pajusalu: None declared, Neeme Tonisson Antegenes OÜ

P20 Population Genetics and Evolutionary Genetics

P20.002.B Short-term effects of heated tobacco product (HTP) use on DNA methylation profiles and whole transcriptome profiles

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Abstract

Background/Objectives: Heated tobacco products (HTP) have rapidly increased in popularity since 2016, advertised as having

fewer health effects than traditional cigarettes. However, the health effects of HTP use on molecular genetics are largely unknown. To investigate these effects, we used blood cell-derived DNA and RNA from a Japanese population cohort study of 11,002 participants.

Methods: Participants were divided into four groups: never smokers (NS), past smokers (PS), current smokers (CS), and HTP users (switched from CS <2 years). We collected peripheral blood mononuclear cells from 52 participants in each group, matched to HTP users using propensity scores. DNA and RNA were purified from these samples and DNA methylation (DNAm) analysis on 17 smoking-related DNAm biomarkers (genes) and whole transcriptome analysis were performed.

Results: DNAm analysis showed that 10 of the 17 genes were significantly hypomethylated in CS and HTP users compared to NS, with *AHRR*, *F2RL3*, and *RARA* showing intermediate characteristics between CS and NS. However, AHRR expression was significantly higher in CS than in the other groups. *LRRN3* and *GPR15* were hypomethylated in HTP users compared to NS, and *GPR15* expression was significantly upregulated in all groups compared to NS.

Conclusion: HTP users (switched from CS <2 years) show abnormal DNAm and transcriptome profiles, although to a lesser degree than CS. However, the molecular genetic effects of long-term use of HTP are still unknown and further long-term molecular epidemiology studies are needed.

Grant References: Grant numbers are JP19ek0210102 (AMED, Japan) and 22H03356 (MEXT, Japan).

Conflict of Interest: None declared

P20.003.C Carrier screening program for BRCA1/BRCA2 pathogenic variants among Ashkenazi Jewish women in Israel: An observational study

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Background: In the last decades, genetic testing has been widely used for the purpose of prenatal screening and preventing severe diseases in offspring. For the first time, a carrier screening has been carried out for healthy adults, with the aim of reducing BRCA1/2-related morbidity and mortality. Evaluation of screening programs is critical for disease prevention.

The prevalence of BRCA1/2 genes is increased in the Ashkenazi Jewish (AJ) populations due to founder variants. The findings in this paper reveal the real-world prevalence of BRCA1/2 carriers in AJ population.

Methods: We performed a cross-sectional study of women who were eligible for BRCA1/2 screening program. Women who self-reported as complete or partial AJ were screened for 14 pathogenic variants in BRCA1/2 genes, following the Israeli Ministry of Health's national screening program.

Results: The study included 13,502 women who underwent screening between June 2020 and June 2022. The prevalence of the pathogenic variants in BRCA1/2 was 0.89% (120/13,502) among the tested women. Of the 14 variants tested, only six variants were detected. Three variants, known as the founder variants among AJ, accounted for 96.6% of identified variants (BRCA2: c.5946del; BRCA1: c.68_69del; BRCA1: c.5266dup). The tested women were younger and of a higher socioeconomic status compared to the eligible non-tested women.

Conclusions: The study provides a new insight into a large carrier screening program for BRCA1/2 pathogenic variants in AJ women in Israel. These findings present real-world prevalence of BRCA1/2 carriers in AJ population and the importance of such programs.

Grant References: Non relevant

Conflict of Interest: None declared

P20.004.D Characterization of structure and evolution of the 17p11.2 region

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Genomic Structural Variants (SVs) have a central role in both evolution and pathological phenotypes. Human 17p11.2 region harbors few polymorphic inversions and five segmental duplication (SD) blocks. SDs can mediate events of Non-Allelic Homologous Recombination (NAHR), leading to duplications and deletions that can cause Potocki-Lupski Syndrome or Smith-Magenis Syndrome, respectively, and other developmental delays. With our work we investigate the frequency of these polymorphic inversions in humans and trace their evolutionary history back to the divergence time between macaque (as representative of the old world monkeys) and the great apes.

Single-cell template strand sequencing (Strand-seq) on a single individual for each of the great apes plus the macaque has been used to detect species-specific rearrangements. To validate the inversions detected with the change of directionality of the sequenced reads in Strand-seq data, we performed interphase Fluorescence In-Situ Hybridization (FISH) on nuclei preparations from the same individuals.

We were able to unravel the genetic structure of the 17p11.2 region in the analyzed species, revealing the presence of several inversions that occurred during our closest primates' evolutive history. Moreover, FISH experiments allowed us to detect nested-inversion that are not noticeable when considering only Strand-seq data, but for which the integration of data from different analyses and previous literature has been essential, such as in the case of Pan genomes.

In conclusion, our study highlights the complexity of the 17p11.2 region and clarifies its evolutive history, helping to trace the origins of SVs that are causative of human pathological phenotypes.

Conflict of Interest: None declared

P20.005.A Comprehensive cardiovascular disease risk assessment across European and US-based longitudinal populations by integrating polygenic risk scores

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Background: Polygenic risk scores (PRSs) help identify individuals at high risk for CAD and improve screening and prevention. However, PRS effects may vary across populations, depending on other risk-factors. This study assessed CAD-PRS associations in relation to traditional risk factors in men and women from European and US-based populations.

Methods: We studied 38,852 people from the Dutch Rotterdam Study (n = 11,001) and US-based MESA (n = 2,685) population studies, and the Sanford Health US-based hospital biobank (n = 25,166), using a standardized CAD PRS based on 181 SNPs in relation to CAD age at onset and lipid medication use. We assessed 10-year CAD risks with PRSs added to traditional risk factors.

Results: The PRS associated with prevalent (OR_{Meta} = 1.4 per SD) and incident (HRMeta = 1.2 per SD) CAD, similarly in women and men. PRS effects on age at CAD onset were similar for men (β_{Meta} = -0.9 [-1.4;-0.5]) and women (β_{Meta} = -0.8 [-1.4;-0.2]). The PRS identified individuals at higher CAD risk, regardless of lipid-lowering medication use (HR_{Meta} = 1.2 [1.1;1.3]). Compared to the middle 50%, individuals in the top 2% of the CAD-PRS distribution had 2.3-fold higher lifetime risk [1.8;3.0] of CAD and a 6.2-fold higher risk [3.1;12.3] before age 60 After adding the PRS the C-index increased by 0.012 [0.002;0.006] in RS and by 0.004 [0.002;0.006] in MESA, and more prominently in intermediate-risk groups.

Conclusion: CAD-PRS predicts prevalent and incident CAD in women and men from different populations. A PRS can help target intermediate-risk individuals for interventions, but clinical guidelines need to be defined.

Conflict of Interest: None declared

P20.006.B Haemochromatosis HFE genotypes: quantifying excess disease outcomes in women in UK Biobank

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Background: The iron overload disorder hereditary haemochromatosis (HH) is a common genetic condition, caused by *HFE* gene mutations (p.C282Y/p.H63D). Morbidity is more common in p.C282Y homozygous men compared to women due to partial protection by menstrual iron losses. However, in women associations between *HFE* genotypes, reproductive factors, and penetrance to disease is unclear.

Methods:244,880 European ancestry women from the UK Biobank (40-70 at baseline) were followed-up via medical records (mean 12.2-years). We tested associations of reproductive factors and HH-associated disease outcomes in *HFE* p.C282Y/p.H63D genotype groups using logistic regression and Cox proportional hazards models.

Results: Female p.C282Y homozygotes (n = 1,604, 3.4% baseline diagnosed HH) had decreased odds of irregular periods at baseline (OR = 0.65, 95% CI:0.47-0.90) vs those without mutations. However, there were no associations with other reproductive factors (menarche/menopause age, live births, sex hormone levels). There were no differences in self-reported fatigue (14.4% vs 13.5%) or diagnosed depression (7.7% vs 7.4%) at baseline between p.C282Y homozygotes and those without mutations. p.C282Y homozygotes had increased risks of incident osteoarthritis (HR:1.46, 95% CI:1.14-1.88) and liver disease (HR:1.52, 95% CI:1.17-1.98), particularly fibrosis/cirrhosis and alcoholic liver disease, but not liver cancer. Stratifying analyses by menopausal status, increased risks of osteoarthritis and liver disease were seen in post-menopausal women specifically, not pre-menopausal women.

Conclusion: In this large community genotyped sample, female *HFE* p.C282Y homozygotes had increased risks of osteoarthritis and liver disease vs those without mutations, with increased risks specifically in post-menopausal women.

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Conflict of Interest: None declared

P20.007.C High prevalence of anxiety and depression in patients with rare diseases in the UK Biobank: exploring the impact of pain and physical wellbeing

Claire Hill[1], *Sonum Shah*[1], *Ashleen Crowe*[1], *Amy Jayne McKnight*[1]

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Rare diseases (RDs), while individually rare (<1 in 2,000), are collectively common, with approximately 6,000 RDs affecting up to 36 million people across the European Union. RD patients report negative mental health and quality-of-life impacts. Scope existed to explore RDs across a population cohort and compare outcomes between individuals with RDs, common diseases (CDs) or no diagnoses (ND).

Harnessing the UK Biobank (UKB) (n = 502,401), ICD10 codes were aligned with the Orphanet Knowledge base to categorise individuals with RDs, CDs or ND. Mental health (professional informed about anxiety/depression), physical activity (types last month), pain (types last month/general pain for 3+ months) and general happiness were assessed. Disease category- and sex-stratified analyses were carried out. Results from two-sample proportion tests (between RD and ND) are described.

A significantly higher proportion of RD patients had informed a professional about anxiety ($p < 0.0001$, effect = 0.29) or depression ($p < 0.0001$, effect = 0.22), with a larger difference observed in females. Significant differences in physical activity were observed across categories, with more RD patients opting for DIY ($p < 0.0001$, effect = 0.07-0.12), and less walking for pleasure ($p < 0.0001$, effect = -0.15) or strenuous sports ($p < 0.0001$, effect = -0.05), compared to ND individuals. General pain ($p < 0.0001$, effect = 0.76) and extreme unhappiness ($p = 0.0026$, effect = 0.03) significantly increased in RD.

This study compares lifestyle and health across RD, CD and ND in the UKB, revealing an increased burden on RD sufferers. Such insights can guide the development of support for patients and their families.

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P20.008.D The ancient genome of Steller's sea cow could open new possibilities for ichthyosis therapy

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Steller's sea cow, an extinct sirenian and one of the largest Quaternary mammals, was described by Georg Steller in 1741 and eradicated by humans within 27 years. Here, we complement Steller's descriptions with paleogenomic data from 12 individuals. We identified convergent evolution between Steller's sea cow and cetaceans but not extant sirenians, suggesting a role of several genes in adaptation to cold aquatic (or marine) environments. Among these are inactivations of lipoxygenase genes, which in humans and mouse models cause ichthyosis, a skin disease characterized by a thick, hyperkeratotic epidermis that recapitulates Steller's sea cows' reportedly tree bark-like skin. While *ALOXE3* and *ALOX12B* are also inactivated in dolphins, their smooth skin may be the result of loose desmosome junctions consequent to additional loss of *DSC1* and *DSG4*. Thus, modulation of *DSC1* and *DSG4* expression in the skin of patients with ichthyosis might improve their symptoms. Using the ancient genome of an extinct mammal as example, we show how evolutionary insights may open new avenues for a better understanding of current pervasive genetic diseases.

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Conflict of Interest: Diana Le Duc University of Leipzig, German Research Foundation

Medical Faculty University of Leipzig, Akhil Velluva University of Leipzig, Johannes Lemke University of Leipzig, Janet Kelso Max Planck Society, German Research Foundation , Michael Hofreiter University of Postdam, German Research Foundation, Torsten Schöneberg University of Leipzig, German Research Foundation

P20.009.A Synteny analyses reveal strong positional conservation and differential expression patterns across evolution

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ABSTRACT:

Background: Several studies have been performed to characterize the transcriptional profile of mammals along their developmental trajectory to identify stage specific changes in gene expression. While these studies focused on the whole organ, we explored chamber-specific changes in the heart across species.

Methods: Bulk RNA-seq data was retrieved from the European Nucleotide Archive (ENA) and processed through our custom pipeline to detect differential patterns in the transcriptional profile. Synteny analyses were then performed to identify gene neighbourhoods showing similar expression traits.

Results: We identified stage-specific expression patterns in the heart along the developmental axis with 109 transcripts showing differential expression in adolescent humans when compared with the neonates ($log2FC > |1.5|$, adj. $p < 0.05$). Similar analyses in mice revealed 1458 transcripts that were differentially regulated in P14 stage (2-week-old mice) compared with P0 stage (newborn). Comparison of the results with the chamber-specific dataset from the Evolutionary Atlas of Cardiac Transcriptome and homologous Genes (EvoACTG), confirmed a subset of genes (32 in humans, 30 in mice) that was differentially expressed in both datasets. The shortlisted genes were assessed by syntenic location, and fifteen of these candidates were found to be conserved across species.

Conclusion: These results demonstrate the presence of genomic regions conserved across evolution implicated in the developmental trajectory of the heart. Further characterization may reveal key drivers in the developmental lineage of the heart in different species.

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Conflict of Interest: None declared

P20.010.B Iron and risk of dementia: Mendelian randomization analysis in UK Biobank

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Background: The genetic iron-overload disease hemochromatosis is associated with increased dementia risk. Whether serum iron is related to dementia is less clear. We aimed to examine the associations between genetically predicted iron biomarkers and dementia risk as well as brain iron deposition and gray matter volumes.

Methods: We analyzed UK Biobank participants of European ($N = 451,284$), African ($N = 7,477$), and South Asian ($N = 9,570$) ancestry. Using Mendelian randomization, we examined the associations between genetically predicted transferrin saturation (TSAT) and risk of dementia (Alzheimer's disease (AD), non-AD, and vascular dementia). In a subset ($n = 39,748$) with brain MRIs, we examined its associations with regional brain iron deposition and gray matter volumes using MR.

Results: In Europeans, one standard deviation higher TSAT increased risk of non-AD dementia (OR:1.27, 95%CI:1.12-1.45, $p = 0.00018$) (including vascular dementia (OR:1.37, 95%CI 1.12-1.69, $p = 0.0023$), but not AD (OR:1.00, 95%CI:0.86-1.15, $p = 0.97$). The association between higher TSAT and increased risk of non-AD dementia was also found in participants of African ancestry, but not South Asian. In the subset with imaging data, higher TSAT was associated with higher brain iron deposition and lower gray matter volumes of the caudate, pallidum, putamen, and thalamus, but not the hippocampus, amygdala, or nucleus accumbens.

Conclusion: Genetic evidence supports a causal relationship between higher transferrin saturation and risk of non-AD dementia including vascular dementia, in participants of European and African and not South Asian genetic ancestry. Treating high transferrin saturation may reduce risk of non-AD dementia.

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Conflict of Interest: None declared

P20.011.C Identifying metabolic features of colorectal cancer liability using Mendelian randomization

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Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Recognizing the early signs of development is vital for informing prevention, early detection, and survival.

Methods: To investigate whether changes in circulating metabolites characterise the early stages of CRC development, we examined associations between a genetic risk score (GRS) describing CRC liability and 231 metabolites on the same individuals aged 8y, 16y, 18y and 25y in the Avon Longitudinal Study of Parents and Children (N = 6,221). We then performed two-sample reverse Mendelian randomization (MR) to investigate the effect of CRC liability (52,775 cases, 45,940 controls) on metabolite levels measured in a random subset of UK Biobank participants (N = 118,466, median age 58y). Finally, we performed conventional (forward) MR to evaluate metabolites for a role in CRC development.

Results: Genetic liability to CRC was associated with 28% of the circulating metabolites at FDR-P < 0.05 across all time points, particularly with higher fatty acids and low-density lipoprotein. Estimates were broadly consistent in reverse MR analyses. In conventional (forward) MR analyses, genetically predicted fatty acid concentrations were most strongly associated with CRC risk (e.g. OR per SD increase omega-3 fatty acids: 1.13, 95% CI = 1.06-1.21, FDR-P = 1.92 × 10-4).

Conclusion: These analyses reveal early alterations in systemic metabolism reflecting increased genetic liability to CRC and suggest that fatty acids may play a role in CRC development.

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Conflict of Interest: None declared

P20.012.D The mediating role of mammographic density in the protective effect of early-life adiposity on breast cancer risk

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Background/Objectives: Observational studies suggest that mammographic density (MD) may have a role in the unexplained protective effect of childhood adiposity on breast cancer risk. Here, we investigated a complex and interlinked relationship between puberty onset, adiposity, MD, and their effect on breast cancer using Mendelian randomization (MR).

Methods: We estimated the effects of childhood and adulthood adiposity and age at menarche on MD phenotypes (dense and non-dense/fatty areas) using MR and multivariable MR (MVMR), allowing us to disentangle their total and direct effects. Next, we examined the effect of MD on breast cancer outcomes, including molecular subtypes. We also explored potential pleiotropy within the MD SNPs. Finally, we performed a mediation analysis based on MVMR results of childhood adiposity and MD.

Results: Childhood adiposity had a strong dense area-decreasing effect, while adulthood adiposity increased the non-dense area. Later menarche had a positive effect on MD, but accounting for childhood adiposity, this effect attenuated to the null. MD had a risk-increasing effect on breast cancer across all subtypes. The MD single SNP estimates were extremely heterogeneous, and examination of the SNPs suggested different mechanisms may be linking MD and breast cancer. Finally, MR mediation analysis estimated that 50% of the childhood adiposity effect was mediated via MD.

Conclusion: In this work, we attempted to disentangle the relationship between factors affecting MD and breast cancer. We

showed that childhood adiposity plays a major role in decreasing mammographic dense area, thereby decreasing breast cancer risk, which presents an opportunity for intervention.

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Conflict of Interest: Marina Vabistsevits: None declared, George Davey Smith: None declared, Tom G. Richardson TRG is full-time employed at GSK for unrelated work. He hold an honorary position at the University of Bristol and was involved in this project at its conception (pre-employment in the industry)., Weiva Sieh: None declared, Joseph Rothstein: None declared, Laurel Habel: None declared, Stacey Alexeeff: None declared, Bethan Lloyd-Lewis: None declared, Eleanor Sanderson: None declared

P20.013.A The impact of genetic heterogeneity in Denmark on the predictive value of family history and polygenic scores

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Background/Objectives: Predicting disease risk and stratifying individuals into risk categories is fundamental to preventive medicine. Family history (FH) and polygenic scores (PGS) have received attention for their promise to improve the accuracy of clinical prediction models. However, the accuracy of PGS is impacted by individual genetic heterogeneity and diminished for individuals of non-European or admixed genetic ancestry. Here, we evaluated the predictive accuracy of PGS and FH for psychiatric disorders as a function of the genetic ancestry continuum in the Danish population.

Methods: Data were obtained from the Danish registers including the iPSYCH2015 cohort, which includes > 134,000 genotyped individuals of which > 80,000 had a psychiatric diagnosis. We estimated PGS and posterior family genetic liabilities (FGL) conditional on family history for all genotyped individuals and compared their predictive performance as a function of genetic distance in the PCA space.

Results: On average, FGL were 19.6% more accurate than PGS across all ancestry groups and ~260% more accurate than binary FH. We observed only limited decay in prediction accuracy for PGS and FGL for psychiatric disorders by genetic distance from the Danish median.

Conclusion: We demonstrate that, on average, FGL are more informative than binary FH indicators and PGS, and that they capture largely independent signals. Our results show that genetic heterogeneity may affect the prediction accuracy of both FGL and PGS for psychiatric disorders.

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Conflict of Interest: None declared

P20.014.B The archaic mutational load predicts the fate of introgressed fragments in humans

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Background and Aims: About 1-4% of the genome of humans living out of Africa entered in the human gene pool through interbreeding with Neanderthals. It has been suggested that Neanderthals accumulated deleterious variants that were swiftly selected against when entered the human gene pool, explaining the depletion of fragments originated from Neanderthals in genes of present-day individuals. Here we leverage the diversity of archaic genomes and deleteriousness measures of mutations to characterize the archaic mutational load along the genome.

Methods: We combined publicly available genomic datasets of present-day humans and archaic hominins and measures of phylogenetic conservations to develop population-genetics aware statistics. These were used to test hypotheses using generalized linear mixed models and resampling-based methods.

Results: We show that regions with more putatively deleterious mutations in archaic populations than in humans were more efficiently removed after introgression than regions with a lower mutational load. We found a similar pattern for variants influencing gene expression and immune-related variants, despite these are overrepresented in fragments of Neanderthal origin.

Conclusion: Fragments carrying an excess of Neanderthal-derived mutations were largely purged by natural selection.

Conflict of Interest: Alessio Gerussi Advanz, Ipsen, Rosanna Asselta: None declared, Viviane Slon: None declared, Pietro Invernizzi: None declared, Fabrizio Mafessoni: None declared

P20.015.C Population genetic distribution of variants in Finland

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Objectives: Genetic population structure is often represented by extracting leading components of genome-wide data via statistical techniques such as principal component analysis (PCA). In contrast, a distribution of a single genetic variant is typically presented by its allele frequency distribution across a geographical map. Here we consider the distribution of a genetic variant within a multidimensional PCA space of genetic population structure and compare the result to the variant's geographical distribution.

Methods: To define the population structure, we extract the first ten principal components from a data set of 49,750 Finnish individuals from THL biobank. We then apply a robust allele frequency estimator to define the distribution of each variant on the PCA space. To informatively visualize the frequencies both on the map of Finland as well as in the PCA space, we introduce a coloring scheme that provides statistical guarantees for frequency differences between different colors.

Results: We illustrate the relationship between the multidimensional PCA space and geographical coordinates by mapping one, two and ten dimensional projections of the PCA space on the map of Finland. As an example of variant-specific distributions, we use rs121964904 causing aspartylglucosaminuria to demonstrate how a rare disease-causing variant can be more tightly structured in the PCA space than on the geographical map.

Conclusion: Our framework provides additional information about the distribution of individual variants on top of geographical frequency maps and contributes to extending future population genetic analyses from the level of individuals to the level of individual variants.

Grant: Sigrid Jusélius Foundation

Conflict of Interest: Juha Riikonen University of Helsinki, Sini Kerminen Nightingale Health Plc, Nightingale Health Plc stock options, Aki Havulinna Finnish Institute for Health and Welfare, and Institute for Molecular Medicine Finland, Helsinki Institute of Life Science, Grant from Academy of Finland (not related to current project), Markus Perola Finnish Institute for Health and Welfare, Veikko Salomaa Research Professor (emeritus) at the Finnish Institute for Health and Welfare, Research grants from the Finnish Foundation of Cardiovascular Research and from Juho Vainio Foundation, Research Collaboration with Bayer Ltd (Not related to the current study), Matti Pirinen University of Helsinki, Academy of Finland (338507, 352795), Sigrid Jusélius Foundation project grant

P20.016.D Disentagling archaic introgression and genomic signatures of selection at human immunity genes

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Pathogens and infectious diseases have been acting as a powerful selective pressure on ancient and modern human genomes and contributed to the current genetic diversity of many genes. There is evidence to suggest that modern humans have acquired immune variants through admixture with ancient hominins.

The main objective of the research was to infer genetic signatures of positive selection that may be involved in adaptation to infectious diseases and to investigate the function of Neanderthal alleles identified within a set of 50 Lithuanian genomes.

Introgressed regions were identified using two statistical approaches: Sprime and ArchIE. Positive selection signatures were analysed using the iHS and XP-EHH. Annotation performed with ANNOVAR in GRCh37 (hg19), RefSeqGene, gnomAD, dbSNP147 and CADD v.1.3. The search of the genetic signatures interaction with protein and genetic interactions from multiple species was performed using The BioGRID database.

We detected high-scoring signals of positive selection at innate immunity genes (*EMB*, *PARP8*, *HLA-C*, *CDSN*), and evaluated their interaction with pathogen proteins. These pathogens are human immunodeficiency virus (HIV) 1, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poliovirus, and human herpes virus (HHV) 8.

Finally, we have identified a strong genomic region comprising *HLA-DRB1*gene introgressed from Neanderthals and under positive selection with three non-synonymous variants: rs9270302: c.C41T:p.A14V; rs9270303:c.A37G:p.T13A; rs707953:c.A14G:p.K5R.

These results reveal some of the genetic footprints left by the pathogens in the Lithuanian genome.

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Conflict of Interest: None declared

P20.017.A Founder effect of a common pathogenic TNNT2 variant responsible for hypertrophic cardiomyopathy in the northwest of Spain

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Background/Objectives: Hypertrophic cardiomyopathy (HCM) is the most common inherited heart disorder, with a prevalence between 1/250-500 individuals. The genetic etiology responsible for HCM remains unknown in more than 60% of patients. At our center, we have identified a recurrent variant in TNNT2, p.Asn271Ile, associated with HCM. Most of the probands came from the northwest of Spain (Galicia), so it has been suggested that they share a common origin. We aimed to determine whether carriers of the p.Asn271Ile variant in TNNT2 share a common haplotype and to estimate the age of the mutation.

Methods: We analyzed 28,000 probands in which TNNT2 was sequenced by NGS at our center. Enrichment of the variant was calculated in our HCM cohort. Eight highly polymorphic microsatellite markers flanking the variant were tested for in 18 Galician HCM probands, compromising a region of approximately 7,300 kb. Custom PCR assays were designed, and fragment length analysis was performed by capillary electrophoresis.

Results: TNNT2 p.Asn271Ile was identified in 49/28,802 probands (0.17%). The variant was significantly enriched in HCM probands (47/11,870; 0.39%) compared to internal non-HCM controls (2/14,804; 0.02%), with an OR = 29.3 (IC95% = 7.1-

120.7). The analysis revealed that the 18 HCM Galician probands shared a common haplotype of 500 kb, estimating that p.Asn271Ile has arisen approximately 26.5 generations ago (650 years).

Conclusions: The pathogenic variant p.Asn271Ile in TNNT2 explains more than 1% of HCM cases in the Galician population. A founder effect has been demonstrated, with the variant arising approximately 650 years ago.

Conflict of Interest: Rosalía Peteiro Full time, Jose María Larrañaga Moreira: None declared, Emilia Maneiro Full time, Roberto Barriales: None declared, Laura Cazón Full time, Iria Gómez Díaz Full time, Marlene Perez Barbeito Full time, Inés Alvariño Full time, Maria Sanchez Full time, Anahi Sanluis Verdes Full time, Guillermo Smith Ramos Full time, Paula Rebolo Full time, Paula Velez Full time, Ivonne Cárdenas Reyes Full time, Diego Cabrera Argaña Full time, Xusto Fernandez Full time, Almudena Amor Full time, María Valverde Full time, Soledad García Hernández Full time, Luis De la Higuera Romero Full time, Martin Ortiz Genga Part time, Juan Pablo Ochoa Full time

P20.018.B Germline genetically predicted body mass index is associated with endometrial cancer somatic transcriptomic, immune, and mutational signatures in The Cancer Genome Atlas

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Background/Objectives: High body mass index (BMI) is a causal risk factor for endometrial cancer but the molecular mechanisms underlying this association remain elusive. We sought to characterise the tumour genomic landscape of endometrial cancers that had developed on a germline genetic background predisposing to higher BMI.

Methods: A polygenic score (PGS) for adult BMI in European-ancestry women was built using 242 previously reported genome-wide significant ($P < 5 \times 10^{-9}$) and independent (r^2<0.05) BMI-associated variants. We assigned this BMI PGS to 354 endometrial cancer cases of genetically inferred European ancestry from The Cancer Genome Atlas (TCGA) using their germline (blood DNA) genotypes. Associations between the BMI PGS, which reflects life-course trajectories of adiposity, and tumour genomic, transcriptomic and immune traits were then identified using generalised linear models adjusted for age, stage, microsatellite status, and 10 genetic principal components.

Results: We ranked 18,458 genes based on the association between their tumour expression and the BMI PGS. Gene set enrichment analysis of the ranked list revealed that the BMI PGS was strongly associated with upregulation of genes in IL6-JAK-STAT3 signalling (false discovery rate (FDR) $= 4.23 \times 10^{-7}$). Endometrial tumours that had developed on a germline background of high BMI were also associated with increased activated mast cell infiltration (FDR $= 8.36 \times 10^{-3}$), and with the single base substitution (SBS) mutational signatures 1 ($p = 0.003$) and 5 ($p = 0.02$).

Conclusion: We combined germline and somatic data using a novel approach to map the multi-omic landscape of endometrial cancers developing on a background of adiposity with implications for precision intervention.

Grant Reference: CRUK [C18281/A29019], UKRI [MR/T043202/1]

Conflict of Interest: None declared

P20.019.C Regionally enriched rare deleterious exonic variants in the UK and Ireland

Mihail Halachev[1], *Elvina Gountouna*[1;2], *Alison Meynert*[1], *Gannie Tzoneva*[3], *Alan R Shuldiner*[3], *Colin Semple*[1], *James F. Wilson*[1;4]

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Geographic clustering of haplotypes appears to have emerged in the UK as a result of differing patterns of immigration and degrees of geographical and/or cultural isolation. However, until recently it has been unclear how such patterns of regional differentiation might impact the protein-coding fraction of the genome.

Here, we exploit UK Biobank and Viking Genes WES data to study regional genetic differentiation across the UK and Ireland in protein-coding genes, encompassing 20 regions of origin and 44,696 unrelated individuals.

We rediscover the strong influence of genetic drift in shaping variation in the Northern Isles of Scotland and among those with full or partial Ashkenazi Jewish (AJ) ancestry. For full AJ, almost half the known rare exonic variants (45%) are at least two-fold more or less frequent than in a European reference group, while the degree of variant frequency differences in Shetland and Orkney are comparable to partial AJ (~20%). We also demonstrate substantial genetic differentiation among several mainland regions of origin, particularly north and south Wales, south-east Scotland and Ireland. With stringent filtering criteria we found 67 recessive variants likely to have adverse biomedical consequences, enriched by at least five-fold in frequency in one or more British or Irish regions relative to a European reference group.

We conclude that regional genetic variation across the UK and Ireland should be considered in the design of genetic studies, and may inform effective genetic screening and counselling.

We acknowledge support from the MRC (MC_UU_00007/10, MC_UU_00007/16) and CSO (Scottish Government: CZB/4/276, CZB/4/710).

Conflict of Interest: Mihail Halachev: None declared, Elvina Gountouna: None declared, Alison Meynert: None declared, Gannie Tzoneva Full-time employees of Regeneron Pharmaceuticals, Inc., Receives salary, stock and stock options as compensation, Alan R Shuldiner Full-time employee of Regeneron Pharmaceuticals, Inc., Receives salary, stock and stock options as compensation, Colin Semple: None declared, James F. Wilson: None declared

P20.020.D Regional genotypic risk scores discover novel molecular phenotypes causally implicated in systemic lupus erythematosus

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Background/Objectives: Systemic Lupus Erythemathosus (SLE) is a systemic autoimmune disease characterized by a complex aetiology with complex genetics in which many factors are involved besides

many molecular intermediates. We aim to find protein levels associated with SLE using individual genotypic information from large SLE cohort and results from large GWAS analyses.

Methods: We used genotypes for 10,180 European ancestry individuals obtained from pre-existing projects studying SLE genetics. We computed genotypic risk scores for biomarkers using the GENOSCORES platform and tested the association between scores and the SLE phenotype by means of logistic regression models. We followed up these results by conducting colocalization and mendelian randomization (MR) analyses. We finally tested associations between the scores and cytokines levels and clinic manifestations.

Results: We detected 7 proteins' scores (FCGR2B, AXIN2, TREML4, AMBN, ATF6, EDA and FIBCD1) significantly associated with SLE risk at a Bonferroni threshold. Furthermore, we showed that gene expression of the *AXIN2* and *TREML4* genes were significantly associated with SLE. Finally, we confirmed a causal role between SLE risk and protein level or gene expression of *TREML4*, *AXIN2* and *FCGR2B* genes. We also discovered significant ($P < 0.05$) associations between proteins scores and IL6, IL1ra and MCP2 levels along with few relevant clinical comorbidities of SLE.

Conclusion: This study expands the list of candidate proteins associated with SLE and regions that might contain novel genes implicated in the SLE phenotype. Our findings exhibit how genotypic scores for molecular traits can be used to identify and characterize genetic associations with complex disease traits.

Conflict of Interest: None declared

P20.021.A Polymorphism dynamics of STRs in 1000 genomes phase 3 trio data

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Short tandem repeats (STRs) are highly polymorphic genomic elements with mutation rates as high as 10^{-4} per generation. Profiling STR variation from population genome datasets helps shed light on their functional roles. Previous large scale studies have genotyped 1.6 million STR loci and linked the polymorphisms of a subset to expression levels of proximal genes. More recently, the UK Biobank consortium reported genotypes of ~2.5 million STR loci from whole genome (WGS) data of 150k individuals. However, studies indicate that the human genome harbors close to 4.5 million STRs, the polymorphism dynamics and mutation rates of which remain largely unknown. Here, we develop an efficient method for genotyping STR loci from large WGS datasets. Using this method, we genotype 4.5 million STRs from 698 trio datasets of the 1000 Genomes Project Phase 3. Comparing trio genotypes, we identify de novo mutation rates and create a catalog of rapidly mutating STR loci in the human genome. We further inspect the dependence of mutation rates on the length of STR, the motif sequence characteristics, repeat purity, sample ethnicity, and genomic context. Our work reports a rapid method to genotype STRs, and serves as a resource to fuel the functional characterization of these versatile elements.

Conflict of Interest: None declared

P20.022.B A benchmarking of human Y-chromosome haplogroup classifiers and a characterization of contemporary patrilineages of the Canary Islands

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Background/Objectives: High-throughput sequencing has profoundly increased the availability of genetic markers in the non-recombinant region of the Y-chromosome (NRY) and has prompted the development of haplogroup classification tools. Here we compared five tools for human NRY haplogroup classification. In addition, the most accurate tool was used to characterize the contemporary patrilineages from the Canary Islands (Spain), a population with significant African influences.

Methods: The benchmarking of the selected tools was done using whole-genome (WGS) and exome (WES) sequencing short-reads from 50 donors of diverse ancestry. To characterize the patrilineages from the Canary Islands, WES from 452 unrelated donors were obtained.

Results: Yleaf offered the best performance for WGS and WES by classifying precisely 96% and 88% of the analyzed samples, respectively. Using Yleaf in WES data from the Canary Islands resulted in the identification of seven macro-haplogroups. Those with a frequency above 10% were E, I, J, and R, the latter being the most predominant in the population (52.65%).

Conclusion: We demonstrate that WES can be an efficient approach to infer the NRY haplogroup, albeit providing a lower level of genealogical resolution than that recovered by WGS. We also provide a characterization of the NRY diversity of the contemporary Canary Islanders at unprecedented resolution.

Grant References: Ministerio de Ciencia e Innovación (RTC-2017-6471-1), co-financed by the ERDF 'A way of making Europe' from the EU; Fundación CajaCanarias and Fundación Bancaria "La Caixa" (2018PATRI20); Cabildo Insular de Tenerife (CGIEU0000219140); ITER Agreement (OA17/008); Consejería de Educación-Cabildo de Tenerife 2021-2025 (CGIAC0000014697).

Conflict of Interest: None declared

P20.023.C To exploit Runs of Homozygosity (ROH) and Identical By Descent (IBD) based methods to identify genetic regions associated to Coronary Heart Diseases

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Cambridge, United Kingdom; [6]AOU Città della Salute e della Scienza, Medical Genetics Unit, Turin, Italy

Background/Objectives: Cardiovascular disease (CVD) is a complex disorder and a leading cause of mortality worldwide.

We aimed at investigating CVD polygenic component by using Runs of Homozygosity (ROH) and Identical By Descent (IBD) methods in a large European cohort.

Methods: We analysed 8932 pre-clinical coronary heart disease (CHD) individuals and 16134 controls, belonging to European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (EPIC-CVD study)[2]. SNPs genotyping analyses were performed with three different chip arrays: Omni Express Exome, Illumina 660Quad array and Human Core Exome.

We investigated the association between CHD and both the count and the size of ROHs and IBDs.

Results: Pre-clinical CHD had less and shorter ROHs when compared to coronary-heart-disease-free control subjects ($p = 7.35e-06$). Despite this, analysing the differences between fatal and not fatal cases, we could observe that fatal cases had more and longer ROHs than not fatal ($p = 0.04$).

Due to the large number of variants carried by each individual, we performed the preliminary likelihood ratio test on IBDs for a subset of 14332 individuals. Regression between cases and controls showed significant results ($p < 2e-16$), showing a lower risk of cardiovascular events based on IBDs presence ($OR = 0.98$).

Conclusion: This study revealed differences in the ROHs levels between individuals with fatal and not fatal CHD events. Nevertheless, the over-representation of ROHs among controls subjects suggested that the accumulation of regions of recessive variants did not increase the risk of CHD, but it might influence disease severity.

Grant References: EPIC-CVD project European Union Framework7 (HEALTH-F2-2012–279233), CARDIATEAM (H2020-JTI-IMI2-n.821508)

Conflict of Interest: None declared

P20.024.D Haemochromatosis HFE genotypes: cumulative incidence of hospital diagnosed complications to age 80 in the UK Biobank

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Introduction: Hereditary Hemochromatosis is caused by the *HFE* genotypes p.C282Y and p.H63D. Iron levels are elevated in individuals with p.C282Y homozygosity, although the extent of related diagnosis and excess mortality is uncertain, particularly in older ages.

Method: We studied European descendants ($n = 451,270$) from the UK Biobank (12.2-year follow-up), including 2,902 p.C282Y homozygotes. Cox Proportional Hazard models adjusted for age, assessment centre, genetic principal components; sex stratified. We estimated cumulative incidence of co-morbidities to age 80.

Results: Male p.C282Y homozygotes had increased risk of multiple health outcomes compared to participants with no *HFE* mutations, including joint replacement surgery (29% incidence by age 80 vs 18% with no *HFE* mutations; Hazard Ratio HR:1.84, 95%CI 1.55-2.18, $P = 2.7 \times 10^{-12}$), any liver disease (20.2% vs 8.0%, HR:2.70, 2.20-3.32, $p = 4.5 \times 10^{-21}$), any brain-related outcome (delirium, dementia, or Parkinson's disease) (19.41% vs 10.02%, HR:1.72, 1.34-2.20, $p = 16 \times 10^{-6}$), any liver cancer (6.71% vs 1.13%, HR:7.63, 5.14-11.33, $p = 9.1 \times 10^{-24}$), and all-cause mortality (33.2% vs 25.6%; HR:1.27, 1.09-1.49, $p = 0.002$). Female p.C282Y homozygotes had higher risk of any liver disease (7.97% vs 6.55%; HR:1.52, 1.17-1.98, $p = 0.002$) and osteoarthritis (13.14% vs 8.75%; HR:1.46, 1.14-1.88, $p = 0.003$). After adjusting for multiple testing, p.C282Y/p.H63D heterozygotes showed no significantly increased risks of health outcomes.

Conclusion: Male p.C282Y homozygotes have a significant excess of hospital-diagnosed complications and all-cause mortality by age 80. Early diagnosis through screening or improved case-finding for *HFE* variants could help to prevent HH-related health outcomes.

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Conflict of Interest: None declared

P20.026.B ASAP - ASsessing Ancestry through Principal component analysis

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The history of human populations has been characterized by admixture events that are reflected in the genome of each population. Therefore, the evaluation of the ancestral components of human populations could be crucial to fully characterize the history of our species, which could also embed relevant information, useful to develop and design efficient medical studies and treatment. Although many algorithms aiming to infer the population's genetic composition have been developed, most of them are characterized by poor reliability when samples with high missingness rate are analyzed, as is often the case for ancient DNA data.

It has been recently shown that F-statistics, harnessed by qpAdm to assess ancestry, are strongly correlated to PCA (Principal Component Analysis), a widely used method in population genetics to infer the genetic variation among populations. Based on this, we propose to leverage on PCA and NNLS (Non-Negative Least Squares) to assess the ancestral composition of admixed individuals. We assessed and tuned our approach on simulated data, including variable missing rate, pseudo-haploid samples and different projection strategies to mirror the quality of ancient DNA.

Our results show that the method we propose, ASAP (ASsessing Ancestry through Principal component analysis), has high accuracy and reliability, similar (and in some cases even better) to that obtained with other already available methods.

Thus, we present a useful tool to assess ancestral compositions of admixed individuals/populations with good accuracy also for ancient samples and without the need to predetermine the proxy sources.

Conflict of Interest: None declared

P20.027.C Genetic correlates of migration patterns within Estonia

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Human migrations are viewed to be predominantly driven by social, demographic and cultural factors. However, little is known about the genetics of migration behaviours in human populations. Behavioral traits were shown to be highly polygenic and a large sample size is required for investigating them with contemporary genetic methods. This became possible only relatively recently with the advent of biobank-scale datasets. As an example, it has been shown that polygenic score (PS) values for some traits related to socioeconomic status and human health are unevenly spatially distributed over the UK. This could be explained by the existence of a genetic component associated with migration behavior, either directly or indirectly, for example, via other traits such as educational attainment (EA).

In our work, we use data from the Estonian Biobank, containing genetic and phenotypic data for more than 200,000 individuals to explore the correlation between genetic structure, social stratification and migration behaviour in Estonia.

We find patterns of uneven distribution of traits and polygenic scores in Estonia. Although contemporary migration attenuates the genetic structure described by principal components, it intensifies geographic stratification specific to some PSs. In particular, individuals who migrated to the most economically developed regions from the rest of the country significantly differ in some PS values from the non-migrating part of the population. The most robust signal we observe is related to traits demonstrating strong genetic correlation with EA.

This work illustrates the potential role of heritable traits in the continuing process of shaping human fine-scale population structure.

Conflict of Interest: None declared

P20.028.D Population specific circulating proteins influencing diseases and traits in African ancestry

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Background and Objectives: Individuals of African ancestry exhibit a unique genetic architecture that extends to a multitude of African-specific diseases and traits. As such, it is therapeutically important to identify population-specific circulating proteins that may act as drug targets.

Methods: Cis-pQTLs from 1,567 circulating proteins in African Americans were identified from publicly available data and used as exposure instruments to estimate their causal effect on 13 traits in different African ancestry cohorts using two-sample Mendelian randomization (MR). In parallel, population branch statistics (PBS) were computed to identify cis-pQTLs with strong allele frequency differentiation to detect putative signals of natural selection in African populations.

Results: Following MR, circulating protein levels in individuals of African ancestry were significantly associated with height, lipid traits, diabetic retinopathy, and post-traumatic stress disorder after multiple-testing correction. In the PBS analysis, cis-pQTLs from 804 circulating proteins revealed 41 putative natural selection candidates. Among the 41 candidates exhibiting the largest PBS values (i.e., top 95th percentile), five proteins instrumented by African-specific cis-pQTLs were prioritized from the MR findings, including inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4). Interestingly, ITIH4 was causally associated with height (beta = -0.048, SE = 0.012, $P = 5.64 \times 10^{-5}$) and demonstrated a putative signal of positive selection (rs2710344, African MAF = 0.35; Non-Finnish Europeans MAF = 0.001).

Conclusion: In this analysis we have identified African-specific circulating proteins causally influencing diseases and traits, including ITIH4, of which large frequency differentiation was putatively explained by natural selection.

Grant References: None.

Conflict of Interest: None declared

P20.029.A Selection bias in the estimation of causal effects within family studies using Mendelian randomization

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Background: Selection bias is a substantial concern for analyses of epidemiologic studies as samples do not typically represent a true random sample of the population of interest. This reduces the internal validity of a study and biases results. There is increasing awareness that selection bias can affect studies using population samples, we do not know how selection bias affects studies using samples of family members such as within family Mendelian randomization (MR) studies.

Methods: We generate and compare selection bias in estimates using trio data from three models: a phenotypic model, a population MR model, and a within family MR model. We attribute selection bias to conditioning on confounders of the exposure outcome relationship. Simulations were conducted to demonstrate the differences in estimates over increasing selection into study. Subsequently, these models were applied to available trio data in MoBa to investigate the impact of this bias on causal offspring estimates.

Results: The mean bias in estimates for all approaches reduced as selection into the study increased and all methods were affected by selection bias. The within-family estimates were less biased, relative to the phenotypic and typical MR models. Applied analyses to determine whether these differences in estimated are demonstrated are ongoing.

Conclusions: Our simulated results demonstrate selection bias has a substantial effect on causal estimates and this may be mitigated through restriction of study samples to family trios.

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Conflict of Interest: Ciarrah-Jane Barry Wellcome Trust (218495/Z/19/Z/WT), Medical Research Council (MC_UU_00011/1/), Neil Davies Research Council of Norway (295989, 300668), Medical Research Council (MC_UU_00011/1, MR/V002147/1), National Institute of Mental Health (MH130448), National Heart Lung and Blood Institute (R01 HL105756-11), European Social Science Genetics Network (HORIZON-MSCA-2021-DN-01), National Institutes of Health (1R01NS107607-01A1),

P20.030.B Disease prevalence, health-related and socio-demographic factors in the GCAT cohort. A comparison with general population of Catalonia

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Background/Objectives: Population-based cohorts play a key role in genetic studies. It is known that these cohorts usually include a healthy volunteer bias. Assessment and characterization of this bias would help to extrapolate cohort studies to the general population. Here, we assess the bias of the population-based cohort GCAT, encompassing 20,000 participants from Catalonia, aged 40-65 years at recruitment (2014-2018). Due to the characteristics of the recruitment, it has an overrepresentation of usual blood donors (94.88%). Including a 59.2% of females, and with an 83.3% of white/Caucasian participants. The aim of this study is to compare the individuals of GCAT cohort with all the Catalan population from the same age range, to assess their differences and the representativeness of this cohort for the entire population, as well as determining the weights to make it representative.

Methods: The comparison was done using Information System for Research in Primary Care (SIDIAP) data, a database of population-wide primary care electronic health records, including the 75% of Catalan population. Prevalence comparison was done using two one-sided t-test. We used weighting techniques accounting for age, sex, and other socio-demographic factors.

Results: GCAT Cohort is healthier than general population. Individuals of this cohort live in less deprived areas, have healthier lifestyle habits, lower mortality rate and lower disease prevalence. Individual weights were obtained to correct this bias.

Conclusion: Although this bias may not directly affect exposure-disease associations, it is important to acknowledge this source of bias, and be able to make results generalisable using weighting techniques.

Conflict of Interest: None declared

P20.031.C A BRCA1 pathogenic founder variant in Orcadians justifies targeted screening

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Founder pathogenic variants in BRCA genes are well described in European populations. Screening for these variants has been reported to be cost-effective, and NHS England is planning to start offering targeted testing to women with at least one Jewish grandparent, regardless of cancer family history, this year.

The Northern Isles of Scotland have the most divergent and isolated of British and Irish populations. We recurrently observed the *BRCA1* pathogenic variant c.5207T>C p.(Val1736Ala) in clinical investigation of breast and ovarian cancer families from Orkney, and demonstrated relatedness through clinical genealogy. Twenty of 2,088 ORCADES population cohort participants have the variant (1%). Genealogy records indicate an origin in the outer isle of Westray, c. 1600s, and all carriers share a common haplotype around the variant.

Within ORCADES, 19/20 variant carriers report a Westray-born grandparent. We estimate that 1 in 25 (4%) individuals with three or four Westray grandparents carry the variant, as do 1 in 50 with one or two Westray grandparents.

Offering a saliva-based test for p.(Val1736Ala) to those who self-report a Westray grandparent, with genetic counselling follow-up for those ascertained, would be a cost-effective way to target *BRCA1* testing to the ~20% of Orcadians most at risk. Planning for a community based self-administered saliva testing service is underway.

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Conflict of Interest: Zosia Miedzybrodzka Professor of Medical Genetics

Service clinical director Medical Genetics, NHSS Grampian, Application to Friends of ANCHOR for pilot of screening of Westray population is under review, Awards to institution not wrt this project: MEGS award from AMGEN for FH data analysis, AKCEA for FCS testing, AstraZeneca funds somatic testing for some breast cancer patients tested in our lab. Not related to this project.

, Attended professional symposia on lipids part-funded by

Educational visit to Oslo newborn screening lab funded by Novartis Jan 2023

Speaker at PTC therapeutics meeting on neuro-developmental disorders

Attended meetings of Scottish Lipid Forum jointly funded by AstraZeneca, Novartis, MSD, AMGEN, Crest,

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P20.033.B Leptin-melanocortin pathway associated childhood obesity

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Background/Objectives: Monogenic obesity is severe, genetically determined disorder that affects up to 1/1000 newborns. Recent

new therapeutics have highlighted the need for early identification of individuals with rare genetic variants that can alter the functioning of the leptin-melanocortin signalling pathway, to speed up clinical intervention and reduce the risk of chronic complications.

Methods: NGS of genes in hypothalamic leptin-melanocortin pathway was performed in 1508 participants with and without obesity, aged 2-19 years. The model-estimated effect size of rare genetic variants in this pathway on longitudinal weight gain between carriers and non-carriers was derived.

Results: In total, 21 (1.4%) participants had known disease-causing heterozygous variants (DCVs) and 62 (4.1%) participants were carriers of rare variants of unknown clinical significance (VUS). The estimated frequency of potential genetic variants associated with obesity ranged between 1/150 (VUS and DCV) and 1/850 (DCV) and differed significantly between participants with and without obesity. On average, the variants identified would result in 7.6 kg (7.0-12.9 kg at the 95th percentile of body weight) (girls) and 8.4 kg (8.2-14.4 kg) (boys) of additional weight gain in carriers at age 18 years compared with subjects without obesity.

Conclusion: Children with a genetic predisposition to obesity can be promptly identified and may account for approximately 6% of obesity cases. Early identification of variants in the *LEPR*, *PCSK1*, *POMC*, *MC3R* and *MC4R* genes could reduce the societal burden and improve the clinical management of early severe childhood obesity and its implementation should be further investigated.

Grant references: SRA J3-9282, Z3-7412, P3-0343.

Conflict of Interest: None declared

P20.034.C The Genome of Europe: A Europe-wide Reference Database of Genomes

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Background / Objectives: The mission of the 1 million genomes (1 + MG) initiative, declared in 2018 by 26 signatory countries, is to make >1 million whole genome sequences (WGS) accessible for use in research, health care and prevention. A 1 + MG working group, named Genome of Europe (GoE), started in 2019 to establish a European Reference Genome Database of >500k WGS.

Methods: The rationale for creating GoE is to have a large, Europe-wide collection of subgroup-specific reference genomes (with minimal phenotype data). These can be used: a) to analyse genetic diversity across European populations, b) to interpret potentially clinical/pathogenic genetic variants in comparison to disease-specific genomes, c) to recalibrate genetic risk profiles to ancestral backgrounds, d) as a reference panel for imputations in lower resolution but larger scale array genotyping efforts.

Results: The GoE working group has provided recommendations on: a) composition of the samples contributing to GoE, b) proportional sample number contributions per country, c) sample recruitment protocols, d) WGS technology, and e) data requirements (together with the EU Genomic Data Infrastructure (GDI) project. We have identified 40 ancestries across 27 EU member states, and proposed a distribution of WGS tasks. We identified WGS capacity and technology preferences, and formulated consent requirements.

Conclusion: The GoE WGS database will create a reference dataset for genomic health programs of the European countries and beyond.

Conflict of Interest: None declared

P20.035.D The prevalence and genetic spectrum of familial hypercholesterolemia in Qatar based on whole genome sequencing of 14,000 Subjects

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Familial hypercholesterolemia (FH) is an inherited disease characterized by a reduced efficiency of low-density lipoprotein-cholesterol (LDL-C) removal from the blood and consequently, an increased risk of life-threatening early cardiovascular complications. In Qatar, the prevalence of FH has not been determined and the disease, as in many countries, is largely underdiagnosed. In this study, we combined whole genome sequencing data from the Qatar Genome Program with deep phenotype data from Qatar Biobank for 14,056 subjects to determine the genetic spectrum and estimate the prevalence of FH in Qatar. We used the Dutch Lipid Clinic Network as a diagnostic tool and scrutinized 11 FH-related genes for known, and possible, pathogenic mutations. Results revealed an estimated prevalence of 0.8% (1:125) for definite/probable cases of FH in the Qatari population. We detected 16 known pathogenic/likely pathogenic mutations in *LDLR* and one in *PCSK9;* all in a heterozygous state with high penetrance. The most common mutation was rs1064793799 (c.313+3A>C) followed by rs771019366 (p.Asp90Gly); both in *LDLR*. In addition, we identified 18 high penetrant *possible pathogenic* variants, of which 5 were Qatari-specific, in *LDLR*, *APOB*, *PCSK9*, and *APOE* which are predicted to be among the top 1% most deleterious mutations in the human genome, but further validation is required to confirm their pathogenicity. We did not detect any homozygous FH or autosomal recessive mutations in our study cohort. Currently we are investigating the polygenic architecture of hypercholesterolemia in Qatar. This study is funded by a QNRF grant (PPM 03-0324-190038).

Conflict of Interest: Ilhame Diboun Sidra Medicine, Qatar Foundation, Yasser Al-Sarraj Qatar Foundation, Salman Toor Hamad Bin Khalifa University, Qatar Foundation, Shaban Mohammed Hamad Medical Corporation, Nadeem Qureshi University of Nottingham, Moza Al-Hail Hamad Medical Corporation, Amin Jayyousi Hamad Medical Corporation, Jassim AlSuwaidi Hamad Medical Corporation, Omar Albagha Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar., Qatar National Research Fund (PPM 03-0324-190038).

P20.036.A Unraveling the interplay of genetic predisposition to aging-related phenotypes, air pollution and sex on cognitive performance

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Background: Air pollution is a known risk factor for aging-related conditions, including cognitive impairment. However, the underlying biological mechanisms are not yet fully understood and may depend on individual biological characteristics such as age, sex and genetic background. In this study, we investigated the combined effect of genetic risk scores (PRS) of human aging traits, air pollution, and sex on cognitive function.

Methods: We studied 2,377 cognitively unimpaired adults from the ALFA study. Long-term exposure to air pollutants (NO_2, $PM_{2.5}$ and PM_{10}) was estimated using land-use regression models. PRS of human aging traits, including life expectancy, longevity, health-span, perceived age, and epigenetic age acceleration, were calculated using PRSice-2. A cognitive test battery was administered to assess episodic memory (EM), executive function (EF) and Preclinical Alzheimer Cognitive Composite (PACC). Models were adjusted for age, years of education and *APOE-ε4* status. Sex-specific effects were examined by fitting interaction models.

Results: Genetic predisposition to longer health-span was associated with better EM and PACC in women exposed to lower NO_2 and $PM_{2.5}$ levels. Genetic predisposition to younger perceived age was associated with better EF and EM, but only at low NO_2 levels. In women, genetic predisposition to younger perceived age had a positive effect on PACC when exposed to low PM_{10} levels, whereas it had a negative effect in men.

Conclusion: The effect of air pollution on healthy aging depends on complex gene-environment interactions that differ among sexes. Further analyses will be conducted to characterize the biological pathways involved in these associations.

Conflict of Interest: Blanca Rodríguez-Fernández: None declared, Gonzalo Sánchez-Benavides: None declared, Carolina Minguillón: None declared, Marta Cirach: None declared, Mark Nieuwenhuijsen: None declared, Jordi Sunyer: None declared, Karine Fauria: None declared, Jose Luis Molinuevo JLM is currently a full-time employee of Lundbeck, JLM has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences, JLM has previously served as a consultant or at advisory boards for the following for-profit companies, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences, Manel Esteller: None declared, Arcadi Navarro: None declared, Juand D. Gispert JDG has received speaker's fees from Philips and Biogen and research support from GE Healthcare, Roche Diagnostics and Hoffmann-La Roche., Aleix Sala-Vila: None declared, Marta Crous-Bou: None declared, Natalia Vilor-Tejedor: None declared

P20.037.B The POPGEN project: diving into the French fine-scale population genetic structure in the time of our grandparents to build a reference panel of genomes

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Background/Objectives: The POPGEN project was launched as part of the French genomic medical initiative to build a catalogue of variants found in the different regions of metropolitan France and provide allele frequencies in order to help filter out neutral variants from patient genomes.

Methods: Individuals from the population cohort Constances were asked to complete a questionnaire on birthplaces and birth years of their parents and grandparents. Based on their answers, 15,000 individuals were selected to cover the different regions of metropolitan France and were posted saliva collection kits. DNA was extracted from the 10,245 kits returned with proper consents and 9,862 were sent for genotyping. Genotyping was successful for 9,772 individuals. Different clustering methods were used to study fine-scale population structure and rare variants were imputed using public reference panels enriched by 856 whole genomes from the FranceGenRef project.

Results: We describe the results obtained by different methods to cluster individuals and show how these clusters correlate with geography and could impact allele frequency estimation of rare variants. We also show how we selected a subset of individuals for whole genome sequencing to capture the maximum of the genetic diversity and to provide a reference panel for imputation.

Conclusions: This study proposes a design to sample individuals from the general population to create reference panels that could help improve imputation accuracy for geographically clustered variants. The POPGEN project will contribute to the "Genome of Europe" project.

Grant: French Ministry of Research PFMG2025 and ANR IA-10-LABX-0013 FranceGenRef

Conflict of Interest: None declared

P20.039.D 20 years of genetic testing for hereditary transthyretin amyloidosis (ATTR) provide new evidence for a founder event in the endemic focus of the Balearic Islands

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Background: TTR amyloidosis (ATTR) can affect multiple organs and systems, including the nervous and gastrointestinal systems, the heart, kidneys and eyes. Val30Met (V30M) is the most common pathogenic variant of the TTR gene worldwide and also in the island of Mallorca (Balearic Islands, Spain) where ATTRV30M amyloidosis is considered endemic.

Results: Since 2002 we have tested 1168 patients from the four Balearic Islands (0.1% of a population of 1.2 million inhabitants). The main referral reasons have been a family history (presymptomatic testing), polyneuropathy and cardiac involvement. In total, the diagnostic yield was 25% and we detected 287 carriers for the V30M variant and 7 carriers for other potentially pathogenic variants (H31N, S77Y, E89K, T119M and V122I). The prevalence for the V30M variant based on these results for each island, are 1/3500-4000 for Mallorca and Menorca. However, Ibiza shows a much lower prevalence of 1/25000. Furthermore, we find that 93% of patients carry V30M and another variant in the same gene, G6S, in cis conformation.

Conclusion: This is the first time that Menorca is described to have a similar V30M prevalence to that of the island of Mallorca. G6S has been considered a benign variant, however, we hypothesize it could play a role as a phenotype modifier and contribute to the specific phenotypic traits of the Balearic population. In addition, the unique presence of a G6S and V30M haplotype argues in favor of a founder event in the Mallorca and Menorca population that has not affected the island of Ibiza.

Conflict of Interest: None declared

P20.040.A A genetic perspective on the recent demographic history of Ireland and Britain

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Motivation: The demographic histories of fine-scale genetic communities within Ireland and Britain are largely unclear.

Methods: We assembled genotype data from Irish and British individuals with ancestry from specific regions. Using networks of Identity-by-Descent segment sharing, we detected genetic communities within Ireland and Britain, and inferred regional demographic histories by estimating: (1) changes in effective population size (Ne), (2) recent migration rates, (3) degrees of haplotype sharing across length categories, and inferred European ancestry proportions within Ireland and Britain over time scales.

Results: We find evidence of recent population decline in the Orcadian, Manx and the north and south Welsh communities (89, 72, 28, 24 generations ago respectively) while Ne trajectories of Irish communities indicate a shared demographic growth throughout Ireland. Further, we observe genetic migration barriers move from central Ireland to east Ulster over time scales while migration corridors between north-east Ireland and south-west Scotland persist. European ancestry principal component projections demonstrate a strong historical signal from north-west France and a more recent north-west Norwegian signal in Irish communities while the British communities have strong Germanic contributions.

Conclusions: We build on existing evidence of fine-scale population structure in Ireland and Britain with new insights into changes in their regional recent effective population sizes and migration surfaces over time. Through this, we can understand the driving forces of rare allele frequencies and disease risk association within these populations.

Grant References: SFI (18/CRT/6214); SFI FutureNeuro Research Centre (6/RC/3948); MNDA (879-791).

Conflict of Interest: None declared

P20.041.B NGS in newborn screening: assessment of a variant classification strategy to identify potential number of false-positive in 4833 healthy individuals

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Background/Objectives: The use of Next Generation Sequencing (NGS)-based genetic testing in expanding the Dutch newborn screening (NBS) has been recognized as promising. The aim of this study was to assess a variant classification strategy to identify the potential number of false-positives (FP) in 99 inherited metabolic disorder (IMD) genes if NGS would be implemented in the Dutch NBS.

Methods: For 4833 healthy individuals, all nucleotide variants present in coding regions and canonical splice sites of 99 IMD genes were classified according to ACMG/AMP:2015 guidelines. For all (likely) pathogenic variants ((L)P), variant allele frequency and mode of inheritance was evaluated. If a heterozygous (L)P was found in an autosomal recessive (AR) gene, the presence of a variant of unknown significance (VUS) in that gene was checked.

Results: Only three individuals (0.06%) were identified with a homozygous (L)P in an AR gene; these variants were previously described with mild disease or even in asymptomatic individuals, in genes that also have a severe early-onset phenotype. In addition, four individuals (0.08%) had a (L)P + VUS in an AR gene.

Conclusion: This variant classification strategy has the potential to result in low false-positive rates, and especially for diseases without a biomarker, NGS could be used as a first-tier test to identify individuals that might need follow-up in the setting of a NBS program.

NGS can furthermore reduce FP rates for current NBS diseases if used as a second-tier test, especially in diseases with aspecific biomarkers or clear genotype-clinical phenotype correlation.

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Conflict of Interest: Dineke Westra: None declared, Emma van Berkel: None declared, Galuh Astuti: None declared, Gea Kiewiet: None declared, Martijn Dollé: None declared, Marleen Jansen: None declared, Rebecca Heiner Advisory committee Newborn Screening for Metabolic Diseases, Dutch Society for Pediatrics, Francjan van Spronsen - Advisory committee Newborn Screening for Metabolic Diseases - Dutch Society for Pediatrics

- Program committee for Newborn Blood Screening - Dutch National Institute for Public Health and the Environment, B. Sikkema-Raddatz: None declared, Els Voorhoeve: None declared, Marcel Nelen: None declared

P20.042.C Causal effects of plasma proteome on MRI-quantified visceral adipose tissue within the UK Biobank

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Background: Excess visceral adipose tissue (VAT) is strongly associated with detrimental cardiometabolic outcomes. By leveraging results from burgeoning large-scale proteo-genomic studies through Mendelian randomization (MR), we aimed to gain insight into the potential druggability of VAT.

Methods: As candidate instrumental variables for circulating protein levels, we used cis-variants for OLINK-measured proteins, previously reported on by the UK Biobank Pharma Proteomics Project (UKB-PPP). Specifically, we included 1,156 proteins with ≥1 primary cis-pQTLs attaining multiple-corrected genome-wide significance ($p < 3.4 \times 10\text{-}11$) in discovery ($n = 35{,}571$ EUR) with additional nominal significance and directional consistency in replication ($n = 18{,}181$). SNP-exposure weights were obtained from the UKB-PPP discovery-phase pQTL analyses. SNP-outcome weights were obtained by performing a GWAS on Magnetic Resonance Imaging (MRI)-derived VAT in up to 36,664 UKB participants of White-British descent.

Results: Only 1 protein had more than 1 cis-pQTL available as an instrument, with (mean) F-statistics ranging from 45 to 25,094 across proteins. After FDR-correction for multiple testing, we observed evidence of a potential causal effect of R-spondin 3 (RSPO3) levels on VAT (Wald ratio: 0.22 (95% CI: 0.12, 0.31) SD increase in VAT per unit increase in RSPO3 plasma protein levels, non-FDR p-value $6.6 \times 10\text{-}6$). Given the potentially sex-specific nature of VAT, sex-specific MR- and colocalization analyses will be undertaken when individual-level UKB protein data becomes available (expected release March 2023).

Conclusion: With previous literature suggesting that RSPO3 may suppress adipogenesis in a depot-specific manner, our findings provide tentative confirmatory proteo-genomic evidence for this protein as a cardiometabolic risk factor and a potential drug target.

Conflict of Interest: None declared

P20.043.D How assortative mating coupled with shared familial environment impacts SNP-heritability and polygenic scores

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Background/Objectives: Assortative mating is widespread in human populations; shortly put, it occurs when there is positive correlation between mates' phenotypes. It induces a positive correlation between the genetic and the environmental values of parents. If in addition parent-offspring environment is shared, it leads to a correlation between the offspring's genetic value and his/her environment. This latter correlation will build up generation after generation, until an equilibrium point is reached. We aim to explore the consequences of this gene-environment correlation on SNP heritability and polygenic score performances.

Methods: In the framework of the polygenic additive model, we assume that there is a correlation r between mate phenotypes, and a correlation ν between parent-offspring environments. We derive the equations governing the evolution of the gene-environment correlation ρ, and its value at equilibrium. The validity of these results is confirmed by realistic genome-wide simulations. We give estimates for the impact of ρ on estimated SNP effect sizes, SNP-heritability estimates, and polygenic score performances.

Results: The gene-environment correlation ρ increases with r and ν, and can exceed $\rho = 0.5$ when r and ν reach high values (>0.7). The SNP effect sizes estimates and the SNP-heritability estimates can be severely impacted. The prediction ability of polygenic scores is less affected but essentially because polygenic scores are correlated with the environment.

Conclusion: The combination of assortative mating and shared familial environment can induce sizable gene-environment correlations in a population, which affects genetic epidemiology methods.

Conflict of Interest: None declared

P20.044.A Genetic architecture of body size change from childhood to adulthood

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Background: Genome-wide association studies (GWAS) have identified numerous genetic loci associated with body size in childhood or adulthood. However, no genetic loci have been identified for the change in body size from childhood to adulthood.

Methods: We conducted a GWAS for body size change from childhood to middle age in 418,139 individuals of European ancestry from the UK Biobank. Participants self-reported their body size at age 10 as "thin", "average" or "plump" as compared to their peers. We then created a corresponding three-category variable for body size in adulthood, utilizing measured adult BMI. Body size change was calculated as the difference in body size category between age 10 and middle-age.

Results: We identified 12 loci that were associated with body size change ($P < 5 \times 10^{-9}$) that have not been identified in previous GWAS of child or adult BMI. Three of the loci were previously reported for association with psychiatric traits in adulthood, such as depression, neuroticism, and insomnia. Five loci were associated with body fat distribution, body composition, or height in adulthood. Three loci were linked to sexual maturation, including age at menarche and testosterone levels.

Conclusion: Our findings suggest that the genetic mechanisms responsible for regulating changes in body size over time are distinct from those involved in controlling body size at a single point in time. Deeper understanding of these mechanisms will be important to design more effective interventions for promoting healthy body size from childhood to adulthood

Grant references: Novo Nordisk Foundation (NNF18 CC0034900, NNF17SA0031406, NNF17OC0026848) and Horizon2020 MSCA (No846502).

Conflict of Interest: None declared

P20.045.B Mitochondria-specific signature of oxidative damage in human cancers: an excess of A>G on heavy chain of the mitochondrial genome in slow-dividing normoxic tissues

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Mitochondrial genome may be affected by oxidative damage being tightly involved in aerobic energy production. We recently hypothesised that other rules of evolution act in mtDNA compared to nDNA: the oxidative damage driven G>T substitutions are rare and independent of age in mtDNA (https://doi.org/10.1038/s41588-019-0557-x). Moreover, our mammalian study demonstrated that A>G substitutions can be a signature of oxidative damage (https://doi.org/10.1093/nar/gkac779). Thus, we assumed that different tissues can be characterised by different oxidative damage and reanalyzed a collection of 7600 somatic mtDNA mutations obtained by Yuan et. al 2020 focusing on three factors known for each somatic mutation of each cancer (variant allele frequency (VAF), time spent single-stranded (TSSS) and tissue turnover rate (TTR)). We observed that the probability of a substitution $A_H>G_H$ (heavy chain notation) is a positive function of VAF, TSSS and TTR. We propose, that all these factors are positively associated with oxidative damage: high VAF marks mutations, originating in the early stages of cancer (when tissues are more normoxic); high TSSS is associated with increased oxidative damage; low tissue turnover rate is associated with a high level of normoxia. Analysing mutational spectra with context (192-component mutational spectra) we demonstrated that the recent mutations in normoxic tissues (low VAF and low TTR) and the recent mutations in hypoxic tissues (low VAF and high TTR) demonstrate the highest cosine dissimilarity, suggesting that the level of aerobic metabolism is an important factor shaping the mtDNA mutational spectrum. Supported by RSF №21-75-20143.

Conflict of Interest: None declared

P20.046.C Bioinformatic analysis of DNA from the post-Scythian Oglakhty cemetery in South Siberia

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Background/Objectives: The Tashtyk archaeological culture is one of the post-Scythian Iron Age cultures of Southern Siberia that left numerous archaeological sites. To date, information about the origin and genetic diversity of Tashtyk culture has been limited.

Methods: We performed the whole-genome sequencing of two human mummies buried in the Oglakhty cemetery using samples of bone powder. Pseudo-haploid SNP calls were generated from randomly selected reads. For the further analysis (PCA, ADMIXTURE, f3, f4 statistics) the ancient and modern populations potentially related to the Tashtyk culture were selected from the 1240K + HO datasets. Furthermore, we determined the mitochondrial DNA and Y-chromosome haplogroups and tried to perform kinship analysis.

Results: MtDNA haplogroup analysis showed that individuals had a potential maternal kinship and belonged to the same I4a1 subclade of mtDNA haplogroup I. The Y-chromosome haplogroup was identified for the male individual as R1a1a subclade. Based on the PCA plots and admixture analysis, we showed that the Tashtyk specimens were genetically close to individuals from the local Siberian cultures Karasuk and the Tagar, as well as to inhabitants of Eastern Kazakhstan and Kyrgyzstan.

Conclusion: We present the reconstruction and analysis of the ancient genomes of two individuals – male and female – buried in the Oglakhty cemetery (early Tashtyk culture, 2nd to 4th centuries AD). Our pilot study provides fresh paleogenomic data on the ancient societies of Southern Siberia

Conflict of Interest: None declared

P20.048.A Expanded newborn screening in Ukraine: four-month experience

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Introduction: Newborn screening for selected endocrine, metabolic, and genetic disorders has been part of public health systems for more than 50 years with all developed countries worldwide. In October 2022, expanded newborn screening for 21 diseases, including metabolic disorders, SMA, and SCID, began in Ukraine.

Aim: Despite the martial law and the occupation of part of Ukraine, we were laying the groundwork for the rapid introduction of expanded neonatal screening in Ukraine.

Methods: The service is free for all babies born in Ukraine. The entire process of neonatal screening is monitored and recorded in the electronic health care system: from the registration of the newborn and the taking of blood samples by the doctor to the processing of the referral by the laboratory technician and the recording of the diagnostic report.

Results: The pilot launch started in 12 regions of Ukraine – northern and western parts of the country. Laboratory tests according to the neonatal screening program are carried out by two regional centers of neonatal screening in Kyiv and Lviv. Pre-war population 130 000 newborns per year. During four months of work, approximately 32,000 newborns were screened, as a result 20 patients were identified. Transitory metabolic disturbances were found in 10 patients.

Conclusions: Expansion of the neonatal screening program and digitalization of processes will make it possible to timely identify the risks and timely treatment of orphan diseases in infants and prevent their clinical manifestations as soon as possible creating conditions for a long and fulfilling life for patients.

Conflict of Interest: Nataliia Olhovich Sanofi, Takeda, Biomarin, Roche, Sanofi, Takeda, Biomarin, Roche, Nataliia Samonenko

Sanofi, Takeda, Biomarin, Roche, Sanofi, Takeda, Biomarin, Roche, Oksana Barvinska: None declared, Nataliia Mytsyk: None declared, Yuliia Zhyvytsia: None declared, Yuliia Tymruk: None declared, Olena Kutsyk: None declared, Iryna Nagnibeda: None declared, Nataliia Fruncevich: None declared, Marina Patsora: None declared, Tetiana Shklyarskaya: None declared, Mariia Haidei: None declared, Nataliia Gorovenko: None declared

P20.050.C Genome wide association amplified through broad-scale proteomics to identify causal therapeutic targets in large population cohorts like the UK Biobank

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Understanding the broad scale dynamics of the human proteome is crucial for identifying biomarkers to be used as indicators for disease severity and progression, patient stratification, and mechanistic insight in drug development. These data also amplify power to detect genetic supported therapeutic target discovery. The Proximity Extension Assay (PEA) is a technology that translates protein information into short DNA signals that can be sequenced across large samples sizes in both healthy and disease samples. The high-throughput nature of the assay is enabled by linking protein-specific antibodies to DNA-encoded tags that can be read out on a next generation sequencer. We have combined the PEA technology described above with automated sample preparation and a high-throughput sequencing readout for parallel measurement of ~3,000 proteins for up to 384 samples at a time, generating over 1 million data points per run. Characterizing the proteome alongside genetic and clinical data enables a pQTL framework to not only validate known clinical targets and identify new clinical targets but to also suggest repurposing opportunities of clinical candidates for new indications. Here we will summarize results where proteomics is impacting large population health studies (e.g., UK Biobank, SCALLOP) to advance epidemiology and precision medicine.

Conflict of Interest: Cindy Lawley Employee of Olink, Stockholder of Olink, Philippa Pettingill Employee of Olink, Klev Diamanti Employee of Olink, Lotta Wik Employee of Olink, Niklas Nordberg Employee of Olink, John Broberg Employee of Olink, Johan Björkesten Employee of Olink, Erika Assarsson Employee of Olink, Sara Henriksson Employee of Olink, Ida Grundberg Employee of Olink, Shareholder in Olink, Christina Westerberg Employee of Olink, Elin Liljeroth Employee of Olink, Adam Falck Employee of Olink, Martin Lundberg Employee of Olink, Lasse Folkersen: None declared, Anders Malarstig Employee of Pfizer

P20.052.C Separating the causal effects from the exposure-indicator aspects of height in relation to coronary heart disease

Si Fang[1], *Tom G. Richardson*[1], *Tom Gaunt*[1], *George Davey Smith*[1]

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Background/Objectives: Height serves both as a causal factor and as an indicator of early-life exposures in its association with coronary heart disease (CHD). Mendelian randomisation studies indicate that greater height reduces CHD risk, perhaps through influencing coronary arteries and other allometric processes. Conversely, height is influenced by environmental exposures acting from the intrauterine period through until early adulthood. Nutritional, infectious, disease-induced and psychosocial insults are all postulated to influence both final height and CHD risk.

Methods: We hypothesise that the exposure-indicator component of height can be indexed through outputting the residuals from a regression of height on a polygenic score (PGS) for height. This produces two component scores for each individual: PGS-height (PGSH) and residual height (RH). We expect that RH would be more strongly associated with the early-life environmental exposures that influence final height than PGSH would.

Results: Analyses of UK Biobank data (N = 283,534 including 26,633 incident CHD cases) showed RH is associated with incident CHD risk (HR[95%CI] per SD 0.91[0.897,0.924] in males, 0.87[0.845,0.885] in females), with associations that are stronger than with PGSH (HR[95%CI] per SD 0.95[0.897,0.924] in males, 0.96[0.940,0.983] in females). RH also showed a stronger association with education attainment, income and Townsend deprivation index than PGSH in both males and females.

Conclusion: Comparing the associations of genetic and residual components of height suggests the latter have stronger links to both socially patterned exposures and to CHD outcomes, indicating the importance of environmentally driven influences on CHD risk.

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Conflict of Interest: Si Fang: None declared, Tom G. Richardson TGR is an employee of GlaxoSmithKline outside of this work., Tom Gaunt TRG receives funding from Biogen for unrelated work., George Davey Smith: None declared

P21 Functional Genomics and Epigenomics

P21.001.A Sex specific epigenetic association of serum urate after BCG vaccination

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Background: Vaccination is a widely used and effective method of preventing infections. However, some routine vaccines have been reported to increase the risk of gout flares, and the underlying mechanism is not yet understood.

Methods: We conducted an epigenome-wide association study to investigate the association of whole blood DNA methylation and serum urate concentration in a cohort of 321 healthy individuals before, two weeks and three months after receiving anti-tuberculosis vaccine Bacillus Calmette-Guérin (BCG). We then replicated our findings in an independent cohort, and explored the functionalities of the identified CpG sites by integrating DNA methylation with matched inflammatory proteins, circulating metabolites and hormone levels.

Results: Our study found that serum urate concentration increased in a sex-specific manner after BCG. DNA methylation was associated with serum urate and the change in urate induced by BCG in a sex-specific manner. In males, urate-associated CpG sites were related to neuroprotection and immune activation and implicated in genes like *LST1*, *AIF1*, *KLF11*, *LY6G5C*, *PRRC2A*, *LTA*, and *NFKBIL1*. In females, the urate change-associated CpG sites were related to lipid and glucose metabolism and annotated to genes such as *PCYT1A*, *ALDH3B2*, *DHCR7*, *SPNS2*, *GC*, *PGS1*, *SETD4, PRKAG2, RHEB, YWHAQ, and SOCS1*. Hormones, such as cortisol, 11 deoxy cortisol, and 17 hydroxy progesterone, may modulate the sex-specific impact on long-term change in urate after BCG.

Conclusion: Our findings highlight the importance of developing sex-specific interventions targeting epigenetic regulators to better benefit from urate related immune activation after vaccination while reducing the risk of gout and hyperuricemia.

Conflict of Interest: None declared

P21.002.B Methylome profiling of immune cells highlights the impact of short-term antigen stimulation in peanut allergy

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Background/Objectives: Peanut allergy is a potentially life-threatening complex disease mediated partly by epigenetic mechanisms. Studies regarding food allergic individuals from a DNA methylation (DNAm) perspective are, however, sparse. Here, we examine the DNAm signals underlying peanut allergy as well as the response of peripheral blood mononuclear cells (PBMCs) to peanut-stimulation.

Methods: PBMCs were isolated from a cohort of peanut allergic individuals (cases, n = 67). The control group included unaffected, non-sensitized individuals (controls, n = 57). PBMCs were cultured in vitro for 48 hours in the presence and absence of peanut proteins to mimic an allergic response. DNA was profiled using the Illumina EPIC BeadChip and analyzed with Meffil.

Results: Comparing cases and controls, we observed 11 genome-wide differentially methylated probes and 110 regions (DMPs/DMRs) post-peanut-stimulation (nearest genes include: *ABCC1, PHTF2, BANP, CCL8, PLEKHH3, PNLDC1*). No DMPs and only 11 DMRs were detected pre-stimulation. Comparing unstimulated and peanut-stimulated PBMCs, we found 162 DMPs and 12 DMRs unique to the cases while 70 DMPs and 3 DMRs were unique to the controls. We also observed an overlap of 135 DMPs and 6 DMRs between cases and controls. Lastly, we show that genes linked to DMPs and DMRs are involved in allergy-related gene ontologies including IL2/STAT-, NOTCH-signaling, and focal adhesion.

Conclusion: Overall, our data suggests some DNAm sites are amenable to short-term alterations that are only detected post-peanut-stimulation. We therefore provide further insight into the complex underlying mechanisms of food allergy.

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Conflict of Interest: None declared

P21.003.C Understanding how immune oxidants can drive epigenetic change

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Oxidative stress is a common feature of inflammation-driven cancers, and promotes genomic instability and aggressive tumour phenotypes. It is known that oxidative stress transiently modulates gene expression through the oxidation of transcription factors and associated regulatory proteins. Neutrophils are our most abundant white blood cells and accumulate at sites of infection and inflammation. Activated neutrophils produce hypochlorous acid and chloramines, which can disrupt DNA methylation by oxidising methionine. In this study, we investigated whether chloramine exposure corresponds with changes in genomic DNA methylation that drive transcriptional output. Proliferating Jurkat T-lymphoma cells were exposed to sublethal doses of glycine-chloramine and DNA methylation patterns were compared using the IlluminaE-PIC850Karray. We observed decreased genome-wide methylation four hours after exposure, which correlated with altered RNA expression for 24 and 48 hours, indicating sustained impacts on exposed cells. A large proportion of the differentially methylated CpG sites were situated towards chromosomal ends, suggesting that these regions are most susceptible to inhibition of maintenance DNA methylation. This may contribute to epigenetic instability of chromosomal ends in rapidly dividing cells, with potential implications for the regulation of telomere length and cellular longevity.

Conflict of Interest: Annika Seddon: None declared, Andrew Das: None declared, Mark Hampton: None declared, Aaron Stevens PI on the grant that funded the research

P21.004.D Epigenome-wide association study of DNA methylation in blood and coagulation factor VIII and von Willebrand factor plasma levels

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Background: Limited information is available on how DNA methylation of cytosine-phosphate-guanine (CpG) sites across the genome regulate circulating factor VIII (FVIII) and von Willebrand Factor (VWF) levels.

Methods: We performed an epigenome-wide association study to examine the association of leukocyte DNA methylation levels at 483,735 CpG sites with circulating FVIII activity and VWF antigen levels in 2,690 Black and 1,085 White participants from the Atherosclerosis Risk in Communities (ARIC) study. FVIII activity and VWF antigen levels were measured at baseline, while DNA methylation was measured with the Illumina 450K Beadchip at

visit 2 (mean time difference: 3.4 years). Discovery analysis was performed in the Black population, with replication in the White population.

Results: We identified 46 and 55 significant CpG sites associated with FVIII and VWF ($p < 1.03E-7$) in the discovery analysis, respectively. Among these, for FVIII, 12 associations were replicated which mapped to 1 novel locus (*B3GALT4*) and 2 known but previously unreplicated loci (*ABO* and *CORO1A*). For VWF, 13 were replicated, mapping to one novel (*NBEAL2*) and one known (*ABO*) locus. When we adjusted for the previously reported top genetic variant at the *ABO* locus (rs687289) in the Black population, all the associations at the *ABO* locus were attenuated ($P < 0.05$).

Conclusion: We identified novel epigenetic associations with FVIII and VWF levels at the *B3GALT4* and *NBEAL2* loci. *B3GALT4* is involved in glycosylation of glycoproteins like FVIII and VWF, while *NBEAL2* is critical for the biosynthesis of platelet alpha granules, which store proteins that enable platelet adhesion initiation, including VWF.

Conflict of Interest: None declared

P21.005.A CHD8 missense variants cause a variable neurodevelopmental disorder with incomplete penetrance

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Background: Loss-of-function (LoF) *CHD8* variants cause a neurodevelopmental disorder with autism and macrocephaly. *CHD8* missense variants (MVs) have not been studied systematically.

Methods: *CHD8* MVs were compiled from multiple sources, classified according to ACMG/ACGS guidelines, and modelled using AlphaFold/PyMOL. From selected individuals with *CHD8* MVs, phenotype information was collected systematically and DNA methylation (DNAm) arrays and Episign analysis performed.

Results: We found ~75% of >200 *CHD8* MVs across public databases were annotated as variants of uncertain significance (VUS). In our cohort of 39 patients with 35 rare *CHD8* MVs, 33 were categorised as VUS (7 tepid, 8 warm and 3 hot) and 2 as likely pathogenic. High-confidence *CHD8* 'LoF' episignatures were detected in 8/25 patients. Episignature-positive *CHD8* MVs were located in functionally important and/or predicted highly-structured domains. Group episignature comparison showed generally weaker profiles for *CHD8* MVs compared to LoF variants. Two episignature-positive MVs were inherited from sub-clinically affected, but episignature-positive mothers. Clinical features of *CHD8* MV episignature-positive individuals were variable and included intellectual disability (78%), autism (55%), macrocephaly/tall stature (50%), hypotonia (30%), and seizures (20%). Overall, these were similar to the known *CHD8* LoF phenotype.

Conclusions: Our work demonstrates that classification of *CHD8* MVs is challenging, that DNAm analysis can help to resolve their pathogenicity, and that *CHD8* MVs located in functionally important/highly-structured domains cause a variable neurodevelopmental disorder. We also show that a '*CHD8* episignature' can be detected even in sub-clinically affected patients, demonstrating the utility of Episign in discovering incomplete penetrance of the clinical phenotype.

Conflict of Interest: None declared

P21.006.B Cell organization and organ development defects in Kabuki-KMT2D mutant stem cells

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Background/Objectives: Kabuki syndrome is characterised by distinct facial dysmorphism, intellectual disability, developmental delay, and a range of internal organ malformations such as congenital heart defects, skeletal defects, cleft palate and genitourinary malformations. Loss-of-function (LoF) *KMT2D* variants cause KS Type 1 (KS1). *KMT2D* encodes a H3K4 methyltransferase, but the underlying disease mechanism in KS is unknown.

Methods: To explore the role of KMT2D in stem cells, we generated induced pluripotent stem cell lines from fibroblasts of 3 patients with KS1; and a LoF heterozygous *KMT2D* in a wildtype human embryonic stem cell line using CRISPR-Cas9. We performed RNASeq, H3K4me1 ChIPSeq in KS1/ KMT2D$^{+/-}$ stem cells and compared them with controls.

Results: Analysis of RNASeq and ChIPSeq data revealed significant differences in expression of genes involved in cell cycle, oxidative phosphorylation, MAPK/ERK pathway and cytoskeleton organization in KS1/KMT2D$^{+/-}$ stem cells. Additionally, a set of genes related to skeletal muscle show a positive correlation with the level of H3K4me1 while a set of genes related to immune system show an inverse correlation with H3K4me1 level.

Conclusion: KS1 causing *KMT2D* variants result in defects in several genes related to cell organization and organ development already at the stem cell stage suggesting that correct dosage of *KMT2D* and downstream related genes are essential for normal progression of cell differentiation in KS1.

Grant References: Great Ormond Street Hospital (GOSH) charity, NewLife

Conflict of Interest: None declared

P21.007.C Role of BMI1 in retinal cell survival in a retinitis pigmentosa mouse model

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Our previous observations have shown that *Bmi1* loss results in photoreceptor survival and delays retinal degeneration in *Rd1* mice. However, the exact mechanisms behind this effect are not yet understood. We hypothesize that *Bmi1* loss leads to epigenetic induction of transcriptional program, that promotes retinal cell survival given that it's part of the Polycomb Repressive Complex 1(PRC1).

To uncover the genes and pathways affected by *Bmi1* loss, we analysed RNA-seq data from *Rd1* and *Rd1;Bmi1*$^{-/-}$ mouse retinas. Specifically, we used data from WT mice to determine whether *Bmi1* loss restored expression of genes disrupted in *Rd1* or triggered separate program. To further examine the function of *Bmi1* and PRC1, we assessed global or specific modifications in H2AK119Ub and H3K27me3 markers in WT, *Rd1*, *Rd1;Bmi1*$^{-/-}$ mice retinas.

Gene expression data showed *Rd1;Bmi1*$^{-/-}$ specific deregulation of transcription factors, namely, EN2, SIX1, SIX2, and NEUROD6, which are involved in neuron survival and development. Interestingly, expression of genes deregulated in *Rd1* versus WT was rescued in *Rd1;Bmi1*$^{-/-}$ mice. *Guca1a* and *Guca1b* were downregulated in *Rd1* versus WT mice, but further upregulated in *Rd1;Bmi1*$^{-/-}$ retinas; corresponding to decreased accumulation of cGMP, which is known to contribute to photoreceptor death. There was gene set enrichment for PRC2 members and H3K27me3 markers in *Rd1;Bmi*$^{-/-}$ *versus Rd1 mice*. We also confirmed epigenetic regulation on some targeted genes either directly or indirectly.

Our research confirms *Bmi1*'s role in controlling photoreceptor death in *retinitis pigmentosa* model and underscores *Bmi1*'s epigenetic control of key pathways crucial for photoreceptor stability and survival.

Conflict of Interest: None declared

P21.008.D Using eQTL to identify the regulatory networks and drivers of variation in the individual response to sepsis

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Background/Objectives: Sepsis is a clinical syndrome caused by a dysregulated response to infection. Inter-patient heterogeneity is a major obstacle to the development of targeted treatments. We have previously identified gene expression-based disease subgroups (Sepsis Response Signatures: SRS), but the factors driving inter-individual variation are unknown. We therefore aimed to investigate the role of genetic variation in the host response and delineate regulatory networks underlying SRS.

Methods: Using genotyping and RNA-seq data on 638 adult patients with lung or abdominal sepsis, we mapped expression (eQTL) and co-expression module (modQTL) quantitative trait loci in the disease context. We tested for interactions between SRS and genotype, and combined transcription factor (TF) binding site information (SNP2TFBS) and predicted regulon activity (DoRothEA) to identify candidate upstream regulators. We estimated per-sample cell proportions with a sepsis reference dataset (CIBERSORTx) to identify relationships between these TFs and specific cell types.

Results: We report 16,054 independent cis-eQTL and 31 modQTL, and found a significant genotype-SRS interaction (FDR<0.05) for 1,578 SNP-gene pairs. We identified 41 TF motifs enriched in SRS interaction QTL for which the TF also had differential activity between SRS. These included HIF1A and CEBPB, which were associated with progenitor and immature neutrophil subsets respectively, supporting our recent single cell phenotyping experiments implicating these subsets in SRS.

Conclusion: Our eQTL interaction approach has identified factors putatively linking host genetic variation, cell subtypes, and the individual transcriptomic response to infection. Understanding the regulatory networks underlying patient heterogeneity could inform development of immunomodulatory treatments and personalised medicine in sepsis.

Conflict of Interest: None declared

P21.009.A Circulatory miRNAs and multiple sclerosis: possible biomarkers for conversion from relapsing-remitting to secondary progressive form

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Background and Objective: Multiple Sclerosis (MS) is a neurodegenerative disorder of the central nervous system. One of the most common form of MS is the relapsing-remitting form (RRMS), characterized by acute attacks followed by recovery. RRMS patients can remain in this form for the whole life, but often the disease can aggravate, with a gradual worsening after a relapsing-remitting phase: in this case the disease is defined as secondary progressive (SPMS). At now no biomarkers are found to distinguish RRMS patients that will convert in SPMS vs. those that will remain RRMS. The aim of the present study is to verify if

particular circulating miRNAs can predict the conversion when patients are still in a relapsing-remitting phase.

Methods: For this retrospective study, we analyzed miRNAs expression in sera of 85 RRMS patients. After 10 years, 41 patients remained RRMS, whereas 44 converted in SPMS (cSPMS). Sera of 8 RRMS and 8 cSPMS were used for the serum miRNome analysis performed by qPCR, finding 8 miRNAs significantly deregulated in the two groups. Next, we analyzed the expression of these miRNAs in the serum of the remaining patients by ddPCR.

Results and Conclusion: Results showed that miR-34a-5p, miR-103a-3p and miR-376a-3p were significantly up-regulated in cSPMS compared to RRMS ($p < 0.05$ for all). These results show that the serum concentration of miR-34a-5p, miR-103a-3p and miR-376a-3p is deregulated in SPMS patients already 10 years before the conversion, suggesting their possible use, together with other parameters, as biomarkers to predict the conversion from RRMS to SPMS.

Conflict of Interest: Simone Agostini: None declared, Roberta Mancuso: None declared, Lorenzo Agostino Citterio: None declared, Domenico Caputo: None declared, Mario Clerici IRCCS Fondazione Don Carlo Gnocchi University of Milan

P21.010.B Epigenome-wide association study of asthma with severe exacerbations in African American and Latino youth

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Background/Objectives: African Americans and some Latino subgroups report the highest asthma morbidity and mortality related to severe asthma exacerbations. However, there is a lack of biomarkers for asthma exacerbations and the influence of DNA methylation (DNAm) remains unknown. We aimed to identify DNAm markers associated with asthma with severe exacerbations.

Methods: Genome-wide DNAm was profiled in whole-blood using the Illumina MethylationEPIC array. An epigenome-wide association study (EWAS) of pediatric asthma with severe exacerbations was conducted on 502 African Americans (327 non-asthmatics and 175 exacerbators). The replication stage included 466 Puerto Ricans and 217 Mexican Americans. Models were corrected for age, sex, ancestry, tissue heterogeneity, and batch effect. Genomic inflation was corrected using a Bayesian method and multiple comparisons were adjusted using a false discovery rate (FDR)<0.05. A case-only EWAS was conducted in 219 African American non-exacerbators and 175 exacerbators. Differentially methylated regions (DMRs) were assessed and included in gene-set enrichment analyses.

Results: 236 CpGs were significantly associated with asthma exacerbations in African Americans (FDR<0.05; $\lambda = 1.04$), and 161 were replicated in both Latino subgroups ($p < 0.05$). These included CpGs annotated to asthma-related genes (e.g., *GLCCI1*, *IL18RAP*, *APOBEC3H*, and *ELAVL1*). Three CpGs were associated with severe asthma exacerbations in the case-only analysis: cg07026010 (*NUDCD3*), cg1427040 (*CDC7*), and cg24811432 (*LINC01478*). A total of 48 DMRs were identified (FDR<0.05), which showed enrichment in IL-2, IL-9, TGF-β, HDAC, and CEBP signaling pathways (FDR<0.05).

Conclusions: We revealed novel methylation loci for asthma with severe exacerbations in pediatric admixed populations.

Grant references: NHLBI; NHGRI; NIEHS; NIMHD; MCIN/AEI/10.13039/501100011033 (PID2020-116274RB-I00).

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P21.011.C Hypernetwork analysis: A novel approach for epigenome analysis, with Kabuki syndrome as an exemplar

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Background/Objectives: Kabuki Syndrome 1 (KS1) is caused by loss-of-function variants in KMT2D H3K4-methyltransferase. KMT2D regulates thousands of genes. A systems-approach,

therefore, is essential for understanding pathomechanisms underlying KS1.

Methods: DNA methylation data generated from blood was used to reveal differentially methylated points (DMPs), which were used for system-based analyses.

Results: In KS1 ($n = 22$), compared to controls ($n = 138$), we identified 2,002 DMPs (adjusted $p < 1 \times 10\text{-}4$, 753 hypermethylated and 1,249 hypomethylated) across the epigenome. Focussing on genomic regions with >7 contiguous (in cis) hypermethylated or hypomethylated DMPs, we identified 17 significant differentially methylated regions (FWER<0.05, 11 hypermethylated and 6 hypomethylated) associated with embryonic morphogenesis and anterior/posterior pattern specification pathways. As this traditional approach failed to extract of the functional relevance of >90% DMPs in our data, we used hypernetwork analysis. This revealed 986/2,002 DMPs to be highly co-ordinated (strongly correlated but majority in trans and not necessarily methylated in the same direction) in KS1. These DMPs were enriched for genes associated with extracellular matrix organization, cartilage development and neuronal migration. Finally, using an iterative analysis of 1000 network simulations we detected significantly lower entropy in KS1 compared to controls ($p < 1 \times 10\text{-}4$). This suggests a more ordered and less diverse co-ordination by DMPs implying lower information content of the KS1 epigenome.

Conclusions: Hypernetworks approach is useful in quantifying network-level differences, and in extracting deeper mechanistic insights into the fundamental pathophysiology of genetic disorders. This is especially important with rapid increase in patient-derived epigenome data, as this approach may have significant translational potential.

Conflict of Interest: None declared

P21.012.D Changes in expression of m5C RNA effector proteins in Alzheimer's disease

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Background: The study of modified RNA species known as epitranscriptomics, has become increasingly relevant in our understanding of disease modifying mechanisms. 5-methylcytosine (m^5C) on RNA regulates multiple RNA metabolic processes and is abundant in the mammal brain. The methylation system is controlled by effector proteins which 'write', 'read', or 'erase' the modification mark. As RNA regulatory processes are known to be dysregulated in the aging brain, we propose that changes in effector protein abundance may be contributing to neurodegenerative disease.

Methods: To examine the contribution of m^5C effector proteins in Alzheimer's disease (AD) and neuropathology, we analysed RNA sequencing data of 31 effector proteins from 51 AD samples and 56 non affected controls across four brain regions. Data was obtained from the Aging, Dementia and Traumatic Brain Injury Study. Gene expression profiles were compared between both groups, and between the scales of neuropathological assessment, Braak and CERAD. Expression was also compared across individuals with a history of traumatic brain injury (TBI).

Results: We observed significant differences in expression of RNA methylation writers *NSUN6* and *NSUN7* across AD and controls and, along with the reader *ALYREF*, differences in expression on neuropathologic ranking. In the TBI assessment, we observed *NSUN6* to be significantly decreased in individuals with a history of TBI.

Conclusion: Our findings indicate that changes in m^5C writer and reader proteins are associated with dementia and/or TBI and highlight the potential for m^5C epitranscriptomic processes contributing to cognitive diseases.

Funding: Nottingham University; Neuroscience Support Group, UK; CONACYT PhD scholarship.

Conflict of Interest: None declared

P21.013.A Detection of radiation-induced mutations in bulk human gingival fibroblasts using NanoSeq

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Background/Objectives: Ionizing radiation causes DNA damage in exposed cells which can lead to genomic changes such as single nucleotide variants (SNVs) and insertions/deletions (InDels) due to misrepair. Assessing these mutational changes in bulk cell samples by next-generation sequencing (NGS) is difficult, as mutated genomic loci vary in every cell and standard NGS protocols produce an appreciable number of sequencing errors that could be mistaken for radiation-induced DNA mutations. A recently published duplex sequencing-based method called NanoSeq enables detection of somatic mutations in bulk samples with an extremely low error rate. We applied this method to investigate irradiation-induced mutations in human gingiva fibroblasts and compare them to the mutational background of non-irradiated cells.

Methods: For this pilot study, DNA from gingiva fibroblasts exposed to 10 Gy ionizing radiation and a non-irradiated control was used to prepare NanoSeq libraries. The libraries were sequenced on an Illumina NextSeq 550 instrument and the data analyzed according to the published NanoSeq documentation.

Results: We found no apparent difference in the number or mutational rate of somatic SNVs in the irradiated and non-irradiated sample but observed a 2-fold increase in the number of InDels in irradiated cells (32 in the control, 64 in the irradiated sample). The insertion/deletion ratio was unchanged between samples.

Conclusion: Our preliminary findings show a moderate increase in the number of InDels, but not SNVs, in irradiated fibroblasts. This is unexpected, given the highly mutagenic potential of ionizing radiation. Follow-up studies will be necessary to expand on these results.

Conflict of Interest: None declared

P21.014.B Glutamate receptor regulation by RNA methylation mechanisms

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Background/Objective: Epitranscriptomic processes involving N-6-methyladenosine (m^6A) modification influence gene expression through regulating where and when transcripts are translated and degraded. This study aimed to investigate human brain m^6A methylation patterns on selected transcripts which encode glutamate receptor subunits (GRS) and also to examine the

spatio-temporal profiles of m^{6}A-modified RNA during neuronal quiescence and after activation of GRS.

Methods: m^{6}A-methylated RNA datasets generated from human brainstem, cerebellum, hypothalamus, cerebrum and parahippocampus grey and white matter tissues were mapped, and transcripts known to encode ionotropic GRS were pre-selected and modification sites along the transcripts annotated. Differentiated SHSY-5Y cells were activated with NMDA and changes in co-localisation between modified RNA and effector protein abundance assessed by immunocytochemistry and confocal microscopy.

Results: GRS transcripts showed high differential methylation across the brain regions with variations in transcript topology, i.e., along 5'UTR, exons and 3'UTR. Kainate receptor subunit transcripts were particularly m^{6}A-methylated within the cerebellum. Neuronal activation showed significant differences ($p < 0.05$) in the abundance of m^{6}A with readers, YTHDF1, YTHDF2 and YTHDF3, and with the eraser, ALKBH5, at post-synaptic sites during early plasticity (after 5 minutes NMDA) and during late plasticity (after 30 minutes NMDA).

Conclusion: The findings indicate that key GRS transcripts are differentially m^{6}A methylated in the human brain and that the role of effector proteins at synapses during glutamate-associated plasticity maybe context specific. Such mechanisms are predicted to have important consequences for the regulation of local protein synthesis at synapses and could be contributing factors in the development of cognitive diseases.

Conflict of Interest: None declared

P21.015.C Blood- and brain-based epigenome-wide association studies of smoking

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Background/Objectives: Self-reported smoking is often incorporated into disease prediction tools but suffers from recall bias and doesn't capture passive exposure. Blood-based DNA methylation (DNAm) is an objective way to assess smoking. However, studies have not fully explored tissue-specificity or epigenome-wide coverage beyond array data.

Methods: A blood-based epigenome-wide association study (EWAS) of smoking was carried out in 18,413 Generation Scotland individuals at ~850k DNA methylation (DNAm) sites. For 24 pairs of smokers and non-smokers a high-resolution approach was implemented (~4 million sites, TWIST methylome panel). An EWAS-derived biomarker of smoking was tested in the independent Lothian Birth Cohort 1936 (n~900). Lastly, we ran EWASs of smoking across 5 brain regions for 14 individuals to identify tissue-specific signals.

Results: An array-based Bayesian EWAS in 18,413 individuals identified 24 independent smoking-associated DNAm loci with posterior inclusion probability > 95%. In the case/control subset of 48 individuals, 44 significant associations were identified via linear regression for the array-based analysis, compared with 97 associations for the TWIST data ($p < 10^{-5}$) – overlap of 14 associations. Prediction analyses are ongoing. Several loci showed near perfect discrimination of smoking status in both blood and brain but these loci do not overlap across tissues.

Conclusion: Sequencing-based approaches provide novel insights into the epigenetic architecture of smoking. Whereas individual loci offer excellent discrimination of cases and controls, these vary by tissue. Future work will explore if tissue-specific signals identify pathways and mechanisms by which smoking influences brain health.

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P21.016.D Identification of DNA methylation markers and biological pathways associated with asthma exacerbations

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Background/Objectives: Patients with asthma may experience episodic flare-ups, known as exacerbations, which may be life-threatening, and significantly contribute to the asthma burden. There is a potential application of changes in DNA methylation (DNAm) patterns as biomarkers of asthma outcomes. However, no previous studies have evaluated the possible role of DNAm on asthma exacerbations at genomic level. Our aim was to identify changes in DNAm and biological processes related to the development of exacerbations among asthma patients.

Methods: We examined epigenome-wide methylation levels using the Illumina MethylationEPIC array in whole blood from 307 Spanish individuals from the Canary Islands (167 non-exacerbators/ 140 exacerbators). After identification of differentially methylated CpG sites in the discovery, we performed a validation study on 74 asthma patients from mainland Spain (30 non-exacerbators/ 44 exacerbators). We used linear regression to compare methylation at CpG sites between non-exacerbators and exacerbators, and models were adjusted by age, sex, tissue heterogeneity, and batch effect. Multiple comparison correction was performed adopting a false discovery rate (FDR)<0.05. Differentially methylated regions (DMRs) were assessed and examined in gene-set enrichment analyses.

Results: Four CpGs were identified in the discovery phase (FDR<0.1; λ = 1.02). One of these CpGs (cg25345365), located in a previously asthma-related gene (*ZBTB16*), was replicated ($p < 0.003$). Additionally, a total of 23 DMRs were identified, which showed enrichment in B cell survival, cholesterol, TMTC3, APOL2, TPST2, and CCND1 signaling pathways (FDR<0.05).

Conclusions: CpGs and DMRs may have a role in asthma exacerbations which needs to be further studied.

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Conflict of Interest: Elena Martin-Gonzalez: None declared, Javier Perez-Garcia: None declared, Esther Herrera-Luis: None declared, Fabian Lorenzo-Diaz: None declared, Ruperto González-Pérez: None declared, Paloma Poza-Guedes: None declared, Olaia Sardón: None declared, José M. Hernández-Pérez: None declared, Paula Corcuera: None declared, Javier Korta-Murua: None declared, Elena Mederos-Luis: None declared, Inmaculada Sánchez-Machín: None declared, Jesús Villar Public grants from Instituto de Salud Carlos III CB06/06/1088), Mario A. González-Carracedo: None declared, Maria Pino-Yanes Grant PID2020-116274RB-I00 by Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033)

P21.017.A Circulating epigenetic biomarker profiling for early diagnosis of coronary artery disease

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Background/Objectives: Cardiovascular diseases (CVD) are the major cause of death worldwide. In coronary artery disease (CAD), one example of CVDs, typically atherosclerotic plaques are narrowing coronary arteries (stenosis), resulting in an impairment of blood flow which subsequently decreases the heart's blood and oxygen supply. Untreated, this leads to damaged heart muscle tissue and cardiac dysfunction and can cause acute events like myocardial infarction. Coronary angiography (CA) is the state-of-the art procedure to diagnose stenosis, achieved by injecting contrast agent into coronary arteries and performing X-ray imaging. Aim of this study was to develop novel, minimally invasive epigenetic biomarkers for stenosis prediction to reduce unnecessary invasive CAs as right now 40% of patients undergoing CA do not display stenosis in the end.

Methods: We recruited 146 patients who had undergone coronary angiography, 77 displayed significant stenosis whereas the other 69 did not have stenosis. We collected whole blood, plasma and cell-free saliva from all patients and performed biomarker discovery studies analyzing around 30-40 patients per experimental group and omics layer. These discovery analyses included genome-wide DNA methylation profiling from whole blood via Illumina EPIC microarrays as well as small RNA sequencing from plasma and cell-free saliva derived extracellular vesicles.

Results: We identified a variety of statistically significant differences in DNA-methylation and small RNA profiles between stenosis and non-stenosis patients and will outline the details of the outcome of these multi-omics biomarker discovery studies. We will further present preliminary biomarker verification analysis performed on the total set of 146 patients.

Conflict of Interest: None declared

P21.018.B Functional evaluation of a novel nonsense variant of the CASR gene identified in a patient with chronic hypocalcemia

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Background/Aim: The *CASR* gene encodes for a G-protein coupled receptor sensing extracellular calcium levels. It has been long established that *CASR* gain-of-function variants cause hypocalcemia, while loss-of-function variants lead to hypercalcemia. The aim of this study is to verify the functional impact of a de novo nonsense *CASR* c.2897_2898insCTGA, p.(Gln967*) variant identified in a 16-year-old girl with chronic hypocalcemia.

Methods: We generated a model using site-directed mutagenesis on a plasmid carrying the *CASR* gene tagged with green fluorescent protein (GFP). We then induced WT and mutated *CASR* expression in non-expressing HEK293T cells through lipofectamine-based transient transfection. As functional readouts we determined responses of WT and mutated *CASR* to variations in the extracellular calcium concentrations at different stimulation times.

Results: The correct expression of WT and mutated CASR was verified by western blot analysis showing the presence of a full length or a truncated protein, respectively. Specifically, the mutated construct resulted in the presence of a 12 KDa lighter protein (corresponding to 111 aminoacids). These data indicate that mutated cells escape nonsense mediated decay and produce a truncated protein. Furthermore, extracellular calcium stimulation showed increase of downstream MAPK activity for mutant HEK293T transfected cells compared to WT.

Discussion: We demonstrated that the *CASR* p.(Gln967*) variant causes the formation of a truncated protein with a gain-of-function effect, consistently with the patient phenotype. This allows to speculate that *CASR* nonsense terminal variants lead to the loss of the intracellular C-terminal regulatory domain with enhanced receptor response to serum calcium.

Conflict of Interest: None declared

P21.019.C scMetaBrain: federated single-cell consortium for cell-type specific eQTL analysis of neurological disease variants

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Background/Objectives: Recently we have completed large scale eQTL meta-analyses in blood (eQTLGen; Võsa *et al*. 2021) and brain (MetaBrain; de Klein *et al*. 2021), providing insight into the

downstream effects of disease-associated genetic risk factors. Although having substantial sample sizes, these studies lack the cell type context that single-cell RNA sequencing (scRNA-seq) can provide. To pinpoint these cellular contexts and better interpret neurological diseases, we have initiated the scMetaBrain study, which aims to enable a federated scRNA-seq eQTL analysis in human brain.

Methods: To harmonize and compare with our single cell eQTL efforts in blood, we have adapted our recently developed pipeline (scEQTLGen, van der Wijst *et al*. 2020) to brain. This includes robust containerized pipelines that perform quality control, demultiplexing, cell type classification, and eQTL analysis per cell-type and dataset. By applying a meta-analysis of the summary statistics, we enable easy inclusion of new datasets without the need of sharing person-identifiable data.

Results: As proof of concept, we have applied our pipeline to automatically analyze 35 samples from Mathys *et al*. 2019. We report good replication statistics compared to the largest brain single-cell eQTL study (Bryois *et al*. 2022).

Conclusion: Here we introduce scMetaBrain, in which we aim to setup a single-cell brain consortium for the identification of downstream consequences of trait-related genetic variants in specific brain cell types. We envision that this consortium will enable a unique opportunity to disentangle tissue and cell type specific regulatory effects in the brain.

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P21.021.A Blood methylome associates with fractional exhaled nitric oxide and bronchodilator drug response in pediatric asthma

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Background/Objectives: Bronchodilator drug response (BDR) and fractional exhaled nitric oxide (FeNO) are clinical biomarkers widely used in the management of asthma. DNA methylation (DNAm) contributes to asthma pathogenesis, capturing both genetic and environmental factors. However, the influence of DNAm on these biomarkers is understudied. We aimed to identify DNAm markers in whole blood associated with BDR or FeNO in moderate-to-severe pediatric asthma.

Methods: We analyzed 121 samples from European children with moderate-to-severe asthma whose DNAm levels were profiled with the EPIC microarray (Illumina). The association of DNAm with BDR and FeNO was assessed through limma regression models adjusted for age, sex, ancestry, and cell composition. A false discovery rate (FDR)<0.1 and a genome-wide significance threshold of $p < 9 \times 10^{-8}$ were used to control for false-positive results. Cross-tissue validation was assessed in nasal samples. We also assessed differentially methylated regions (DMRs) and enrichment in traits and biological pathways.

Results: The CpG cg12835256 (*PLA2G12A*) was genome-wide associated with FeNO (coefficient = -0.015, $p = 2.53 \times 10^{-9}$) and validated in nasal samples ($p < 0.045$). Three CpGs were suggestively associated with BDR (FDR<0.1). We identified multiple DMRs associated with FeNO or BDR including *EGR3*, *AURKC*, and the *HOXA* family (FDR<0.05). Epigenetic markers associated with FeNO or BDR were enriched in asthma-related traits and pathways, including allergic and inflammatory responses, smoking, and aging.

Conclusion: We reported novel associations of DNAm markers associated with BDR and FeNO enriched in asthma-related traits and pathways. These findings would allow the discovery of new biomarkers for asthma management.

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P21.022.B Characterising the CNS expression of genes implicated in Multiple Sclerosis progression

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Background/Objectives: Most people with Multiple Sclerosis (MS) develop inexorably worsening disability, termed progression, which continues even when inflammation is suppressed. In a recently completed genome-wide association study of MS progression, we identified the first genetic variant (rs10191329) significantly associated with outcome. Here, we aimed to characterise the central nervous system (CNS) expression of the genes flanking this intergenic variant: *ZNF638* and *DYSF*. Understanding how rs10191329 exerts its biological effects will accelerate the identification of rational targets for drug discovery.

Methods: Using existing bulk and single cell RNA sequencing (scRNA-seq) data, we examined *ZNF638* and *DYSF* expression in CNS tissue and human cerebral organoids. We explored existing single nucleus RNA-seq data from post-mortem brain tissue (55 controls, 28 MS patients) for *ZNF638* and *DYSF* expression. We are generating neuronal progenitor cells and cortical neurons from human pluripotent stem cells and will analyse ZNF638 and DYSF expression using immunofluorescence staining and confocal imaging.

Results: In RNA-seq data, *ZNF638* and *DYSF* showed enhanced expression in oligodendrocytes, and expression in neurons. In cerebral organoids, scRNA-seq data revealed higher *ZNF638* expression at later stages of organoid development, whereas *DYSF* was predominantly expressed at the earliest stages.

Conclusion: Analysing the cell-type-specific expression of *ZNF638* and *DYSF* assists in developing assays to next assess the function of these genes in MS-relevant tissues. Understanding this will inform the design of CNS models to study the biological effects of the MS progression-associated variant.

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P21.023.C Global transcription is profoundly impacted in primary fibroblasts obtained from pediatric patients affected by leukodystrophy harboring mutations in Pol III-encoding genes

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Background/Objectives: Leukodystrophies (LDs) are genetic disorders affecting the white matter in the central nervous system, with a deficiency in myelin development. POLR3-related LDs are caused by mutations in RNA Polymerase III (PolIII) subunits, such as POLR3A and POLR3B. PolIII is involved in the transcription of small ribosomal units, tRNAs, 7SL RNA, U6 spliceosomal RNA, and more. tRNAs are implicated in translation, whilst small RNAs can perform regulatory functions on mRNA transcripts. We thus aimed to identify the transcriptional

dysregulations present in POLR3-mutated patients and to also assess alterations in the translation process.

Methods: Fibroblasts were obtained from skin biopsies of 2 POLR3A and 1 POLR3B mutated patients and matched controls. RNA was extracted with Trizol reagent and Total RNA sequencing was performed with the CORALL Total RNA-Seq Library Prep Kit using Illumina NextSeq 500 Sequencing. Differential expression analysis was performed with DESeq.2 package and enrichment analyses were performed on differentially expressed genes (DEGs). Click-iT Protein Labeling approach was used to analyze newly synthesized proteins.

Results: RNA-seq analysis highlighted a strong dysregulation in POLR3 patients, identifying 159 DEGs with the comparison to LDs patients to controls. Moreover, when comparing each patient to its matched control, a specific DEGs signature was also observed, suggesting caution when grouping patients harboring different mutations in the same gene. Moreover, nascent protein synthesis analysis highlighted a down-regulation in nascent protein synthesis.

Conclusions: our results highlight a profound impact on transcription and translation processes, in patients specific primary cells used as pre-clinical experimental model of the disease.

Conflict of Interest: None declared

P21.025.A Epigenome-wide integrative studies in COVID19 reveal a respiratory environmental component, a distinct genetic regulation of DNA methylation and a shared epigenetic signal with autoimmune diseases

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Background/Objectives: DNA methylation is known to play an important role in the regulation of the immune processes behind COVID-19. In this study, we aim to evaluate the implication of epigenetic changes in COVID-19 progression and severity and its environmental and genetic drivers.

Methods: Whole blood samples from controls (N = 101) and positive lab tested individuals (N = 473) were obtained. We performed a genome-wide DNA methylation analysis to reveal DNA methylation changes associated with COVID19 susceptibility and severity, as well as meQTL mapping analyses.

Results: Our analyses reveal the existence of epigenomic regulation of functional pathways associated with COVID-19 shared with autoimmune conditions. We find an environmental signature that discriminates mild from severe cases and regulates IL-6 expression via the transcription factor CEBP. We elucidated the mediation role of DNA methylation in genetic risk, and identified a group of genes which DNA methylation is genetically regulated with dependence on severity or infection status.

Conclusions: The findings of this work suggest that an interaction between environment, genetics and epigenetics play a role in triggering the cytokine storm. Our results illustrate an autoimmune epigenetic signature as well a distinct genetic regulation of DNA methylation depending on COVID19 severity.

Conflict of Interest: None declared

P21.027.C miRNAs in nephroblastoma

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Background/Objectives: Nephroblastoma, also known as Wilms tumor (WT), is a type of pediatric cancer with an incidence rate of approximately 1 in 10,000 children. Treatment strategies involve surgery, chemotherapy, and radiation therapy, and provide a successful survival rate of over 90% at WT early detection. Despite the high survival rates, biomarkers for early diagnosis are needed to develop more effective treatments. Therefore, our study aimed to identify miRNAs as potential epitranscriptomics early WT biomarkers.

Methods: Small RNA sequencing was used to detect miRNA profiles in WT fresh frozen tissue (FFT). A group of Formalin-Fixed Paraffin-Embedded (FFPE) samples was utilized to confirm significantly expressed miRNA in an independent group of tissue WT samples. miRNA role was predicted with pathway analysis and the FFT mRNA sequencing was performed to evaluate the potential miRNA effect on tissue transcriptome.

Results: Comparative miRNA analysis of FFT and FFPE revealed 27 miRNAs with decreased and 14 miRNAs with increased expression in WT compared to normal kidney tissue. Pathway analysis indicated the involvement of significantly differentially expressed miRNA in cancer pathogenesis, while mRNA sequencing analysis indicated altered expression of genes involved in cell and drug metabolism.

Conclusion: Wilms tumors are heterogeneous tumors composed of stromal, regressive, blastemal, epithelial or anaplasia tissue types. By utilizing next-generation sequencing, we analyzed the entire miRNA expression profiles and identified universal miRNAs independently of the histological type of WT. Like miRNAs, mRNAs are significantly differentially expressed in WT, but mRNAs are not directly associated with cancer pathology.

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Conflict of Interest: None declared

P21.028.D Dissecting the molecular mechanisms underlying therapy resistance and sensitivity in HER2-positive gastric cancer

Joao M. Fernandes Neto[1;2], *Bárbara Mesquita*[1;3], *Carla Pereira*[1;4], *Diana Martins*[1;5;6], *Joana Carvalho*[1], *Dina Leitão*[7;8], *Marta Ferreira*[1;9], *Pedro Ferreira*[1;10], *Fátima Carneiro*[1;7;8], *Gabriela M. Almeida*[1], *Carla Oliveira*[1;8]

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Background/Objectives: HER2 amplification drives the progression of many cancers, including gastric cancer (GC). Not all patients with HER2-positive tumors benefit from anti-HER2 therapy, and overall survival remains below 4-months compared

to chemotherapy alone. We aimed at elucidating the reasons for suboptimal response to anti-HER2 therapy and identifying novel targets for combination therapy in patients with HER2-positive GC.

Methods: Primary and metastatic cancer lesions (n = 41) from 15 treatment-naïve GC patients were evaluated by Immunohistochemistry (IHC) for 8 targetable MAPK and PI3K pathway-related proteins and by deep and/or shallow whole-genome sequencing. A pooled genome-wide CRISPR screen was performed in HER2-positive GC cells treated with anti-HER2 therapy.

Results: 41% (17/41) of the neoplastic lesions from HER2-positive GC patients were HER2-negative. VEGFA and mTOR drug-targetable proteins were highly expressed in 37% and 78% of lesions, respectively, without HER2 expression correlation. Shallow WGS recapitulated the deep WGS copy number variant (CNV) profile, identifying amplification of HER2, even in HER2-negative lesions (by IHC), as well as other IHC-positive drug-targetable alterations. CRISPR screens (currently being sequenced), will potentially identify further synthetic lethal targets for testing in this setting.

Conclusion: Our data provides new targets for combination therapy in highly heterogeneous HER2-positive GC tumors, while showing the value of low-cost shallow WGS to identify targetable alterations (HER2 or other), even if lesions are HER2-negative using current molecular diagnostics approaches.

Conflict of Interest: None declared

P21.030.B Unmasking the disease risk hidden in haematopoietic enhancers

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Background/Objectives: Enhancers are non-coding sequences that regulate gene expression via chromatin looping. Most genetic variants associated with complex diseases lie in enhancers but understanding their impact on gene expression is challenging because: I) Genes can be regulated by multiple enhancers and II) enhancers can be active in some cell types while repressed in others. Therefore, to contribute to the first challenge, we generated maps of enhancers activity patterns across cell types.

Methods: We used 648 ChIP-seq datasets from the BLUEPRINT Consortium to produce chromatin state maps across 31 human haematopoietic cell types. Fisher tests were done to find GWAS trait-associated variants enriched in enhancers with the same activity patterns across cell types. Enhancer gene targets were identified using RNAseq, eQTLs and HiC datasets.

Results: We defined ~1.5M enhancer regions across the genome and classified them based on their activity across the 31 haematopoietic cell types. For each group of enhancers with the same activity pattern, we identified those regions enriched in GWAS variants. For example, we found that enhancers active in the neutrophil lineage are associated with ulcerative colitis. Enhancers specifically active in macrophages are enriched in variants associated with cardiovascular disease risk and their target genes are consistently involved in lipid metabolism processes.

Conclusion: We provide the most complete chromatin state resource for human haematopoietic cells, including patterns of enhancer activity across cell types. This will be a valuable resource for the interpretation of the non-coding variants associated with disease.

Grant references: Barbour Foundation PhD studentship.

Conflict of Interest: None declared

P21.031.C Stability selection enhances feature selection and enables accurate prediction of gestational age using only seven DNA methylation sites

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Background: DNA methylation (DNAm) is robustly associated with gestational age (GA) in newborns and with chronological age in children and adults. This property has enabled the development of several epigenetic clocks that can accurately predict chronological age and GA. However, the lack of overlap in predictive CpGs across different epigenetic clocks remains a puzzle. Moreover, the large number of CpGs used for prediction of GA may limit the clinical utility of the epigenetic clocks.

Methods: To identify CpGs that are stably predictive of GA, we applied a statistical approach called 'stability selection' to DNAm data from 2,138 newborns in the Norwegian Mother, Father, and Child Cohort study. Stability selection combines subsampling with variable selection to restrict the number of false discoveries in the set of selected variables.

Results: We identified 24 CpGs that were stably predictive of GA. Furthermore, only up to 10% of CpGs in previously published GA clocks were stably selected in our study. Using these stably selected CpGs, we were able to construct a new GA clock consisting of only seven CpGs that showed a similar prediction performance as that of established GA clocks ($R^2 = 0.653$, median absolute deviation = 4.19 days).

Conclusion: We introduce a methodological framework for feature selection that is broadly applicable to any trait that can be predicted from DNAm data. A new and highly performant GA clock based on only seven CpGs creates new opportunities for a more efficient and targeted use of DNAm-based GA estimations in research and clinical settings.

Conflict of Interest: None declared

P21.032.D Genetic loci that disconnect obesity and abdominal fat distribution impact adipocyte differentiation and function

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Obesity is closely associated with abdominal fat distribution, characterized by a high waist-hip ratio (WHR), which can negatively impact cardiometabolic health. However, the relationship between overall and abdominal fat can vary greatly among individuals, potentially due to genetic factors. Intriguingly, we have recently discovered several genetic loci that invert the relationship between general and abdominal adiposity: They are associated with higher body mass index (BMI) but lower WHR. These genetic loci often show a favorable effect on insulin sensitivity and lower risk of type 2 diabetes, despite an increase in body fat. To better understand the biological mechanisms behind these genetic associations in adipocytes, we aimed to identify and

experimentally follow up on putative causal genes that invert the relationship between BMI and WHR. We have performed in vitro perturbation using siRNA on six prioritized candidate genes in white adipocytes from both mice and humans. Our initial results suggest that four of these genes – *ADAMTS9*, *ABHD15*, *EMILIN2* and *COL18A1* – may lead to decreased differentiation of fat cells, affecting cell growth, lipid storage, and the expression of adipogenic marker genes. Currently, we are investigating the effects of these genes on lipid metabolism, insulin signaling, and glucose uptake. Through these experiments, we hope to identify new targets for the development of therapeutics that can invert the relationship between obesity an abdominal fat distribution, improving cardiometabolic health. Grants from the Novo Nordisk Foundation (NNF18CC0034900, NNF20OC0063707).

Conflict of Interest: None declared

P21.033.A Investigation of the possible role of CITED2 transcription factor in the circadian clock mechanism

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Background: The circadian clock regulates several behavioral and physiological processes. Therefore, disruption of the circadian clock by mutation of core clock genes is associated with several diseases such as sleep disorders. At the molecular level, the circadian clock is generated by transcriptional and translational feedback loop (TTFL) mechanisms. In mammals, TTFL is determined by the interaction of four main clock proteins: BMAL1, CLOCK, CRYs, and PERs. BMAL1 and CLOCK form dimers and initiate the transcription of clock-controlled genes by binding an E-box element within the promotor genes. Although the core clock components have been discovered, genetic, biochemical, and computational studies suggested that additional clock components are likely that act as core clock components or regulate the molecular clock as tissue-specific processes.

Result: To identify new core clock genes or modifiers, we developed a high-throughput screening method in which about 1400 mammalian transcription factors were screened based on BMAL1/CLOCK transactivation assay. We identified a protein (CITED2) that inhibits BMAL1/CLOCK transactivation. Our results indicated that CITED2 interacts with CLOCK and inhibits BMAL1/CLOCK-driven transcription in a dose-dependent manner. We also discovered that deleting the CITED2 gene in U2OS cell line increases the period length of the circadian rhythm.

Conclusion: CITED2 is a transcription co-regulator that plays important roles in normal hematopoiesis and has a role in modulation of the hypoxic response. Our preliminary findings suggest CITED2 as clock mechanism component and help us to better understand the pathogenesis of circadian clock-related diseases in hypoxic conditions.

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Conflict of Interest: Ibrahim Barış Full time, PI, TUBITAK 221Z119, Cagla Cakmak: None declared, Basak Velioglu: None declared, Elif Uyanik: None declared, İbrahim Halil Kavaklı: None declared

P21.034.B Changes in DNA methylation associated with type of delivery

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Background/objectives: DNA methylation is an epigenetic process by which nonsequence-based regulatory information could be mitotically transformed from mother-to-daughter cells. Recently, studies showed that changes in methylation occur also in the process of childbirth in type-of-delivery dependent manner, vaginal/cesarean delivery (VD/CD). Delivery represents an epigenetic process with potential impact on DNA methylation state with possible impact on health later in life.

Methods: DNA samples, obtained from umbilical-cord and peripheral blood of 11 newborn infants with different types of childbirth (VD/CD), were used to generate target-enriched DNA libraries by Twist targeted methylation sequencing protocol for sequencing on Illumina next-generation sequencing systems.

Results: In VD/CD comparison, 3017 CpG islands were significantly differentially methylated in umbilical cord blood samples and 2124 CpG islands in peripheral blood samples. These were localized into 168 differentially methylated regions (78 hypermethylated/90 hypomethylated) in umbilical cord blood samples and into 157 regions (79 hypermethylated/90 hypomethylated) in peripheral blood samples. Moreover, in comparison of umbilical cord and peripheral blood, identified differentially methylated loci were associated with multiple pathways: receptor activation in carcinogenesis, cAMP and cGMP-DKG signaling pathways and Ca-signaling pathways.

Conclusions: Observed changes in DNA methylation state between umbilical cord and peripheral blood samples could be used for understanding of processes during development. Our results suggest any changes in DNA methylation for vertical analysis - vaginal vs cesarean delivery.

Grants

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Conflict of Interest: Patrik Krumpolec Medirex Group Academy full time employee, Erik Dosedla: None declared, Zuzana Turcsanyiová: None declared, Dominik Kodada Medirex Group Academy part-time employee, Oliver Petrovic Medirex Group Academy full time employee, Dominik Hadžega Medirex Group Academy full time employee, Gabriel Minarik Medirex Group Academy full time employee

P21.035.C Quantitative expression assay of GNAS gene: a reliable functional analysis in various clinical and molecular situations

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The complex *GNAS* locus encodes the α subunit of the stimulatory G protein and additional imprinted transcripts. The *GNAS-A/B* differentially methylated region is methylated only on the maternal allele. Paternal A/B transcript physiologically inhibits *GNAS* expression in *cis* in endocrine gland tissues. Three main phenotypes are associated with genetic anomalies in this locus: Pseudohypoparathyroidism type 1a (PHP1a), type 1b (PHP1b) and pseudopseudohypoparathyroidism (PPHP). Identification and functional analysis of genetic modifications can be challenging in some cases.

We investigated eight French families presenting various *GNAS*-associated phenotypes: 5 PHP1a, 1 PHP1b, 1 PPHP and 1 partial PPHP phenotype. *GNAS* gene sequencing, methylation and CNV analysis were realised following the routine diagnosis protocol. Whole genome sequencing was performed in three cases. A quantitative expression analysis of *GNAS* gene using quantitative Real-Time PCR and droplet digital PCR was performed on whole blood RNA in comparison with a sex and age matched control.

We identified 5 novel variants: 2 synonymous, 1 missense, 2 intronic and 3 novel chromosomal rearrangements: 1 inversion in the regulatory region upstream the *GNAS* gene and two different deletions localised in 5'UTR. In 6/8 of cases (75%) a haploinsufficiency was revealed. The seventh case has an abnormal RNA present, without haploinsufficiency. The last case with partial PPHP phenotype presents a synonymous *GNAS* variant with no impact on RNA splicing.

The quantitative whole blood expression assay of *GNAS* gene is a reliable functional analysis that should be considered even without knowing the exact molecular cause whenever the patient has a *GNAS*-associated phenotype.

Conflict of Interest: None declared

P21.036.D Epigenetic signatures of smoking, alcohol abuse and BMI

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Background/Objectives: Recognizing changes in DNA methylation acting as a mediating mechanism between lifestyle and the body's response in the form of disease development can have a significant impact on public health. In addition, a donor of biological traces can be described by lifestyle-associated DNA methylation, which can improve forensic investigation. The goal of the study was to identify differentially methylated positions (DMPs) correlated with lifestyle factors in a Polish population.

Methods: The study covered a group of 757 people from Poland aged >20 with a defined lifestyle (cohort 1). The set of samples was enriched with 200 postmortem blood samples from individuals aged 30-60, including extreme alcoholics and a control group (cohort 2). Epigenome-wide data was obtained for both cohorts using the Infinium HumanMethylationEPIC BeadChip (EPIC array).

Results: Analysis indicated 43 EWAS-significant CpGs for smoking and 18 CpGs for BMI (P value $<1\times10^{-6}$). In addition, the 9 EWAS-significant CpG markers identified in the group of extreme alcohol abusers (cohort 2) were replicated in an independent group of people with declared no or moderate alcohol consumption (cohort 1).

Conclusions: Our study validated known markers and identified novel epigenetic signatures of various lifestyle factors in the human genome.

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Conflict of Interest: None declared

P21.037.A Pregnancy stressors such as exposure to the COVID-19 pandemic or fetal congenital heart defect are associated with genome-wide epigenetic changes in the newborn

Kristen Kocher[1], *Surajit Bhattacharya*[2], *Nickie Niforatos-Andescavage*[1], *Eric Vilain*[3], *Catherine Limperopoulos*[1], ***Emmanuèle Délot***[2]

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Background/Objectives: Our quantitative MRI studies show that prenatal stress is associated with brain growth and maturation differences in newborns, with potential life-long cognitive, learning, and behavioral consequences. Stressors to the fetus can fundamentally alter the neonatal epigenome. To understand how epigenetic marks may predict susceptibility or resilience to negative developmental consequences, we aimed to characterize the episignatures in newborns exposed to stress during pregnancy.

Methods: A methylation array approach was used to assess genome-wide differences between a control and several stress-exposed cohorts: babies with congenital heart defect, pregnancies in the first or second trimester of gestation during the first year of the COVID-19 pandemic, pregnancies complicated by placental defects or premature birth. We designed and validated an analytic platform for the Infinium EPIC array (Illumina) across cohorts. Maternal mental health was captured longitudinally during and after pregnancy using standardized instruments. Quantitative brain MRI was performed on fetuses and newborns.

Results/Conclusions: Striking genome-wide methylation differences were seen between control babies born before and those in gestation around the start of the COVID-19 pandemic, regardless of whether mother actually got infected or not. Differential episignatures between pregnancies occuring early vs. late in the pandemic suggest acute and chronic stress may have different impact. A specific episignature was also found in babies with cyanotic congenital heart defects, including genes involved in hypoxia metabolism such as *HIF1a* and *EPO*. A subset of differential methylation sites were common to the various stressed cohorts, providing critical insight into the impact of stress on the neonatal epigenome.

Conflict of Interest: Kristen Kocher: None declared, Surajit Bhattacharya: None declared, Nickie Niforatos-Andescavage: None declared, Eric Vilain Stocks in Bionano. This is not relevant here. No Bionano equipment or technology is used for this project., Catherine Limperopoulos: None declared, Emmanuèle Délot: None declared

P21.038.B Evaluation of available markers and algorithms for age prediction in semen

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Background/Objectives: Age prediction based on DNA methylation is well established in many somatic cells and tissues, however in sperm cells it remains challenging. In this study, we evaluated the performance of known epigenetic age estimators for semen and selected the most age-informative CpGs for sperm cells.

Methods: Semen samples were collected from over 200 males from Polish population. EPIC microarray technology and SBE method were used to measure DNA methylation. Age predictions were done using the ComprehensiveGLACode.R code and the model described in Lee et al. 2015. Statistical analyses including prediction modelling was conducted with IBM SPSS Statistics 28.

Results: The highest prediction accuracy with mean absolute error of 2.8 years was obtained using the GLA Calculator - a genomic predictor based on 51 genomic regions. The selection of the 8 most informative CpGs allowed the development of a regression model that explains 73.4% of the age variation observed in Polish population and allows to predict epigenetic age with an MAE of 3.22 ± 2.35 years.

Conclusion: The 8-CpG model for age prediction in semen offers simplicity and accuracy which makes it useful in forensics. To fully characterize the newly developed model, further tests were performed on the influence of various lifestyle factors e.g. physical activity, health status on the accuracy of age prediction.

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Conflict of Interest: None declared

P21.039.C Modelling Epigenetic Age Over Time

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Background/Objectives: Epigenetic age (EA), an age estimate based on methylation states across the genome, is associated with various exposures and health outcomes. The recent growth in longitudinal DNA methylation data collection has led to an increase in research on EA over time. Here, we compare and evaluate methods frequently used in such studies to help guide future studies to least biased results.

Methods: Using a simulation study based on longitudinal ALSPAC data, we evaluated the robustness of different approaches to (i) models, (ii) outcomes, (iii) time variables, and (iv) the scope of data. To further validate our simulation results in a real-life example, we applied all models using maternal smoking as an exposure variable with longitudinal EA as the outcome.

Results: The most accurate exposure coefficient estimates were obtained through linear mixed models (LME) and generalized estimating equations (GEE) that accounted for time using chronological age. Models that included timepoint as their time variable showed underestimated results. By contrast, accounting for years between measures or categorical time resulted in inflated estimates. LME and GEE also achieved higher accuracy in estimating age-exposure interactions, accounting for time through either chronological age or years between measures. Our applied analyses of maternal smoking and epigenetic aging confirmed these findings.

Conclusion: In summary, our results provide guidance for future studies evaluating associations between exposures and longitudinal EA. Ultimately, more consistent methods in epigenetic aging studies will improve the robustness and reproducibility of findings.

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Conflict of Interest: None declared

P21.040.D Genome-wide DNA methylation profile of maternal and cord blood in spontaneous and egg-donation pregnancies

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From fertilization through implantation and during pregnancy, the epigenetic profile of the embryo changes dynamically and environmental exposure may trigger methylation modifications that have long-lasting impacts on health and disease. According to the DoHaD hypothesis, fetal epigenetic modifications may persist after birth as an "epigenetic memory" and influence postnatal health. ART (Assisted Reproductive Technology) is a potential factor that could influence the epigenetic pattern of the fetus; nevertheless, the role of ART on fetal epigenetics remains unclear.

We performed methylation profiling by NGS in cord blood and maternal peripheral blood from 17 spontaneous pregnancies (SPPs) and 14 egg-donation pregnancies (EDPs) in order to: 1) evaluate the methylation profile in maternal compared to cord

blood; 2) evidence methylation differences in cord blood of SPPs and EDPs; 3) compare the methylation profiles of mother and child pairs. Our results highlighted: 1) a specific methylation pattern in cord compared with maternal blood; we observed 10510 CpG sites methylated only in cord blood and 3941 CpGs exclusively methylated in maternal blood. In cord blood about 50% of hypermethylated sites were found in open sea regions, while most of hypomethylated sites were within CpG islands. Pathway enrichment analysis of differentially methylated regions in promoters and 5′ UTRs highlighted the involvement of the WNT/B-catenin pathway and neuronal differentiation processes; 2,3) Methylation profile in cord blood of SPPs did not differ from the profile of EDPs, but we evidenced that the mean methylation differences between mother and child pairs were higher in EDPs compared to SPPs.

Conflict of Interest: None declared

P21.041.A A functional single cell screen demonstrates epigenetic control of cancer cell heterogeneity

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Cellular heterogeneity is integral to biology, and provides cell populations with plasticity to respond to external cues. In cancer however, elevated transcriptional heterogeneity is malicious: it increases the likelihood for cells to survive the selective pressures of therapy, supports resistance acquisition and causes disease recurrence. Despite this paramount importance, drivers of intratumoural heterogeneity are poorly understood. Recent studies have indicated a role for epigenetic enzymes in regulating transcriptional heterogeneity. To investigate this systematically, we applied joint single cell RNA sequencing and CRISPR gene inactivation to study effects of knock down of epigenetic enzymes. Focusing on the notoriously heterogenous non-small cell lung cancer (NSCLC), we targeted all 267 known epigenetic enzymes in two in vitro models for NSCLC, A549 and PC9. Remarkably, integrative analysis showed that loss of epigenetic enzymes influences stochastic rather than biological heterogeneity, indicating a key role for epigenetic enzymes in controlling random cell to cell variability. Biological and stochastic heterogeneity appear uncoupled, with knockdown of some enzymes simultaneously decreasing stochastic but increasing biological variability. When stratifying the heterogeneity effects for each enzyme per chromatin state, we observe more significant impact of enzymes on genes marked by the modification they control, whether it be repressed, silent or active. In conclusion, our work highlights a novel role for the epigenome, with epigenetic enzymes controlling transcriptional stochasticity in genetically uniform populations.

Grant reference: Foundation against Cancer and FWO.

Conflict of Interest: None declared

P21.043.C The interplay of DNA methylation and H3K36 methylation: a model of epimutation-mediated transcriptional interference silencing

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Transcriptional interference (TI) is the in cis suppression of one transcriptional process by another, and is known to affect several human genes through different mechanisms. Here, we investigate TI-mediated gene silencing and the resulting epigenetic changes at the *MMACHC/PRDX1* locus, two flanking genes on opposite strands. In a subgroup of cblC patients, a PRDX1 mutation at the canonical splice acceptor site of intron 5 disrupts normal splicing, thereby causing antisense transcription and silencing of *MMACHC*, generating an aberrant H3K36me3 mark and DNA methylation (DNAme) at its promoter. Using a mouse stem cell model, we recapitulate *MMACHC* silencing through ablation of the *PRDX1* intron 5 splice acceptor site, and knockout of the de novo DNA methyltransferases, DNMT3A/B, leads to partial expression of *MMACHC*. H3K36 methylation has been implicated in the recruitment of DNMT3A/B, and since these results show a partial restoration of gene expression after their depletion, this intriguingly suggests that this TI-mediated silencing may not only be dependent on the presence of DNAme, but implicates the presence of aberrant H3K36me marks. SETD2 is the only mammalian enzyme known to deposit H3K36me3, while H3K36me2, which also recruits DNMTs, is deposited by the NSD family of methyltransferases. Therefore, we have generated SETD2-NSD1/2/3-QKO cells depleted of all H3K36me to further investigate the underlying mechanisms of this TI-mediated silencing. While the mutation of *PRDX1* is necessary for the establishment of silencing, its maintenance may be dependent on the interplay between other epigenetic factors.

Grant reference: Funded by the Canadian Institute of Health Research (CIHR)

Conflict of Interest: None declared

P21.044.D Validation of rs7132908 as a causal variant at the childhood obesity chr12q13 locus

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Background: Our GWAS effort with the Early Growth Genetics (EGG) Consortium has revealed the *FAIM2* locus on chr12q13 as among the strongest associations with childhood obesity. Given our existing 'confluence of evidence,' we hypothesize that rs7132908 is causal at this locus and impacts expression of at least one gene.

Methods: We performed luciferase reporter assays using human primary astrocytes and HEK293Ts. We also generated human embryonic stem cell (hESC) lines homozygous for either allele. Each hESC line was then differentiated to a heterogeneous model of hypothalamic arcuate nucleus neurons and used for single nucleus RNA-seq.

Results: We characterized allele-specific *cis*-regulatory activity of the region harboring rs7132908 with genes implicated by our chromatin-based variant-to-gene mapping efforts in astrocytes. With the *FAIM2* promoter, the non-risk allele region increased expression (fold change 1.75), while the risk allele decreased expression (fold change 0.53); in HEK293Ts, a decrease in expression was limited to just the risk allele (fold change 0.60). With the *RACGAP1* promoter, only a decrease in expression was observed with the risk allele (fold change 0.13). In addition, hESCs with the non-risk allele differentiated to 62% neurons and 19% fibroblasts, while the hESCs with the risk allele differentiated to 3% neurons and 86% fibroblasts. Within the fibroblast population, 3,511 genes were differentially expressed, while within the neuron population, just 75 genes were differentially expressed.

Conclusion: rs7132908 resides in a *cis*-regulatory element influencing *FAIM2* and *RACGAP1* expression. Furthermore,

rs7132908 regulates genes involved in hypothalamic neurodevelopment.

Grant References: R01HD056465 and F31HD105404

Conflict of Interest: None declared

P22 New Treatments for Genetic Disorders

P22.001.A Effects of clonazepam in patients with ARID1B-related intellectual disability

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Mutations leading to *ARID1B* haploinsufficiency frequently cause intellectual disability (ID). In mice with Arid1b haploinsufficiency, treatment with clonazepam showed a positive effect on object recognition, anxiety, and sociability novelty in these mice. Therefore, this study evaluated the effects of clonazepam in ARID1B patients.

This was a randomized, double-blind, placebo-controlled, two-way crossover study. Patients received doses of clonazepam(max 0.5 mg, twice daily) or a placebo for 22 days with a 3-week washout period. Safety and tolerability parameters, pharmacokinetics and pharmacodynamic effects on neurocognitive tasks, and behaviour and cognitive function measured by the Aberrant Behaviour Checklist and Clinician's Global Impression(CGI) scale were evaluated.

Sixteen patients received clonazepam, of which fifteen patients completed both study periods. For seven patients (44 %), an improvement in CGI was reported on clonazepam, compared to two on placebo (13 %). Examples of self-reported improvement include fewer emotional outbursts, increased expressive speech, and self-reported mental calmness. One patient scored 'no change' on CGI after clonazepam treatment(two on placebo), whilst a deterioration in CGI was observed in seven patients on clonazepam (44 %) (none on placebo). This deterioration was usually linked to side effects($n = 6$).

The results suggest that clonazepam may affect some *ARID1B* patients. That is to say, half of the patients reported a positive effect on the CGI scale, whilst the other half reported a deterioration in global functioning. Furthermore, there appears to be a correlation between side effects and deterioration, suggesting that a lower dose might result in a better benefit-risk ratio.

Funders: ZonMW Project number 10140261910002

Conflict of Interest: None declared

P22.002.B 1 Mutation 1 Medicine: a European platform for ASO development and treatments for individuals with severely debilitating or life threatening nano-rare neurological diseases

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Introduction: The goal of 1M1M is to develop single patient tailored ASO mediated exon skipping treatments for individuals with severely debilitating or life threatening (SDLT) nano-rare neurological diseases (RND).

Methods: Using the recently provided proof-of-concept that development of a mutation-specific, intrathecally delivered splice-switching ASO treatment is feasible within 18 months (PMID: 31597037) as a blueprint, 1M1M is currently building a European platform. Based on EMA advice, processes and infrastructure are being established, validated and improved.

Results: 1M1M has been developing workflows for: potential splice-altering variant interpretation; ASO design and testing; patient identification including variant, disease and patient perspective; regulatory-like process towards start/stop of development and treatment decision including patient dossier, gene groups and treatment board.

Fit-for-purpose and sustainable infrastructures such as a 1M1M-registry are being set-up. A European 1M1M network of >20 hospitals has been formed.

The following numbers and facts reflect the 1M1M achievement: >2.000 RND genes-of- interest identified; >40 potential cryptic splice mutations of patients investigated; ASO development programs underway for six genes; full patient dossiers have been used to organise *ATM*, *POLR3A* and *PLP1* gene group meetings; EMA advice received in March 2022 (PMID: 36669889); first treatment of an eligible patient in Europe has started in September 2022 at the University Hospital Tübingen.

Conclusion: 1M1M has successfully started to build a European platform for ASO development and treatments for individuals with nano-rare SDLT RNDs. Some open issues such as reimbursement by healthcare insurances remain to be addressed.

Conflict of Interest: None declared

P22.003.C Orphan designations (ODs) granted to gene therapy (GT) in Europe: analysis and evolution over the last two decades

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Background/Objectives Methods: Gene therapy (GT) has developed considerably thanks to knowledge and techniques continuously improving over the past 20 years, and now provides a unique therapeutic option for rare diseases (RD). Orphanet (www.orpha.net), the European RD knowledge base, aims to gather and improve knowledge on rare diseases providing high-quality information, including data on orphan drugs and clinical trials. Data can be retrieved on Orphadata.com. An analysis of the ODs in Orphadata and granted to GT by EMA up to 31/12/2022 was performed.

Results: As of 31/12/2022, 356 ODs had been granted to 320 GTs. 306 ODs were still active indicating that 14% of ODs were irreversibly removed from the Community Register of orphan medicinal products. GTs represented about 16% of all orphan medicinal products. Diseases targeted by Orphan GTs were mainly rare inborn errors of metabolism, ophthalmic, neurologic and neoplastic diseases. Thirteen orphan GTs had an approved marketing authorization (MA), mainly in hematology (5/13), corresponding to 4% of all Orphan GTs. Among them, 4 had neither MA nor OD in US. An increase of OD granted to GT over the last 20 years was observed with a global acceleration since 2014 despite a marked decrease for 2019. Median time between OD and corresponding MA was 1579 days. The correlation

between preclinical research on GT and clinical applications will be presented.

Conclusion: This analysis confirms the increased number of GT but also points out the restricted range of rare diseases for which GTs have been developed.

Grant References: AFM-Téléthon, EJP RD

Conflict of Interest: Julie BRUYERE-ZRELLI Full time: INSERM US14, collaborator : AFM téléthon, Melania Cruciani full time: INSERM-US14-Orphanet, Collaborator : EJP-RD, Florence Sauvage Part-time : INSERM-US14-Orphanet, Charlotte Rodwell Part-time : INSERM-US14-Orphanet, Collaborator: EJP RD; Solve-RD, Maladies Rares Info Services, Valerie Serriere-Lanneau Full time: INSERM-US14-Orphanet, Collaborator: EJP-RD, OD4RD2, Marc Hanauer Full-time: INSERM-US14-Orphanet, Collaborator: OD4RD2; EJP RD, ELIXIR-FR; Solve-RD; EHDS Pilot 2; TEHDaS JA;, Scientific Advisory board national support group "Aide aux Jeunes Diabétiques"., Support group Chairman (Association ENT'RED-paris, réseau enfance diabète), Ana Rath Full-time: INSERM-US14-Orphanet, Principal investigator: OD4RD2 ; collaborator: EJP RD; Solve-RD; EHDS Pilot 2; TEHDaS JA, advisory board: ERN-LUNG; ERN-BOND; Share4Rare

P22.004.D Repurposing with purpose - from discovery to rapid drug treatment of Bachmann-Bupp Syndrome as a model of the potential to treat and cure rare diseases

Caleb Bupp[1;2;3], *Elizabeth VanSickle*[1;3], *Julianne Michael*[1;3], *Marlie Vipond*[1], *Abby Dalman*[1], *Chad Schultz*[2], *Andre Bachmann*[2;3]

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Background/Objectives: Mutations in the C-terminus of ornithine decarboxylase 1 (*ODC1*) cause Bachmann-Bupp Syndrome (BABS, OMIM #619075) characterized by developmental delay, hypotonia, and non-congenital alopecia. ODC is a rate-limiting enzyme in the polyamine pathway that plays a key role in embryogenesis, organogenesis, and neoplastic cell growth. Difluoromethylornithine (DFMO) is a well-tolerated FDA-approved drug that irreversibly inhibits ODC enzyme activity.

Methods: Three BABS patients were treated on FDA investigational new drug protocols with DFMO starting at ages 4 years, 6 years, and most recently 3 months. Changes in hair, muscle tone, and development were tracked with side effects monitoring. Samples were collected before and during treatment for polyamine analysis.

Results: All patients showed hair, eyebrow, and eyelash regrowth. Muscle tone, coordination, and cognition improved with accelerated development. Patients previously unable to even sit unsupported began assisted walking and the patient started on early treatment at 3 months old showed rapid recovery with near normal development and cognition now at age 1 year, 2 months. There were no significant adverse effects. The first patient treated showed near immediate improvement in her polyamine (N-acetylputrescine) levels. Results from additional treated patients are currently pending and will be presented.

Conclusion: BABS treatment with DFMO results in significant clinical improvement and biochemical normalization. This example showcases the potential of rapid genetic diagnosis and drug repurposing as well as the challenges that must be addressed with ultra-rare disorder treatment.

Grant References: Leveraging modulation of polyamine metabolism for therapeutic advantage in genetic disorders. R01HD110500-01 – National Institutes of Health, USA

Conflict of Interest: Caleb Bupp 1. Leveraging modulation of polyamine metabolism for therapeutic advantage in genetic disorders. R01HD110500-01 – National Institutes of Health, USA - Bupp/Bachmann as principal investigators

2. International Center of Expertise for Polyamine Disorders - Spectrum Health/MSU Alliance, 2019-2023. Bupp/Bachmann - primary investigators, DFMO provided by Sanofi at no cost, Patent: "Methods and compositions to prevent and treat disorders associated with mutations in the ODC1 gene". Inventors: Bachmann AS, Bupp C, Rajasekaran R. Patent No. US 11,273,137 B2. Date of Patent: March 15, 2022, licensed to Orbus Therapeutics, Inc., Consultant for Orbus Therapeutics, Elizabeth VanSickle: None declared, Julianne Michael: None declared, Marlie Vipond: None declared, Abby Dalman: None declared, Chad Schultz: None declared, Andre Bachmann 1. Leveraging modulation of polyamine metabolism for therapeutic advantage in genetic disorders. R01HD110500-01 – National Institutes of Health, USA - Bupp/Bachmann as principal investigators

2. International Center of Expertise for Polyamine Disorders - Spectrum Health/MSU Alliance, 2019-2023. Bupp/Bachmann - primary investigators

, Patent: "Methods and compositions to prevent and treat disorders associated with mutations in the ODC1 gene". Inventors: Bachmann AS, Bupp C, Rajasekaran R. Patent No. US 11,273,137 B2. Date of Patent: March 15, 2022, licensed to Orbus Therapeutics, Inc.

P22.005.A The EDELIFE clinical trial, the first investigation of a signalling molecule to treat in utero a human developmental genetic disorder

Holm Schneider[1], *Smail Hadj-Rabia*[2], *Florian Faschingbauer*[3], *Christine Bodemer*[2], *Dorothy Grange*[4], *Encarna Guillén-Navarro*[5], *Gianluca Tadini*[6], ***Angus Clarke***[7]

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Males affected by X-linked hypohidrotic ectodermal dysplasia (XLHED) cannot sweat, have few teeth, sparse hair and fewer mucous glands in the airways. Over-heating impacts day-to-day activities and, with the patients' susceptibility to severe infections, mortality is increased. XLHED is caused by pathogenic variants of the ectodysplasin A gene (*EDA*), with most affected males being hemizygous for null mutations of the signalling protein ectodysplasin A1 (EDA1). A treatment given to affected mice was developed more than 20 years ago; it was effective but administration was time-critical. This was a recombinant protein combining the receptor-binding domain of EDA1 and part of the IgG1 F_C domain. After further animal studies, a trial in newborn males with XLHED showed the recombinant protein to be safe but ineffective.

First prenatal treatments of affected humans were reported in 2018. The drug product administered intra-amniotically, ER004,

represents a first-in-class protein replacement molecule that acts as a substitute for the missing EDA1 in unborn patients. This is the first use of drug therapy to trigger a developmental step that then corrects – at least partially – a developmental genetic disorder. Other protein-based therapies require prolonged, and often lifelong, application.

The administration of ER004 *in utero* during the late second and third trimesters of pregnancy on a named-patient basis has confirmed significant benefits to the development of sweat glands and perspiration (sustained >6 years) and permanent teeth. We report experience to date with this treatment and with the EDELIFE trial, a prospective, genotype-controlled, multicentre study, to which recruitment continues.

Conflict of Interest: Holm Schneider Holm Schneider is inventor on a patent related to the prenatal treatment of XLHED. He signed, however, a Remuneration Waiver Agreement with the Free State of Bavaria to relinquish any personal financial gain from this invention., Smail Hadj-Rabia: None declared, Florian Faschingbauer: None declared, Christine Bodemer Amryt, Sanofi, Abbvie, Novartis, Dorothy Grange Member of the Scientific Advisory Council for the National Foundation for Ectodermal Dysplasia, Encarna Guillén-Navarro Third generation sequencing, in vitro and in vivo functional characterization and drug repositioning screening in Ectodermal Dysplasias, PI21/01082-ISCIII and FEDER Funds (PI: Encarna Guillen-Navarro), Infraestructura de Medicina de Precisión asociada a la Ciencia y Tecnología (IMPaCT) de la Acción Estratégica en Salud 2017-2020. IMPaCT Genómica, IMP/00009 (PI: Angel Carracedo; CI: EGN) Administrations of ER004 in Male Subjects With X-linked Hypohidrotic Ectodermal Dysplasia (EDELIFE) (NCT04980638). ER004-CLIN01 / F60082AI201. EspeRare Foundation and Pierre Fabre Medicament, Unpaid Advisory Board member of Spanish Society of Ectodermal Dysplasia (AADE), Gianluca Tadini: None declared, Angus Clarke Ethics Advosry Group and Newborn Ethics Group of Genomics England Ltd; Medical Advisory Board of Ectodernal Dysplasia UK; medical advisor to Rett UK; Pfizer Inc (advsory board on role of medical geneticists in gene therapy); Bloodspot Advisory Group to the (UK) National Screening Committee

P22.006.B Novel siRNA-based therapeutic approach targeting ACTG2-R178C mutant mRNA for the treatment of Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS)

Isabella Baldini[1], ***Riccardo Ottalevi***[2], *Antonio Infascelli*[1], *Greta Valoti*[1], *Antonio PM Graziani*[1], *Noemi Sabatini*[1], *Maria Chiara Masciovecchio*[1], *Chiara Ciace*[1], *Davide Clementoni*[1], *Anna Lo Iacono*[1], *Gianluca Iodice*[1], *Juan Caceres*[2], *Michela Luciano*[3], *Riccardo Paone*[1]

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Background: MMIHS is a rare form of functional intestinal obstruction in the newborn. Mutations in the ACTG2 gene, leading to disruption of γ-2 enteric actin filaments and impaired contraction of intestinal smooth muscle cells, have been linked to MMIHS in multiple studies. Even though survival has improved in recent years, MMIHS is a condition that requires invasive palliative treatments. To meet the need for novel therapeutic approaches for MMIHS, we designed a group of therapeutic siRNA to specifically target the ACTG2 mutant allele.

Methods: WGS was performed on DNA isolated from chorionic tissue of a 13th week pregnant patient presenting an echography with alteration of gut and bladder of the newborn. EXTENSA™ software has been used to identify the pathogenic ACTG2 variant (NP_001606.1:p.Arg178Cys) and a Machine Learning (ML) software has been used as prediction tool for the identification of 8 siRNAs with the most discriminatory power between ACTG2-R178C and ACTG2-WT mRNA. In vitro preclinical studies have been performed on HEK293 cells to evaluate the silencing of the ACTG2-R178C mRNA highly specific manner.

Results: Preliminary data suggest that our selected siRNA specifically target the ACTG2-R178C mRNA and reduce the expression of mutant γ-2 enteric actin while showing no effect on ACTG2-WT, thus enabling a condition of haplosufficiency.

Conclusion: Our study proposes a therapeutic approach based on siRNA as a novel treatment for MMIHS, identified siRNA sequences as good candidates for the development of a new drug and underscore a translational impact for future strategy to cure this disease.

Conflict of Interest: None declared

P22.007.C SMN is a genetic modifier of GEMIN5-mediated syndrome

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GEMIN5 is essential for core assembly of small nuclear Ribonucleoproteins (snRNPs), the building blocks of spliceosome formation. Biallelic mutations in *GEMIN5* lead to a neurodevelopmental syndrome among patients presenting with developmental delay, motor dysfunction and cerebellar atrophy by perturbing snRNP complex protein expression and assembly. Currently, molecular determinants of GEMIN5-mediated disease have yet to be explored. Here, we identified SMN as a genetic suppressor of GEMIN5-mediated neurotoxicity in vivo. We discovered that an increase in SMN expression by either SMN gene therapy replacement or the antisense oligonucleotide (ASO) Nusinsersen, significantly upregulated the expression of GEMIN5 in mammalian cells and mutant GEMIN5 derived iPSC neurons. Further, we identified a strong functional association between the expression patterns of SMN and GEMIN5 in patient Spinal Muscular Atrophy (SMA) derived motor neurons harboring loss of function mutations in the *SMN* gene. Interestingly, SMN binds to the C-terminus of GEMIN5 and regulates GEMIN5 expression through the Tudor domain. Lastly, we show that SMN upregulation ameliorates defective snRNP biogenesis and alternative splicing defects caused by loss of GEMIN5 in iPSC neurons and in vivo. Collectively, these studies indicate that SMN is a potent regulator of GEMIN5 expression and neuropathologies.

Conflict of Interest: None declared

P22.008.D Patient and variant stratification for personalized genetic treatments of nano-rare diseases

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Around 6 % of the world's population is affected by at least one of the 7,000+ known rare diseases, most of which are genetic in origin. No disease-modifying treatments are yet available for over 90 % of these disorders; new therapeutic strategies are urgently needed. In recent years, it has been shown that antisense oligonucleotides (ASOs) are one option to treat rare inherited diseases, and splice-switching ASOs have been approved for disorders like spinal muscular atrophy and Duchenne muscular

dystrophy. Notably, individual patients with nano-rare mutations are now being treated with mutation-specific ASOs. These efforts are coordinated by national (Dutch Center for RNA Therapeutics), European (1Mutation1Medicine), and global (N = 1 collaborative) networks and consortia.

Selecting which patients and genetic variants are eligible for such personalized treatment is challenging. Yet, prioritization of patients and variants is crucial to facilitate treating those that benefit most and not treating those unlikely to benefit.

To offer guidance and streamline the development of tailor-made ASOs, the Dutch Center for RNA Therapeutics has developed a set of practical guidelines for patient stratification and prioritization of genetic variants. The guidelines take the clinical presentation as well as the variant itself into account and can be used as an aid for clinicians and researchers alike.

By using explicit examples for eligible and non-eligible cases, we here present our guidelines in a hands-on manner, providing clinicians and researchers with the knowledge necessary to evaluate their own patients for personalized ASO-based treatments.

Conflict of Interest: None declared

P22.009.A Drug repurposing for Cohen Syndrome

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Cohen Syndrome (CS) is a rare autosomal recessive disorder caused by biallelic loss-of-function mutations in the *VPS13B* gene. Over 500 pathogenic mutations have been identified, and an estimated 30'000 to 50'000 individuals are affected worldwide. CS is characterized by multiple clinical features, including microcephaly, developmental delay, intellectual disability, epilepsy, hypotonia, retinal dystrophy and neutropenia.

The precise function of VPS13B is unknown, but it is part of the chorein-domain-containing protein family and is proposed to act as a lipid transporter at inter-organelle membrane contact sites. VPS13B is localized to the Golgi complex and the most evident phenotype of VPS13B deficient cells is the fragmentation of the organelle.

Using the Golgi fragmentation phenotype, we screened a library of 1280 FDA-approved compounds and identified two classes of compounds that efficiently recovered Golgi morphology in VPS13B knockout cells: Glucocorticoid receptor agonists and functional inhibitors of acidic sphingomyelinase and ceramidase.

From lipidomic analysis, we found very specific lipid dysregulations in VPS13B knockout cells, mainly a decrease of the C18 n-acyl species of sphingomyelin (SM). These SM species have been shown to play a crucial role in Golgi to ER transport and fragmentation of the Golgi complex. Interestingly, the acidic sphingomyelinase-inhibiting drugs were able to recover the level of these lipid species in VPS13B KO cells, indicating a potential mechanism of action of these drugs.

In conclusion, we developed a robust cell-based assay for drug screening in CS and propose drug repurposing by phenotypic screening as a valuable option for drug discovery in rare genetic disorders.

Conflict of Interest: None declared

P22.010.B The Long QT syndrome in patients with cardiac arrest is associated with a substrate in the pericardial cavity

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At least one fifth of sudden cardiac deaths from patients worldwide are explained by Long QT syndrome. The phenotype of Long QT syndrome has been considered since decades as a pure electric disorder with no detectable heart structural abnormalities. In spite of that, many authors reported the presence of epicardial electrical instability among patients affected by Long QT syndrome. We studied eleven consecutive patients affected by severe Long QT syndrome, genetically confirmed, all implanted with dual chamber device in secondary prevention of ventricular fibrillation with a high frequency of appropriated shocks. Using a high-density electro anatomical mapping, we demonstrated an arrhythmogenic substrate characterized by low-voltage, fragmented and prolonged electrograms clustering in the epicardium of the right ventricle exclusively. Catheter ablation allowed the homogenization of this substrate, with a shortening of QT interval in all studied patients, regardless for genetic mutations. This treatment successfully rescued the Long QT syndrome clinical phenotype preventing ventricular fibrillation recurrence.

Conflict of Interest: None declared

P23 Genetic Counselling/Services/Education

P23.001.A Evaluating a protocol for communicating melanoma personalised risk scores: a pilot study

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Background/Objectives: Personalised risk scores (polygenic and non-genetic risk factors) can facilitate risk-stratification, which could inform targeted melanoma screening. Methods for effective personalised risk communication are needed for clinical implementation.

Aim: Assess attitudes, acceptability, and psychosocial impact of a protocol for communicating personalised melanoma risk.

Methods: Affected and unaffected adults enrolled in ongoing melanoma studies were recruited. Participants received a personalised risk booklet and attended a genetic counselling appointment (online or in-person). This mixed-methods approach used questionnaires at baseline and 1-month post-results (analysed with paired t-tests and Fisher's Exact tests) and semi-structured interviews (analysed thematically).

Results: 35/73 consented to participate; 31 and 30 completed baseline and follow-up questionnaires respectively. Participants rated the information was useful and motivational for favourable health behaviours. They were satisfied with the quality, quantity,

format, and understandability of the booklet. Low- and high-risk groups felt highly empowered at both baseline and follow-up. High-risk participants' overinflated perceived melanomas risk at baseline decreased appropriately post-results ($p = 0.018$) while still reporting 'above average risk' more frequently than low-risk participants at both timepoints ($p = 0.007$ and $p < 0.001$ respectively). No between group differences in perceived personal control existed at baseline, but at follow-up, low-risk individuals reported greater perceived control than high-risk individuals ($p < 0.001$). Genetic-specific distress, and uncertainty was low for all participants post-results. Qualitative interviews supported quantitative findings and highlighted the importance of access to a clinician for results interpretation and risk management.

Conclusions: This personalised melanoma risk protocol was acceptable and associated with

improved understanding and increased perceived control.

Grant References: NHMRC (APP2009136)

Conflict of Interest: Courtney Wallingford: None declared, Matthew Law: None declared, Adam Mothershaw: None declared, Astrid Rodriguez Acevedo: None declared, Hans Peter Soyer H.P.S. is a shareholder of MoleMap NZ Limited and Ederm-

consult GmbH and undertakes regular teledermatological reporting for both companies., H.P.S. is a shareholder of MoleMap NZ Limited and Ederm-

consult GmbH and undertakes regular teledermatological reporting for both companies. H.P.S. is a medical consultant for Canfield Scientific, Inc; MoleMap Australia Pty Ltd; Blaze Bioscience, Inc; and Revenio Research Oy and is a medical advisor for First Derm., Aideen McInerney-Leo: None declared, Tatiane Yanes: None declared

P23.002.B Whole genome sequencing in the English NHS Genomic Medicine Service: An exploration of service delivery using process mapping

Nastazja Laskowski[1], *Angus Clarke*[2], *Christine Patch*[3], *Amanda Pichini*[4], *Melissa Hill*[5;6], *Sinead Whyte*[7;8], *Celine Lewis*[5;9]

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Background/Objectives: The NHS is the first national healthcare system to offer whole genome sequencing (WGS) as part of routine care. The aims of this research are to (1) process map and (2) identify similarities/variations in the delivery of WGS for paediatric rare disease diagnosis across 7 NHS trusts.

Methods: Twenty-six observations of clinical encounters were conducted in 12 departments (7 genetic, 3 neurology, 1 cardiology, 1 general paediatric) across 7 NHS trusts in England. Observations were accompanied by field notes and interviews with 19 healthcare professionals. Qualitative data were analysed deductively to understand the processes for patient referral, consent, test ordering and results delivery.

Results: Differences in maps between departments include (1) whether eligible patients are referred to genetics or seen in mainstream service, (2) whether, how and what information parent(s) receive before WGS appointment, (3) professional background of person obtaining consent, (4) whether additional consent discussion takes place separately from clinic appointment, (5) format for recording consent, (6) when and who completes required paperwork, (7) actions regarding missing samples/consent forms, (8) when and where bloods are taken. Similarities include (1) specialties referring for WGS, (2) content of consent discussion, (3) required paperwork for submission to laboratory, (4) communication of results.

Conclusion: Our findings highlight variation in how WGS is being delivered across England. The next stage is to identify which steps benefit from a flexible approach and which may benefit from standardisation to optimize patient care.

Grant References: Celine Lewis is funded through an NIHR Advanced Fellowship Grant (NIHR300099).

Conflict of Interest: Nastazja Laskowski: None declared, Angus Clarke: None declared, Christine Patch Christine Patch was an invited member for a new born screening panel organized by Illumina., Amanda Pichini Amanda Pichini is employed by Genomics England, a company wholly owned by the Department of Health and Social Care, which supports the NHS to deliver whole genome sequencing in the Genomic Medicine Service., Melissa Hill: None declared, Sinead Whyte: None declared, Celine Lewis Celine Lewis is funded through an NIHR Advanced Fellowship Grant (NIHR300099).

P23.003.C Mapping the patient world in predictive Huntington's testing

Matilda Bradford[1], *Angus Clarke*[2]

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Consultations were recorded for patients referred for predictive testing for Huntington's disease (HD). Of these, five representative participants were selected for analysis of transcripts through a specifically social lens.

Transcripts were analysed to identify all instances where an additional figure was referenced by the patient. From this a 'map' was developed representing the frequency at which other figures became present in the patient's consultation. The use of reported speech by patients was also recorded.

Patients were found to have significant variation in the figures they describe when navigating their test process. Some patients describe few external figures, their test appearing to exist in isolation from the rest of their lives. Others have extensive references, demonstrating an anticipation of how the test intersects with their work, social and familial identities. Reference to other figures emerged as a mechanism through which patients model their future, negotiate their wishes and attribute meaning to their test. Reported speech appears as a feature for some, functioning as a means of protection where they may anticipate judgement or rejection.

By focusing on the figures referenced in consultation, professionals can help patients to situate their test meaningfully within their social and relational experience. We theorise that this could help patients who struggle to navigate abstract questions about the impact of testing. The 'social map' for a patient can also be depicted visually, providing an opportunity to extend family tree work and facilitate patient-centred consultations, highlighting areas of vulnerability and opportunities for support.

Grant reference: ES/R003092/1

Conflict of Interest: Matilda Bradford Royal Devon University Healthcare NHS Foundation Trust, Projected funded by ESRC grant: ES/R003092/1, Angus Clarke Cardiff University, ES/R003092, GSK (completed project) EspeRare clinical trial (no personal funding)

P23.004.D Genome Access, a digital solution for genetic counseling

Marco Crimi[1], *Marta Plebani*[1], *Francesca Ronzoni*[1]

[1]*Kaleidos, Bergamo, Italy*

As part of genetic counselling (GC), people learn how to deal with the medical, psychological, and familial implications of genetic contributions to disease as well as adapt to them. We have recently released "Genome Access" (GA), a platform developed to virtualize several steps of the GC process so that people can be better informed about their genetic health status and be more confident when making decisions about it.

In order to virtualize the counselling process for patients with genetic diseases, the platform is based on Artificial Intelligence algorithms developed ad hoc. In addition to being embedded in secure communication protocols, GA is entirely encrypted and compliant with GDPR to protect personal identity.

GA is built with three interoperable modules:

1) Through the "Information & Educational" module, the process of delivering privacy information, collecting consent from the patient, and explaining DNA test results and explaining what they mean to patients is made easier. There are several interactive videos created for genetics and DNA risk recurrence that the patient can watch and interrogate "Genebot", a custom chatbot made for genetics.
2) The "Data retrieval" module provides tools for guided self-analyses and for collecting the genogram, which is essential for GC. In addition, this second module facilitates the physician by suggesting specific DNA tests and providing a bioinformatic platform for clinical and genetic data interpretation.
3) Tele-Genetics. Lastly, the platform provides software for releasing a final clinical report as well as "virtualizing" the relationship between patients and healthcare providers through a televisit solution.

Conflict of Interest: Marco Crimi Project Manager, This study was supported by the grants Artemisia (CESVI-Bergamo) and SI4.0 2021 (Unioncamere Lombardia), Marta Plebani Part Time, Francesca Ronzoni Full Time

P23.005.A APOGeE launch: a European medical genetics e-textbook by ERN-ITHACA

Nicholas Szeto[1], *jonathan berg*[2], *Maurizio Genuardi*[3], *Martin Krahn*[4;5], *Ute Moog*[6], *Edward S. Tobias*[7], *Peter Turnpenny*[8], *Johannes Zschocke*[9], *Alain Verloes*[1]

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Launched in April 2023 as a public beta, APOGeE (A Practical Online Genetics e-Education) is a free online interactive medical genetics textbook built with Moodle. Written by various authors from the ITHACA network and other ERNs, APOGeE covers topics in biological genetics, formal genetics, clinical and physiological approaches to genetic diseases, precision medicine, genetic disease treatment and bioinformatics.

As a full specialty training programme in medical genetics and rare diseases, the project aims to provide free learning materials for everyone. Doctors and researchers from Europe and beyond, from all socioeconomic backgrounds, can access the book as well as different learning modules, self-evaluation tools and discussion forums. Authored and edited by international experts, the online platform will continue to receive updates with state-of-the-art knowledge and courses shared in the network.

As a companion for the European Certificate in Medical Genetics and Genomics (ECMGG) examination organised by the European Union of Medical Specialists (UEMS), the main content will be supplemented by more than 200 medical vignettes of diseases with medical illustrations, labelled diagrams and pathways, all linked to their relevant chapters. Moreover, the Moodle platform will include links to external reference materials such as articles, guidelines and videos for further reading.

Grants: The project is co-funded by the CEF, Connecting Europe Facility of the European Union, under the action number 2020-FR-IA-0128.

Conflict of Interest: None declared

P23.006.B 'Steps to Delivering a genetic test result': Using patient perspectives to improve future care

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As genetic testing in mainstream healthcare setting becomes more common practice genetic counsellors and clinical geneticists have adopted a support and training role in this transition of genetic testing practices. Health Education England Genomics Education Programme (HEE GEP) competency framework for communicating germline genomic results was developed to support training of HCP in these next steps of genomic communication. Using this competence framework 'Return of Germline genetic test results' one hour education training package was developed to support mainstream healthcare professionals (HCP) who have been/will be involved in communicating germline genetic/genomic test results.

Although case examples were used in this programme from a genetic counsellor perspective it was evident that the patient experience in receiving these results was lacking. With input and experiences from North Thames GMSA Patient, Public and Carer Panel, Education and Training Sub-group a 'Steps to Delivering a genetic test result' guide was developed for healthcare professionals. Receiving a genetic test result is potentially a life changing moment for the patient and the family. The intention of this document is to provide a stepwise guide for HCP delivering germline genetic test results from a patient perspective. Therefore, healthcare professionals should endeavour to deliver the results like it is the first time one has ever given one 'First time, each time' approach.

Conflict of Interest: Pooja Dasani Principal genetic counsellor, Great Ormond street hospital, Genetic Counsellor Registration Board (GCRB) board member

P23.007.C Working towards the ERN ITHACA international consensus statement on the Diagnosis and Management in Rubinstein-Taybi Syndrome

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Background/Objectives: ERN ITHACA is the European Reference Network for Intellectual disability, TeleHealth, Autism and Congenital Anomalies. As a clinical research network, ERN ITHACA connects patient representatives and medical experts to develop best practices on diagnosis and management of rare developmental anomalies. During the last two years, ERN ITHACA has supported the drafting of the consensus statement on the Rubinstein-Taybi Syndrome (RTS).

Methods: From 20 January 2021 to 07 June 2022 the RTS consortium had four digital meetings to discuss the contents and the progress of the statement. Through 29 to 30 September 2022, the consortium met face-to-face in order to discuss the recommendations and to strengthen the collaboration around the syndrome. An anonymous digital voting process on the strength of the recommendations followed. 46 experts voted in total and recommendations mainly obtained the grade A (general agreement allow full agreement with the recommendation). An open-access publication of the consensus statement is foreseen.

Results: A series of recommendations on clinical diagnostic criteria for RTS, molecular investigations, long-term management of various particular physical and behavioural issues, and care planning were outlined by the group of international experts and patient representatives.

Conclusion: The consensus statement is expected to contribute to improving the quality of care for RTS patients. ERN ITHACA provides methodological and logistic support to experts interested in writing a consensus statement on rare developmental syndromes. These consensus statements are equally requested from the patient representatives' community.

Grant References: EU4Health Programme, Grant Agreement nr. 101085231

Conflict of Interest: None declared

P23.008.D Educating the healthcare workforce in genomic variant interpretation: Two Massive Open Online Courses (MOOCs) for the rare disease and cancer NHS workforce

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Background: The rigorous process of genomic variant interpretation requires highly trained healthcare professionals (HCPs). To meet this training need, we designed two Massive Open Online Courses (MOOCs) for HCPs involved in germline genomic rare disease and inherited cancer testing.

Methods: The MOOCs were created by a multidisciplinary working group of subject matter experts. An evaluation cohort of genomics HCPs (consultant geneticists, genetic counsellors, clinical scientists and trainees) and non-genomics clinicians (oncologists, surgeons, haematologists, paediatricians and cardiologists) completed surveys and quizzes to assess learner satisfaction, confidence and knowledge gained in variant interpretation.

Results: Between baseline and follow-up, total confidence scores for each MOOC improved by 15.2 points (95% confidence interval [CI] 12.4–18.0) and 18.9 points (95%CI 15.5–22.5) ($p < 0.0001$ for both). In six formative variant classification exercises 80% of respondents classified the variants such that correct clinical management would be undertaken. More participants from the non-genomics workforce rated the material as "Too Complex" when compared to the specialist genomics workforce.

Conclusions: After completing one or both MOOCs, self-reported confidence in genomic variant interpretation significantly increased ($p < 0.0001$), and most respondents could correctly classify variants such that appropriate clinical management would be instigated. Genomics HCPs reported higher satisfaction with the level of content than the non-genomics clinicians. The MOOCs provided foundational knowledge, and improved learner confidence, but future work is needed to adapt content for clinicians working in specialties outside of genetics.

Grant Reference: CRUK Catalyst Award CanGene-CanVar (C61296/A27223)

Conflict of Interest: Beth Coad: None declared, Katherine Joekes: None declared, Alicja Rudnicka: None declared, Amy Frost: None declared, Kate Tatton-Brown: None declared, Katie Snape CRUK Catalyst Award CanGene-CanVar (C61296/A27223)

P23.009.A ClinGen HHT Variant Curation Expert Panel's modified variant interpretation and classification guidelines

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Background/Objectives: Hereditary Hemorrhagic Telangiectasia (HHT) is a genetic vascular disease. It is diagnosed according to Curaçao Criteria. Genes associated to HHT are *ENG*, *ACVRL1*, *SMAD4*, *GDF2*. In 2015, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) published a consensus scoring process for systematically analyzing pathogenicity evidence and classifying variants. Many clinical laboratories have implemented these guidelines, without additional gene/disease rule specifications. There is reviewer subjectivity leading to discrepancies in variants interpretation and classification. ClinGen is an NIH-funded program that works to standardize and implement clinical relevance criteria for human disease genes and variants. ClinGen has established expert panels to adapt the ACMG-AMP guidelines for particular genes/diseases. In 2019 a ClinGen HHT Variant Curation Expert Panel (VCEP) was approved and began actively working.

Methods: Adapt ACMG-AMP guidelines for standardized HHT variant interpretation. Resolve ClinVar classification discrepancies for variants in *ENG*, *ACVRL1*, and *SMAD4*. Provide 3-star level expert panel classification for HHT variants in ClinVar. Curate ARUP hosted database and submit variants to ClinVar for one centralized HHT variant database.

Results: The HHT VCEP has proposed modified variant interpretation and classification guidelines that include rules with HHT-specific modifications, rules determined not applicable to HHT, and rules that required no modification from the original 2015 guidelines.

Conclusion: This work will aid in the standardization of variant interpretation and data sharing of HHT variants, which will provide a centralized curated resource where clinicians and researchers can go to find the significance of variants associated with HHT.

Conflict of Interest: None declared

P23.010.B Impact of a novel mainstreaming genetic counsellor role in endoscopy services: Optimising surveillance pathways

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Introduction: To understand the impact of a novel genetic counsellor (GC) role in endoscopy service to optimise surveillance pathways and provide formal family history (FHx) assessment for patients reporting a FHx of colorectal cancer (CRC).

Methods: Patients under colonoscopy surveillance solely due to self-reported FHx of CRC between 2018-2022 at the local Endoscopy Unit were identified. Records were reviewed retrospectively. Patients were invited to complete an online FHx questionnaire (https://fhqs.org/), formally analysed by a GC. Relevant genetic investigations were undertaken based on current

practice guidelines, and surveillance was adjusted. Change in surveillance was audited.

Results: Since implementing the GC role in December 2021-2022, **208** patients were identified as undergoing colonoscopy surveillance due to FHx. Of these, **49%** (102/208) were recognised as undergoing inappropriate surveillance after reassessment of FHx. Surveillance was downgraded for **45.6%** (95/208) and increased for **3.3%** (7/208) found to have cancer predisposition syndromes or at high-moderate risk of developing CRC. Additionally, **32.69%** (68/208) patients were discharged as ineligible or at population risk. Multiple cases where referral to clinical genetics was required but not expedited were also identified.

Overall, **95** patients had their surveillance frequency reduced, preventing **336** unnecessary colonoscopies at a cost saving of ~ **£278,880**.

Conclusions: Identifying patients eligible for colonoscopy screening is inconsistent and relies on self-presentation. Lack of training and input from clinical genetics/FHx nurses results in patients receiving inappropriate surveillance. This novel role highlights the need for FHx training for endoscopists, multidisciplinary working and integrating genetic counsellors in endoscopy units to optimise FHx-based patient surveillance.

Conflict of Interest: None declared

P23.011.C The use of narrative therapy and community work methodologies for group supervision in genetic counselling

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Genetic counsellors are provided with regular space for individual or, preferably, group supervision, which helps shape their complex and demanding practice.

Genetic counselling is about communication, psychoeducation, and up-to-date knowledge of medical genetics and psychosocial understandings. It is rooted in the medical model and is performed in social interactions with patients and families. Since its beginnings, genetic counselling has been shaped by the changes in the technical factors of genetics, as well as the socio-historical and cultural factors of each location of practice. More than two decades on, there remains a paucity of research investigating the genetic counselling process. Supervision provides a window into this process. Could new approaches and research methodologies help clarify this question?

As a counselling supervisor with a background in clinical psychology, systemic supervision and narrative therapy, I am currently researching the use of narrative therapy and community work methodologies in group supervision. This approach assumes that genetic counsellors bring diverse beliefs and values to their work, influenced by social discourses such as gender, class, and race.

In this presentation, I will explain the specific structure of the group supervision sessions I facilitate at two UK hospitals to 28 counsellors.I will provide examples and demonstrate recordings that will capture the experiences of counsellors who receive this type of group supervision.

I will argue that, in a highly scientific and medical context, it is possible and necessary to explore multiple perspectives that help to create meaning and innovate the practice of genetic counsellors.

Conflict of Interest: Mariangels Ferrer Duch Part-time, Currently, part-time PhD student with the university of Vic in Spain.

P23.012.D The concept of Cinemeducation in Human Genetics teaching for undergraduate Biomedical Science students

Katja Eckl[1]

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Background: Edge Hill University has introduced a new Biomedical Science programme in academic year 2021-2022. Following the recommendations of the Institute for Biomedical Science (IBMS), we developed an elective Human Genetics module for year 2 students permitting students to further their knowledge in this clinical laboratory specialty. This module employs the concept of cinemeducation assisting students to critically reflect on ethical dilemmas and personal emotional which can arise from human genetics concepts learning. Cinemeducation is the use of film in (bio-)medical education to highlight ethical debate and enable students understand and embrace personal conflicts which can arise from their subject.

Methods: Students are taught weekly in human genetics concepts through lectures and interactive seminars. Cinemeducation is enabled by regular film screenings. All screenings are critically evaluated and discussed. All teaching for this module is regularly surveyed (JISC online surveys, Blackboard anonymous surveys and polls) analyzing students' perception of new learning approaches.

Results: This is an on-going study. Preliminary results from the student cohort 2021-2022 show an overall positive response to cinemeducation. Students having attended all cinemeducation film screenings and debates, improved their patient-directed writing style (evident in coursework: Case Report). Survey data indicate students benefit significantly from the opportunity to correlate individual patient experiences with fact-based learning, which enables students to develop empathy and understanding towards a given patient group.

Conclusion: The concept of cinemeducation benefits not only medical but also biomedical students in developing empathy and patient-directed language, preparing students for their career in the NHS or related roles.

Conflict of Interest: Katja Eckl I am a full-time employee at EHU.

P23.013.A Pre-amniocentesis group counselling session- an effective platform for providing women with essential pretest information

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Objective: Prenatal genetic testing options increased dramatically with the introduction of new technologies. Couples opting for invasive testing are required to make choices based on information that is complex and not easy to comprehend, especially for individuals with no prior knowledge. We aimed to assess whether a pre amniocentesis group counselling session is an effective way for providing couples with adequate information required for decision making.

Methods: Women opting for amniocentesis for various indications received information regarding the procedure and regarding various genetic testing options (chromosomal microarray, karyotype, rapid chromosomal analysis whole exome sequencing) WES)). The information was presented as a lecture delivered by a genetic counselor. Women were asked to fill a pre and post

lecture questionnaire aimed to assess comprehension and to evaluate pretest decisions and how the counselling session was perceived.

Results: The cohort included 55 Women; 36 with prior genetic consultations (group A) and 19 who received the information for the first time (group B). Both groups improved their test scores with a significantly higher improvement for group B. Both groups scored high for the question aimed to assess comprehension of WES, while regarding the understanding of low penetrance copy number variations the score was poor for both groups. 64% opted not to be informed on variables of unknown significance. 91% reported that the amount of information provided was adequate.

Conclusion: Pre amniocentesis group consultation is an effective platform for providing couples with essential pretest information even for those receiving the information for the first time.

Conflict of Interest: None declared

P23.014.B Gaps in the phenotype descriptions of ultra-rare genetic conditions: Systematic review and recommendations

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Background/Objectives: Genome-wide sequencing and genetic matchmaker services are propelling a new era of genotype-first ascertainment of novel ultra-rare diseases. The degree to which reported phenotype data address informational priorities for families and clinicians is unclear. We hypothesize that current phenotype descriptions limitedly address questions of adaptive functioning, feeding and growth, medication use, and proxies for quality of life.

Methods: We systematically reviewed the literature to identify reports of novel genetic conditions published from 2017-2021 where ascertainment was genotype-first. Reports were assessed by two independent raters regarding the adequacy of phenotype data provided in these domains: (I) Development, cognition, adaptive functioning, behavior, and mental health; (II) Feeding, growth, and nutrition; (III) Medication use and treatment history; and (IV) Pain, sleep, and quality of life.

Results: In total, 200 of 3243 screened publications met inclusion criteria. Preliminary analysis of the reported phenotype data revealed superficial descriptions and numerous gaps in the four phenotype domains in the majority of publications. Common issues included lack of detail regarding the severity of developmental delays / intellectual and developmental disabilities, use of general descriptions like "feeding difficulties" and "behavior problems", and little to no information about past treatment trials (e.g., anti-epileptic and psychotropic medications).

Conclusion: Phenotype information relevant to clinical management, genetic counseling, and the stated priorities of patients and families, is lacking in many descriptions of new ultra-rare genetic diseases. We propose a checklist to guide phenotype data collection and reporting.

Grant References: SickKids Research Institute, University of Toronto McLaughlin Centre

Conflict of Interest: None declared

P23.015.C Informing relatives about hereditary cancer: what is the effect of customized support from a family consultant on the percentage of at-risk relatives referred for DNA testing?

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Background: People with a hereditary predisposition for cancer may experience major health benefits from preventive measures. To maximize the dissemination of correct information on hereditary predisposition within affected families, in September 2019 we introduced a family consultant providing customized support to cancer patients with hereditary predisposition to inform their at-risk relatives. In this study we compared the effect of this new method to the former situation without extra support. The question is whether the new method leads to more referrals for DNA testing of at-risk relatives.

Methods: We performed a retrospective study at the genetics department of the Radboud University Medical Center, Nijmegen, The Netherlands, amongst cancer patients with a newly diagnosed pathogenic DNA variant in the BRCA1, BRCA2, MLH1, MSH2, MSH6 or PMS2 gene. Patients with a DNA test result between 1/9/2017 and 30/8/2019 were included in the control cohort, between 1/9/2019 and 30/8/2021 in the intervention cohort (with customized support). Follow-up for both cohorts ended October 2022. The percentage of referred first degree relatives that may benefit from DNA testing was compared between both cohorts.

Results: There was no statistically significant difference between control ($N = 63$) and intervention cohort ($N = 76$) in percentage of first degree relatives referred per patient (81% vs. 75%, $P = 0.16$).

Conclusion: Customized support by the family consultant did not increase the percentage of at-risk relatives being referred. We recommend in-depth interviews with non-referred relatives to establish their motives to help us optimize our support of new index patients and their at-risk relatives.

Grant References: Not applicable.

Conflict of Interest: None declared

P23.016.D Genomic Results Booklets to support families after sequencing

Patricia Birch[1;2], *SHELIN ADAM*[1;2], *Patricia Gombas*[1], *Michelle Demos*[2;3], *Mary Connolly*[2;3], *Cyrus Boelman*[2;3], *Kyrstin Lavelle*[1], *Jan Friedman*[1;2], *Alison Elliott*[1;2;4]

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Background/Objectives: Families undergoing genomic sequencing in our hospital sometimes described feeling "abandoned and lost" after receipt of results. Therefore, we collaborated with sequencing-experienced parents to develop customizable Genomic Results Booklets (GRBs), describing each family's specific results, relevant resources, and supports. GRBs are printable eForms with dropdown menus and auto-population of some fields for customization efficiency. Versions for both informative and non-informative (negative) results are available in five languages. 100 families received GRBs in a genomic research study. Feedback was overwhelmingly positive: GRBs empowered families to understand and navigate their genomic results. https://doi.org/10.1016/j.pecinn.2022.100039

We are testing the effectiveness and impact of GRBs, using an adapted clinical version, in a busy pediatric neurology clinic with no access to genetic counsellors.

Methods: Neurologists notify our team of each new sequencing result, and we prepare the GRB, which is emailed to the family. We are using questionnaires and interviews to evaluate GRB's impact on patients.

Results: Sample booklet: https://www.bcchr.ca/sites/default/files/group-gencounsel/sample-booklet.pdf To date, 27 GRBs have been distributed in English, Punjabi or Arabic. Most families found GRBs useful, and some referred back to them later, showed them to family members and care providers, and made suggestions for additional content, such as how to access psychological counselling. This was true whether results were informative or not: "*I was confused after my doctor conveyed the results as negative… The booklet explained to me the reasoning…*"

Conclusions: GRBs can help provide equitable access to information, and support families in clinics without genetic counsellors.

Grant References: GenomeCanada LSARP to AE

Conflict of Interest: Patricia Birch University of British Columbia, Department of Medical Genetics, GenomeCanada collaborator, SHELIN ADAM University of British Columbia, Department of Medical Genetics, Research Grant collaborator, Patricia Gombas: None declared, Michelle Demos University of British Columbia, Department of Neurology, Mary Connolly University of British Columbia, Department of Neurology, Cyrus Boelman University of British Columbia, Department of Neurology, Kyrstin Lavelle University of British Columbia, Department of Medical Genetics, Jan Friedman University of British Columbia, Department of Medical Genetics, Alison Elliott University of British Columbia, Department of Medical Genetics, Research Grant, principal investigator

P23.017.A Evaluation of the South West Peninsula BRCA support groups

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Background/Objectives: Guidelines recommend that woman with a positive BRCA diagnosis should have access to ongoing support beyond that of genetics counselling, due to the associated lifelong psychosocial impact of the diagnosis. To address this, the Peninsula Clinical Genetics Service offers a quarterly BRCA support group.This service evaluation aims to understand the experiences of woman who attend the groups, evaluate the format of the groups and to receive feedback on possible additions to the service.

Methods: Five attendees of the South West Peninsula BRCA support groups participated in this evaluation and semi-structured interviews were conducted to gain insight into their experience and obtain feedback of the BRCA support groups. Thematic analysis was undertaken on the transcripts of the interviews and recurrent and valuable themes were deduced.

Results: Three major themes were identified within the data: 1) Peer support received in the BRCA support groups; 2) Other sources of support available to participants; 3) Session structure and format of the BRCA support groups, including feedback on possible additional services.

Conclusion: The data highlights the importance of peer support within the BRCA support group to address the psychosocial and practical ongoing needs associated with a positive BRCA diagnosis and aid decision making, as well as the importance of professional facilitation of the group. It has also addressed ways the service could be improved to further support these individuals. This includes: Refining the groups structure and format of the BRCA support groups and integrating additional services such as family education days.

Conflict of Interest: Lisa Massimo Genetic Counsellor, Royal Devon and Exeter Hospital, Dissertation project, as part of MSc for genomic counselling masters with the university of Manchester

P23.018.B Clinical genetics and genetic counselling external quality assessment provision

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Background/Objectives: Clinical Genetics educational case scenarios were introduced by GenQA to complete the exclusive provision of External Quality Assessment (EQA) across the entire clinical genomics service from patient counselling, sample preparation, testing processes, result interpretation and reporting. Clinicians across the world can now review their clinical practice, learn from peers and demonstrate continuing professional development. EQAs covering dysmorphology, cardiovascular disorders, monogenic disorders and oncogenetics have been offered since 2014. Pilot EQAs for genetic counselling and inborn errors of metabolism were recently introduced.

Methods: Annual online multi-stage case scenarios which follow the patient pathway and reflect real clinical cases are provided. The focus of the clinical genetics EQAs is the correct selection of genetic testing based on the clinical presentation and family history and then the correct interpretation of test results to provide a clinical diagnosis. The genetic counselling EQA has more emphasis on provision of relevant genetic counselling. An expert panel of international clinicians mark the submissions based on professional guidelines and a tailored score report with feedback comments is provided to each participating centre.

Results: Clinicians can use the EQA Summary reports, which provide the expected answers and learning points for the case scenarios together with an overview of performance, to benchmark their centres against participants from other countries. Centres with sub-optimal performance are offered support.

Conclusion: Optimal patient care and promotion of best practice is facilitated by these EQAs, which provide a valuable educational opportunity for Clinical Geneticists and Genetic Counsellors.

Conflict of Interest: Melody Tabiner GenQA, Ros Hastings GenQA, Katrina Rack GenQA, Zandra Deans GenQA

P23.020.D Building resilient Patient-Clinician partnerships in the European Reference Networks: Results from the Team building pilot project of ERN ITHACA

Dorica Dan[1;2], ***Anne Hugon***[1;2;3], *Rita Francisco*[4], *Lenja Wiehe*[1], *Ioel Detton*[2], *Ammi Andersson*[2], *Gabor Pogany*[2], *Sue Routledge*[2], *Ernithaca consortium*[2]

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Background: The European Reference Networks (ERNs) are multi-stakeholder healthcare networks. A key building block to ensure ERNs' responsiveness to patient needs is the development and consolidation of a strong patient-clinician partnership culture. Teamwork is associated with several challenges, many being

transversal to all multidisciplinary teams. In addition to time and continuous effort, it is fundamental to create opportunities for patients and clinicians to learn how to collaborate more effectively, acquiring skills for setting common goals, co-implementing and evaluating ERN activities. EURORDIS organized and co-financed together with ERN ITHACA, pilot team building sessions for clinicians and patient representatives.

Methodology: The team-building pilot consisted of 3 consecutive online sessions lasting a total of 6h led by a professional facilitator. The agenda and objectives of the sessions were co-develop by the coordination team, patient representatives and the trainer. The sessions were interactive learning experiences including practical exercises, tips and technique-sharing.

Results: A total of 18 participants attended (9 patient, 7 clinicians and 2 ERN project managers) of which 11 replied toa satisfaction survey (58% response rate). Generally, participants indicated a high satisfaction with the team building overall and felt that it met its objectives. A clear area for improvement is the participation of clinicians and defining a concrete outcome jointly such as a collaboration roadmap.

Conclusion: ERN ITHACA piloted an innovative and successful patient-clinician team building activity. Although there is room for further improvement, participants reported high satisfaction with the sessions which open new and effective avenues towards improving patient-clinician collaboration.

Conflict of Interest: None declared

P23.021.A Translation and Adaptation of the Genomics Outcome Scale into the Czech language

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Background/Objectives: The Genomic Outcome Scale is a validated six-item Patient Reported Outcome Measure (PROM) which enables to quantify the outcome of genetic counselling. We set out to translate and adapt the original English version into the Czech language.

Methods: The methodology reflected the recommended principles of good practice for the translation and cultural adaptation process for Patient Reported Outcomes Measures. Two forward translations were prepared by the authors. These were reconciled by consulting with five English content-naïve speakers. The reconciled version was discussed in turn with a content-aware certified translator and four clinical geneticists. An amended version was created, which contained several versions of the translated statements and was used in telephone interviews with patients of the genetics department. After six interviews further amendments were made. In the following four interviews better understanding was noted.

Results: We created a pilot Czech version of the Genomic Outcome Scale which will be used for a further refinement and will allow us to conclude the process of creating a validated tool for quantifying an outcome of a genetic consultation in Czech.

Conclusion: Having a tool to quantify an outcome of a genetic consultation can serve a multitude of purposes from improvement of the clinical genetics service to teaching to informing public health care policies.

Grant References: Supported by MH CZ – DRO (FNOl, 0098892)

Conflict of Interest: None declared

P23.022.B Designing the Newborn Genomes Programme in the UK

Dalia Kasperaviciute[1], *David Bick*[1], *Dasha Deen*[1], *Katrina Stone*[1], *Mathilde Leblond*[1], *Amanda Pichini*[1], *Augusto Rendon*[1], *Sue Hill*[2], *Alice Tuff-Lacey*[1], *Richard Scott*[1]

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Background/Objectives: Whole genome sequencing has the potential to screen for hundreds of rare genetic conditions in a single test providing opportunities for earlier treatment. Our research programme explores (i) the benefits, challenges, and feasibility of sequencing the genomes of 100,000 babies within the NHS, alongside current newborn screening; (ii) use of babies' genomes for discovery research and (iii) storing this data over their lifetimes. We describe the programme development, focusing on our research to inform our analytical approaches.

Methods: With parents, and in partnership with NHS experts, policymakers, and other stakeholders, we co-created a recruitment and consent process, and are defining which conditions will be screened for. We are establishing the optimal sampling process through studies involving, first, 40 adults; and, second, 600 babies. We modelled the conditions detection algorithms in a cohort of ~35,000 individuals to maximise positive predictive value.

Results: We established four principles for conditions' selection: (A) strong evidence that the genetic variants cause the condition; (B) the condition significantly impacts quality of life; (C) intervention in early childhood could improve outcomes and (D) those interventions are equitably accessible.

We showed that high specificity is achieved by prioritising variants based on a curated list of known pathogenic/likely pathogenic variants and loss of function variant predictions. We expect that ~1% of results will require manual review of pathogenicity.

Conclusions: We identified the most feasible methods for running a large-scale study of genome-led newborn screening within the NHS. We continue to define results confirmation, feedback, and patient care pathways.

Conflict of Interest: Dalia Kasperaviciute Genomics England, David Bick Genomics England, Dasha Deen Genomics England, Katrina Stone Genomics England, Mathilde Leblond Genomics England, Amanda Pichini Genomics England, Augusto Rendon Genomics England, Sue Hill: None declared, Alice Tuff-Lacey Genomics England, Richard Scott Genomics England

P23.023.C Reviewing our prenatal referral pathway: Improving equity of care

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Northeast Thames Regional Genetics service serves a population of 5.5 million. Our prenatal patients are managed by a small team of genetic counsellors (GCs) with a mixed caseload. GCs were allocated specific hospitals within our geography to manage prenatals on an ad-hoc basis in addition to their usual clinical workload. In 2020/21 GCs reported an increase in prenatal workload, exacerbated by staff shortages and changes to the test directory such as Non-invasive prenatal diagnosis and Whole exome/genome sequencing. We therefore aimed to reduce ad-hoc prenatal workload whilst still managing prenatal cases in a timely manner based on patient needs.

In order to meet the increased clinical need with a small workforce, we implemented a new way of triaging prenatal referrals. Whilst continuing the allocation of specific hospitals for each GC, a weekly clinic was created and covered by a GC on a rota basis. A standard operating procedure (SOP) was developed to differentiate between cases that were suited for an ad-hoc discussion or for a clinic appointment.

This clinic has reduced ad-hoc prenatal work. Consequently, each GC can improve continuity of care for prenatal patients already known to them, whilst maintaining relationships within their referring hospitals. Use of clinic slots has also alleviated discrepancy in prenatal workload across GCs based on different numbers of referrals from each hospital. The referral SOP has ensured all cases are reviewed against departmental criteria, reducing inequity of care. We wish to share our learnings with the clinical genetics' community as a good practice example.

Conflict of Interest: None declared

P23.024.D Evolution of Ehlers-Danlos Syndrome consultations in the university hospital of Liege

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[1]*CHU of Liege, Human Genetics, Liege, Belgium*

Background/Objectives: In 2018, we evaluated the number of appointment requests for suspected Ehlers-Danlos Syndrome (EDS) and related collagenopathies, as well as the indication to receive these patients in the genetics department of the University Hospital of Liege. We found that in 97.8% of the cases no genetic consultation was necessary. Therefore, we tried to better screen the patients we received by sending them an explanatory letter and a clinical form to fill in to apply for an appointment. Three years after the implementation of this system, we wanted to evaluate it.

Methods: We performed a retrospective observational study of the patients from the University Hospital of Liège who had come to a genetic consultation for EDS suspicion and related collagenopathies since the implementation of our pre-screening system and compared the data to those we had collected before.

Results: We received 634 appointment requests: 149 were suggestive so a consultation was directly scheduled. We sent our questionnaire to the remaining 485 requests: only 99 patients completed it. A total of 248 patients (39.11%) were received: the percentage of patients without signs of collagenopathy was lower as was the number of hypermobile EDS, and the percentage of molecular diagnosis was higher, compared to 2018.

Conclusion: After having been informed, 79.6% of patients did not continue their request. The proportion of patients without signs of collagenopathy has decreased but the rate of hypermobile EDS is still very high. Introducing guidelines for referral to genetic consultation among Physicians of EDS patients remains a challenge.

Conflict of Interest: None declared

P23.025.A Patient experience of the preimplantation genetic testing service at Guy's and St Thomas' NHS Foundation Trust

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Background: The Guy's and St Thomas' (GSTT) Pre-implantation Genetic Testing (PGT) service provides the majority of NHS funded PGT and 37% of all PGT cycles across the UK. This patient pathway involves the co-ordinated care of health care professionals across clinical genetics and assisted conception departments, from one commissioned primary centre (GSTT) and three satellite centres; Exeter, Leeds and Sheffield. PGT is an emotionally demanding process and an often difficult journey for couples. Patient evaluation is the current promoted model of feedback to ensure improvement to patient care and positive experiences. However, there are few existing reports of the PGT patient experience in the UK and none regarding service delivery in the current NHS PGT model.

Methods: A PGT patient experience survey was designed and then refined following patient focus group. Surveys were sent to all PGT patients with a scheduled embryo biopsy appointment in 2021 at GSTT. 65 participants responded. Experiential qualitative data was interpreted using thematic analysis and combined with quantitative survey data to provide a comprehensive view of patient experience.

Results: We identified core themes related to the patient's preparation for PGT, communication needs and the impact of PGT on emotional wellbeing. Patients highlighted both positive experiences and raised potential areas for improvement.

Discussion: Potential areas of service improvement related to aspects of continuity of care; information, management and relationships. Improved emotional care and access to information was called for. These results may be helpful to other PGT centres seeking to improve patient experience.

Conflict of Interest: None declared

P23.026.B Where does risk for clinical genetics occur? Developing a patient pathway and identifying risks using a process map for comparisons across Europe

Deborah Lambert[1], *Dearbhla Butterly*[1], *Alana J Ward*[1], *Ioana Streata*[2;3], *Florin Burada*[2;3], *Anca Riza Costache*[2;3], *Christine Poole*[4], *Sally Ann Lynch*[1;4]

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Background: Objectively pinpointing risks along the Clinical Genetics patient care pathway can identify areas where controls could improve safety and quality.

Methods: Creation of a 22-step Clinical Genetics process map spanning 5 stages: patient and family history assessment, clinical management of genetic testing, sample processing and analysis, result transmission and result discussion. A 6-week anonymised audit of events/near-misses associated with clinical risk using a process map tool was completed by the clinical genetics teams in Children's Health Ireland and Regional Centre of Medical Genetics Dolj.

Results: Clinical risks were identified in 55/551 (10.0%) appointments (Ireland) and 15/74 (20.2%)(Romania). Risk frequency was >1 per month in all 5 process map stages in Ireland and in all but 'report transmission' in Romania. Cases with ≥2 care steps broken: 41.8% (n = 23, Ireland) and 20.2% (n = 3, Romania). Patient journey risk hotspots differed by centre- Ireland: substandard test interpretation; inappropriate test selection; report not received by referring clinician; inappropriately worded result generated. Romania: incorrect test ordered and incorrectly recorded phenotype and diagnosis. Root causes of risk were IT system deficiencies (35%) and excessive waiting times for Clinical

Genetics (20%) (Ireland), and inappropriate test selection and sub-optimal consent (Romania).

Conclusions: Accessible, inter-connected IT infrastructure, staff resourcing and genomic education for tertiary referrers to Clinical Genetics are possible means to reduce risk. Additional ERN ITHACA and UK centres will complete the audit in 2023 to broaden the perspective and capture successful patient-safety control measures.

Grant: Adelaide Health Foundation R22808.

Conflict of Interest: None declared

P23.027.C A patient's perspective: understanding experiences of a nurse led cancer risk assessment service model

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Background/Objectives: Breast cancer risk assessments using a family history have well-established clinical utility. However, the service model by which this risk assessment is provided, varies across England. NICE guidelines recommend that this risk assessment service should be provided at a secondary care level. The Cancer Risk Assessment Service (CRAS) model was designed to fill this gap by providing a family history-based assessment to patients in South East London by skilled nurses. Few studies have explored different models of breast cancer risk assessment provision from a patient perspective.

Methods: This service evaluation used structured patient questionnaires to gain an understanding of patient experience from those who had accessed the CRAS between 2017 and 2019 inclusive; 182 individuals completed the survey. Open questions and free text were used for thematic analysis.

Results: We identify key factors affecting patient experience, that are distinct to this model of service provision. Access to formal risk assessment services for cancer in secondary care is effective and resource efficient.

Conclusions: The service evaluation elicits patient's expectations and needs from a breast cancer risk assessment service for the future. CRAS is an exemplar of embedding genomics for point of care risk assessment and delivers safe and equitable care outside clinical genetics.

Conflict of Interest: Ailidh Watson Great Ormond Street hospital NHS Foundation Trust, vishakha tripathi: None declared, Michelle Weston: None declared

P23.028.D Genetic counselling in paediatrics: does the acute paediatric setting influence the decision of opting-in or out of secondary finding return?

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Background/Objectives: Genomic medicine in the acute paediatric setting has great potential, as rapid results from whole genome sequencing (WGS) can significantly influence the treatment and care of critically ill children. The emerging role of genetic counsellors in the acute setting presents novel genetic counselling issues, such as the importance of a rapid response, the emotional distress families experience and the unfamiliar environment where genetic counselling is provided. With this analysis, we aim to assess whether there might be any observed differences in the number of families that opt in to receiving secondary findings, between the acute and non-acute settings.

Methods: We compared the number of individuals that chose to receive secondary findings (both associated with carrier status and predisposition to disease) between two groups of patients from a research project that aimed to assess the clinical utility of WGS. One group consisted of 228 individuals recruited in the acute paediatric setting, and a second group consisted of 518 individuals recruited in a non-acute setting (children with autism spectrum disorder).

Results: We observed statistically significant differences between the two groups, with a tendency of families seen in the acute paediatric setting to opt out more often of secondary finding return.

Conclusion: This analysis suggested that the challenges and considerations arising in the acute paediatric setting might influence familial decisions regarding genetic testing. Further research might help us to understand how to support families appropriately.

References: Lynch, F *et al*., 2021

Grants: Proyecto estratégico I + D S3 2020-2022. GEMA IV Gobierno de Navarra

Conflict of Interest: None declared

P23.029.A Implementation and evaluation of personal genetic testing as part of genomics analysis courses in German universities

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Background/Objectives: Due to the increasing application of genome analysis and interpretation in medical disciplines, professionals require adequate education. Here, we present the implementation of personal genotyping as an educational tool in two genomics courses targeting Digital Health students at the Hasso Plattner Institute (HPI) and medical students at the Technical University of Munich (TUM).

Methods: We compared and evaluated the courses and the students' perceptions on the course setup using questionnaires. 13 questionnaires with a total of 174 responses were conducted.

Results: During the course, students changed their attitudes towards genotyping (HPI: 79% [15 of 19], TUM: 47% [25 of 53]). Predominantly, students became more critical of personal genotyping (HPI: 73% [11 of 15], TUM: 72% [18 of 25]) and most students stated that genetic analyses should not be allowed without genetic counseling (HPI: 79% [15 of 19], TUM: 70% [37 of 53]). Students found the personal genotyping component useful (HPI: 89% [17 of 19], TUM: 92% [49 of 53]) and recommended its

inclusion in future courses (HPI: 95% [18 of 19], TUM: 98% [52 of 53]).

Conclusion: Students perceived the personal genotyping component as valuable in the described genomics courses. The implementation described here can serve as an example for future courses in Europe.

Grant References: The research leading to these results has received funding from the Horizon 2020 Programme of the European Commission under Grant Agreement No. 826117.

Conflict of Interest: None declared

P23.031.C Resilience and burnout of Australian Regional Genetic Counsellors

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Background/Objectives: Genetic counsellors, like other medical professionals have been shown to be at high risk of burnout. Regional genetic counsellors in New South Wales, Australia, are generally sole practitioners working at high capacity with limited resources. The Oldenburg Burnout Inventory (OLBI) was used to measure work disengagement and exhaustion, providing a predictor for burnout amongst regional genetic counsellors.

Methods: OLBI was measured in February 2020, before completion of a professional resilience workshop and postworkshop in September 2020, January 2021 and June 2022. 11-8 regional genetic counsellors completed the OLBI across 11 regional genetics services.

Results: Disengagement levels were high and exhaustion score close to high prior to completion of the professional resilience workshop, indicating high levels of burnout amongst regional genetic counsellors. Various interventions (resilience buddies, weekly worksheets, self-care, mental health checks) were put in place postworkshop which reduced both disengagement and exhaustions levels, even during the COVID19 pandemic. Disengagement and exhaustion levels slowly rose in 2021 and 2022. This study is limited in its small cohort and limited information regarding sociodemographic and external factors which may influence exhaustion and disengagement levels amongst genetic counsellors.

Conclusions: Practical interventions did improve levels of exhaustion and disengagement in regional genetic counsellors, reducing the likelihood of burnout. Further studies are needed to explore which interventions were most useful long-term and how these can be implemented in the workplace.

Grant References: Genetic counsellor attendance at the professional resilience workshop was supported by The Health Education and Training Institute (HETI), NSW Health, Australia.

Conflict of Interest: None declared

P23.032.D Genetic syndromes hiding behind art masterpieces: an intriguing relationship between medicine and arts

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Background: Iconodiagnosis is the search for clinical signs suggestive of diseases in artworks depicting the human figure. From a clinical point of view, this approach helps clinical geneticists improve their knowledge and ability to diagnose rare diseases and genetic syndromes.

Methods: We analyzed art catalogs and published papers to identify the presence of possible syndromes in paintings. The 236 selected artworks were added to a custom-made database containing information regarding the picture and the phenotypical features, detailed with HPOs terms. This information was discussed within a multidisciplinary team of geneticists from five Italian hospitals.

Results: The artwork analysis allowed us to identify 272 HPOs and 11 genetic syndromes from different historical periods. Among them, the most interesting are: 1) Fifteen examples of Down Syndrome from the Toltec culture (terracotta statue from 500 A.D.) to the Renaissance (three "Madonna with the Child" by Mantegna); 2) Angelman and Prader-Willi Syndromes in two paintings by Caroto and Carreño de Miranda where the subjects are two young children displaying their most emblematic phenotypical features: stereotypical smile and severe obesity; 3) Marfan Syndrome in an abbot with long hands and slender fingers; 4) Pycnodysostosis affecting the painter Henry de Toulouse-Lautrec; 5) Noonan syndrome in "Among those left" by Ivan Albright. Interestingly, the diagnosis hypothesized in the blacksmith was confirmed from a molecular point of view in his great-grandson (Cole.,1980).

Conclusions: This project allows clinicians, students, and patients to explore medicine and genetics through art, highlighting how painters have always depicted these conditions as nature's spectacle.

Conflict of Interest: None declared

P23.033.A ACCESS RD: Leveraging artificial intelligence to enhance knowledge mobilization and access to targeted interventions in rare neurodevelopmental disorders

***Francois Bolduc**[1], Manpreet Kaur[1], Jeremy Costello[1], Tania Cuppens[2], Mickael Leclercq[2], Ashwani Singla[1], Ritvik Khanna[1], Ajay Kumar[3], Ian Dunham[3], Arnaud Droit[2], Marek Z Reformat[1]*

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Objective: We assessed how artificial intelligence (AI) could be used to navigate but also leverage the complexity seen in rare neurodevelopmental disorders (NDD) and facilitate access to information for families and health practitioners.

Method: We developed a corpus containing online information from public sources or medical literature related to phenotypes seen in individuals with NDD from the Deciphering Developmental Disorder (DDD) and Orphanet datasets. We then used natural language processing (NLP) to extract entities and relations between them and build a hybrid knowledge graph (KG). In parallel we developed a novel annotation algorithm using Topic modeling for free-text vocabulary and named entity recognition for standard vocabularies such as Human Phenotype Ontology(HPO), Unified Medical Language System(UMLS), ERIC thesaurus and AIRs taxonomy for education and services terminology respectively.

Results: We found that variability in ontologies was a significant issue for automated labeling of the corpus. Comparison of source specific KG identified overlapping entities but interestingly differences in relative weight. Importantly the KG allowed us to identify shared entities between distinct RD. The resource

annotation tool allowed for ranking of targeted interventions and the development of a user interface: ACCESS RD.

Conclusion: Using AI will provide a scalable and more accessible path to knowledge mobilization between the various stakeholders involved in rare disorders (RD) but our study also identified several barriers (harmonization, labeling) which will need to be addressed. Moreover, we show how KG could allow clustering of RD and enhance access to interventions.

Grant: UK Research and Innovation and CIHR (Reference ES/T013435/1)

Conflict of Interest: None declared

P23.034.B Carrier status in prenatal molecular testing: should specific guidelines be promoted?

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Rationale: Genetic testing reports are formal documents issued by laboratories to the referring clinician describing the outcome of a molecular genetic investigation on a case. Specific reporting descriptions might be needed to conform to specificities of local laboratory and clinical departments, National rules, regulation and legislation. Stating carrier status on a prenatal diagnosis report conveyed to the expecting parents is against the principle of recognising the right not to know one's genetic status. At the theoretical level, such right can be evaluated in relation to clinician's duty to inform user as well as solidarity with family relatives who, without that information, could be deprived of preventive or therapeutic measures.

Methods: The *European Molecular Genetics Quality Network* (EMQN) is an External Quality Assessment organization that aims to improve genomic testing quality. This study gathers information among EMQN assessors on: i) the existence of national specific guidelines for carrier genetic testing; ii) reporting heterozygous variants/carrier status in prenatal diagnosis context, and iii) the question whether we should improve existing guidelines to cover this diversity among countries.

Results and discussion: By gathering preliminary data, it became evident that the national guidelines/policies of each lab differ significantly. Consequently, we recognize that it might be difficult to develop broadly applicable reporting/wording guidelines but we would initially like to draw attention to this important issue to global health and untimely attempt to propose wording guidelines. Explicitly addressing trade-offs between the individual right not to know and genetic information delivery in an accurate, unambiguous and succinct report.

Conflict of Interest: Paula Jorge Centro Hospitalar Universitário de Santo António, EMQN FraX EQA Assessor, carolina sismani Professor | The Cyprus Institute of Neurology & Genetics, EMQN FraX EQA Assessor, Katrin Hinderhofer Fachhumangenetikerin (GfH) Clinical Laboratory Geneticist (EBMG), EMQN FraX EQA Assessor, Minna Toivonen Sairaalageneetikko

Lääketieteellinen genetiikka, Genomiikka, EMQN FraX EQA Assessor, Dieter Gläser Genetikum, Neu-Ulm, FraX EMQN EQA Assessor, Valerie Biancalana Centre de référence des maladies neuromusculaires, FraX EMQN EQA Assessor, Gabriele Wildhardt: None declared, Sherryl Taylor: None declared, Michael Morris: None declared

P23.035.C Shaping the research agenda in genomics. Perspectives of Nurses Midwives and Allied Health Professionals (NMAHPs) in genomics research

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Background: Nurses, midwives and allied health professionals (NMAHPs) in the UK are not a highly visible discrete group in the genomics research landscape, to date, yet they have important contributions to make to the research agenda. NMAHPs are represented at the National Institute for Health Research (NIHR) National Genomics Specialty Group (NGSG) by the authors who wanted to identify and characterise those they represent. No data was previously available about this group.

Methods: A survey questionnaire comprising multiple choice answers and free text was shared via email across:

Association of Genetic Nurses and Counsellors (AGNC) email group,

NGSG Clinical Research Networks,

NHS England newsletter for nurses interested in genomics.

Respondents were encouraged to share with colleagues via professional networks.

Results: 45 responses were received, from Genetic Counsellors, Nurses, Clinical Scientists and 3 others which showcased a breadth of research skills and experience. Grounded in their practice, NMAHP's concerns they have for patients included: understandings of the impact of genomic testing on their patients' lives; complexity of genomic information for patients and clinicians; how best to support patients to make decisions that are right for them. These formed the central themes and priorities of their research focus. Respondents expressly identified that they would like to develop and lead qualitative research.

Conclusion: The survey provides evidence of skilled and motivated UK NMAHPs who, with additional support, could make a significant contribution to research. This research community can provide a valuable resource for understanding how the mainstreaming of genetics is impacting patient care.

Conflict of Interest: None declared

P23.036.D Genetic counsellors in the Republic of Ireland – workforce and professional quality of life surveys

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Background/Objectives: Approximately 18 genetic counsellors (GCs) work in the Republic of Ireland. Professional and individual vulnerability is a risk to Irish GCs without official recognition as a

health care profession, career framework or established professional association. We aimed to evaluate current landscape by survey.

Methods: Workforce and professional quality of life surveys (https://proqol.org/proqol-health-1) were developed. Surveys were promoted via the national Human Genetics Society, department posters, and direct mail to lone practitioners. ProQOL scores were calculated by published methodology. Univariate statistics were calculated in Excel.

Results: Response rates were 83.3%(15/18) for both surveys. Responder characteristics: 86.6% female, 63.3% ≤40 years old. 93.3% have an MSc in Genetic Counselling. 86.6% are GCRB and/or EBMG registered; the remainder (13.3%) working towards GCRB registration. 93.3% are in public-sector employment and 33.3% work in mainstreamed GC roles. 93.3% have access to counselling supervision: 86.6% individual, 40.0% group, 46.7% self-funded, and 13.3% partially employer funded. 73.3% have participated in audit or research in the past 3 years. Employers did not wholly support continuing professional development financially (86.6%) nor in protected time (73.3%). Regarding wellbeing, overall burnout scores were 'moderate', 73.3% felt that their work exhausts them sometimes/often/very often, and 80.0% felt unable to provide care that they believe should be provided sometimes/often/very often.

Conclusion: These initial surveys benchmark the GC profession in Ireland. While this specialized workforce is highly trained, formal Health Service recognition is necessary for safeguarding to prevent professional harm and individual GC burnout.

Grant Reference: Adelaide Health Foundation R22808

Conflict of Interest: None declared

P23.037.A Genetic consultation on hereditary cancers organized through nongovernmental organisation "Everything for her" prepares users for genetic testing procedure in clinical systems

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Background: The translation of genomics into clinical medicine would benefit from patient-oriented programs that provide a strong foundation in core genetics principles and means to access, identify, and use reliable decision-making information. Therefore, to prepare hereditary cancer patients for easier mastering through the complicated process of genetic testing, in the nongovernmental organization (NGO) "Everything for her" we have organized a program for genetic consultations.

Methods: 34 female patients and their family members were included in the study. All of them received a 13-item questionnaire organized to assess their satisfaction with the genetic consultation procedure and the information received.

Results: Out of 34 patients contacted, 21 fulfilled the questionnaire. For 90 % of them, genetic consulting resulted in better preparedness for upcoming clinical interviews. 86 % confirmed a better understanding of the genetic nature of their disease and decreased tension for genetic testing. 95% reported psychological support and felt that the geneticist understood and heard them, while all answered that no genetic term was used without explanation.

Conclusion: Although most users access genetic counseling/testing in clinical settings, consultation with a geneticist/psychotherapist before a hospital appointment reduced psychological pressure, and even 85.7% of them stated that they felt better afterward. We believe that this form of support for the classical medical treatment of hereditary cancer patients enables easier mastering of arduous cancer-related clinical procedures and that its implementation should be continued.

Grants: This research was co-financed by grant agreement No. KK.01.1.1.01.0008.

Conflict of Interest: None declared

P23.038.B The collaboration between the European Society of Human Genetics-Young Committee (ESHG-Y), UNIQUE and ERN-ITHACA: increasing the knowledge on rare genetic disorders for non-native English speakers

Juliana Miranda Cerqueira[1;2;3], ***Ana Raquel Gouveia Freitas da Silva***[3;4], *Silvia Kalantari*[3;5], *Celia Azevedo Soares*[6;7;8], *Francesca Tonini*[9], *Anna Pelling*[10], *Claire Andersen*[10], *Anne Hugon*[11], *Alain Verloes*[11], *Ernithaca consortium*[12], *Unique - Rare Chromosome Disorder Support group*[10], *European Society of Human Genetics - Young Committee*[3]

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Background/Objectives: UNIQUE supports families and caregivers of anyone affected by a rare chromosome disorder associated with developmental delay and intellectual disability. UNIQUE produces freely available patient-oriented guides most of which are only available in English, limiting the access of non-English speaking families and healthcare professionals. The European Society of Human Genetics-Young **(**ESHG-Y) committee and Young Geneticist Network (YGN), in partnership with UNIQUE and European Reference Network (ERN-ITHACA), aimed to recruit clinical human geneticists working in the field of rare genetic disorders to proofread UNIQUE guides into various languages.

Methods: Native speakers volunteers of eight languages were recruited between December 2020 and August 2022. UNIQUE guides for nine rare disorders were selected for auto-translation proofreading: *FOXP2* syndrome, 1q21.1 microduplications, 7q11.23 duplication, 8p inverted duplication and deletion, 15q13.3 microduplications, 16p11.2 duplications, 22q13 deletions Phelan-McDermid syndrome, 22q11.2 deletion and Xq28 duplications.

Results: A total of 32 volunteers were recruited from Europe, North America and South America. The guides were mostly revised into Portuguese (n = 6, 75%), Spanish (n = 5, 62.5%), Italian (n = 5, 62.5%), and Polish (n = 4, 50%). Other guides were revised into French (n = 2, 25%) and Dutch (n = 2, 25%), among others.

Conclusion: The ESHG-Y/YGN experience with gathering volunteers reflects the interest of young geneticists in broadening accurate and simplified information on rare genetic disorders for non-native English-speaking families. A set of translated guides may also be useful for families speaking less common languages and non-European countries in which French, Spanish and Portuguese are the first languages.

Grant reference: EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516

Conflict of Interest: None declared

P23.041.A Direct-to-consumer genetic tests and Canadian genetic counsellors: An exploration of professional roles in response to novel biotechnologies

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Background/Objectives: Genetic counsellors' role is evolving in response to health-related direct-to-consumer genetic tests (DTC tests). While there is consensus in the literature that pre- and post-DTC genetic counselling would benefit consumers, genetic counsellors have reservations about DTC tests, and there is a paucity of research on providing DTC counselling services.

Methods: A pilot quantitative survey investigated genetic counsellors' concerns regarding informed consent and privacy in relation to DTC tests and identified policies that genetic counsellors/counselling associations could implement. This is the first study to examine Canadian genetic counsellors' views on DTC tests and how this disruptive biotechnology affects their traditional roles.

Results: The findings indicate that genetic counsellors are cognizant of the harm to informed consent and consumer privacy associated with DTC tests but are hesitant to engage directly with consumers and wary of misusing clinical time and resources. However, counsellors are open to producing educational materials on DTC tests and collaborating with other stakeholders and the DTC industry to support consumers.

Conclusion: Practical considerations for DTC counselling sessions are discussed, including the unique needs of DTC patients and the challenges posed by DTC tests to the genetic counselling duty to inform. This research benefits genetic counsellors and physicians in other health jurisdictions, including Europe, by examining how to best utilize genetic counsellors' professional skills in the DTC context, to minimize burdens on the healthcare system and support DTC test consumers.

Grant References: GenCOUNSEL: Optimization of Genetic Counselling for Clinical Implementation of Genome-Wide Sequencing, Genome Canada/Canadian Institutes of Health Research

Conflict of Interest: None declared

P23.042.B Evaluation of nurse-coordinated transitional genomics clinics for young people with intellectual disability: views and experiences of parents

Chris Jacobs[1], *Theo Hanson*[2], *Jessica Planner*[3], *Olivia Hoeper*[3], *Alyssa Wilson*[2], *Carey-Anne Evans*[2], *Mary Ruth Sheahan-Endries*[4], *Meredith Wilson*[4], *Clara Chung*[4], *Sulekha Rajagopalan*[4], *Alison McEwen*[3], *Tony Roscioli*[2]

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Background/Objectives: Genomic sequencing has the potential to facilitate the transition from paediatric to adult care for young people with intellectual disability. We aimed to evaluate the views and experiences of parents of young people undergoing genomic sequencing for intellectual disability via nurse-coordinated transitional genomics clinics.

Methods: English-speaking parents of young people attending the clinics were invited to participate. 37 parents completed a pre/post clinic survey measuring hope, empowerment, readiness to transition and overall satisfaction. Following results disclosure, semi-structured interviews were conducted with a sub-set of participants.

Results: The most frequent pre-clinic hope was explanation for the cause of the condition (71%). All empowerment measures increased following clinic attendance (mean score increased from 21.2 to 23.6). Confidence in the ability to prepare for transition to adult care increased from a mean score of 5.4 to 6.5. Overall satisfaction was high. Qualitative themes supported the quantitative findings: (i) receiving a genetic diagnosis provided empowerment and confidence to navigate the child's health condition, (ii) attendance at the clinic enabled access and awareness of information and support, (iii) the clinic experience provided hope and relief for families, including those without a diagnosis or receiving an uninformative result.

Conclusion: Attending transitional genomics clinics was empowering for parents of young people with intellectual disability, even when the outcome of genomic testing was uninformative. Embedding a specialist nurse into transitional genomics clinics helps parents prepare their child for the transition to adult care.

Grant references: Sydney Partnership for Health Education Research and Enterprise, Genome-Connect Clinical Academic Group

Conflict of Interest: None declared

P23.044.D Genetic Counsellors Belgium: the evolution of professional recognition

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Background: Genetic counselling (GC) is a fast-growing profession in Belgium. Yet, GC lacks legal recognition, that if not properly regulated, can expose the public to the risk of harm. This study aims to report the evolution of recognition of genetic counselling as a distinct health profession in Belgium.

Methods: The number of European countries with practicing genetic counsellors was reported, as well as the number officially recognized as a health profession. The education of 25 counsellors was explored. The evolution of the educational program in Belgium from postgraduate to an interuniversity master's degree was described.

Results: In 2023, genetic counsellors were employed in 19 European countries. Only 5 European countries offer a recognized training and only 2 countries provide legal recognition of the profession. In Belgium, the number of genetic counsellors employed in the eight genetic centers increased from 19 in 2019 to 25 in 2023. Since 2017, a GC postgraduate course (not officially recognized) was launched in Ghent, in which a maximum of 10 students could attend. All centers in Belgium attempt to launch an interuniversity master's education program, but the process was discontinued in 2023.

Conclusion: The number of practicing genetic counsellors is increasing, whereas the recognition of the profession and controlled qualitative education is lagging behind. Legal recognition would ensure safer, high-quality provision of genetic

counselling services to the patients by establishing educational and professional standards for GC, limiting the use of the title 'genetic counsellor' to qualified practitioners.

Conflict of Interest: Virginie Szymczak Genetic counsellor, Lena KUKOR Genetic counsellor, Aude Lombard Genetic counsellor, Julie Crèvecoeur Genetic counsellor, Ileen Slegers Genetic counsellor

P23.045.A Genomic medicine education initiatives at the Tbilisi State Medical University (Tbilisi, Georgia), the past ten years of experience

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A multifaceted approach to education is required to improve the genetic and genomic knowledge among medical students and non-genetic healthcare professionals. Here we describe ten years of experience in genomic medicine education at the Department of Molecular and Medical Genetics, Tbilisi State Medical University. Since the founding of the department in 2006, its main priorities have been to ensure the compliance of the educational programs with core competencies in Genetics and Genomics developed by the ACGME and APHMG, as well as the availability of professional literature in the Georgian language.

Several new initiatives have been developed over the past ten years in the department in order to prepare students for future medical careers. These initiatives include case-based study approaches that have been used with; the development of a course in laboratory Genetics and Genomics. This course is fully compatible with project-based learning principles. Since there are three research projects in the department's laboratory being funded by the national scientific foundation, dedicated students now have opportunities to be engaged in real research work and present their findings at scientific conferences. Additional extra-curricular activities include encouraging students to participate in the international project Unique. The department regularly organises a Journal Club, and events dedicated to the Rare Disease Day and DNA Day. Professional education initiatives include development of continuing medical education courses in laboratory genetics.

In conclusion, our efforts will equip future physicians with competences in genetics and genomics so they can apply the new technologies and discoveries in their clinical practice.

Conflict of Interest: None declared

P23.046.B Parental perceptions and expectations of exome sequencing in children with neurodevelopmental disorders

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Background: With the implementation of exome sequencing in routine diagnostics and the increasing number of disease-associated genes more families with children, with a neurodevelopmental phenotype, receive a genetic diagnosis. Due to the significance of de novo variants in this context, trio exome sequencing is usually applied. However, studies about parental perceptions towards exome sequencing, in particular in German-speaking countries, is lacking.

Methods: In retrospect, 21 parents from 125 families were interviewed about their expectations, fears and consequences of trio exome sequencing. The content of the semi-structured interviews was analysed qualitatively and three report groups were compared: positive results (7/16), negative results (7/16), uncertain results (2/16).

Results: Almost all probands hoped for a genetic diagnosis to improve their child's situation. This concerned mainly therapeutic options, prognosis, cause, contacts with other parents, and health care support. Half feared that a result might have negative impacts on the children later in life. But regardless of the outcome, obtaining results from exome analysis was a relief. Receiving a genetic diagnosis often freed parents from guilt and blame. Negative results did not end the search for a (genetic) diagnosis, but parents came to rest. Families with uncertain results were torn, they reported more frustration the more they perceived the result of their child as uncertain.

Conclusion: Genetic testing was mostly perceived abstractly as "just taking blood". The explorative research approach showed that families wish not to be left alone with the decision and genetic results. This highlights the importance of genetic counselling in the process.

Conflict of Interest: None declared

P23.047.C Human Genome Organisation (HUGO International) Education Committee: development of greater worldwide access to genomic educational resources, training and courses

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Background: The Human Genome Organisation (HUGO) Education Committee, established in 2021, aims to facilitate state-of-the-art human genomic education and training, worldwide. The international members of the committee are each active members of other national and international genetic and genomic organisations, including: the European, African, Australasian, Asia Pacific and American Societies of Human Genetics; the European Certificate in Medical Genetics and Genomics (ECMGG) exam; Human, Heredity and Health in Africa (H3Africa); and the Genomic Medicine Foundation UK.

Methods: The HUGO education committee now comprises six active sub-committees, covering: genome sequencing and technology; variant interpretation and genome databases; computational genomics and bioinformatics; clinical genomics and genomic medicine; genetic and genomic counselling; and genomic education for the general public.

Results: Here we provide results of a recent international survey, identifying genomic education needs of non-genetics health professionals, globally, covering preferred future course content and delivery modes plus the various perceived advantages and potential difficulties associated with next-generation sequencing, worldwide. We also provide updated information and data relating to a wide range of other very recent activities being undertaken, including: development of a genetic counselling international training curriculum; creation of a catalogue of genomics training modules; running the VEPTC and HGVS training courses; developing the new custom-designed HUGO educational web pages, accessed from 75 countries, facilitating access to numerous worldwide resources, courses and organisations; and

the development of closer links with other genetic/genomic organisations worldwide, including the ESHG.

Conclusions: many activities are underway, facilitating global genomics education by HUGO, with links to ESHG EduComm.

Conflict of Interest: None declared

P23.048.D The European Society of Human Genetics - Young Committee - our achievements in the last 5 years and goals for the future

Delia Sabau[1;2;3], *Mridul Johari*[3;4;5], *Ana Raquel Gouveia Freitas da Silva*[3;6], *Silvia Kalantari*[3;7], *Ana Rita Matos*[3;8], *Rhys Dore*[3;9], *Magdalena Mroczek*[3;3], *Juliana Miranda Cerqueira*[3;10;11], *Elena Avram*[3;12;13], *European Society of Human Genetics - Young Committee*[3]

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The European Society of Human Genetics – Young (ESHG-Y) committee represents the young researchers and specialists in the field of Human Genetics in Europe.

In the last five years, our representatives have had a supportive role in the EBMG, ESHG Board, the ESHG Scientific Programme Committee, the ESHG Education Committee and the European Journal of Human Genetics editorial team. Our board members also participate in external organizations like ERN-Ithaca, Unique, Orphanet, EuroGEMS and MOOC BIG. In 2023, we co-organized the Young Investigative Forum (YIF) meeting at the International Conference of Human Genetics 2023 (ICHG). Board members of the ESHG-Y chaired various YIF and ICHG sessions. In January 2023, we launched the "ESHG-Y VIRTUAL LIVE Sessions: the voice of young human geneticists" project. This project aims to discuss exciting topics whilst sharing educational and training opportunities with the young generation. In the first episode, "ESHG-Y & YIF - links between Europe and Africa in Human Genetics", we invited representatives from the YIF.

The ESHG-Y also organized different Educational Sessions, Symposia and Workshops at the ESHG Annual Conference 2023. In collaboration with the European Board of Medical Genetics (EBMG), we organized a virtual live session: "Becoming European Board Certified in Medical Genetics and Genomics".

The ESHG-Y has successfully achieved its objectives by developing ongoing projects and partnerships. We will continue coordinating projects for the young generation through our European and International collaborations.

Conflict of Interest: None declared

P23.049.A Cultural competence within Clinical Genetics Services: A Comparative Analysis of Services within Australia (AUS), New Zealand (NZ) and the United Kingdom (UK)

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The concept of cultural competence was first identified in the 1980s to address the effects of cultural and linguistic barriers on the delivery and access to health services (Somnath *et al* 2008). More recently, this includes competence around religious, sexual & gender identity (Bass B, Nagy H, 2022). In order for a healthcare system to be equitable, it should be crafted to fit the specific political, social and cultural circumstances of the population it serves.

European nations have influenced the ethos of healthcare systems globally, which has resulted in systems that do not meet the needs of the underrepresented ethnic users. The Health Services of NZ and AUS are key examples of this intrusion (Durie, M. 1994, 2004, 2005, 2006, Truong *et al*, 2021).

The UK NHS is built on a western model of medicine, which prioritises patient-centred care and individualised-decision-making. This naturally poses potential challenges, given approximately 1 in 5 individuals in the UK population identify with one or more ethnic minority groups. This is particularly challenging for Clinical Genetics given the nature of genetics involves family units, and thus encompasses both individual- and shared-decision making.

In order to deliver effective clinical care, it is integral for healthcare providers to improve their awareness of implicit biases through reflection of their own beliefs and behaviours. This analysis attempts to depict this further across three nations, providing some constructs to which services can work towards to better meet the needs of the diverse UK population.

Conflict of Interest: Subhashini Crerar Clinical Genetics, Nandini Somanathan* Clinical Genetics

P23.050.B The European Society of Human Genetics' innovative international educational initiatives for young human geneticists – a collaboration between ESHG-Y and EduComm

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Norway; [11]EURORDIS - Rare Diseases Europe, Barcelona, Spain; [12]Erasmus University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands; [13]Children's Health Ireland at Crumlin and Temple Street, Department of Clinical Genetics, Dublin, Ireland; [14]GENYO Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government, Liquid Biopsy and Cancer Interception Group, PTS Granada, Granada, Spain; [15]National Centre for Inherited Metabolic Diseases, Mater Misericordiae University Hospital, Dublin, Ireland; [16]University of Surrey, Department of Clinical and Experimental Medicine, Guildford, United Kingdom; [17]Imperial College London, Department of Metabolism, Digestion and Reproduction, London, United Kingdom; [18]Facultad de Medicina, Universidad de Zaragoza, Catedrático Vinculado Director Departamento de Microbiología, Pediatría, Radiología y Salud Pública, Zaragoza, Spain; [19]Hospital Clínico Universitario "Lozano Blesa", Unidad de Genética Clínica Servicio de Pediatría, Zaragoza, Spain; [20]Centro de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar Universitário do Porto, Porto, Portugal; [21]Universidade de Aveiro, Departamento de Ciências Médicas, Aveiro, Portugal; [22]Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar (UMIB/ICBAS) and Laboratory for Integrative and Translational Research in Population Health (ITR), Universidade do Porto, Porto, Portugal; [23]Academic Unit of Medical Genetics and Clinical Pathology, Laboratory Medicine Building, Queen Elizabeth University Hospital, University of Glasgow, Glasgow, United Kingdom; [24]School of Medicine, Dentistry and Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom; [25]Clinical Genetics, West of Scotland Centre for Genomic Medicine, Laboratory Medicine Bldg., NHS Greater Glasgow and Clyde, Queen Elizabeth University Hospital, Glasgow, United Kingdom; [26]Division of Evolution Infection and Genomics, School of Biological Sciences, University of Manchester, Manchester, United Kingdom; [27]Manchester Centre for Genomic Medicine, Saint Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom

Background/Objectives: The European Society of Human Genetics (ESHG) together with our international partners aims to support young human geneticists from all over the world and offer them relevant educational opportunities. Within the ESHG, two committees have a pivotal role in implementing this task: the ESHG Education Committee (EduComm) and the ESHG – Young Committee (ESHG-Y). The objective of this poster is to present the ESHG educational programmes and our international partnerships involving young human geneticists.

Methods: Data from all ESHG educational programmes and international partnerships since 2020 involving young human geneticists were analyzed, emphasizing the number of young attendees involved.

Results: The main educational programmes developed by the ESHG dedicated to junior human geneticists are: the ESHG International Mentorship Programme, the ESHG International Observership Programme and the ESHG Courses. The ESHG International Mentorship and Observership Programmes aim to offer funding to visit highly regarded human genetics departments each year. Regarding the ESHG Courses, in the last 3 years the number of attendees has increased, and in 2023 nine courses will be co-organized in some cases with pre-meeting courses. The ESHG-Y was involved in the YIF Meeting (AfSHG), in episodes of the ESHG Podcast "Genetic Sounds" and in translating the EuroGEMS website.

Conclusions: The ESHG has successfully implemented educational projects that have engaged young human geneticists across the world. In the future, we will be seeking to develop further activities with our international partners like the Education Committees of the Canadian College of Medical Geneticists and the Human Genetic Society of Australasia.

Conflict of Interest: None declared

P23.051.C Teaching and training programs in rare genetic disorders - Romanian Medical Genetics Network experience

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Background/ Objectives: Rare genetic disorders (RDs) represent an important medical, financial, and social burden for all healthcare systems. RDs affects >1.000.000 patients in Romania, more than 90% of cases being undiagnosed.

Objectives: To increase the awareness and improve knowledge of rare genetic diseases among Romanian medical specialists (medical doctors and nurses), through specific training programs targeting different medical specialties to teach them the methodology needed to diagnose and manage patients affected by a rare genetic disorder.

Methods: Between February 2018 - December 2021, the Regional Centre of Medical Genetics Dolj, part of Clinical Emergency County Hospital Craiova, together with University of Medicine and Pharmacy from Craiova and Romanian Prader Willi Association, Zalau, implemented at national level the training project "Improving the professional skills of medical personnel from relevant specialties for the multidisciplinary management of rare genetic diseases – ProGeneRare" (108073/ POCU/91/4/8/ 01.09.2016). The project involved experts from the entire Romanian Network of Medical Genetics.

Results: During the project "Improving the professional skills of medical personnel from relevant specialties for the multidisciplinary management of rare genetic diseases", 786 medical professionals (664 medical doctors and 122 nurses) from all the Romanian regions benefit from training in rare genetic diseases field. Also, 60 young medical geneticists were trained in genetic diagnostic based on next generation sequencing methods.

Conclusions: Implementation of ProGeneRare project had a significant contribution in improvement understanding by specialists of rare genetic disorders and increased involvement of national specialists in clinical management of rare diseases.

Grant References: PROGENERARE Project - 108073/ POCU/91/ 4/8/01.09.2016

Conflict of Interest: Ioana Streata Human Genomics Laboratory, University of Medicine and Pharmacy from Craiova, Regional Centre of Medical Genetics Dolj, Clinical County Emergency Hospital, Craiova, Romania, Anca Riza Costache Human Genomics Laboratory, University of Medicine and Pharmacy from Craiova, Regional Centre of Medical Genetics Dolj, Clinical County Emergency Hospital, Craiova, Romania, Razvan Plesea Human Genomics Laboratory, University of Medicine and Pharmacy from Craiova, Regional Centre of Medical Genetics Dolj, Clinical County Emergency Hospital, Craiova, Romania, Mihai Cucu Human Genomics Laboratory, University of Medicine and Pharmacy from Craiova, Regional Centre of Medical Genetics Dolj, Clinical County Emergency Hospital, Craiova, Romania, Ana-Maria Buga University of Medicine and Pharmacy from Craiova, Regional Centre of Medical Genetics Dolj, Clinical County Emergency Hospital, Craiova, Romania, Amelia Dobrescu Human Genomics Laboratory, University of Medicine and Pharmacy from Craiova, Regional

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P23.052.D The worldwide use of the ESHG's guide to online educational resources (EuroGEMS.org) and its new Portuguese and Spanish translations

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Background/Objectives: In view of the increasing requirement, worldwide, for reliable educational genomics resources, a free-to-access website (www.EuroGEMS.org) was created for the European Society of Human Genetics (ESHG). It provides a user-friendly English-language guide to 115 high-quality online worldwide resources for genetics specialists, non-genetics-professionals, students and the public. At the suggestion of the ESHG, it has since been translated into Spanish (the first language of 480 million people and most recently also Portuguese (with 200-250 million native speakers).

Methods: Full translation and meticulous cross-checking of each EuroGEMS.org web-page was undertaken by Spanish-speaking and Portuguese-speaking bilingual genetics professionals. The Portuguese web-pages were launched in June 2022.

Results: In total, the www.EuroGEMS.org website has been visited from 136 countries. It has been endorsed by the ESHG Board and by the Human Genome Organisation (HUGO-International). The Spanish-language pages have been accessed from 39 countries (including 16 in South and Central America). The more-recently launched Portuguese-language pages have already been visited from 12 countries, including, most frequently, Portugal, France, Brazil, UK, Angola, Uruguay and the Czech Republic (in descending order). There has been a marked (2.5-fold) increase in the number of Portuguese speakers using EuroGEMS since launch. Additional data will be presented.

Conclusion: The EuroGEMS.org website is being used in an increasing number of countries. Adding to the English and Spanish-language pages, the new Portuguese-language pages have greatly increased its accessibility and readership, in Portugal, Brazil and elsewhere (including Angola). A French translation, now underway, should further facilitate access to genetics and genomics resources, worldwide.

Conflict of Interest: None declared

P23.053.C The ESHG-Young & the Young Investigator Forum: Partnership, networking and innovation

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Background/Objectives: The young generation of human geneticists has become more involved in creating strong international partnerships that aim to build ongoing collaboration with young international organizations and work together on different projects. Working together to establish impactful projects that sustain the young generation will have a significant impact on the development of the human genetic field all over the world. The objective of this poster is to present the collaboration between the European Society of Human Genetics –Young Committee (ESHG) and the Young Investigator Forum (AfSHG) in organizing the YIF 2023 Meeting.

Methods: Results from the ESHG-Y and the YIF partnership are presented in this poster.

Results: In 2023, the ESHG-Y assisted the organization of the YIF Meeting as part of the International Conference of Human Genetics 2023 (ICHG) and representatives of the ESHG-Y were invited to be chairs at the ICHG sessions. In January 2023, the „ESHG-Y VIRTUAL LIVE Sessions: the voice of young human geneticists" project was launched. This project aims to discuss common topics of interest whilst sharing educational and training opportunities for the young generation. In the first episode "ESHG-Y & YIF - links between Europe and Africa in Human Genetics" we invited representatives from the YIF.

Conclusions: The ESHG-Y and YIF is an ongoing partnership connecting young geneticists from Europe and Africa. ESHG-Y aims to expand this collaboration and engage other international young human genetics committees with the purpose of sharing resources and having a positive impact on the young generation of human geneticists.

Conflict of Interest: None declared

P24 Ethical, Legal and Psychosocial Aspects in Genetics

P24.001.A Recontact following withdrawal of minors from research or due to attrition

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Background: The participation of minors in research is critical for generating knowledge that can translate into positive health outcomes. The participation of minors in research, however, raises distinct ethical challenges, including the issue of recontact at the age of majority or medical maturity to obtain consent for the minor's continued participation in the research. This is especially relevant in longitudinal studies. One question that has not received much attention is that of re-contacting minors who have been withdrawn from the research or who have been lost to follow-up.

Methods: We considered the ethical permissibility of recontact for 3 scenarios in which the minor's participation in research may end, as identified in the literature: 1) parental withdrawal; 2) withdrawal by the minor; and 3) loss to follow-up (attrition). Our analysis of permissibility was based on our review of biomedical ethical guidelines and bioethical scholarship.

Results: We argue that recontact is permissible in cases of parental withdrawal and loss to follow-up, based on respect for the minor's developing autonomy and right to choose. However, where the minor had asked to be withdrawn from the research, we argue that recontact is not ethically permissible, based on their prior wishes and right to be heard.

Conclusion: As paediatric longitudinal studies become more common, researchers may wish to recontact participants for numerous reasons of scientific interest or utility. Our research aims to help researchers navigate the ethical complexities of recontact following withdrawal or attrition.

Grant References: Canadian Institutes of Health Research (PJT 148721)

Conflict of Interest: None declared

P24.002.B Retention of genetic family records and genomic data - a code of practice

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Genetic family records are health records relating to multiple individuals linked as a family. The results of genomic tests form part of this record, but the genomic data itself and records of how it was interpreted are usually held separately. There is often a tension between the costs of storage (whether electronic or paper, and including carbon footprint), the data protection risks of retention, and the benefits for individuals and society of retaining the records. Typically, individual health records are retained for 3 - 8 years after the last contact with the patient or after their death, according to current UK codes of practice. Exceptions exist such as pathology records. For genetic family records, which may have transgenerational implications for genetic risk assessment, selection and interpretation of genomic investigations, and for decisions about clinical screening programmes, a longer period is needed. After consultation with the British Society for Genomic Medicine, the Association for Clinical Genomic Science, the Royal Colleges Joint Committee on Genomics in Medicine and the NHS Clinical Reference Group in Clinical Genomics, we propose that genetic family records should be retained for a minimum of 30 years (1 generation) since last contact with a family member. Data files generated in the process of genomic sequencing are regarded as working documents, which should be retained for at least 5 years. The final written genomic report should be retained for 30 years. Consideration should be given to maintaining and sharing database(s) of classified variants to support future variant interpretation.

Conflict of Interest: None declared

P24.003.C Perspectives of parents of children with genetic conditions on reproductive genetic carrier screening

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Background: Little attitudinal research concerning reproductive genetic carrier screening (RGCS) has focused on the perspectives of parents of children with a genetic condition. As this particular group is expected to already have genetic and experiential knowledge, their perspectives could enrich the public and professional discussion on the responsible implementation of RGCS.

Methods: This interview study is part of a larger research project focusing on perspectives on RGCS of different stakeholders. We performed 14 semi-structured in-depth interviews ($n = 22$) with parents of children with a known genetic condition. Data were analyzed through thematic analysis.

Results: Overall we found positive attitudes among interviewees towards RGCS. Commonly cited reasons in support of RGCS were to enable informed reproductive choices, to be able to prepare in advance for the possibility of having a child with a genetic condition, the possibility to inform relatives, etc. Most parents supported the idea to offer RGCS to all couples planning a family, but critical considerations were also raised (e.g. low genetic health literacy in the general population, false reassurance to have a 'healthy' child, stigmatization of those living with a genetic condition, etc.).

Conclusion: Parents of children with a genetic condition support the implementation of RGCS but also raised some concerns based on their own lived experiences. This shows the added value of including perspectives of this particular group within the ongoing debate to implement RGCS in a responsible way.

Grants: FWO (G094518N)

Conflict of Interest: None declared

P24.004.D The relationship between psychiatric symptom severity and quality of life in patients with beta-thalassemia major: North Cyprus experience

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Background/Objectives: The term "beta-thalassemia" refers to a class of hereditary hemoglobin diseases that are distinguished by diminished or absent beta-globin chain synthesis (1, 2). Percentage of carriers of beta thalassemia is around 15% in the Turkish Republic of Northern Cyprus (T.R.N.C.). In the past decade, T.R.N.C. has prevented the birth of thalassemic children and brought the sick birth frequency to 0% thanks to a legislation for detection of carriers before marriage. Our aim in this study is to examine the relationship between psychiatric symptom severity and quality of life in adult patients with β-thalassemia major.

Methods: In this study, the distribution of the participants with and without beta-thalassemia major according to their socio-demographic characteristics, general health status, and disease-related characteristics were determined by Pearson chi-square test to compare the groups. Descriptive statistics and normality tests were performed and Diagnostic and Statistical Manual of Mental Disorders of the participants with and without a diagnosis of beta-thalassemia major was calculated.

Results: A statistically significant correlation was found between the severity of psychological symptoms, quality of life and general health status of individuals with and without a diagnosis of beta-thalassemia major.

Conclusion: To the best of our knowledge, our study is the first to examine the severity of psychological symptoms and quality of life in individuals with and without beta thalassemia major in the T.R.N.C. It is important to conduct further studies that reduce the severity of psychological symptoms and increase the quality of life of individuals with beta thalassemia major.

Conflict of Interest: None declared

P24.005.A Developing a best practice approach to ethics in genomics healthcare and research

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Background: In its Genome UK strategy, the Government committed to establishing a 'gold standard UK model' for how to apply strong and consistent ethical standards in genomics healthcare and research. In partnership with Office for Life Sciences and genomics leads in Scotland, Wales and Northern Ireland, the Nuffield Council on Bioethics facilitated discussions on how to achieve this.

Methods: We gathered case studies on how people across the UK are considering the ethical issues raised by genomics, and held workshops with clinicians, researchers and people with personal experience of genetic conditions.

Results: Participants agreed that the development of a gold standard model – or best practice approach – for ethics in genomics could help those working in the field to negotiate ethical issues, promote consistency of approach and, ultimately, create better, more equitable experiences for patients and research participants. It would need to incorporate different components such as ethical principles, professional guidance, discussion fora, and practical tool kits. It should be transparent and inclusive, both in how it is produced and developed and in who has access to it. A UK approach to genomics ethics would need to be sensitive to the international context and specific to UK audiences.

Conclusion: The next step will be to create a map of existing resources to understand what is already available and identify areas where further work is needed.

Grant references: Nuffield Council on Bioethics

Conflict of Interest: Catherine Joynson Nuffield Council on Bioethics, Member, UK National Screening Committee Blood Spot Task Group

P24.006.B The myth of the "Genetic Wallflower": in reproductive carrier screening, for every Papageno there is always a Papagena

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Background/Objectives: Many professional bodies, including ACMG, ESHG, HGSA and RANZCOG, have developed clinical practice guidelines recommending offering pan-ethnic carrier screening to assess reproductive risk. Technological advances permit simultaneously testing >1,000 genes with associated autosomal recessive (AR) conditions. Concerns have been raised that widespread carrier screening will leave people at "risk of being genetic wallflowers, rejected by all suitors because of the recessive genes they carry" (Francis S Collins, 2013). We have explored whether carrier screening at population scale might carry this risk.

Methods: We performed Monte Carlo simulations on in silico populations, mimicking reproductive partner choice with and without carrier testing for AR conditions. Using data from gnomAD and ClinVar genomic databases, we have recently shown that the optimally sized "Goldilocks" panel for reproductive carrier testing contains ~550 genes, and will detect ~99.7% of clinically important variants relevant to carrier screening. For modelling, we used this 550 gene panel, as well as a larger ~1,200 gene panel.

Results: For both 550 as well as the larger 1,200 gene panels, we found no evidence for emergence of "genetic wallflowers". There always remained sizable pools of potential reproductive partners, regardless of the number of pathogenic carrier variants present in an individual.

Conclusion: We have demonstrated there remain large numbers of potential reproductive partners following population-scale reproductive carrier testing for AR conditions, regardless of the size of the carrier panel. Our conclusions reinforce the message that carrying a genetic variant is "no-one's fault" and testing is beneficial not stigmatising.

Conflict of Interest: Leslie Burnett Employee - Invitae Australia, Takeda Pharmaceuticals Investigator Initiated Grant;

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University of NSW Sydney (Honorary), Mia Gruzin Employee - Invitae Australia

Employee - Garvan Institute of Medical Research, Eric Lee Employee - Virtus Diagnostics, Thermo-Fisher scientific master-class webinar (carrier screening), Royal College of Pathologists of Australasia (RCPA): Genetics Advisory Committee (Honorary)

RCPA Quality Assurance Programs Pty Ltd: Molecular Genetics Advisory Committee (Honorary), Matthew Hobbs Employee - Garvan Institute of Medical Research, Sarah Poll Employee - Invitae Corporation, Stockholder of Invitae, Nicole Faulkner Employee - Invitae Corporation, Stockholder of Invitae, Swaroop Aradhya Employee - Invitae, Stockholder - Invitae, Advisory Board - Biomarin

P24.007.C What to consider in PGT-P guidelines? Perspectives of healthcare professionals

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Background/objectives: Preimplantation genetic testing using polygenic risk scores (PGT-P) has recently been introduced commercially. Many ethical concerns have been raised and guidelines are still largely absent.

Methods: We performed qualitative interviews with 31 healthcare professionals in the field of genetics and reproductive medicine in Europe and North-America.

Results: Healthcare professionals were largely in favour of guidelines and regulation for PGT-P, e.g. nationally or via scientific organizations. Most were in favour of limiting PGT-P to serious medical conditions and not allowing screening for non-medical traits. Risk requirements were seen as useful by many, and most preferred screening for one condition over a panel of conditions and favoured ranking embryos over embryo selection. Furthermore, the target group of PGT-P was considered, i.e. people with a polygenic condition in the family, people already using PGT/IVF or the general population, with the first target group generally seen as the most ethical. Lastly, many healthcare professionals were in favour of joint decision-making, but a few healthcare professionals thought that either patients or professionals should be primarily in charge.

Conclusion: If PGT-P is implemented in clinical practice, it is important that guidelines consider the topics 1) for which conditions and traits PGT-P would be desirable, 2) for which aims to offer PGT-P, 3) which target group(s) to offer PGT-P to, and 4) who makes decisions concerning the offer of PGT-P.

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Conflict of Interest: None declared

P24.008.D Making genomic research results meaningful: lessons learned from community engagement around the world

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Background/Objectives: The lack of results sharing from studies in genomics and its precursors in biomedical research has played an important role in eroding trust in the scientific relationship and withholding valuable information from communities who have shared their data with scientists. Here, we highlight an approach that seeks to address colonial and ongoing legacies of extractive research by returning population-level genomic and health results first and foremost to participating communities.

Methods: We performed in-depth community engagement reinforced by a commitment to reciprocity, transparency, and equity in research partnerships. Locally determined health interests, cultural norms, and community belief systems were taken into consideration before returning data.

Results: Through case studies from French Polynesia, New Zealand, and Madagascar, we demonstrate how community engagement ensures that results return is productive in diverse communities. We show that local engagement also informs how to best communicate data in each unique study setting, including among geographically dispersed populations and those with low literacy rates.

Conclusion: Ultimately, these case studies highlight the critical importance of community engagement in shaping a locally meaningful results return process, thereby contributing to greater equity and justice for those participating in genomic studies.

Conflict of Interest: None declared

P24.009.A Breaking Perceptions: How to effectively support Healthcare Professionals to participate in research projects

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Background/Objectives: Despite evidence demonstrating benefits for healthcare, when Healthcare-Professionals participate or lead research projects within their organization, research engagement remains suboptimal.

We aimed at understanding the main enablers and barriers Healthcare-Professionals face to successfully engage in research, how they perceive organizational support, and how this influences their research engagement.

Methods: A preliminary systematic literature-review identified the main enablers and barriers Healthcare-Professionals face to successfully engage in research, that were used to create and validate a survey questionnaire. 888 Healthcare-Professionals, working in Portugal, anonymously replied. Answers were analyzed with SPSS and Multiple-Linear-Regression.

Results: 75.7% Healthcare-Professionals are female, 64.2% are aged 26-35 (33%) or 36-45 (31.2%) years. Medical doctors are the most represented category with 35.8% of the sample, followed by nurses(34.8%), and diagnostic and therapeutic technicians are the least represented (29.4%). Among 45 different clinical specialties or health services in this study, there were 34 Healthcare-Professionals working in molecular and/or clinical genetics. The analysis of the questionnaire identified 'motivation' and 'personal interest' as main enablers for Healthcare-Professionals research engagement, while 'lack of time', 'lack of recognition' and 'low organizational support' represents the main barriers. The level of research engagement is higher for individuals working at university-hospitals or at health organizations linked to research institutions. Low levels of 'perceived organizational support' negatively affects Healthcare-Professionals research engagement.

Conclusions: This pioneer study reveals the importance to align human-resource management practices to strategically reinforce research engagement of Healthcare-professionals. Time-management-systems integrating and acknowledging involvement in research activities research and research-support offices emerge as probable solutions.

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Conflict of Interest: Liliana Sousa PhD fellowship, Ana Carvalho Professor, Carla Oliveira Principal Investigator, PI in national and international research grants

P24.010.B Meaning making, mastery, and mental health: how patients with inborn errors of immunity adapt to their illness

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Introduction: Genetic professionals aim to facilitate adaptation to illness. However, research is limited on *how* individuals with inborn errors of immunity (IEI) adapt. We aimed to fill this gap by interviewing participants with IEI regarding adaptation to their illness.

Methods: We contacted 35 participants with IEI (ages 18-40) from an existing sequencing study with available baseline psychological data who exhibited at least mild anxiety/depression by PROMIS 29v2.1 and above-average scores on an adaptation scale (better adaptation). Twenty participants (57.1%) completed semi-structured interviews. The interview guide was based on Taylor's Theory of Cognitive Adaptation and focused on meaning making, mastery, and mental health. Interviews were recorded, transcribed, and coded. Transcripts are being analyzed using an inductive, semantic thematic approach.

Results: Interviewees were 32 years old on average and 65% were female. Common diagnoses were GATA2 deficiency (n = 5) and common variable immune deficiency (n = 5). Seven (35%) participants underwent hematopoietic stem cell transplant.

Codes related to meaning making included helping others and accepting the person one has become. Many codes about mastery described discerning what can and cannot be controlled. Mental health codes included sources of anxiety and depression, connections between physical and mental health, and the desire for clinicians to ask about mental health. Additional codes included social factors, comparison to others, life course, and reproductive decision-making. Results of thematic analysis will be reported at the meeting.

Discussion: Adaptation to IEI is contextual and multifactorial. Further analysis will explore targets for facilitating adaptation, including identifying unmet support needs.

Conflict of Interest: None declared

P24.011.C Between desire and fear: A qualitative interview study exploring the perspectives of carriers of a genetic condition on human genome editing

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Background/Objectives: Human genome editing technologies are advancing rapidly. Their potential disruptive implications lead to ethical and societal questions that cannot be answered by scientists alone. Further consideration of different stakeholders' views on human genome editing is crucial to translate society's values into thoughtful regulations and policies. We therefore explored the worldview-based (secular or religious) views of carriers of autosomal dominant disorders on somatic and heritable genome editing (SGE and HGE).

Methods: Ten in-depth semi-structured interviews were conducted and analyzed using reflexive thematic analysis.

Results: We found three main themes: 'The benefits of SGE and HGE for individuals', 'the societal consequences of using HGE', and 'the consequences of interfering with nature through HGE'. All participants were positive towards a safe use of SGE regardless of the severity of conditions, and most participants were positive towards the use of HGE, but only to prevent severe genetic conditions. Based on their religious beliefs, a few participants were against using HGE in any case, regardless of the severity of a condition. However, most participants, with religious or secular worldviews, reported similar views on HGE, both regarding their desire to prevent serious genetic disorders and their fear of the potential harmful impact on society and nature if HGE would be implemented more widely.

Conclusion: Reflecting on HGE often involved ambivalent worldview-based views. When engaging different stakeholders, space is needed for ambivalence and the weighing of values.

Grant References: Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by the Dutch Research Council [NWO/NWA.1389.20.075].

Conflict of Interest: Wendy Geuverink As of 1st September 2022 Wendy Geuverink is involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by the Dutch Research Council (NWO) With project number [NWA.1389.20.075]., Carla van El As of 1st September 2022 Carla van El is involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by the Dutch Research Council (NWO) With project number [NWA.1389.20.075]., Martina Cornel As of 1st September 2022 Martina Cornel is involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by the Dutch Research Council (NWO) With project number [NWA.1389.20.075]., Bert Jan Lietaert Peerbolte: None declared, Janneke Gitsels - van der Wal: None declared, Linda Martin: None declared

P24.012.D The hard and stony path to classification: historical, ethical, social, and methodological considerations of PCA to represent human genomic diversity

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Background: Human genetics researchers often rely on principle component analysis (PCA) and linear or logistic regression to make inferences about human population diversity. However, the conclusions of PCA and other dimensionality reduction and regression techniques are often poorly understood, undertheorized and mis-interpreted in the field of human genetics. This is contrasted with other disciplines, in which such methods have been highly scrutinized.

Objectives: Show how the emergence of PCA was driven by historical and political motivations to classify humans; and how other disciplines have avoided over-interpreting PCA.

Methods: Examined historical motivations and conceptual underpinnings of methods which persist in human genetics but are contested and improved upon in other fields. Evaluated the use of PCA in past and current human genetic studies.

Results: PCA was initially developed because existing linear regression models could not handle complex systems like human physical features. This approach was predicted to offer utility in other disciplines but was at the time used as a statistical device to support eugenic reasoning. Today, many disciplines use PCA to visualize complex systems while preserving key features of the data. Genetic investigators often treat principal components as unproblematic finished products, denoting real entities as opposed to statistical devices. This interpretation inadvertently perpetuates underlying eugenic reasoning, enabling the weaponization of peer-reviewed genetics research to fuel harmful agendas.

Conclusion: Insights about dimensionality reduction techniques from other disciplines may help the field of human genetics improve the scientific validity of group-level representations, thus limiting their utility in shaping harmful social narratives.

Conflict of Interest: None declared

P24.013.A The regulation and governance of lifetime genomic data—priorities for action

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Introduction: Whole genome sequencing of newborns is being considered for diagnosis and care of children with genetic conditions, to facilitate research and potentially, to provide a genomic record which can be used to care for an individual throughout their lifetime. Commissioned by the Genomics England Newborn Genomes Programme, we aimed to evaluate the foreseeable data law and governance issues that may arise with the lifetime storage, interrogation and reanalysis of human genomes.

Methods: We conducted targeted reviews and analysis of the ethical and legal frameworks governing the generation, storage and use of genomic data for health purposes.

Results: We identified a wide range of considerations to be addressed from the outset, in the medium term or requiring sustained focus into the long-term. We group these around four overarching priorities: (i) Developing appropriate approaches to reanalysis, updating genomic data and recontacting families (ii) preserving a child's right to an open future, managing a transition in decision-making from parents to children and managing conflicting views (iii) incorporating genomic data in the clinical record and implications of learning healthcare approaches, and (iv) anticipating and setting policies for novel uses of genomic data, including potential non-medical applications.

Conclusions: While we do not identify a fundamental challenge to using a genome as a lifetime medical resource, a range of complex and specific issues need to be addressed by policy-makers, regulators and healthcare professionals. Proactive consultation with young people and families will be crucial to the development of appropriate policies and careful stewardship of genomic data.

Conflict of Interest: Elizabeth Redrup Hill: None declared, Tanya Brigden: None declared, Alison Hall Member of the Genomics England (GEL) Ethics Advisory Committee, the GEL Newborn Ethics Committee and the GEL Conditions Framework Working Group (all unpaid)., Colin Mitchell The PHG Foundation was commissioned by Genomics England for this work

P24.014.B Policy guidance for direct-to-consumer genetic test services: key aspects that trigger policy issues

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Direct-to-consumer genetic tests (DTC-GT) are accessible for citizens worldwide to autonomously assess varying health outcomes. DTC-GTs often comprise various service elements, including: DNA-analysis, and medical or lifestyle advice. Risks and benefits have been debated and studied extensively, but evidence regarding individual or societal impact is lacking. Uncertainty arises among policy makers, law enforcers, and regulators about how to ensure and balance public safety and autonomy. This study aims to outline aspects that trigger policy issues and to provide policy guidance.

Potential risks and benefits of DTC-GT services for consumers and society, including and beyond medical implications, were mapped via a systematic scoping review. These findings were structured into phases, following the steps that consumers take. A checklist was designed to offer policy guidance. All phases of DTC-GT services were included in the checklist.

Potential risks and benefits of DTC-GT services were mapped and structured into six phases, summarized as *consumer journey*: exposure, pretest information, DNA-analysis, data management, posttest information, individual and societal impact. The checklist consists of 8 themes, covering 38 items that may raise policy issues in the following aspects: general service content, validity and quality assurance, potential data and privacy risks, scientific evidence and robustness, and quality of the provided information.

Both the *consumer journey* and the checklist break the DTC-GT offer down into key aspects that may impact and compromise individual and public health, safety, and autonomy. The tools may help policy makers, regulators, and law enforcers to interpret, assess and act in the DTC-GT service market.

Conflict of Interest: None declared

P24.015.C Public perspectives on genomic newborn screening: a qualitative approach

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Newborn screening (NBS) is one of the world's most successful population screening initiatives. As several large-scale studies assessing the feasibility of integrating genomic sequencing into NBS launch internationally, it is timely to explore public perspectives with the view to integrating these into study designs.

We conducted eight online focus groups with members of the Australian public. Transcripts were deidentified and analysed using inductive content analysis.

Of the 38 participants, most (85%) were female and the majority (82%) had children. Participants were generally highly in favour of genomic NBS, citing the potential for more children to benefit from early treatments/interventions. They felt parents should be informed of the option of genomic NBS during pregnancy, allowing more time to understand the information and make an informed choice. In-person information delivery was preferred. Participants agreed that consent should be explicit, although opinions differed as to whether it should be obtained during pregnancy or the time of sample collection. Regarding which conditions should be screened, many participants felt severity and treatability should not be requirements for inclusion in genomic NBS panels. However, they believed these factors should be prioritised if funding limitations required condition numbers to be constrained. Return of findings related to adult-onset conditions was generally not supported.

Integrating public perspectives into decisions about public health initiatives is critical to increase acceptance and uptake.

These findings are being used to inform the design of a model for implementing genomic NBS in Australia. Preferences will help optimise costs and outcomes to provide high-value care.

Conflict of Interest: Danya Vears Employed part time by a grant administered by the Australian Government on genomics newborn screening, CI on a grant administered by the Australian Government on genomics newborn screening, Fiona Lynch: None declared, Christopher Gyngell CI on a grant administered by the Australian Government on genomics newborn screening, Stephanie Best CI on a grant administered by the Australian Government on genomics newborn screening, Ilias Goranitis CI on a grant administered by the Australian Government on genomics newborn screening, Alison Archibald CI on a grant administered by the Australian Government on genomics newborn screening, Clara Gaff CI on a grant administered by the Australian Government on genomics newborn screening, Lilian Downie CI on a grant administered by the Australian Government on genomics newborn screening, Sebastian Lunke CI on a grant administered by the Australian Government on genomic newborn screening, Zornitza Stark CI on a grant administered by the Australian Government on genomic newborn screening

P24.016.D Citizen engagement and public trust in genomic data sharing: recommendations for trustworthiness from an experts workshop organised by B1MG

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Background/Objectives: The '1+ Million Genomes' (1 + MG) initiative, coordinated by the Beyond 1 Million Genomes (B1MG) project, aims to enable secure access to genomic and corresponding clinical data across Europe for research, personalised healthcare, and policy making. It is a joint initiative of 24 EU countries, the UK, and Norway. Citizen engagement and public trust have been raised as critical components in the B1MG stakeholder forum meetings and country visits. To discuss these components, an experts workshop was facilitated.

Methods: Experts workshop: 14 participants (expertise including bioethics, ELSI, governance, human genetics, patient representation, citizen engagement) shared insights and formulated recommendations to participating 1 + MG countries.

Results: Lessons learned from various engagement activities were shared under three subthemes: "When an ELSI framework is in place, what is the (additional) role of citizen engagement in fostering data sharing and public trust?"; "How does citizen engagement relate to (interests of) other stakeholders?"; "When and how to engage and at what level?". Recommendations stressed the need for dedicated resources, acknowledging different views and interests, good and transparent governance for enabling trustworthiness, capacity building, collaboration with key stakeholders, and meaningful participation through early engagement with a careful choice of engagement strategy.

Conclusion: Citizen engagement needs sustained resources and attention across projects and national and EU initiatives. Trust depends on citizens and patients, so a trustworthy data sharing infrastructure needs transparent governance to consider and incorporate citizen views.

Grant References: B1MG: EU's Horizon 2020 Research and Innovation programme, grant agreement 951724, https://b1mg-project.eu/

Conflict of Interest: Carla van El Senior researcher part-time AmsterdamUMC, PROPHET (PeRsOnalized Prevention roadmap for the future HEalThcare): collaborator

ExACT (Marie Curie RISE - European network staff eXchange for integrAting precision health in the health Care sysTems): collaborator

PRESAGE (Public Realm Entrance and Societal Alignment of Germline Editing): collaborator

B1MG (Beyond 1 Million Genomes) collaborator for organising expert workshop on citizen engagement and public trust, IC2PerMed (expert advisor in a working group), I am secretary general of the Public and Professional Policy Committee of ESHG, Eric Vermeulen: None declared, Mark Bale Public Policy Projects

Ireland Health Service Executive, IQVIA (external consultant), Miriam Beusink: None declared, Lise Bitsch Danish Board of Technology Foundation, full time, PI, Human Brain Project, SGA3 (GRANT_NUMBER: 945539)

PI, Societal Engagement with Key Enabling Technologies (SocKETs) (GRANT_NUMBER: 958277)

Collaborator, Human Brain Project (SGA2) (GRANT_NUMBER: 785907)

Collaborator, Human Brain Project (SGA1) (GRANT_NUMBER: 720270)

PI, Governing nanotechnologies through societal engagement (GoNano) (GRANT_NUMBER: 768622)

Consultant, contract research Kavli Foundation, no grant number, Advisory Board EU project EnvironMental, Nikki Coutts: None declared, Hans van Delden: None declared, Věra Franková First Faculty of Medicine, Charles University, Edith Gross: None declared, Wannes van Hoof I work on public engagement projects, I work on public engagement projects, Denis Horgan: None declared, Helena Machado: None declared, Michaela Mayrhofer: None declared, Arshiya Merchant ELIXIR (Hub), Project Officer in the following grants: Beyond 1 Million Genomes; HealthyCloud; Personalised Prevention Roadmap for the future of healthcare in Europe, European Health Data Space 2 pilot (HealthData@EU), Christine Patch Wellcome Connecting Science, New Born Screening Advisory Panel, Ilumina, Borut Peterlin Pfeizer, Thermofisher, Carina Pittens: None declared, Barbara Prainsack For the sake of full disclosure (I do not think it is a CoI but it could seem one) I am the Chair of the European Group on Ethics which advises the European Commission; and I am a member of the Austrian National Bioethics Committee., Gabby Samuel Full time employed.

, 2022-2023 £61,692. Collaboration agreement with University of Leeds project UKRI Net-Zero Digital Research

Infrastructure Scoping. Work at KCL will support stakeholder engagement opportunities.

2022-2023 £100,792.00. Samuel G. Farley, M. Research in environmentally sustainable life science & medical practice. MRC

2022-2024 £125,164.00. Rae, C. Selvan, R, Samuel G. Developing environmentally sustainable best practices for human brain imaging. MRC

2021- present £883,623. Blair G (PI), Jirotka M, Knowles B, Lucivero F, Samuel G (CI), Sorrell S, Widdicks K, Webb H. Design Principles and Responsible Innovation for a Sustainable Digital Economy (Paris-DE). ESPRC

2021-present £246,365 Samuel, G. The environmental sustainability of data-driven health research: a case-study of genomics and digital phenotyping in the UK. Wellcome Research Fellowship

, One day a week as a Senior Research Fellow for the UK Biobank Ethics Advisory Committee (via Oxford Uni)., Ruben Kok Stichting DTL projects & Stichting Health-RI, B1MG (CSA), GDI (Digital Europe), Serena Scollen: None declared

P24.017.A Case (dis)closed: crafting points to consider for policymakers on the return of genetic test results with familial consequences

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Findings from genetic testing can have important implications for patients and their relatives. When a genetic risk is present in a family, disseminating that information to all family members who are at risk can play a vital role in connecting them with preventive, treatment, and reproductive options. However, research indicates that patients do not always disclose these results. Many countries lack specific regulation related to genetic testing adjudicating what can be done in cases of nondisclosure of genetic risk. In light of this ethical challenge, we developed points to consider for policymakers on the issue of nondisclosure of genetic results with familial consequences. This guidance is informed by our previous analyses of guidelines and legislation, as well as our empirical work with clinicians and the public. These points to consider balance the rights and responsibilities of patients, at-risk relatives, and clinicians, while also accounting for practical considerations, based on the resources and organization of healthcare services. Such guidance will assist clinicians to navigate this ethically fraught area in their practice, which will improve patient care.

Grant references: Horizon Europe grant: 101057721

Conflict of Interest: None declared

P24.018.B Preserving individual rights in international genetic research: from consent to a systemic approach to governance

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The internationalisation of genetic research is currently facing several challenges, including the preservation of individual rights which has traditionally been based on the informed consent of research participants or on the anonymisation of data.

Due to the international evolution of research, which has increased the (re)use and sharing of genetic data for various purposes, and the known limitations of consent and anonymisation, this traditional approach is no longer valid. These data are now seen as a collective resource serving the public good. However, this new approach does not diminish the need to respect the individual rights of research participants, but requires a rethink.

In this presentation, we will use the example of the CINECA project (Common Infrastructure for National Cohorts in Europe, Canada and Africa) to show that the research community has organised itself to integrate and take account of these developments, basing this legal and ethical requirement not only on the informed consent of individuals, but also on governance measures: transparent information, an important role for ethics and data access committees, innovative privacy technologies, contractualisation of ethical requirements, etc.

These governance measures allow for responsible research, taking into account the different approaches to privacy, given the partners in CINECA, their different legal systems and the value of informed consent in each region. They also compensate for the lack of clarity of the legal framework for the re-use of health data (GDPR), which is still uncertain on the eve of the creation of the European Health Data Space.

CINECA, GANo825775

Conflict of Interest: None declared

P24.019.C Clinicians' experience and views about the challenges of genomic testing of gamete donors, as well as pregnancies or offspring conceived by gamete donation

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Background: advanced genomic technologies often yield uncertain and probabilistic data. The use of gamete donation exacerbates the complexity when the DNA and full phenotypic information of the donor are missing. Even once a definite result is obtained, it may have implications beyond the specific tested individual/fetus, including the donor, past, present, and future recipient parents (and their children).

We aim to provide empirical data on clinician's experience with and attitudes towards donors' consent, re-contact for additional genetic testing, and return of results to donors and recipients (past, present, future).

Methods: In-depth interviews were conducted with 12 clinicians involved in gamete donation and genetic testing and were analyzed using the Grounded-theory approach.

Results: participants indicated that broad consent at donation for any future genetic testing is the easiest option for clinicians, yet re-contacting donors for new tests seams the right thing to do. Nevertheless, they acknowledged that reaching donors years after donation may not be practical. When a pathogenic finding is identified in a donor/fetus/child, clinicians were in favor of informing past and present recipients, even if penetrance is incomplete. Views differed regarding late-onset diseases.

Conclusion: with the ever-growing use of genomic medicine, consent given at time of donation may not reflect new possible tests. In parallel, the use of gamete donation is on the rise. Consequently, new ethical, legal, and social challenges will arise

regarding donors, recipient families and providers of gamete donation services and require address. Findings from our study may serve to guide the development of professional guidelines.

Conflict of Interest: None declared

P24.020.D Individual attitudes towards identity, privacy and health insights from personal genomics: Evidence from a full archival search on Twitter

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Background: In recent years, the massive growth of direct-to-consumer genomics testing services (DTCG) has raised the attention of the public and posed pressing regulatory questions. However, to date, very little is known about how the newly found accessibility of personal genome information is perceived by individual consumers, as well as accompanied concerns on privacy, self-identity and health insights.

Methods: We collected public tweets related to two major DTCG companies from 2006 to 2020 (n = *1.6M*, posted by 757,750 individual users), and analysed word, terminology, topic and sentiment prevalence over time.

Results: Online discussions on DTCG experienced a massive year on year increase ever since 2014, in line with the steady and rapid growth of new genetic tests on the market. The percentage of organic tweets gradually declined over the years, and in contrary, the proportion of retweets was growing. This trend correlates with the annually increased direct to consumer medical marketing spendings in the US since 77% of geo-tagged tweets in our datasets are in the US, followed by the UK and Canada.

Conclusion: Among the sentiment analysis on English written tweets, we discovered that on average that most tweets are sentimentally positive, even among concerning topics that are privacy related or classified as hate speech or offensive language. This is possibly due to the fact that Twitter enforces relevant policies and restriction to minimise harmful contents on its platform.

Grant References: European Research Council ERC Advanced Grant CHRONO 835079, the Leverhulme Trust, Leverhulme Centre for Demographic Science to PI Mills.

Conflict of Interest: None declared

P24.021.A Crowdsourcing smartphone data for biomedical research and algorithm training: ethical and legal questions

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Background: Smartphone health apps are increasingly used as a platform for collecting and sharing large volumes of crowd-sourced health data for research, including in genomics, and algorithm training. Using this data presents opportunities to expand biomedical knowledge, though it also entails certain risks, including privacy, data protection, and human subjects research protections. A better understanding of how apps are crowdsourcing data is therefore necessary.

Methods: We conducted a search of the Apple App and Google Play stores in North America and Europe for apps which might be used to crowdsource health data. We then reviewed their privacy policies, terms of use, and other mobile or web-based disclosures to better understand how data were being used and whether they might be repurposed for research or algorithm training.

Results: Based on the search results, we will develop an App Atlas that helps elucidate this poorly understood legal space. To date, we have identified 52 apps available in the European and Canadian markets that either openly crowdsource health data for research or algorithm training or retain the legal or technical capability to do so. We found that there is a lack of consistency and transparency in the documents we consulted, which will be outlined in this presentation.

Conclusions: Numerous smartphone apps are currently crowdsourcing data for research or algorithm training. This raises ethico-legal issues which require further attention to ensure a balance between protecting individual interests and maximizing the scientific utility of crowdsourced data.

Grant References: Fonds de Recherche du Québec (FRQ-NT G0E3721N).

Conflict of Interest: None declared

P24.022.B Attitudes, concerns and motivation of the Latvian general population and researchers towards citizen-science research projects in the field of genomics

Alise Svandere[1], *Signe Mezinska*[2], *Katrina Kaleja*[1], *Normunds Kante*[1], *Helena Daiga Litvina*[1], *Helvijs Niedra*[1], *Raitis Pečulis*[1], ***Vita Rovite***[1]

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Citizen-science is research carried out by citizens or amateur scientists in cooperation with scientists or within the framework of specific scientific projects, based on scientifically developed methods. Examples of citizen-science include bird watching and counting, collecting environmental data, performing astronomical observations and reporting these data to scientists. Citizen-science makes science accessible to the public and promotes public trust. The study aimed to evaluate the concerns and motivation of the Latvian general population and researchers towards citizen-science research projects in the field of genomics.

We developed a specific survey that consisted of seven citizen-science research project examples (*vignettes*) that included various levels of participant involvement, use of data and relation to genetic research. After each research project *vignette* description survey participants need to answer questions about their motivation and concerns to participate. The same *vignettes* were used for the researcher survey, but the researchers were asked to answer questions on their potential motivation or concerns using such citizen-science research data for their research studies.

The survey data was analysed according to specific *vignettes* where we analysed what was the most frequent motivation and concerns in specific types of studies for potential research participants from the general population. We also looked at how the concerns and motivation differed for the same individual for different types of studies and what were the main principal differences in opinions of researchers and citizens.

Overall, citizen-science studies could give valuable data for research and increase public trust in science.

Conflict of Interest: None declared

P24.023.C Whole genome sequencing inadvertently reveals first degree consanguinity: A clinical case

Maya Atanasoska[1;2], *Radoslava Vazharova*[1;3], *Lubomir Balabanski*[1], *Slavyana Staykova*[1], *Irena Bradinova*[1;4], *Daniela Avdjieva-Tzavella*[5], *Draga Toncheva*[1;6]

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Introduction: Generally, each person carries 4-5 recessive variants associated with rare and complex disorders. The chance that unrelated parents will have a child with a birth defect or disability is between 2-3%. This risk is much higher in the offspring of consanguineous marriages, which are one of the most common causes for rare, recessive and lethal disorders in children.

Materials and Methods: Our patient is a 1-year-old girl with complex clinical features and skeletal dysplasia, her older brother passed away at 40th day after birth as a result of complex heart anomaly. She was examined through whole genome sequencing on a BGISEQ-500 platform. Targeted bioinformatic analysis was performed followed by analysis for detection of regions with absence of heterozygosity using Gemini tool-Galaxy platform.

Results: We identified co-occurrence of two homozygous pathogenic variants: one missense in the gene *SIK3* (c.950T>G) associated with spondyloepimetaphyseal dysplasia, Krakow type and one nonsense variant in *P4HA1* (c.1084C>T) associated with P4HA1-congenital disorder of connective tissue. Besides that, more than 10% of the patient's genome showed absence of heterozygosity suggesting very close parental consanguinity.

Conclusion: Ten percent of the world's population are of consanguineous parentage and in some societies, as a result of cultural and religious manners, more that 60% of all marriages are consanguineous. Through WGS we revealed two ultra-rare disorders in our patient and a first-degree consanguinity in her adult parents. Consanguineous unions are a complicated and multi-layered problem, difficult to manage mostly due to a lack of consideration for cultural, social, and geographical variables.

Conflict of Interest: None declared

P24.024.B Screening for severe genetic conditions: ethical implementation requires a multifaceted approach

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Background/Objectives: Severity of genetic conditions is often a criterion for undertaking genetic testing or further interventions, however it is complex to define and apply. Determining a condition's severity requires the integration of various factors such as clinical features, impact on quality of life, and healthcare requirements. This presentation examines severity in the context of reproductive genetic carrier screening (RGCS).

Methods: Our mixed-methods research integrates a bioethics analysis of the concept of severity with empirical findings about factors affecting the implementation of large-scale population RGCS, drawing on outcome data from the Mackenzie's Mission project and international bioethics literature.

Results: At the level of a screening program, a generalised approach to severity is necessary for assessing what genetic conditions to screen for. There is evidence that screening participants expect that conditions included have been selected judiciously by trusted experts. However, individuals making reproductive decisions based on an "increased chance" result in RGCS require more nuanced information that incorporates diverse perspectives, for example from people with lived experience, their family members, and clinicians. Early findings from Mackenzie's Mission suggest that severity does influence reproductive choices, so describing severity requires careful consideration.

Conclusion: Offers of RGCS should be cognisant of the trust potential participants place in those who choose the conditions to screen, and providers should be prepared to support a decision-making process that responds to the complexity of determining the severity of genetic conditions.

Grant References: Australian Government GHFM73390 (MRFF-G-MM)

Conflict of Interest: Lisa Dive: None declared, Alison Archibald Employed by Victorian Clinical Genetics Services, a not-for-profit genetic testing provider, Lucinda Freeman: None declared, Erin Tutty: None declared, Ainsley Newson: None declared

P24.025.A Obstacles and expectations of rare disease patients and their families in Türkiye: ISTisNA project survey results

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The information on the experiences during disease processes and daily life of rare disease patients is still limited. Here we present a comprehensive survey analysis conducted among the patients and their families within the scope of the Istanbul Solution Platform for Undiagnosed and Rare Diseases-ISTisNA project. A total of 498 individuals responded to the survey, and 58% of the participants answered all questions. The age range of 1-10 years (44.7%), and 91% of all the patients had a precise diagnosis. The diagnosis rate in the first 6 months was 69%, and almost 10% of the patients remained undiagnosed. The mothers were the primary caregivers (72%). 30% of the caregivers had to quit their jobs and 25% of the patients (0-18 years) had to leave school. Accessing physicians with relevant specialization and reaching treatments/medications/supplements were the two main obstacles the participants mentioned, with a frequency of 81% and 73%, respectively. Around 50% of participants faced difficulties at work/school and in their social lives. The highest expectation or priority was the establishment of rare disease-specific diagnosis and treatment centers, accurate and detailed information on diseases in the Turkish language, and easy access to physicians, treatments, and supportive therapies. This is the most comprehensive survey conducted on the rare disease community in Türkiye. These results show that the individuals affected by rare diseases and their families have similar problems and expectations. On the other hand, regional and country-specific issues are still in the line to be solved.

Conflict of Interest: Ugur Ozbek Istanbul Development Agency ISTKA

P24.026.D Ethical and practical challenges of Open Science in human genetics research

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Sharing data, protocols and experiences in human genetics is facing legal restrictions and ethical dimensions regarding personal

or sensitive data. Open Science is a challenge as regards society involvement, data protection, intellectual property rights, research career and research responsibilities. After a period of declarations of principles, recommendations (UNESCO 2021) and institutional positioning, Open Science policies implementation require education, training and practical tools. In this context several initiatives of interest for the human genetics community may help addressing such challenges.

We will present a map of initiatives and tools pertinent for human genetics and helping addressing ethical and practical challenges of Open Science.

Some of these are:

1. the European Open Science cloud, EOSC Future project, funded by the European Commission (EC) has organised with the Research Data alliance (RDA), a network of ambassadors in different domains, one being human genetics and health ethics; various tools are made available;
2. The FAIRplus project has produced a Cookbook with recipes addressing technical and regulatory aspects in making data FAIR and a training online programme
3. National initiatives exist such as the French National Plan for Open Science
4. Groups from international organisations are actively exploring how Open Science activities are valued as research outputs (e.g.RDA-ShARC -Sharing research and credit group).

In the field of human genetics, Open Science may present multiple advantages not only for research and health care but also for society as a whole, provided it is managed carefully.

Grants: https://fairplus-project.eu/ **GA 802750**
EOSC Future project GA 101017536

Conflict of Interest: Anne Cambon-Thomsen funding from European Commision for two grants related to the abtract, advisory board for ethics in several EU funded projects, Ethics evaluation of European projects proposals

P24.027.C Should non-invasive prenatal testing be used for fetal sex determination? Perspectives and experiences from healthcare professionals

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Background: Fetal sex can be accurately determined with non-invasive prenatal testing (NIPT) very early in the pregnancy. However, whether fetal sex should be reported after NIPT is ethically contentious. In Belgium, NIPT is very accessible because it is offered to all pregnant women as a first-tier screening and it is almost fully reimbursed. Fetal sex is reported upon request of the expectant parents.

Methodology: We conducted a semi-structured interview study using reflexive thematic analysis with 33 healthcare professionals in Belgium, representing several specialties (geneticists, obstetricians, midwives, laboratory specialists, counsellors, pediatricians) to assess their experiences with and attitudes towards early fetal sex determination with NIPT in Belgium.

Results: Many healthcare professionals did not consider it problematic to determine and report fetal sex if the expectant parents want to know. Because fetal sex is highly attractive for expectant parents to know, the concern was raised that fetal sex determination with NIPT compromises informed decision making; and that it may distract from the primary aim of NIPT. Other issues participants identified were that a non-medical trait is reported at no extra cost in a public healthcare test-offer and that this information could be used for sex selective TOP. The primary proposed solution for many of the ethical issues addressed by participants was improving pre-test counseling both in terms of quality and availability.

Conclusion: This study can inform the ethical debate as well as the development of policy and guidelines on the expanding scope of NIPT.

Grant: Internal funds KU Leuven

References: -

Conflict of Interest: None declared

P24.028.D Cancer worry and psychological well-being in individuals with Neurofibromatosis 1

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Background/Objectives: Neurofibromatosis 1 (NF1) is a progressive autosomal dominant inherited condition, with variable expression and uncertain prognosis. The condition affects ~ 1 in 3,500 people worldwide and is associated with characteristic skin and other manifestations. Previous studies have demonstrated poorer psychological well-being in individuals with NF1, often in a clinical setting. Increased cancer worry has been reported in women with NF1 attending early breast screening; but little is known about cancer worry in the broader NF1 population. Therefore, the aim of this study was to evaluate cancer worry and psychological well-being in individuals with NF1.

Methods: Individuals with NF1 and controls were invited to participate in an online survey. Questions included an 8-item cancer worry (CWS), 6-item anxiety (STAI-6) and 16-item skin related quality of life (QoL) (Skindex-16) scales.

Results: Fifty individuals with NF1 (40% did not attend an NF clinic) and 40 controls completed the online survey. Compared to controls individuals with NF1 reported increased cancer worry ($p = 0.002$), anxiety ($p = 0.004$) and poorer QoL ($p = 0.001$). Increased cancer worry and poorer QoL correlated with more severe symptoms, with cancer worry also correlated with younger age.

Conclusion: These findings suggest cancer worry is an important concern for many in the wider NF1 community. As adult attendance at NF clinics is low and cancer surveillance is known to be important for young adults with NF1; these results support the need for better awareness and access to NF genetics services and increased psychological support for those with NF1.

Conflict of Interest: None declared

P24.030.B The cost of rejection: an internal audit of the clinical genetics service active triage pathway at CHI Crumlin, Ireland

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Aims: To evaluate the reasons for referral rejection in the triage pathway. To identify the time and cost implications.

Methods: A retrospective analysis of rejected referrals triaged by one consultant was undertaken over an 18 month period (01/

01/2021-30/06/2022). Calculation of costs used data from a previous study.

Results: The consultant rejected 8.3% of referrals. Reasons included: 75% had not included the genetic report (6% of all referrals), 10% were conditions not accepted by our service, 8% redirected to other specialities, 3% given alternative written advice and 4% for other reasons.

Follow-up information was requested on 101/128 (78%) of rejected referrals. 57% of referrers responded; in 43% no response was receive. Median response time was 33 days. Of those who sent back information, 39% remain on waiting-list, 50% attended or were given appropriate advice, 5% did not attend and 4% had alternative follow up.

The estimated timeframe from referral to triage response is 41.5mins/referral. For rejected referrals this equated to 59hrs/year. Our departmental cost for managing repeat referrals is €34.80. Using this as the cost of rejection letter, this costs €4454.40 in 18 months for one consultant or overall €11,878.4/year departmental cost.

Conclusion: The majority of referrals are rejected for non-enclosure of genetic reports. Many referrals would have accepted to the waiting-list otherwise. This means patients are not accessing clinical services because the referrer isn't providing the necessary information to allow triage. Should similar rejection rates exist in other specialities this would equate to a cost of €2,714,214.40/year to the HSE.

Conflict of Interest: None declared

P25 GWAS

P25.001.A Age-specific transcriptional risk scores improve patient stratification and outcome predictions for early-onset acute myeloid leukemia

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Background/Objectives: Early-onset acute myeloid leukemia (AML) (age at initial pathologic diagnosis ≤ 40) represents a unique subtype associated with poor prognosis and relapse risk. We hypothesized that the summation of risk-allele associated gene expression, i.e., a transcriptional risk score (TRS) may identify a prognostic gene expression signature specific to patients with early-onset AML.

Methods: We integrated summary-level GWAS data with eQTL studies to identify candidate causal genes (eGenes) for constructing a TRS for early-onset AML. We evaluated the performance of this TRS in stratifying patients with early-onset AML into risk groups, as well as to predict relapse risk.

Results: We identified eGenes whose expression was linked to risk loci for early-onset malignancies through eQTL analysis. We show that a TRS based on these eGenes was significantly prognostic for early-onset AML cases in a discovery cohort; OHSU-AML, as well as two replication cohorts; TARGET-AML and TCGA-AML. AML cases were stratified by TRS, and patients with a low TRS had significantly better event-free (EFS) and overall survival (OS) compared with patients with a high TRS. Additionally, the TRS stratified patients of intermediate clinical or cytogenetic risk, and low TRS remained an independent positive predictor of EFS and OFS in multivariate Cox model analyses (OS: HR = 0.18 (95% CI = 0.05-0.65), $P = 0.009$, EFS: HR = 0.28 (95% CI = 0.12-0.66), $P = 0.003$).

Conclusion: Together, this work describes novel prognostic gene signatures specific to patients with early-onset AML and may provide insight for the clinical management of these patients.

Conflict of Interest: None declared

P25.002.B GnomADmerging: merge whole genome sequencing of controls with genotyping array of cases to perform genome-wide association studies

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Background: Whole Genome Sequencing data (WGS) are becoming unique genetic resources. Using such extensive genetic data as convenience controls to perform genome-wide association studies (GWAS) increases the overall power. To avoid false positive associations due to distinct genotyping strategies (WGS/arrays), markers have to be carefully filtered. Classical filtering methods are based on the comparison of control allele frequencies between technologies. If cases and controls are genotyped on a different technology, classical methods lead to filtering out disease associated markers. Here, we propose *GnomADmerging* which filters markers without considering the allele frequency differences.

Methods: In *GnomADmerging*, markers are filtered using their gnomAD annotations and classical quality control of genotyping arrays (QC). Two other methods are compared to ours, a method based on chi-square test of allele frequencies (CHISQ) and QC and a method using only QC. Two genotyping array datasets are merged separately with a control WGS dataset, one with cases and controls simultaneously genotyped (Data1) where CHISQ can be applied and one with only cases genotyped (Data2) where CHISQ will discard also real associations.

Results: Applying the QC method on Data1 selects 350,000 markers whereas CHISQ and *GnomADmerging* methods select 280,000 markers. Regarding Data2, QC method selects 310,000 markers whereas *GnomADmerging* selects 250,000 markers. For both datasets, the QC method leads to several isolated GWAS significant markers, likely false positives, whereas CHISQ and *GnomADmerging* discard them.

Conclusion: *GnomADmerging* filters variants as efficiently as the classical filtering method CHISQ. *GnomADmerging* doesn't filter real associations when only cases are genotyped using array.

Conflict of Interest: None declared

P25.003.C Uncovering genes affecting ageing in diverse human populations: a multi-ancestry genome-wide association study

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Background: Identifying genes associated with human longevity can improve our understanding of the fundamental mechanisms of ageing and ageing-related diseases. However, previous genome-wide association studies (GWAS) on ageing have mostly been conducted in individuals of European ancestry.

Method: We used data from 7,398 samples of African, 2,144 East Asian, 7915 South Asian, and 345,666 European ancestry from the UK Biobank. We carried out a GWAS of parental lifespan within a survival analysis framework, followed by a multi-ancestry meta-analysis, to identify novel ageing-related loci across different populations. We also assessed the transferability of established genes associated with ageing in European ancestry groups to African, East Asian and South Asian ancestry groups.

Results: We identified two novel loci (one near *SNTB1*) in the analysis of the African ancestry groups; two novel loci near *CWH43* and *TNKS* in the analysis of the European ancestry group; and three novel loci near *DCUN1D4/LRRC66*, at *STK35* and near *MANBAL/SRC* gene in the multi-ancestry meta-analysis. Our results suggest that the association of rs429358 at *APOE*, the most significantly associated and robustly replicated variant in the European ancestry groups, may not be transferable to African ancestry groups analysed in this study ($P_{het} = 0.021$).

Conclusion: This multi-ancestry GWAS is the first attempt to unveil the genetic architecture of ageing in diverse human populations rather than in a single ancestry group. Our study showed that the inclusion of diverse ancestry groups facilitates the discovery of novel loci associated with ageing.

Conflict of Interest: None declared

P25.004.D Variant-to-function translation of obesity-associated loci through multi-omics data integration

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Background: Genome Wide Association Studies (GWAS) have identified hundreds of loci associated with body mass index (BMI). However, the variant-to-function translation of only a handful of these loci has been successful.

Objectives: Here, we aim to prioritize (causal) genes within BMI-associated loci to ultimately provide new biological insights into obesity pathogenesis.

Methods: We integrated GWAS summary statistics of BMI (Yengo et al, 2018) with multi-omics QTLs, such as gene expression (eQTLs) and protein levels (pQTLs), across different tissues. We performed colocalization analyses to detect shared genetic signals between BMI and -omic traits. We implemented a two-sample Mendelian Randomization approach to assess the causal relationship between gene expression and/or protein levels and BMI.

Results: Cis-e/pQTLs of 916 genes colocalized at 259 of the 536 BMI-associated loci. In 18 of the 259 loci, the same genes colocalized both at the gene expression- and protein-level (e.g., *TTC12* and *LYZ*). Trans-e/pQTLs and/or metabolite-QTLs colocalizing in 181 of the 259 loci pointed to molecular mechanisms. For example, we found that genetic variants in *GLP2R* that associate with higher BMI, were associated with lower gene expression levels of *GLP2R* in brain and adipose tissue, and with lower protein levels of glucagon and higher levels of citrulline in plasma. Furthermore, the protein levels in plasma of 62 genes, such as *SNX1* and *PRKCB*, were causally associated with BMI, which may provide new biomarkers of obesity risk.

Conclusion: We identified candidate genes for regulating body weight within half of the BMI-associated loci identified by the GIANT Consortium.

Conflict of Interest: None declared

P25.005.A Genome-wide structural equation modelling underpins common genetic architecture of kidney function traits

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Background: Identifying molecular targets for chronic kidney disease, a global health issue affecting >10% adults, is challenging due to disease complexity and lack of specific biomarkers. Genomic structural equation modeling (GenomicSEM) has proven successful to unravel genetic loci associated with latent traits underlying multiple biomarkers. To identify kidney-specific genetic variants, we applied GenomicSEM to European-ancestry genome-wide association study summary statistics of four kidney function traits released by the CKDGen Consortium and UK Biobank (n≥343,836).

Methods: We processed ~6 million genetic variants across traits (minor allele frequency≥0.005; imputation quality score≥0.6), estimating pairwise genetic correlations via linkage disequilibrium-score regression. Using the "commonfactorGWAS" function in "GenomicSEM" v0.0.5c, we identified one latent factor (F_kidney) and estimated genetic associations via weighted least squares. Loci were functionally characterized using FUMA v1.5.0.

Results: We identified 195 loci (+/-250kb around the most associated variant) encompassing 1,108 independent variants (LD $r^2<0.6$; $P < 5.0 \times 10^{-8}$) associated with F_kidney: 5 new loci were significantly associated with F_kidney but not with any single biomarker and 39 were significantly associated with all four biomarkers. Gene-set analysis using MAGMA v1.07 showed higher tissue-specific enrichment for genes associated with F_kidney compared to single-biomarker analyses, in the kidney cortex ($P = 2.2 \times 10^{-19}$) and medulla ($P = 5.7 \times 10^{-19}$).

Conclusion: GenomicSEM of multiple kidney traits can help underpin genetic architecture of the unobservable kidney function. Further integration of kidney-specific tissues and deeper biological annotations are warranted to identify relevant molecular targets.

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Conflict of Interest: None declared

P25.006.B Rare CNVs in biologically plausible candidate genes found by WGS in COVID-19 patients

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Background: It is well known that the main pathomechanism in severe COVID-19 is an immune imbalance and systemic inflammatory response. Common polymorphisms and rare variants in the IFN-pathway genes have been identified as predisposing factors. Here, we exploited WGS data to identify rare variants, including SNVs/indels CNVs and SVs, impacting genes involved in biological mechanisms plausibly implicated in COVID-19.

Methods: DNA from 460 COVID-19 hospitalized patients, characterized by varying degrees of severity was collected in the frame of the ORCHESTRA project and high-coverage WGS was performed. WGS reads were processed and variants called using DRAGEN Germline v3.10.4. Variants in the multisample VCFs for SNVs/Indels and SVs/CNVs were functionally annotated and prioritized according to the impacted genes being implicated in the IFN-pathway or associated with the HPO term "recurrent respiratory infections". Finally, we selected variants never described in general population.

Results: In IFN-pathway genes we found heterozygous pLoF variants in IFNAR1 (p.Q329X), IRF7 (p.A177CfsX15) and STAT2 (c.1035-2A>G). In HPO-filtered genes we found deletions ranging from 21 to 126 kb in CD4 (MIM:619238), CD36 (MIM:608404), MAVS (involved in virus-triggered innate immunity) and DNAJB13 (MIM:617091) and duplications (65-535 kb) in RORC (MIM:616622), DOCK8 (MIM:243700) and CSF2RA (MIM:300770).

Conclusion: We did not find any variants that could be considered explicatory of COVID19 severity in genes belonging to the IFN-pathway, but we found CNVs potentially impacting on genes implicated in susceptibility to recurrent respiratory infections. Our findings point to an underinvestigated potential source of genomic variation predisposing to COVID19.

Grant References: H2020-ORCHESTRA G.A. n.101016167

Conflict of Interest: Alessandro Mattiaccio: None declared, Paola Dimartino: None declared, Edoardo Spagnolo: None declared, Alessia Fiorentino: None declared, Valerio Carelli: None declared, Maddalena Giannella: None declared, Pierluigi Viale H2020-Orchestra, Zaira Palacios Baena: None declared, Jesús Rodríguez-Baño: None declared, Marco seri: None declared, Tommaso Pippucci: None declared

P25.007.C Common variants affect cell-free DNA: A genome-wide association study using data from over 100,000 non-invasive prenatal tests

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Cell-free DNA (cfDNA) is a biomarker of increasing importance. Functionally, the biology behind cfDNA is linked to different cell-death mechanisms and auto-immune diseases. Technically, it enables non-invasive screening applications, such as Non-invasive Prenatal Testing (NIPT) and Cancer Liquid Biopsies. In these applications, changes in fragmentation patterns of cfDNA are increasingly used in ever more complex predictive models. However, little is known about how these patterns arise and whether they are affected by genetic variation between samples.

We performed multiple genome-wide association studies to investigate the effect of common genetic variants on plasma cfDNA. For this, we used data from over 100.000 Dutch NIPT screens to impute SNPs and derive concentration and fragmentation properties.

We find that all properties have significant, partially distinct, heritable components. A missense variant in DNASE1L3 (R206C) has the strongest effect on cfDNA. Especially in homozygous carriers of this variant, sequenced fragments were larger, less frequently ended in CC/GG, had lower plasma cfDNA concentrations and were much more likely to receive inconclusive NIPT results due to limited sequencing output. However, DNASE1L3 is not the only genetic determinant of plasma cfDNA as we detect many other genome-wide significant loci. Furthermore, we find significant genetic correlations between our cfDNA traits and disease GWA studies. Finally, for the application of NIPT, we show that all commonly used 'fetal fraction' predictors are affected by these variants and that their accuracy can be improved by a 'personalized' model. We expect that similar results can be obtained for other applications of cfDNA.

Conflict of Interest: None declared

P25.008.D Identification of pathobiologically relevant cell types for male-pattern hair loss

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Male-pattern hair loss (MPHL) is a heritable and prevalent form of hair loss. The phenotype is characterized by a strict androgen-dependency and altered hair cycle dynamics (i.e. shorter growth/prolonged resting). Genome-wide association studies (GWAS) have identified 389 risk loci, implicating numerous genes and pathways. However, the cell types and hair cycle stages in which these genes and pathways exert their effects have not been elucidated.

We applied the single-cell disease relevance score (scDRS) approach, which identifies cells exhibiting excess expression of disease-associated genes. We used MPHL GWAS data (Yap, 2018) with independent scRNA-Seq datasets from (i) the Tabula Muris Senis (TMS) FACS atlas and (ii) the murine hair follicle (HF) during growth and rest (Joost, 2020) to identify MPHL-relevant cell types in both a broad and HF-specific context.

Analysis of the TMS FACS atlas revealed significant excess expression (FDR<0.05) of MPHL-associated genes in 18 cell types. The highest proportion of significant cells was observed in dorsal skin. Further associations included gonadal, marrow and subcutaneous adipose tissues. The analysis of HF-specific data identified nominally significant ($P < 0.05$) associations with 7 cell populations, including (i) root sheath keratinocytes and dermal sheath fibroblasts during hair growth and (ii) the dermal papilla and skin fibroblasts during hair rest. Gene-set analyses identified 191 pathways (FDR<0.05) that seem to be relevant in specific (ferroptosis, adipogenesis) or across hair cycle stages (androgen signalling).

Together, our data provide novel insights into MPHL-relevant cell types and cellular processes and demonstrate the utility of the scDRS approach for GWAS follow-up.

Conflict of Interest: Sabrina Henne: None declared, Nicole Engelmann: None declared, Stefanie Heilmann-Heimbach S.H.-H. is a part-time employee at Life&Brain GmbH.

P25.009.A Pathway-specific analysis of the burden of rare variants in complex phenotypes

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Background: Complex phenotypes are affected by both environmental and genetic factors. Moreover, the underlying genetic factors are difficult to interpret due to power issues at single variant level and because biological effects often arise at pathway-level. Pathway-analysis of the burden of rare deleterious variants can potentially uncover significant biological associations with complex traits.

Methods: Pathway-based scores were generated for each individual using the GenRisk, a tool computing the gene-based burden scores. These scores are the sum of gene-based scores which are normalized by the number of genes in a pathway. Pathway-based gene sets were taken from Human MSigDB Collections. Association analyses and risk prediction models were generated for different complex traits in UK Biobank.

Results: Association analysis identified pathways significantly associated with corresponding phenotypes. For instance, the HDL assembly and plasma lipoprotein assembly pathways were found to be strongly associated with cholesterol levels ($P = 3.71 \times 10^{-12}$ and 1.08×10^{-11} after FDR correction, respectively). Furthermore, alanine aminotransferase levels show significant associations with alanine and aspartate metabolism, and urea cycle associated pathways ($P = 9.25 \times 10^{-31}$ and 9.89×10^{-20} after FDR correction, respectively).

Conclusion: pathway-specific analysis can reveal novel insight on the genetics of complex traits and can help in prioritizing the relevant biological processes associated with a phenotype.

Conflict of Interest: None declared

P25.010.B Uncovering gene-trait associations through non-coding rare variant analysis on 125,075 UK Biobank genomes

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Background: Genome-wide association studies (GWAS) have associated >10.000 common variants with traits, but these fail to account for the entire trait heritability. The study of rare variation promises to be complementary to GWAS in linking genotype to phenotype given their expected stronger effects on phenotypes. However, while the study of rare variation in exonic regions proved instrumental in associating hundreds of genes to human traits, the impact of rare variation in non-coding regions – where most GWAS hits are found – remains unassessed.

Methods: We perform association tests on non-coding rare variants (SKAT-O tests, MAF<1%) regulating >18.000 protein-coding across human blood-related traits using whole genome sequencing data from 125,075 UK Biobank individuals. We evaluate the use of different state-of-the-art gene regulatory annotations in blood cell types including: (1) regulatory regions from promoter capture Hi-C data, (2) cis-regulatory domain predictions based on ChIP-seq data and (3) predicted gene-enhancer associations from multimodal single-cell data.

Results: Across the 3 regulatory annotations, we identified >250 gene-trait associations (>190 distinct genes) among 12 blood traits tested so far. Importantly, ~50% of the gene-trait associations were replicated in rare variant exome studies or GWAS studies. Moreover, >80% of the association were kept when conditioning for known fine-mapped common variants, indicating that rare variants reveal independent signals.

Conclusion: Like rare coding variants, non-coding variation shows to be highly informative of genotype-to-phenotype signals. We expect these analyses to improve our understanding of complex human traits and ultimately aid to develop pertinent therapeutic agents.

Grant References: SNF:PP00P3_176977

Conflict of Interest: None declared

P25.011.C Germline genetic risk factors in breast, prostate, colorectal and skin cancer can be linked to established somatic driver genes through tissue-specific gene regulatory networks

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Background/objectives: Somatic mutations of genes are known to cause and drive cancer, while GWAS of cancer traits indicate genetic loci which are associated with a cancer trait. However, the overlap between known somatic driver genes and positional candidate genes from such loci is surprisingly small. We hypothesized that genes inside susceptibility loci could converge through tissue-specific networks on cancer-specific driver genes.

Methods: Using 300,000 RNA-seq profiles we separately generated gene regulatory networks for 57 different tissues using profiles derived from cancer and non-cancer tissue. We took summary statistics for recent GWAS of breast, prostate, colorectal and skin cancer traits. We subsequently adapted our Downstreamer method to use tissue-specific networks to determine 'key' genes that are co-regulated with other genes that map within different cancer susceptibility loci.

Results: The 'key' genes identified by Downstreamer were strongly enriched for cancer drivers (as defined by COSMIC and IntoGen). Unexpectedly, the enrichment of relevant known cancer driver genes is strongest when performing the Downstreamer analysis using gene regulatory networks derived from non-cancer samples from the relevant tissue of origin.

Conclusion: We show how genetic loci identified by GWAS of cancer traits can be linked to known driver genes through tissue-specific gene regulatory networks, providing an important explanation why seemingly unrelated sets of genes that either harbour germline risk factors or somatic mutations cause the same type of disease.

Grant references: NWO VICI 09150182010019, Oncode Senior Investigator, NWO VENI 9150161910057

Conflict of Interest: None declared

P25.012.D : Using genetics to explore the role of BMI as a shared risk factor in multimorbidity

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Background: Multimorbidity, defined as the co-occurrence of multiple chronic conditions, has become an increasingly important area of research in ageing populations. Observational studies have already highlighted the importance of obesity as a risk factor in multimorbidity. In this work, we use genetics to investigate the role of BMI in the co-occurrence of 48 conditions.

Methods: We leverage existing statistical genetics tools such as GenomicSEM and Mendelian Randomization, to quantify the contribution of BMI to the genetic correlation between each pair of conditions. We develop a novel approach that uses block-jackknife to assess the significance of the attenuation when adjusting for BMI genetics.

Results: We identify several pairs for which the genetic correlation is significantly attenuated after adjusting for BMI genetics. For example, the genetic correlation between osteoarthritis and type 2 diabetes is entirely explained by BMI genetics (rG = 0.22,rG-adjusted = 0.01,p-attenuation = 2e-06), and we observe a two-fold reduction for the genetic correlation between type 2 diabetes and asthma (rG = 0.23,rG-adjusted = 0.11,p-attenuation = 8e-04), as well as sleep apnoea and hypertension (rG = 0.43,rG-adjusted = 0.23,p-attenuation = 2e-05). For all pairs, the hypothesis of BMI causally affecting both conditions and acting as a confounder is supported by significant causal effect estimates in Mendelian Randomization analyses.

Conclusion: While these results confirm the role of obesity as a shared risk factor for several pairs of conditions, most of the genetic correlations remain significant after adjustment. This suggests shared causal pathways beyond BMI and further analyses are needed to adjust for other risk factors and account for bi-directional effects within a pair.

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Conflict of Interest: Ninon Mounier: None declared, Bethany Voller: None declared, Luke Pilling: None declared, Timothy M. Frayling Tim Frayling has received funding from GSK and consulted for Sanofi and Boehringer Ingelheim, Jack Bowden Jack Bowden is a part time employee of Novo Nordisk, engaged in work unrelated to this project

P25.013.A Improvements to the GWAS Catalog user interface to meet growing data volumes

Elliot Sollis[1], *Abayomi Mosaku*[1], *Ala Abid*[1], *James Hayhurst*[1], *Sajo John*[1], *Maria Cerezo*[1], *Peggy Hall*[2], *Elizabeth Lewis*[1], *Santhi Ramachandran*[1], *Daniel Suveges*[3], *Fiona Cunningham*[1], *Lucia Hindorff*[2], *Michael Inouye*[4;5;6], *Laura Harris*[1], *Helen Parkinson*[1]

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Background: The GWAS Catalog is a highly accessed and richly annotated open-source knowledgebase of human genome-wide association studies (GWAS). In February 2023, the Catalog contains data for 70,190 GWAS, including 471,482 curated top associations and 55,228 full summary statistics datasets - a 2-fold increase for GWAS, 1.4-fold for associations and 3.9-fold for summary statistics, over February 2022. While rapidly increasing data volumes show the great success of the GWAS community in generating and disseminating new results, the scale of the data requires improved tools for access and visualisation.

Methods: We identified major requirements of Catalog users, including efficient display of data from large publications, quick access to summary statistics, and flexible visualisation of associations across the genome. We diagnosed obstacles to these goals caused by increased data volumes. An in-depth code review was conducted, followed by iterative software improvements and user testing.

Results: Publications with thousands of GWAS are challenging to visualise and query efficiently. Redesigned interactive data tables now enable efficient loading of the largest publications. As users increasingly seek access to summary statistics, we enhanced their visibility with more prominent links. The GWAS Diagram has become difficult to interpret as it plots ~100,000 data points. We present a redesigned Diagram allowing flexible query, filtering and download options.

Conclusion: The GWAS Catalog interface now handles increased data volumes more efficiently, allowing users to access data quickly and easily. This will also facilitate the inclusion of future large sequencing-based datasets from deeply phenotyped cohorts.

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P25.014.B Genome-wide association study and polygenic risk score analysis of nocturnal enuresis provides novel biological insights

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Background/Objectives: Nocturnal enuresis (NE), commonly known as bedwetting, affects 10-16% of 7-year-old children. We have shown that common genetic variants play a substantial role in NE risk and identified the first two genome-wide significant loci. However, the genetic aetiology of NE remains to be further delineated. Here we present an updated genome-wide association study (GWAS) of nocturnal enuresis using the Danish iPSYCH2015 population-based Case–Cohort sample established to investigate mental disorders.

Methods: NE cases between 5 and 25 years of age were identified based on register information on ICD-10 diagnoses and redeemed desmopressin prescriptions. Controls were the remaining individuals in the same age range. The GWAS was conducted

in unrelated and genetically homogenous individuals (7,971 cases and 65,795 controls) using among others psychiatric diagnoses as covariates. Nine previously generated polygenic risk scores (PRSs) for psychiatric, sleep and metabolic phenotypes were analysed for association with NE.

Results: We identified three loci significantly associated with NE ($p < 5 \times 10^{-8}$). One of these was novel (rs6908136, $p = 4.11 \times 10^{-9}$) mapping three new NE risk genes (*LRRC1*, *FAM83B* and *HCRTR2*) based in both eQTL and chromatin interaction data. Gene set analysis of all NE risk genes mapped ($n = 28$) showed enrichment for genes associated with being a morning person ($p_{adj} = 1.05 \times 10^{-5}$). We further found that PRSs for ADHD, BMI and urine potassium were associated with NE ($p_{adj} < 0.05$). Findings await replication in an independent cohort.

Conclusion: Our study suggests that genetic factors regulating sleep, BMI and electrolyte excretion might be important in NE.

Conflict of Interest: None declared

P25.016.D Genome-wide interaction study with smoking for colorectal cancer risk identifies novel genetic loci related to tumor suppression, inflammation and immune response

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Background: Tobacco smoking is an established risk factor for colorectal cancer (CRC). However, genetically-defined population subgroups may have differentiable susceptibility to smoking-related effects on CRC.

Methods: A genome-wide interaction scan was performed including 33,756 CRC cases and 44,346 controls from GECCO, CORECT and CCFR genetic consortia.

Results: Evidence of an interaction was observed between smoking status (ever vs never smokers) and a locus on 3p12.1 (rs9880919, $p = 4.58 \times 10\text{-}8$), with higher associated risk in individuals carrying the GG genotype (OR = 1.25, 95%CI = 1.20-1.30) compared with the other genotypes (OR = 1.17 for GA; OR = 1.12 for AA). Among ever smokers, we observed interactions between smoking intensity (increase in 10 cigarettes smoked per day) and two loci on 6p21.33 (rs4151657, $p = 1.72 \times 10\text{-}8$) and 8q24.23 (rs7005722, $p = 2.88 \times 10\text{-}8$). Individuals carrying the rs4151657 TT genotype showed higher risk (OR = 1.12, 95% CI = 1.09-1.16) compared with the other genotypes (OR = 1.06 for TC; OR = 0.94 for CC). Similarly, higher risk was observed among individuals carrying the rs7005722 AA genotype (OR = 1.17, 95% CI = 1.07-1.28) compared with the other genotypes (OR = 1.13 for AC; OR = 1.01 for CC). Functional annotation revealed that SNPs in 3p12.1 and 6p21.33 loci were located in regulatory regions, and were associated with expression levels of nearby genes. Genetic models predicting gene expression revealed that smoking variables were associated with lower CRC risk with higher expression levels of CADM2 (3p12.1) and ATF6B (6p21.33).

Conclusions: Our study identified novel genetic loci that may modulate the risk for CRC of smoking status and intensity, linked to tumor suppression and immune response.

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Conflict of Interest: None declared

P25.017.A UGT1A1 is the major locus influencing serum bilirubin levels in Native American ancestry, confirming its pan-ethnic relevance

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Background/Objectives: Bilirubin is the final product of heme degradation. It is conjugated in the liver with glucuronic acid by the UDP-glucuronosyltransferase 1A1 (UGT1A1) enzyme to allow its elimination via bile. It is reported that circulating bilirubin levels are strongly associated with *UGT1A1* gene variants; however, it is unknown whether the Native American ancestry may display a similar pattern or contribute to new gene variations associated with bilirubin.

Methods: We measured total serum bilirubin in 707 adolescents of the Chilean Growth and Obesity Cohort Study (GOCS), in addition to >1.7 million genotypes using the Illumina Multi-Ethnic

Global Array (MEGA). Subsequently, we constructed a local ancestry reference panel with participants from the 1000 Genomes Project, the Human Genome Diversity Project, and the GOCS cohort. We inferred haplotype tracts of Native American origin to perform an ancestry-specific GWAS.

Results: We found that *UGT1A1* variants, including the rs887829 variant upstream *UGT1A1*, are the unique signals achieving genome-wide statistical significance, both in the entire GOCS (beta = 0.30; p-value = 3.3×10^{-57}) and in the stratified cohort according to Native American ancestry (beta = 0.35; p-value = 1.2×10^{-16}). This variant explained 37.6% of the variation of bilirubin levels in the Native American ancestry, and TT carriers averaged 4-fold higher bilirubinemia than those carrying the CC genotype (p-value = 2.82×10^{-12}).

Conclusions: Our results indicate for the first time that UGT1A1 is also the main regulator of bilirubin levels in Native American ancestry and confirm its pan-ethnic relevance.

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Conflict of Interest: None declared

P25.018.B Phenome-wide association studies of copy number variations in UK Biobank whole genomes

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DNA copy number gains and losses have profound functional consequences in human population diversity and genetic disorders. Previous phenome-wide association studies (PheWAS) using microarray-based and WES-based CNVs have revealed many clinically important genotype-phenotype associations. However, due to technology-specific limitations (e.g., microarray-based CNVs have poor resolution and sensitivity, whilst WES-based CNVs only cover the coding regions), the full potential of CNV analysis in understanding the causes of human diseases is yet to be explored. To advance human genomics research, UK Biobank conducted whole genome sequencing of all ~500K participants, which is the world's largest whole genome sequencing project. WGS data have much better coverage than array and WES data and outperform them in CNV detection with unprecedented higher accuracy. In the present study, we first called CNVs from ~500K UKB WGS and then performed bespoke post-hoc filtering and re-genotyping to generate a high quality CNV call set for downstream analysis. We systematically characterised the CNV landscape in the genome and conducted CNV PheWAS analysis using ~16K binary and ~1400 quantitative phenotypes. Through different genetic models, we detected novel associations in coding and noncoding regions, in addition to well-known associations including 16p11.2 deletions with obesity and 22q11.2 deletions with the risk of malignancy. Our study not only generates and characterises the largest WGS-based CNV call set, a rich resource for further functional and mechanistic investigations on CNVs, but also presents the largest phenome-wide survey of dosage-sensitive regions in the human genome.

Conflict of Interest: Xueqing Zou AstraZeneca, AstraZeneca, Fengyuan Hu AstraZeneca, AstraZeneca, Oliver Burren AstraZeneca, AstraZeneca, Xiao Jiang AstraZeneca, AstraZeneca, Santosh Atanur AstraZeneca, AstraZeneca, Samuel Lewis AstraZeneca, AstraZeneca, Katherine Smith AstraZeneca, AstraZeneca, Quanli Wang AstraZeneca, AstraZeneca, Slavé Petrovski AstraZeneca, AstraZeneca, Keren Carss AstraZeneca, AstraZeneca

P25.019.C Genome-wide meta-analysis identifies novel loci conferring risk of acne vulgaris

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Introduction: Acne vulgaris (acne) is a common skin disorder characterized by comedones and inflammatory lesions on the face and upper torso. The multifactorial pathogenesis of acne is not fully understood. Genome-wide association studies (GWAS) and acne-associated single-gene disorders and syndromes highlight processes occurring during sebaceous gland (SG) maturation with links to the tissue microenvironment and remodeling. This study aimed to ascertain novel genetic loci predisposing to acne development.

Materials and methods:

We performed GWAS in Estonian Biobank cohort (30,194 cases, 94,694 controls), followed by meta-analysis with two independent cohorts - FinnGen and Lifelines, comprising altogether 34,422 cases and 364,991 population controls of European ancestry. Functional characterization of the new loci was carried out with FUMA and polygenic risk was calculated by PRS-CS-auto.

Results: We identified four novel genome-wide significant loci: 11q12.2(*FADS2*); 12q21.1(*LGR5*); 17q25.3(*FASN*); and 22q12.1(*ZNRF3*) and replicated 19 previously known loci in Europeans, bringing the total number of acne susceptibility loci to 50. All common SNPs across the genome explained 9.4% of the phenotypic variance (SNP-based heritability) in acne liability. Individuals in the top 5% percentile of polygenic risk scores had their acne risk elevated by 1.62 compared to individuals with average risk (20-80%).

Conclusion: The identification of novel acne susceptibility genes belonging to Wnt signaling pathway, known determinant of stem and progenitor cell differentiation during hair follicle morphogenesis and regeneration, highlights imbalanced SG homeostasis in acne pathogenesis. The detection of loci, containing genes, which encode key enzymes in lipid metabolism, emphasizes the etiological link of acne to metabolic diseases.

Conflict of Interest: None declared

P25.020.D Puzzling out the genetic landscape of Hearing Function (HF): a combined approach of Genome-Wide Association Studies (GWAS) and Transcriptome-Wide Association Studies (TWAS)

Aurora Santin[1], *Stefania Lenarduzzi*[2], *Giuseppe Giovanni Nardone*[1], *Beatrice Spedicati*[1;2], *Anna Morgan*[2], *Mariarosaria Persichillo*[3], *Amalia De Curtis*[3], *Simona Costanzo*[3], *Alessandro Gialluisi*[3;4], *Licia Iacoviello*[3;4], *Paolo Gasparini*[1;2], *Maria Pina Concas*[2], *Giorgia Girotto*[1;2]

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Background: To date, little is known about the genetic bases of HF. Deepening the genetics of HF is relevant not only to detangle the molecular mechanisms of the hearing system, but also to identify novel genes involved in hearing loss (HL) complex forms. Although several GWAS have been performed, a lot of work still needs to be done.

Methods: Three Italian cohorts (1887 individuals) with available audiometric and genotyping data were included in this study. GWAS meta-analyses were performed with METAL software to investigate HF as a set of nine quantitative traits, as previously reported. TWAS were conducted with FUSION software based on meta-analysis summary statistics and Genotype-Tissue Expression data in nine brain tissues.

Results: Meta-analyses results allowed the identification of ~190 genes across all nine traits, reaching suggestive and significant *p*-values. In particular, the most interesting results include:

SLC1A6 ($p < 10^{-9}$), encoding a glutamate transporter expressed in the brain. Changes in glutamate-related genes expression are involved in HL mechanisms.

ASTN2 ($p < 10^{-9}$), which encodes a brain protein previously associated with HL.

ITGBL1 ($p < 10^{-6}$), which is expressed in the surrounding cells of the murine inner ear.

Preliminary TWAS results revealed additional genes significantly associated with HF after Bonferroni correction, including *ERCC3*, a DNA helicase ($p < 10^{-6}$). Variants within *ERCC3* were already associated with HL.

Conclusions: For the first time, genome-wide significant genes have been associated with HF and a TWAS study was carried out. These findings will significantly contribute to deepen the genetic architecture of HF and related complex disorders.

Conflict of Interest: None declared

P25.021.A A genome-wide association study of hospitalization for pneumococcal community-acquired pneumonia

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Background/Objectives: Pneumococcal community-acquired pneumonia (P-CAP) is a lower respiratory tract infection with a high hospitalization rate. Host genetics plays a critical role in susceptibility and immune response of infectious diseases. Here, we conducted a genome-wide association study (GWAS) to identify loci associated with P-CAP hospitalization.

Methods: The GWAS included 3,765 Spanish individuals, 257 P-CAP patients and 3,508 population controls. We tested the association of 7.6 million of imputed genotypes using a logistic regression model. Subsequently, we prioritized genes based on Bayesian fine mapping and functional evidence using the Variant-to-Gene (V2G) scores.

Results: Six independent variants were genome-wide significant ($p = 5 \times 10^{-8}$), three on 6p21.32, and one for each of the chromosomes 4q28.2, 11p12, and 20q11.22. We prioritized three genes: *C4orf33*, *TAPBP*, and *ZNF341*. No gene was prioritized for 11p12.

Conclusions: We completed the first GWAS of P-CAP hospitalization and identified new susceptibility loci. Deficiencies of *TAPBP* and *ZNF341* were previously described as inborn errors of immunity predisposing to bacterial pneumonia.

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Conflict of Interest: Eva Suarez-Pajes full, Itahisa Marcelino-Rodriguez full, Elisa Hernández Brito full, Eva Tosco-Herrera full, Luis A. Rubio-Rodríguez full, Silvia Gonzalez-Barbuzano: None declared, Melody Ramirez- Falcon full, María Luisa Briones full, Olga Rajas full, Luis Borderías full, Jose Ferreres full, Antoni Payeras full, Leonardo Lorente full, Javier Aspa full, NIEVES CARBONELL full, Jordi Freixinet full, Felipe Rodríguez de-Castro full, Jordi Solé Violán full, Carlos Rodríguez-Gallego full, Carlos Flores full

P25.022.B Assessing univariate facial phenotyping approaches in GWAS

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While human facial shape is highly heritable, it is also complex, which makes elucidating its genetic underpinnings heavily

dependent on a genetically relevant phenotypic description. Simplifying complex 3D facial features into biologically meaningful univariate traits is a frequently used strategy to increase power. Common univariate traits include inter-landmark measurements (i.e., linear distances) and scores resulting from dimension reduction techniques (e.g., principal component analysis or via deep learning approaches). However, no studies have formally investigated the biological value of these alternative phenotypic descriptions. Here, we compare SNP-based heritability and GWAS results of different univariate facial traits with 8,426 individuals. Additionally, we evaluate the performance of the proposed novel phenotyping method, where each face in the dataset is scored with respect to the direction to randomly selected faces, extreme faces, or syndrome average faces. Inter-landmark distances demonstrated the highest mean heritability, followed by the latent scores of an autoencoder; which is a dimension reduction technique using deep learning. Principal components and likeness to randomly selected faces were similar and significantly less heritable than the above-mentioned phenotypes. Interestingly, likeness to extreme and syndromic faces exhibited similar distributions of heritability and were overall least heritable, suggesting that they have a lower relative contribution of common genetic variants. Furthermore, more independent genetic loci were revealed when multiple GWASs of likeness traits based on randomly selected faces were aggregated. Our results suggest that likeness to randomly selected faces, which is a simple phenotyping method, shows the potential to capture genetically relevant shape variation in faces.

Conflict of Interest: None declared

P25.023.C Genomic and transcriptomic data analyses highlight KPNB1 and MYL4 as novel risk genes for congenital heart disease

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Congenital heart defects (CHD) are structural defects of the heart affecting approximately 1% of newborns. CHDs exhibit a complex inheritance pattern. While genetic factors are known to play an important role in the development of CHD, relatively few variants have been discovered so far and very few genome-wide association studies (GWAS) have been conducted. We performed a GWAS of general CHD and five CHD subgroups in FinnGen followed by functional fine-mapping through eQTL analysis in the GTEx database, and target validation in human induced pluripotent stem cell - derived cardiomyocytes (hiPS-CM) from CHD patients. We discovered that the MYL4-KPNB1 locus (rs11570508, beta = 0.24, $P = 1.2 \times 10^{-11}$) was associated with the general CHD group. An additional four variants were significantly associated with the different CHD subgroups. Two of these, rs1342740627 associated with left ventricular outflow tract obstruction defects and rs1293973611 associated with septal defects, were Finnish population enriched. The variant rs11570508 associated with the expression of MYL4 (normalized expression score (NES) = 0.1, P = 0.0017, in the atrial appendage of the heart) and KPNB1 (NES = -0.037, P = 0.039, in the left ventricle of the heart). Furthermore, lower expression levels of both genes were observed in human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) from CHD patients compared to healthy controls. Together, the results demonstrate KPNB1 and MYL4 as in a potential genetic risk loci associated with the development of CHD.

Conflict of Interest: None declared

P25.024.D Parent-of-origin effects on childhood asthma

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Background/Objectives: The risk of childhood asthma is higher if the mother is asthmatic than if the father is. An understudied hypothesis is parent-of-origin (PoO) effects, which occur when the effect of a variant allele in a child depends on whether it was transmitted from the mother or the father. Our aim was to estimate PoO effects on childhood asthma at 7 years of age using the Norwegian Mother, Father, and Child Cohort Study.

Methods: To estimate PoO effects on childhood asthma across the whole genome, Poisson log-linear regression, implemented in the HAPLIN *R* package, was fit to the genotype data of 915 mother-father-child case trios, 603 mother-child case dyads, and 113 father-child case dyads.

Results: Variants at two SNPs, rs3003214 and rs3003211, near *ADSS2* showed significant PoO effects on childhood asthma at the false positive rate of 0.05. When the effect allele G at rs3003214 was maternally inherited, the relative risk of childhood asthma was 1.37 (P = 1.71E-05). In contrast, when the same allele was passed on from the paternal side, the relative risk was 0.81 (P = 4.65E-03). Together, the ratio of the two relative risks was 1.68 (P = 1.13E-08).

Conclusion: We identified genetic variants with significant PoO effects on childhood asthma, providing statistical evidence that genomic imprinting may modify heritable effects on asthma.

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P25.025.A genome wide association study of head and neck cancer

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Background/Objectives: HEADSpAcE is a comprehensive study bring together more than 65,000 participants from Europe, South America, and Asia with the aim to identify new susceptibility loci associated to head and neck cancer risk within European and diverse population. This report summarizes the preliminary results obtained from participants with European ancestry.

Method: TOPMed was used as the imputation reference panel providing information on up to 300 million genetic variants. Population structure was determined by principal component analysis (PCA) and ADMIXTURE analysis. Among all included participants, 50,514 participants-including 15,572 cases and 34,942 controls-were identified as having European ancestry. GWAS was performed after adjustment for sex and the 10 first principal components.

Results: We identified 7 genomic risk loci including a potential novel variant on chromosome 17 (rs78378222) in the untranslated 3' region of TP53 at suggestive significance ($P < 5.0 \times 10^{-8}$), in addition to other genetic risk loci in ALDH2 (4q23), BRCA2 and HLA (6p22) region that previously shown to be linked with head and neck cancer

Conclusion: To the best of our knowledge, this study is the largest head and neck cancer genome wide association study to date and these early results show exciting evidence to continue efforts utilizing larger population in identifying risk loci missed by genetic association studies so far.

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Conflict of Interest: None declared

P25.026.B Deciphering colorectal cancer genetics through multi-omic analysis of 100,204 cases and 154,587 controls of European and East Asian ancestries

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Colorectal cancer (CRC) is a leading cause of mortality worldwide. We conducted a genome-wide association study meta-analysis of 100,204 CRC cases and 154,587 controls of European and East Asian ancestry, identifying 205 independent risk associations, of which 50 were unreported. We performed integrative genomic, transcriptomic and methylomic analyses across large bowel mucosa and other tissues. Transcriptome- and methylome-wide association studies revealed an additional 53 risk associations. We identified 155 high confidence effector genes functionally linked to CRC risk, many of which had no previously established role in CRC. These have multiple different functions, and specifically indicate that variation in normal colorectal homeostasis, proliferation, cell adhesion, migration, immunity and microbial interactions determines CRC risk. Cross-tissue analyses indicated that over a third of effector genes most likely act outside the colonic mucosa. Our findings provide insights into colorectal oncogenesis, and highlight potential targets across tissues for new CRC treatment and chemoprevention strategies.

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P25.027.C Largest genome-wide meta-analyses identify novel risk loci for nonsyndromic cleft lip with/without cleft palate

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Non-syndromic cleft lip with/without cleft palate (nsCL/P) has a multifactorial background and a heritability of >90%. GWAS have identified more than 40 risk loci, which explain about 30% of its heritability. It is expected that additional loci will be identified if the number of samples used in GWAS increases and/or if different population backgrounds are included.

Here we present the largest meta-analysis to date of nsCL/P-GWAS. We combined nine individual GWASs, three of them previously unpublished. The new studies included individuals of Mesoamerican origin, from Yemen, and a large multi-ancestry case-control cohort collected by 23andMe, Inc.. Meta-analyses were performed separately for Europeans (3,586 cases, 206,240 controls and 955 trios), and over all populations (5,324 cases, 270,402 controls, 2,699 trios).

In total we identified 41 and 61 genome-wide significant regions in the European and multiethnic analyses, respectively. Most of the identified regions were previously known, but we have identified 28 new risk regions. Initial analyses of the new association signals identified *FBN2* as a novel candidate gene (chr. 5q23.3, lead variant rs968008, p-value 3,034e-9 in Europeans), previously identified as causative of a marfanoid phenotype with a highly arched palate. Furthermore, we identified risk variants located in the 22q11.2 microdeletion syndrome region (lead variant rs165849, p-value 1,27e-10, maps to regulatory active regions in embryonic stem cells), with a potential regulatory effect on *ARVCF*.

Our study is the basis for clarifying metabolic pathways and gene-gene/environment interactions, and contributes to the clarification of nsCL/P etiology. Ultimately, this is necessary for improved counseling and prevention.

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P25.028.D Genome-wide analysis of short tandem repeat (STR) variation identifies expansions in the leucine aminopeptidase 3 gene (LAP3) associated with idiopathic pulmonary fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is an incurable lung disease characterised by progressive scarring of the interstitium. IPF has a prevalence of between 3 and 60 cases/100 000 with a median survival time of 3 years and is more common in older individuals. Genome-wide association studies have identified more than a dozen genetic variants that implicate genes involved in host defence pathways, telomere maintenance, TGFbeta and mTOR signaling. However, the disease etiology remains incompletely understood.

Short Tandem Repeats (STRs) are tandemly-repeated simple sequence motifs, typically 2-6 bp in size. STRs are highly polymorphic, and are known to cause Mendelian disease, affect gene expression. Their contribution to common disease is not well-understood.

Here we conduct a case-control study using whole genome sequences from 507 IPF cases and 174 control individuals of recent European ancestry, sequenced at ~30x depth using Illumina 150bp paired-end reads, using a PCR-free protocol. We used GangSTR to genotype ~174000 common polymorphic STRs with allele frequency >1% in all samples, 25144 passed quality thresholds (call-rate >=99%) and were used for associations. We discovered 15 intergenic STRs associated with IPF ($p < 2 \times 10^{-6}$) with 7 at genome-wide significance ($p < 5 \times 10^{-8}$). ExpansionHunter Denovo was used to identify rare STR expansions enriched in cases compared to controls across the genome. We detected an STR expansion in the 5′ untranslated region of the leucine aminopeptidase gene (LAP3) significantly enriched in IPF cases ($p < 3.6 \times 10^{-4}$), odds ratio (7.21; 3.38 -15.35).

Pending replication and further mechanistic investigation, this finding represents a new insight into the genetic basis of IPF.

Conflict of Interest: None declared

P25.029.A Unravelling the genetic component of COVID-19 severity in the Italian population by exome-wide analyses

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Background/Objectives: Symptoms associated with coronavirus disease 2019 (COVID-19) hinge on both virus and host characteristics, with host age, comorbidity, male sex, and genetics associated with its severity. Different studies elucidated the role of common variants, while the contribution of rare variants remained unclear. We aim to determine the contribution of rare genetic variants in COVID-19 severity in the Italian population.

Methods: We performed whole-exome sequencing on 215 severe COVID-19 patients and compared their genetic signature with 1755 individuals from the general population. Germline variants were identified and classified in groups based on their pathogenicity. We used an in-house modified version of the EPACTS software and a burden test design to test rare variant contribution to COVID-19 severity.

Results: We set up a reproducible bioinformatic pipeline for rare variants analysis with a modified version of EPACTS. When testing rare variants collapsed by biological process, "glycolysis" and "reactive oxygen species" were among the top results ($P < 9 \times 10^{-7}$ and $P < 0.001$, respectively). When testing rare

variants collapsed by gene and grouped by their predicted functional impact, we found a total of 66 genes with an experiment-wide significant association ($P < 10^{-5}$).

Conclusion: Our work provides a new insight about the hosts genetic signature associated with COVID-19 severity in the Italian population. We aim to integrate these results with bulk-RNA seq and common variants analyses on patients from the same cohort. Overall, these results can link up with other genetic information to define individuals' risk profiles for COVID-19 severity for the Italian population.

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P25.030.B Dissecting the genetic relationship between insulin resistance and adiposity

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Background/Objectives: Insulin resistance (IR) is closely linked to the development of hyperglycemia and type 2 diabetes. Previous genome-wide association studies (GWAS) identified 53 loci by combining three hallmark traits of IR: fasting insulin adjusted for body mass index (FI_{adjBMI}), HDL cholesterol and triglycerides. By adjusting for BMI, this analysis favored identifying IR loci negatively associated with adiposity. Thus, we aimed to identify novel IR loci with FI and FladjBMI to differentiate between BMI-dependent and independent genetic mechanisms.

Methods: To identify IR loci we, first, triangulated GWAS data for FI, HDL cholesterol and triglycerides, then, with FI_{adjBMI}. We computed three polygenic risk scores (PRS) using loci shared by FI and FI_{adjBMI} and those exclusive for each analysis.. To uncover the underlying biology, we performed a tissue enrichment analysis.

Results: We identified 50 and 155 loci exclusive to FI and FI_{adjBMI}, respectively, and 55 shared between the two. PRS analysis showed that the FI_{adjBMI}-exclusive loci were associated with a decrease in gluteofemoral fat and BMI, while the FI-exclusive were associated with an increase in both. The shared loci showed a decrease in gluteofemoral fat, but no association with BMI. The tissue enrichment analysis revealed that FI-exclusive loci were enriched in adipose tissue, the FI_{adjBMI}-exclusive loci in the adrenal glands, and shared were enriched in 12 different tissue sets (FDR<0.05).

Conclusion: IR loci encompass distinct subgroups with varying effects on BMI and fat distribution. In further analyses, we will delve into their biology, identifying the causal mechanisms behind them.

Grant References: None.

Conflict of Interest: None declared

P25.031.C Managing EHR diagnosis coding bias using DRG-adjusted GWAS

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Background: Diagnosis related groups (DRGs), a system based on International Classification of Disease (ICD) codes, are employed globally to classify hospital cases, and determine reimbursement rates. DRGs can cause bias in diagnoses if there are tendencies to assign more expensive codes due to reimbursement incentives. Diagnosis bias in registries, such as the Danish National Patient Registry, has the potential to confound genetic associations in genome-wide association studies (GWAS) where phenotyping relies on registry data. In this study, we investigate the impact of confounding from DRG-coding bias in GWAS of up to 284,000 patients from the Copenhagen Hospital Biobank.

Methods: We analysed DRG-coding bias in electronic health records from all in-hospital patients in Denmark from 2008 until 2016. We then defined the assigned ICD-10 diagnoses as the control phenotype and clustered the DRG-correlated diagnosis as the "corrected" phenotype. The individuals' genetic information was obtained through whole genome sequencing or genotype imputation. GWAS was performed using REGENIE. We assessed the performance difference between the phenotypes by analysing the effect size and p-value of significant associations.

Results: We identified 152 pairs of ICD-10 codes impacted by potential DRG-coding bias. Results comparison revealed differences between the adjusted versus not-adjusted GWAS.

Conclusion: Removing bias and improving the performance of GWAS has the potential to significantly impact our understanding of the genetic basis of complex diseases and traits leading to advances in prevention, diagnosis, and treatment.

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P25.032.D The genetic structure of human complex traits in Southern Italy

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Background: The reservoir of human genetic diversity in Southern Europe has been historically undersampled due to a paucity of large-scale genomic initiatives. In Italy, complex demographic events and a unique geography contributed to high levels of genetic heterogeneity. To characterize factors affecting the architecture of biomedical traits and diseases, we carried out the largest to date genomic survey in the Moli-sani study, a prospective cohort of approximately 25,000 people (age ≥ 35, 49.3% men), recruited from city hall registries in the Molise region.

Methods: We newly profiled genome-wide SNPs and used them to (i) address genetic structure vis-a-vis other Italian and European populations; (ii) describe genetic contributions to over fifty biomedical traits relevant for chronic-degenerative diseases; (iii) assess the portability of polygenic risk scores (PRS) estimated from other European populations in South Italy.

Results: We describe genetic sub-structuring within the cohort. We characterize known and new genetic loci influencing complex traits. While our results confirm a broadly similar architecture for many traits, we estimate that PRSs, on average, explain ~5% less trait variance than other Northern European populations, underlying the importance of exploring unique genetic contributions in these areas.

Conclusions: The genetic profiling of the Moli-sani cohort - coupled with its large size, depth of phenotyping, and prospective design - provides a unique resource to study genetic contributions to common diseases and traits, and the extent to which these are modulated in the context of South Italy's unique history and environments.

Conflict of Interest: Federica Santonastaso full, Simona Costanzo full, Chiara Chiereghin full, Alessandro Raveane full, Edoardo Giacopuzzi full, Davide Bolognini full, Alessandro Gialluisi full, Amalia De Curtis full, Emanuele Di Angelantonio full, Giovanni de Gaetano full, Maria Benedetta Donati full, Nicola Pirastu full, Licia Iacoviello full, Nicole Soranzo full

P25.033.A SURFBAT: a surrogate family-based association test building on large imputation reference panels

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Background/Objectives: Genotype-phenotype association testing studies often rely on existing panels of control individuals from the general population and typically require adjustment for population stratification and/or matching of case and control individuals based on ancestry.

Methods: Here, we develop a method that allows for large reference panels (typically designed for imputation) to serve as control groups with a streamlined adjustment for local ancestry patterns. SURFBAT (a SURrogate Family Based Association Test) performs an approximation of the transmission-disequilibrium test by creating a pseudo-control for each case individual using the haplotype matching algorithms of leading imputation software. The method is suitable when the control panel spans the ancestry spectrum of the case-group population and each control has similar paternal and maternal ancestries. This is the case for recently developed panels in France where individuals were recruited based on grand-parent birthplace data.

Results: We demonstrate the efficacy of our method on simulated data involving 856 individuals with whole-genome sequencing data from France as well as on a real case-control scenario for Brugada syndrome. We show that SURFBAT improves over traditional genome-wide association methods by providing a test inherently robust to fine-scale population stratification. In contrast to alternative methods, SURFBAT does not require a set of pre-defined ancestry groups, nor for local ancestry to be explicitly estimated.

Conclusions: SURFBAT opens up the possibility of efficiently using large imputation reference panels as control groups for association testing. It will soon be incorporated into the imputation software IMPUTE5.

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Conflict of Interest: None declared

P25.034.B Polygenic risk scores predict the onset of 8 diseases better than environmental and clinical risk factors

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Background/Objectives: Polygenic Risk Scores (PRS) have emerged as a promising tool to guide the screening and treatment of complex diseases. However, systematic comparisons with non-genetic risk factors are lacking. Here, we compare the ability of PRS to predict the onset of 18 diseases in FinnGen (N = 342,499). We compared PRS with predictors age, sex, education, general morbidity risk (Charlson index, CCI), and Phenotype Risk Scores (PheRS) capturing disease risk from electronic health records (EHR).

Methods: PRS were calculated using MegaPRS and the latest publicly available genome-wide association study summary statistics. We calculated PheRS using elastic-net models, incorporating up to 287 diagnoses from EHR between the years 1999 to 2009 as predictors. We then fitted separate Cox proportional hazards models on a test set to predict disease onset from 2011 to 2019.

Results: The predictive ability of the model (c-index) with all predictors ranges from 0.6 for Acute Appendicitis to 0.85 for Atrial Fibrillation. For 8 diseases, integrating the PRS significantly increases our ability to predict disease onset over the baseline model with age, sex, CCI, and education. For 5/8 of these diseases, PRS also outperforms PheRS. However, notably for Asthma and Knee Osteoarthritis the PheRS is more predictive.

Conclusion: Overall, both PRS and PheRS add predictive power over commonly used predictors - such as age, sex, and CCI - with the PRS outperforming the PheRS for most diseases. As part of the INTERVENE project, we aim to replicate these findings in other biobanks.

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Conflict of Interest: None declared

P25.035.C Characterization of HLA alleles and improved HLA imputation in a Quebec COVID-19 population

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Associations have recently been reported between HLA alleles and susceptibility or severity of COVID-19 infection. We characterised HLA alleles in individuals from Quebec, Canada, and examined their role in COVID-19 severity. We typed HLA alleles in 2,074 sequenced participants (1,366 European, 210 Middle Eastern, 156 African, 116 East Asian, 98 Central-South Asian, and 77 American) from the COVID-19 Quebec Biobank (BQC19). We identified 403 HLA alleles, 347 of which were in the large multi-ancestry reference panel HLA-TAPAS (N = 21,546). Twenty-three alleles (6 in Europeans and 17 in East Asians) had significantly different frequencies from HLA-TAPAS (P-value threshold $< 14 \times 10^{-5}$). We performed ancestry-specific logistic regressions (covariates: sex, age, 5 PCs) to test for association of HLA alleles with COVID-19 severity and hospitalisation, followed by a multi-ancestry meta-analysis. No associations reached the

Bonferroni-corrected P-value < 1.2×10^{-4}, but HLA-A*32:01 showed evidence of a protective effect against hospitalisation in African, European and Middle Eastern individuals (beta = -0.83 (0.25), P-value = 8.6×10^{-4}). This replicates previous evidence suggesting a protective effect by HLA-A*32:01 against COVID-19 infection in Europeans. HLA-A*32:01 has also been associated with vancomycin-induced drug reactions in Europeans; further investigation into potential confounders is needed. We also constructed a Quebec HLA imputation panel. Our panel correctly imputed, on average, 13.93 of 16 HLA alleles per sample, compared to 13.92 per sample by the HLA-TAPAS panel. Our meta-imputation approach improved this value to 14.3, demonstrating the importance of population-specific reference panels in genotyping-based cohorts.

Conflict of Interest: None declared

P25.036.D Alternative genetic models in IPF susceptibility genome-wide association studies to improve power and accuracy

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a rare chronic progressive lung disease with high mortality and few treatment options. Genome-wide association studies (GWAS) based on an additive genetic model have identified multiple signals of association and highlighted new genes and pathways of interest. We hypothesised that analyses using different genetic models may identify new IPF susceptibility signals where the underlying causal effects are consistent with a dominant or recessive genetic model.

Methods: We conducted GWAS using dominant and recessive genetic models with data from 5,159 IPF cases and 27,476 controls, from seven independent studies imputed using the TOPMed WGS reference panel. We selected signals which met genome-wide significance ($P < 5 \times 10\text{-}8$) and for which results were present in at least two studies. We compared significant signals from the dominant and recessive models with results from an additive model GWAS using the same seven IPF studies.

Results: We obtained 58 genome-wide significant ($P < 5 \times 10\text{-}8$) signals using the dominant model and 25 for the recessive model. Out of those, a total of 14 dominant model signals and 4 recessive model signals were new (not genome-wide significant using an additive model), including 2 recessive signals in exons of *EPN3* and *PMF1*.

Conclusion: The use of dominant and recessive genetic models in GWAS of IPF susceptibility allowed to identify new signals that were not reported at genome-wide significance under an additive model using the same data. These could provide additional insight into the biological mechanisms underlying IPF.

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P25.037.A Genetic landscape of the ACE2 coronavirus receptor

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SARS-CoV-2, the causal agent of COVID-19, enters human cells using the ACE2 (angiotensin-converting enzyme 2) protein as a receptor. ACE2 is thus key to the infection and treatment of the coronavirus and plays important regulatory roles in the cardiovascular and other biological systems. However, the genetic basis of the ACE2 protein levels is not well understood. We conducted the largest genome-wide association meta-analysis of plasma ACE2 levels in >28000 individuals of the SCALLOP Consortium (Systematic and Combined Analysis of Olink Proteins). We identified 10 loci, including 8 novel, capturing 30% of the heritability of the protein. We found significant genetic correlations between ACE2 and various human diseases and traits, including some vascular diseases, severe COVID-19, and certain medications. The silico functional analysis was performed by integrating with other types of omics data. Mendelian randomization analysis of soluble ACE2 on vascular disease outcomes and COVID-19 severity suggested a causal effect of elevated ACE2 levels on COVID-19 severity (odds ratio, 1.63; P = 0.01), hospitalization (odds ratio, 1.52; P = 0.03), and infection (odds ratio, 1.60; P = 0.02). Human plasma ACE2 shares a genetic basis with cardiovascular disease, COVID-19, and other related diseases. The genetic architecture of the ACE2 protein is mapped, providing a useful resource for further biological and clinical.

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P25.038.B Genetic variants associated to type 2 diabetes modulate endocrine enhancers in vivo

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Several type2diabetes (T2D) associated genetic variants overlap with putative endocrine enhancers, suggesting that these variants might impair enhancer activity and consequently, gene expression, resulting in an increased risk of T2D development. We have performed in vivo mosaic transgenesis assays in zebrafish to test the endocrine enhancer activity of human sequences overlapping with T2D associated loci. In an update of a previous report(PMID:32912862), we expanded the number of tested sequences to 35 pairs of T2D risk and non-risk sequences. Seventeen(17) pairs have shown a difference on endocrine enhancer activity when comparing T2D risk and non-risk variants. For seven(7) pairs, the T2D risk variant has shown a decreased enhancer activity, while in the remaining ten(10) pairs, the T2D risk variants have shown increased enhancer activity. One of the latter(rs13266634) is in an *SLC30A8* exon, encoding a tryptophan-to-arginine substitution that decreases SLC30A8 function, which is the canonical explanation for T2D risk association. However, other T2D–associated SNPs that truncate SLC30A8 confer protection from this disease, contradicting this explanation. Here, we clarify this incongruence, showing that rs13266634 boosts the activity of an overlapping enhancer and suggesting an SLC30A8 gain of function as the cause for the increased risk for the disease. We further dissected the functionality of this enhancer, finding a single nucleotide mutation sufficient to impair its activity. Overall, this work assesses in vivo the importance of disease-associated SNPs in the activity of endocrine pancreatic enhancers, including a poorly explored case where a coding SNP modulates the activity of an enhancer.

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Conflict of Interest: None declared

P25.039.C Targeted analysis of rare variant sets within non-coding regulatory regions using whole genome sequence data

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Introduction: The release of high-depth whole-genome sequence (WGS) data by UK Biobank presents a novel opportunity to explore rare variant associations at non-coding regions. Due to the size of the data, a non-targeted approach would be highly inefficient and single variant analysis would be limited because of a low power to detect, exacerbated by a smaller sample size relative to GWAS sample sets derived from genotyping arrays.

Methods: Previous analyses of non-coding regions have employed the use of a sliding window-based approach to test sets of rare variants for association with a given trait. However, this approach does not support the direct targeting of distinct regulatory elements. Using SAIGE-GENE+ we tested ~1 million pre-defined regulatory regions (representing ~8% of the GRCh38 genome) taken from the ENCODE encyclopaedia for association with a collection of complex traits recorded in UK Biobank.

Results: Our analysis of non-coding regions identified a multitude of association signals ($p < 5.0 \times 10^{-8}$) across a collection of complex traits. These results include 44 regions displaying association with HDL cholesterol levels, notably three potential distal enhancer regions: Chr11:116825886 – 116826061 ($p = 1.02 \times 10^{-95}$), Chr11:117208830 – 117208994 ($p = 8.94 \times 10^{-38}$) and Chr16:67656668 – 67656990 ($p = 5.19 \times 10^{-25}$) exhibiting strong association signals.

Conclusion: A strong emphasis has previously been placed upon the analysis of functional mutations within coding regions and their impact on complex traits. Our results suggest that by investigating rare variant sets within non-coding regulatory regions of the genome we can begin to elucidate the role of regulatory regions, their effect on gene transcription and how this relates to complex traits.

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P25.040.D Common protein function-altering genetic variants in vitamin B12 absorption pathway regulate B12 deficiency and non-autoimmune anemia

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Background/Objectives: Vitamin B_{12} (or cobalamin) is an essential nutrient integral to DNA synthesis, fatty acid and amino acid metabolism, myelinogenesis and erythropoiesis. Difficulties in absorbing cobalamin often leads to megaloblastic anemia. Our previous work identified genetic risk factors for autoimmune pernicious anemia, a subcategory of cobalamin anemias. Here we sought to identify the genetic risk factors for the considerably more commonly found non-autoimmune cobalamin deficiency anemia.

Methods: We compiled non-autoimmune cobalamin deficiency cohort based on clinical diagnoses (ICD-10 category D51*), medicinal product use (ATC category B03BA*) and cobalamin levels in blood (LOINC code 14685-2) in the Estonian Biobank. We conducted a GWAS in 4,695 cases and 200,613 European controls and utilize LD score regression analysis to demonstrate the genetic difference between autoimmune and non-autoimmune subtypes of cobalamin anemias.

Results: Among the non-autoimmune subtype of cobalamin anemia we identified genome-wide significant protein function altering missense variants in cobalamin transporter genes (TCN1:D301Y,$p = 1.04 \times 10^{-38}$,OR = 1.52; TCN2:L376S,$p = 9.34 \times 10^{-10}$,OR = 0.77). Protein model structural analyses suggest these variants to result in considerable transporter function changes, which result in differing cobalamin transportation capacities in stomach and bloodstream. Additional regulatory variants were associate in or near other cobalamin absorption genes *CUBN* ($p = 6.67 \times 10^{-19}$, OR = 0.82), *FUT2* ($p = 3.05 \times 10^{-8}$, OR = 0.89) and *MMAA* ($p = 3.47 \times 10^{-8}$, OR = 1.27).

Conclusion: Here we identify five genes that regulate non-autoimmune cobalamin deficiency anemia, which could be used for improved prediction, diagnosis and clinical intervention. Future work will also have to evaluate if the effects of function-altering variants can be addressed with alternative therapeutic approaches.

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Humanity Inc - Scientific Advisor

P25.041.A Reconciling linkage and association studies of complex traits

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Family-based genetic linkage studies have been highly successful in detecting and mapping loci underlying single gene disorders, but mostly a failure when applied to common traits, diseases and their risk factors. In contrast, population-based genome-wide association studies (GWAS) have identified replicable associations between tens of thousands of SNPs and complex traits yet capture less than half of total genetic variance. So far, these two popular methods remained to be reconciled for polygenic traits. Here we conduct a large-scale linkage study in 107,000 sibling pairs with GWAS data and phenotypes on height and body mass index (BMI) and show that linkage signals are consistent, convergent and predictable from GWAS results. We perform linkage with the entire genome to estimate the heritability of height (0.83 ± 0.06) and BMI (0.60 ± 0.07). Our estimates are similar to those obtained from pedigree analyses and imply that a substantial fraction of heritability for both traits has yet to be accounted for by GWAS-identified loci. Locus-by-locus linkage analyses resulted in a large genome-wide inflation of the test statistics, congruent with polygenicity. We show that external GWAS results predict observed linkage for height and adjusting for height polygenic scores reduces linkage signals, again concordant with polygenicity. The apparent detection of "major gene" trait loci from past family-based linkage analyses, including apparent replication yet failure to fine-map, is fully consistent with a highly polygenic architecture of common disease and other complex traits. The genetic architecture of the residual trait-causing genetic variation is also polygenic.

Conflict of Interest: None declared

P25.042.B Genome-wide association analysis identifies two loci and one HLA allele associated with post-TB lung function in African adults

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Background/Objectives: Recovered TB patients suffer from irreversible pulmonary function impairment. Although SNPs associated with TB (~100) and pulmonary function (~6500) were identified by genome-wide association studies (GWAS), the genetic predisposition of post-TB lung function has not been studied extensively. As part of the TB-Sequel study (https://www.tbsequel.org/), this sub-study used GWAS to identify genetic variants associated with post-TB lung function for the first time.

Methods: In this study, post-TB lung function is defined by spirometric measurements after 6-month TB treatment, including forced expired volume in 1 second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio. We tested the association of SNPs with post-TB lung function in 763 genotyped TB-Sequel participants. Furthermore, we imputed HLA alleles and performed association testing. To fully capture factors influencing association signals, we performed stepwise variable selection and lasso regression, and finally adjusted for sex, age, age squared, height squared, TB

history, HIV history, HIV treatment and ancestry principal components in both the SNP and HLA association testing.

Results: As the first GWAS for post-TB lung function, we identified one locus significantly associated with month-6 FEV1 and one with month-6 FVC in TB patients (p-value < 5e-8). Although no SNPs within the HLA region met genome-wide significance, we found that HLA-DRB1*08:04 was significantly associated with increased FEV1 and FEV1/FVC after Bonferroni correction.

Conclusion: Our results suggest that post-TB lung function has genetic components. The affected genes and pathways will be determined in the next stage.

Grant References: DZIF_02.813 and BMBF_01KA2114A

Conflict of Interest: None declared

P25.043.C Genetic factors involved in bruxism: the first Genome-Wide Association Study (GWAS) in isolated populations from North-Eastern Italy

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Background: Bruxism is a multifactorial condition characterized by grinding and involuntary teeth clenching. It has been hypothesized that it is caused by an imbalance in neurotransmission and, to date, little is known about its genetic component.

Methods: Seven hundred and seventy-two subjects (age range 6-89) coming from five genetically isolated villages in North-Eastern Italy (FVG Genetic Park) underwent an accurate/complete odontostomatological visit. Logistic Mixed Model (LMM) was used to study the relationship between bruxism and anxiety. Furthermore, a case-control GWAS was performed on all subjects with available genotyping data (135 cases, 525 controls) to identify possible genetic variants associated with bruxism. A logistic model adjusted for anxiety, sex, age, village, and the first ten principal components was applied using Regenie software.

Results: The LMM revealed that anxiety was a statistically significant risk factor for bruxism (Odds Ratio 2.55; 95% CI 1.21 – 5.39). GWAS results allowed the identification of 102 SNPs within 55 genes reaching a suggestive *p*-value ($p < 10^{-5}$). The most interesting results include *RIMBP2* (13 SNPs with $p < 10^{-5}$) and *NLGN1* (6 SNPs with $p < 10^{-5}$) genes. In particular, *RIMBP2* (topSNP:rs571497947, $p = 4.83 \times 10^{-7}$) encodes a pre-synaptic binding protein involved in neuromuscular synaptic transmission (PMID:32867148), and *NLGN1* (topSNP:rs2046718, $p = 2.54 \times 10^{-7}$) plays a critical role in regulating synapses development, transmission, and plasticity (PMID:33522967).

Conclusion: This is the first GWAS on bruxism, highlighting promising new candidate genes potentially involved in the neurobiological mechanisms underlying the etiopathogenesis of this disorder. Further studies will be needed to deepen their relevance and pathophysiological role.

Conflict of Interest: None declared

P25.044.D Genetic and phenotypic associations between thyroid and reproductive health traits

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Background/objectives: Thyroid synthesizes and releases the thyroid hormones (T3 and T4) responsible for regulating a variety of biological functions, including those related to reproductive health. When the hormone production is unbalanced, this can lead to hyper/hypothyroidism, having a significant impact on reproductive health function. Since thyroid disorders result from the interaction of many genetic variants and are polygenic, mapping the shared genetics and genetic pleiotropy between thyroid and reproductive health, would contribute to the understanding on the correlation between those traits.

Methods: We conducted a large scale genetic analyses of thyroid traits (hypothyroidism, hyperthyroidism and TSH levels) in up to 744,345 European ancestry individuals from six different cohorts to explore their associations with reproductive health traits, both on phenotypic and genetic level. Different analyses and criteria were used to determine the candidate genes for thyroid traits and to evaluate the correlation between the phenotypes.

Results: 200 genome-wide significant associations (33 novel) were obtained for the thyroid phenotypes, where 5/200 loci were previously reported to be associated with both thyroid and reproductive health. 332 genes were considered as likely causal in the thyroid analyses that also have a clear role in reproductive health, providing evidence for pleiotropic effects. Phenotypic analysis showed considerable overlap between thyroid phenotypes and genital tract tissues. Hypothyroidism correlated negatively with age at menopause and sex hormones levels, and positively with genital tract disorders.

Conclusion: We provide a roadmap to understand the shared genetics determinants and genetic pleiotropy between thyroid and reproductive health, both in men and women.

Conflict of Interest: None declared

P25.045.A A genome-wide association study for survival from a multi-center European study identified variants associated with the risk of death due to COVID-19

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Background/Objectives: It has been demonstrated the inter-individual susceptibility to SARS-CoV-2 infection and variability in COVID-19 severity are in part due to host genetics. Among the GWASs so far performed, none looked for variants associated with mortality by analyzing the association between genotypes and time-to-event data.

Methods: We performed a case-only genome-wide survival analysis, 60 days after infection/hospitalization, of 3,904 COVID-19 patients from the GEN-COVID cohort and other European cohorts included in the EGAS00001005304 study. Patients were genotyped using Illumina Infinium Global Screening Arrays. PLINK software was used for data quality check and principal component analysis. Imputation was carried out using the TopMed server. GeneAbel R package was used for survival analysis and age, sex, cohort, time of infection, and the first ten principal components were used as covariates in the Cox model.

Results: Four variants associated with survival at *P*-value$<5.0 \times 10^{-8}$ and 13 at false discovery rate (FDR) < 0.10. Their minor alleles were associated with a higher mortality risk. Five variants are intergenic, the top one is upstream *FGF19* gene, one is intronic of *GPRC5C* gene, and six are intronic of *PSD3* gene. Looking at the 281 variants with a *P*-value$<1.0 \times 10^{-5}$, we found that 19 variants mapped in this latter locus, another 20 mapped in a locus on chromosome 6, and 77 polymorphisms map on chromosome 9, in *WDR5* locus.

Conclusion: These results suggest that COVID-19 mortality risk variants differed from those associated with susceptibility and severity.

Grant References: "PATCOVID" - 2020-2016_Ric_3 - Istituto Buddista Italiano Soka Gakkai

Conflict of Interest: None declared

P25.046.B Joint multi-ancestry and admixed GWAS on 3D facial shape derived from MRI

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Biobanks of structural magnetic resonance (MR) images of the complete craniofacial complex have recently become available. However, as imaging protocols focus on optimally capturing the brain, craniofacial regions are distorted with noise and imaging artefacts due to the MR bias field, subject fixation, and the partial volume effect. Using T1w MRI data from the adolescent brain and cognitive development (ABCD) cohort, we demonstrated an innovative image processing pipeline to extract the facial surface from MR images, mitigating noise and artefacts through inter-MR non-rigid registration. Further, following a global-to-local segmentation of the facial surface into 31 segments, remaining imaging artefacts were detected in a vertex-wise fashion, allowing the selection of artefact-free facial segments for each individual. This increased the number of usable images for a genome-wide association study (GWAS) by 2.3-fold, yielding sample sizes between 4,277 and 7,671 depending on the facial segment. A multi-ancestry admixed GWAS was then conducted on each facial segment, yielding 31 independent genome-wide significant loci, including novel hits near *GLI2*, *ADAMTS18*, *MKX/RAB18*, and *RUNX1*. Additionally, we identified transcription factors with known roles in craniofacial development (*SOX9*, *RUNX2*, *TBX15*, *PAX3*, and *ALX1*). Collectively, the GWAS hits were enriched for processes related to skeletal system development and morphogenesis. In conclusion, we demonstrate that hierarchical facial segmentation in conjunction with a facial surface extraction pipeline can maximize the utility of MRI data for studying craniofacial shape even when imaging artefacts are prevalent. Subsequent GWAS analysis confirmed this by identifying novel and well-known facial shape loci in this multi-ancestry cohort.

Conflict of Interest: None declared

P25.047.C Genetic architecture of direct and indirect effects

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Background: Phenotypes of children are influenced by the parental genotypes beyond the pure transmission of genes from parents to children. These so called indirect genetic effects are generally not considered in Genome-Wide Association Studies (GWAS). Thus, we have little understanding of the number and type of genomic regions associated with maternal, paternal and parent-of-origin effects, or of the correlations among these genetic effects, genome-wide.

Methods: We present a novel method that unbiasedly partitions genetic variance to direct, maternal, paternal and parent-of-origin effects for large scale datasets. This regression approach allows for a joint fit of all effects, selects markers associated to the phenotype, and provides polygenic risk scores for each type of effect. Moreover, the model takes into account covariances between the genetic effects, thus increasing power.

Results: We apply our model to 38,000 families in the Estonian Biobank data to provide the first estimates of the genetic variances and covariances of direct, indirect maternal and paternal, and parent-of-origin effects for annotation groups across the whole genome for 10 different complex traits and common diseases. We detect hundreds of new loci, and determine the genetic correlations of each type of genetic effect with a range of other phenotypes unbiased of assortative mating.

Conclusion: The presented results give a first glimpse at the underlying genetic architecture of direct, indirect and parent-of-origin effects throughout the genome and help to provide an understanding of the relationships between genetic components across the DNA.

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P25.048.D Fine-mapping heart rate loci highlights novel effector genes and causal molecular mechanisms

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Background: Genome-wide association studies (GWAS) for resting heart rate (RHR) have identified common variants at 437 loci[1], but the effector genes and biological pathways at these loci are mostly unknown.

Methods: We performed a multi-ancestry meta-analysis for RHR including 388,237, 6,714 and 8,567 individuals of European, African and South Asian ancestry, respectively, from UK Biobank. Annotation-informed fine-mapping incorporating tissue-specific chromatin segmentation[2] was performed to identify high-confidence causal variants (accounting for >75% posterior probability) and consolidated effector genes for RHR using results from the European-only GWAS.

Results: The multi-ancestry meta-analysis discovered 154 loci, all found in the European-only analyses. Fine-mapping indicated 472 signals; at 116 (25%) of these signals, we identified a high-confidence variant, 17 were missense and 6 had a posterior probability >99.9%. We colocalised 82 signals with cis-eQTLs, and identified promoter interactions and candidate genes for 72 signals across relevant tissues. Additional supportive evidence from mouse model data, human cardiovascular phenotypes, and differential expression highlighted 40 characterised (e.g. *CCDC141*, *MYH6* and *FHOD3*) and new (e.g. *FITM2*) consolidated effector genes for RHR. Local network clustering indicated enrichment with muscle protein and sarcomere organisation processes, and six human cardiovascular phenotypes (FDR $P < 1 \times 10^{-4}$), including left to right shunt and right ventricular failure.

Conclusion: Our fine-mapping and computational approach identified 40 consolidated effector genes for RHR, providing new mechanistic hypotheses for functional validation.

References: (1) Mensah-Kane et al. Front Genet 18; 12:569323 (2021); (2) van Duijvenboden S, Ramírez J et al. bioRxiv 2023.01.26.525702.a

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Conflict of Interest: None declared

P25.049.A Genetic analysis of sleep medication use and variation in the X-chromosome identifies a common missense variant in an orphan GPR101 receptor

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Background/objectives: Genome-wide association analysis provides an opportunity to understand biological mechanisms behind complex traits such as sleep disruption and chronotype. We have earlier described genome-wide association results with sleep, chronotype and fatigue but the role of variants in the X chromosome have remained unexplored in these earlier studies.

Methods: Using FinnGen R10 data (N = 412,181 individuals), we examined the role of variants in the X chromosome. We conducted a GWAS using data from 71,955 individuals with registered purchases of Z-drugs within the ATC code N05CF, and 340,226 controls.

Results: We found a significant association between a missense variant (rs1190736, allele frequency 50%) exonic of *GPR101* and increased use of sleep medications. This association was significant for both males and females (P < 5e-8) but the effect size of the variant was larger for females than males. The *GPR101* gene encodes a G protein-coupled receptor known as *GPR101*. Analysis of rs1190736 with Polyphen suggested the variant to be damaging and accordingly, a follow-up analysis in objective sleep measurement data using activity watches showed a significant association with sleep fragmentation (P < 5e-8).

Conclusion: Our findings imply a possible role of GPR101 in the regulation of sleep. Lacking a known ligand, the orphan GPR101 receptor has been found in previous analyses to be linked to several diseases and health-associated behaviours; these include a causative role in X-linked acrogigantism and acromegaly. Our findings may suggest a role of GPR101 in sleep consolidation.

Grant references: None

Conflict of Interest: None declared

P25.050.B New standards for summary statistics data shared in the GWAS Catalog

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Background: Challenges for researchers in the GWAS field surround access and sharing of summary statistics data, due to lack of standards for data content and file format. The GWAS Catalog is now the largest, most diverse and frequently updated resource of GWAS summary statistics, with more than 55K datasets available (Feb 2023). With data volume growing rapidly, there has been an urgent need to improve minimum information standards, maximising utility of this huge body of data to support downstream uses including Mendelian randomization, generation of polygenic scores and meta-analysis.

Methods: We hosted a series of meetings with summary statistics stakeholders to define key requirements for a standard, and refined the initial schema following wider community feedback. Key requirements comprised (1) key data elements to support a wide range of data analyses, (2) straightforward file access and generation, (3) easily accessible metadata, (4) unambiguous, interoperable data. Mandatory data and metadata fields were identified.

Results: The new standard has been implemented in the GWAS Catalog's submission, data release and harmonisation pipelines, building on the minimal standard we had previously set. Here, we present an overview of submission requirements and demonstrate the improvements in data content for users, including increased sharing of effect size and allele frequency.

Conclusion: We have defined best practices and a minimum information standard for GWAS summary statistics data sharing.

Future application of the standard in the GWAS Catalog and other resources will improve the quality of shared data, benefiting the genomics community.

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P25.051.C Alpha-2A adrenergic receptor (ADRA2A) modulates susceptibility to Raynaud's disease

Anniina Tervi[1], *Markus Räsänen*[2], *Vilma Lammi*[1], *Erik Abner*[3], *Tõnu Esko*[3], *Jacqueline Lane*[4;5;6], *Matthew Maher*[5], *FinnGen Consortium*[1], *Caroline A. Heckman*[1], *Thomas Quertermous*[2], *Samuel Jones*[1], *Hanna Ollila*[1;5;6;7]

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Background/Objectives: The autonomic nervous system controls physiological functions in the body that are not under direct voluntary control and are not typically consciously directed. The targets of the autonomic nervous system include, for example, body temperature, heart rate, respiration, blood pressure regulation and vascular tone. In some instances, however, the autonomic nervous system can malfunction causing symptoms and diseases of dysautonomia and affects many different targets of the autonomic nervous system at once. Raynaud's disease is a dysautonomia where exposure to cold increases the vascular tone of distal arteries causing vasoconstriction and hypoxia particularly in fingers and toes.

Methods/Results: Using genetic and electronic health record data from the UK Biobank, the Estonian Biobank, the Mass-General Brigham Biobank and FinnGen data freeze 10 we identified 10,680 individuals with a diagnosis for Raynaud's disease and over 1 million disease-free controls. Genetic analysis identified the same risk locus at the *ADRA2A* gene region independently in all four cohorts. Meta-analysis identified rs7090046 as the lead variant associated with Raynaud's disease ($P = 1.52 \times 10^{-47}$, beta = 0.22). Functional analysis using RNA expression from GTEx indicated that the genetic variants modulate *ADRA2A* expression in a tissue-specific-manner in the distal arteries (rs7090046, $P = 1.3 \times 10^{-13}$, effect size (NES) = 0.305). Furthermore, co-localization analysis suggests that the lead variant is also the most likely causal variant affecting expression levels of *ADRA2A* in Tibial Artery tissue.

Conclusion: Our results indicate that *ADRA2A* modulates vascular tone in Raynaud's disease and provides the first functional evidence for understanding the mechanisms in dysautonomia.

Conflict of Interest: None declared

P25.052.D Genetic basis of right and left ventricular heart shape

Richard Burns[1], *Julia Ramírez*[2;3], *William Young*[3;4], *Catrin Sohrabi*[3], *Nay Aung*[3;4;5], *Steffen Petersen*[3;4;5;6;7], *Alistair Young*[1], *Patricia Munroe*[3;5]

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Heart shape is a cardiovascular trait that captures variation in cardiac structure and is poorly represented by traditional phenotypes, demonstrating stronger relationships with cardiac disease risk factors (e.g., wall thickness, concentricity) and disease, and its genetic basis has not been studied. To explore this, automated analysis of cardiovascular magnetic resonance (CMR) images was performed in 45,683 participants in the UK Biobank to construct a heart shape atlas from right and left ventricular end-diastolic surface mesh models. The first eleven principal components (PCs) of the atlas were defined as phenotypes, accounting for 83.6% of the total shape variance. We performed heritability and genome-wide association studies (GWAS) on these phenotypes followed by a series of bioinformatics. The eleven PC's all demonstrated substantial levels of heritability (8.5-36.3%). Through GWAS we identified 43 significant loci across ten PCs. Fourteen of these signals had not previously been reported with any ventricular structure, function, electrocardiogram (ECG) or cardiac disease traits. Genetically predicted PCs from polygenic risk scoring were associated with several cardiometabolic diseases, including heart failure and myocardial infarction. In particular, genetically predicted spherical hearts were associated with higher atrial fibrillation risk. By characterising the genetics of heart shape, we have identified new candidate genes and explore the biological pathways implicated in defining cardiac shape and its relationship with cardiometabolic disease.

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Conflict of Interest: None declared

P25.053.A Identifying Gene Function in the DNA Damage Response through the Pleiotropy of Common Genetic Variation

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Background/Objectives: DNA damage is a crucial factor in aging, making the DNA damage response (DDR) an attractive target for aging interventions. In this project, we use the pleiotropic effects of common genetic variation to identify genes responsible for DNA repair, damage signaling, and cell fate in the DDR.

Methods: Our four-step approach involves identifying potential DDR gene loci from UK Biobank Genome Wide association studies, testing their associations with DDR-influenced phenotypes from the UK biobank and the Rotterdam Study, clustering loci and genes based on pleiotropic effect directionality, and finally confirming single-variant and polygenic effects in cell studies.

Results: Our analysis identified 95 independent loci that target DDR genes, with 82 loci associated with age of menopause, 39 loci associated with cancer, and 49 loci associated with physical frailty. By clustering the loci based on pleiotropic effect directionality, we found that loci affecting the same gene did not have opposite patterns. Genes with well-studied functionality showed expected patterns, which supports the validity of our approach. Our current efforts are focused on expanding the DDR loci set, testing previously unknown gene functionality in cell studies, and using proteomics data to explore the relation between frailty and senescent cell inflammation.

Conclusion: This study provides new insights into the genetics of the DDR, which could lead to the development of interventions aimed at improving aging-related health outcomes.

Conflict of Interest: Jard de Vries Full time, Jacinta van de Grint part time, Joris Pothof Full time, Joyce Van Meurs Full time

P25.054.B First genome-wide association study points to HLA-mediated immune mechanisms in the development of lymphatic filariasis

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Objectives: Lymphatic filariasis (LF) represents a parasitic infection caused by filarial nematodes. While the majority of infected are asymptomatic, a considerable proportion of individuals present LF that is characterized by lymphedema, hydrocele, and elephantiasis. Several studies have shown that host genetic factors influence LF susceptibility. This study represents the first genome-wide association study (GWAS) to characterise the genetic risk architecture of LF.

Methods: We analysed genome-wide single-nucleotide polymorphism (SNP) genotype data from 1,508 LF patients and 1,681 asymptomatic controls of West African (Ghanaian) descent.

Results: We identified a genome-wide significant association with LF at a genetic variant (rs7742085) on chromosome 6p21 near the gene *HLA-DQB2* ($P = 3.93 \times 10^{-8}$ (odds ratio (OR) = 1.43 (confidence interval (CI) 1.26-1.63)). We also detected suggestive LF associations ($P < 1.0 \times 10^{-6}$) at three non-HLA loci, namely at *OR5V1* (rs1419637), *RNU6ATAC11P* (rs2243492), and *PAK1* (rs2852388). Moreover, we could not replicate any previously reported LF associations that were drawn from candidate gene association studies. The GWAS data explain 27-48% of heritability, depending on the assumed population prevalence of LF (ranging from 0.5 to 5.0%). Through pathway analysis, we identify an involvement of innate and adaptive immune response in LF development.

Conclusion: Our findings show that genetic variation at *HLA-DQB2* is associated with LF risk pointing to immune-mediated mechanisms in disease pathophysiology. Moreover, our study shows that LF has a polygenic risk architecture and provides the first LF heritability estimate.

Conflict of Interest: Sandeep Grover Fully Employed, Vera Serwaa Opoku Full time, Linda Batsa Debrah Full-time, achim hoerauf Full-time, Carlo Maj Full-time, Alexander Yaw Debrah Full-time, Johannes Schumacher Full-time, kenneth pfarr Full-time, This study was supported by the German Research Foundation (DFG) (PF 673/6-1).

P25.055.C Genetic determinants of human brown adipose tissue activity

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Background/Objectives: White adipose tissue stores excess energy, whereas brown adipose tissue (BAT) is thermogenic, dissipating energy as heat. There is increasing evidence in humans demonstrating that BAT defends cardiometabolic health. However, our understanding of the mechanisms behind human BAT activity is limited, because most research is conducted in mice and small-scale human studies. Therefore, we performed a genome-wide association study (GWAS), in order to identify genes associated with BAT activity in humans through a hypothesis-free approach and to gain insight into the underlying mechanisms.

Methods: We included 759 individuals of African (N = 229), European (N = 226) and Hispanic (N = 304) ancestry from the Mount Sinai Bio*Me* Biobank. BAT activity was measured using ^{18}F-labeled fluorodeoxyglucose positron emission tomography/computerized tomography (PET/CT), and quantified using the PET-CT Viewer software, following the Brown Adipose Reporting Criteria in Imaging Studies consortium guidelines (BARCIST 1.0). The GWAS was performed with REGENIE, adjusting by age, age^2, sex and the first ten principal components.

Results: We identified a locus in *PRXL2A* that is associated with BAT activity in individuals of African ancestry (rs72300118, beta [CI 95%] = 1.09 [1.45-0.73] SD of BAT activity [standardized uptake value mean (MBq/ml) normalized to body weight (kg)] per effect allele, $p = 2.24 \times 10^{-9}$). *PRXL2A* is a redoxin highly enriched in white and brown adipose tissue. We will investigate the functional impact of *PRXL2A* on thermogenic function in human brown fat cells.

Conclusion: This hypothesis-free approach may provide new avenues to elucidate biological mechanisms underlying BAT activity and new therapeutic candidates for improving cardiometabolic health.

Conflict of Interest: None declared

P25.056.D An atlas of genetic contributions to multimorbidity

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Frank Dudbridge[6], Sara Khalid[7], Christopher Fox[8], Sarah Lamb[8], Jack Bowden[1;9], David Melzer[2], Jane Masoli[2;10], Concepción Violán[11], Timothy M. Frayling[1], Joao Delgado[2], Luke Pilling[2]

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Background: Multimorbidity, the co-existence of at least two health conditions, is a common and complex clinical problem. Previous studies have predominantly used observational data to cluster patients and diseases to characterise multimorbidity patterns. Here, we utilize powerful genetic approaches to identify shared genetics between multiple long-term conditions (LTCs).

Methods: With input from expert clinicians and patient advisors we identified LTCs using the CALIBER platform and an established classification of multimorbidity. We estimated prevalence in two population-representative primary care populations. We estimated SNP-based heritability for each LTC and pairwise genetic correlations using LD-score regression, using data from two independent cohorts with genetic data (UK Biobank and FinnGen), and published disease-specific GWAS when available.

Results: Of 97 LTCs, 76 were common (>0.5% in people aged >65 years) in both primary care datasets, of which 48 had significant heritability (Z>4). We found widespread genetic correlation across LTCs; the strongest correlations occur within traditional disease domains, for example in UK Biobank, coronary artery disease and peripheral arterial disease (rG = 0.81, 95% CIs = 0.69-0.92), osteoarthritis and spondylosis (rG = 0.81, 95% CIs = 0.74-0.87). We also identified correlated pairs crossing disease domains, such as type-2 diabetes and osteoarthritis (rG = 0.36, 95% CIs = 0.31-0.41), asthma and rheumatoid arthritis (rG = 0.44, 95% CIs = 0.32-0.57).

Conclusion: We found evidence of shared genetics between multiple LTC pairs. Pairs will be taken forward for more in depth analysis into specific shared causal pathways that could be used to inform clinical interventions.

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P25.057.A Can genetic associations for disease onset be used to predict disease prognosis?

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Background/Objectives: Genetics of disease incidence/onset have been extensively studied using genome-wide association studies (GWAS). However, genetic factors of disease prognosis have been less known. Here, we study if existing polygenic risk scores (PRS) can be used to predict patient's prognosis and if the genetic determinants of disease prognosis overlap with those of disease incidence.

Methods: We selected ten common diseases which are leading causes of death in developed countries and defined disease prognosis as survival time between disease onset and cause-specific mortality. Combining data from 5 major biobanks (N>800k), we

1. examined the association between PRS for disease incidence and patients' survival using a cox proportional hazard model;
2. carried out in-patient survival GWAS to uncover variants associated with disease prognosis;
3. constructed a theoretical framework to better understand our results.

Results: We found incidence PRSs were weakly or not associated with disease-specific mortality as a proxy for patients' prognosis. Overall, we observed an 84.7% reduction of incidence PRSs effects on prognosis compared to their effects on incidence with 6/10 PRSs not associated with prognosis (e.g. breast cancer PRS not associated with patients' survival, HR = 0.9994, P-value = 0.9852). Moreover, most prognosis GWASs, despite reaching the largest sample sizes so far, did not uncover prognosis-specific signals, and showed little heritability (h^2<=0.01), which cannot simply be explained by smaller sample sizes.

Conclusion: Our results suggest that when defined as patients' survival, genetic factors for disease prognosis don't overlap disease incidence/onset. This has important implications for the subsequent development of clinically useful prognostic genetic scores.

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Conflict of Interest: None declared

P25.058.B Independent single nucleotide polymorphisms are associated with clinical and histological non-recovery in long-term treated celiac disease patients

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Background/Objectives: Genome-wide association studies (GWAS) identified numerous genomic regions associated with coeliac disease (CeD). A significant proportion of CeD patients remain symptomatic and histologically unresponsive to a strict gluten-free diet (GFD), for unclear reasons. We investigated whether candidate single nucleotide polymorphisms (SNPs) are associated with these phenotypes.

Methods: Altogether 581 biopsy-proven CeD patients thoroughly phenotyped underwent clinical and endoscopic evaluation after ≥ 12 months on a GFD. Based on a candidate gene approach we tested the associations of 52 CeD risk SNPs and 10 irritable bowel syndrome risk SNPs with incomplete small bowel mucosal recovery and with persistent gastrointestinal (GI) symptoms. A GWAS with each phenotype was performed and pathways enriched by genes connected to the associated SNPs identified. The cumulative effects of 39 CeD SNPs with the phenotypes were also assessed in a genetic risk score (GRS)-tertile model, based on their distribution in 1817 non-celiac controls.

Results: Out of the 62 SNPs tested, *ZFP36L1*-rs4899260 and *SEMA6D*-rs649603 were associated with increased risk of incomplete histological recovery (OR 2.07, 95% CI 1.13-3.80) and with persistent GI symptoms (OR 2.28, 1.24-4.16), respectively. GWAS revealed associations ($P < 5 \times 10^{-6}$) at *LDLR*-rs1799898 and *FOXP2*-rs940468 genes with incomplete histology [OR 4.40(2.41-8.05) and 2.72(1.79-4.15), respectively], and *TBC1D4*-rs9593054 with persistent GI symptoms (OR 2.99, 1.92-4.65). Phenotype-associated genes were enriched in starch and sucrose and cholesterol metabolisms and insulin signaling pathways. No association was identified in GRS.

Conclusions: Independent SNPs, particularly those involved in carbohydrate and lipid metabolisms, might contribute to clinical and histological non-recovery in long-term treated CeD patients.

Conflict of Interest: None declared

P25.059.C Estimating the genomic profile of human complex traits from summary statistics

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Genetic variants across the genome play a crucial role in determining complex traits, which have a polygenic architecture. To better understand this complexity and improve genomic prediction, it's essential to infer the genetic effects distribution throughout the genome. The heteroscedastic effects model (HEM) is a powerful tool that estimates genome-wide SNP effects using a generalized ridge regression framework. However, HEM is limited by its need for individual-level genotype and phenotype data, which makes it challenging to apply in large-scale human genetics studies. To address this issue, we developed a new model called SumHEM, which enables HEM to be fitted using summary statistics from genome-wide association studies (GWAS). We tested SumHEM using simulations and real data analysis and found that it outperforms state-of-the-art methods such as LDpred2 in heritability parameter estimation, genomic effects distribution estimation, and genomic prediction. SumHEM is particularly effective for highly polygenic traits, as demonstrated in our analysis of 300 phenotypes from the UK Biobank. In fact, SumHEM's out-of-sample prediction for more polygenic traits with higher heritability was significantly better than that of LDpred2. Additionally, SumHEM provided comparable heritability estimates to those of LD score regression (LDSC) while revealing a more accurate genome-wide genetic effects profile of each complex trait underlying the heritability model.

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P25.061.A Genetic mechanisms of 184 neuro-related proteins in human plasma

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Understanding the genetic basis of neuro-related proteins is essential for dissecting the disease etiology of neuropsychiatric disorders and other complex traits and diseases. Here, the SCALLOP Consortium conducted a genome-wide association meta-analysis of over 12,500 individuals for 184 neuro-related proteins in human plasma. The analysis identified 117 cis-regulatory protein quantitative trait loci (cis-pQTL) and 166 trans-pQTL. The mapped pQTL capture on average 50% of each protein's heritability. Mendelian randomization analyses revealed multiple proteins showing potential causal effects on neuro-related traits as well as complex diseases such as hypertension, high cholesterol, immune-related disorders, and psychiatric disorders. Integrating with established drug information, we validated 13 combinations of protein targets and diseases or side effects with available drugs, while suggesting hundreds of re-purposing and new therapeutic targets for diseases and comorbidities. This consortium effort provides a large-scale proteogenomic resource for biomedical research.

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P25.062.B Multivariate GWAS on achondroplasia-like craniofacial shape variation in healthy human individuals

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Background/Objectives: Human facial shape is a complex phenotype that is largely genetically determined, whose development involves a series of highly coordinated embryonic events. Naturally occurring mutations that disturb facial development can cause craniofacial dysmorphism. This provides a unique window into the genetics underlying facial variation. In this work, we introduce a novel syndrome-informed facial phenotyping method to identify genomic loci associated with facial variation along a syndromic axis using achondroplasia as an example.

Methods: We compared 3D facial scans from 8,246 healthy European-ancestry individuals and 48 achondroplasia patients to calculate an achondroplasia endophenotypic score. In our healthy control sample, we performed a multivariate GWAS of these scores using canonical correlation analysis and observed 35 independent genetic loci that reached genome-wide significance ($p < 5 \times 10^{-8}$).

Results: Gene ontology analysis showed significant enrichment of genes involved in skeletal development, particularly chondrocyte differentiation and cartilage development. Compared to a GWAS of normal facial variation in the same cohort, this enrichment was specific to our study. Furthermore, by applying these genes to a multivariate genotype-phenotype model in mice, we recovered an achondroplasia-like phenotype, even without the *Fgfr3* mutation that is associated with achondroplasia.

Conclusion: In summary, we identified a polygenic basis for normal facial variation along the achondroplasia trait axis and found an enrichment for developmental processes that are key in achondroplasia pathophysiology. This suggests that both complex and Mendelian genetic variation act on the same biologically determined axes of facial variation, providing novel insights into the genetic intersection of complex traits and Mendelian disorders.

Conflict of Interest: None declared

P25.063.C Novel actionable targets discovered by proteomic GWAS of 1790 largely unstudied proteins

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Genome-wide association study (GWAS) power lies in testing hundreds of thousands of single nucleotide polymorphisms (SNPs) across many genomes for associations with a trait. Recent technological developments in high-throughput proteomic assays allow simultaneous quantitative measurement of thousands of proteins in a single sample.

Blood plasma proteins effectively provide a glimpse into multiple biological systems at once, even those outside the circulatory pathways. Combining broad-capture proteomics with genomic variation across a population is a valuable resource with implications in elucidating complex traits and disease, drug development or repurposing, and precision medicine.

In this study we investigated 6432 plasma proteins using the SomaLogic aptamer-based technology in the Viking Health Study – Shetland, an endogamous population with a relatively low genetic diversity.

A total of 592 significant independent protein quantitative trait loci (pQTL) were found for 366 proteins in plasma (458 cis ($P < 5e-8$), 134 trans ($P < 6.6e-12$)). Of these, 74 pQTL were for 50 previously unstudied proteins. We leveraged this new resource to perform causal inference using bidirectional Mendelian Randomization and Colocalization against complex traits of biomedical importance. Causality was established for 18 proteins, with hitherto undiscovered links to diabetes, depression and total testosterone levels.

Conflict of Interest: None declared

P25.064.D Validation of a polygenic score for Frailty in the Lothian Birth Cohort and English Longitudinal Study of Ageing

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Frailty is a complex trait. Twin studies and a recent Genome Wide Association Study (GWAS) have demonstrated a strong genetic basis of frailty but there remains a lack of genetic studies exploring frailty. This work utilized summary statistics from the most recent and high-powered GWAS conducted in the UK Biobank to create and test the predictive power of frailty polygenic scores (PGS) in two independent samples – the Lothian

Birth Cohort 1936 (LBC1936) and the English Longitudinal Study of Ageing (ELSA). The summary statistics were from a 2021 GWAS on the frailty index, generated from 164,610 individuals aged 60-70 years. Multiple regression models were built to test the predictive power of frailty PGS in five different age groups ranging from 67-84 years. Frailty PGS significantly ($p < 0.001$) predicted frailty at all five time points in LBC1936 and ELSA, explaining 2.1% ($\beta = 0.15$, 95%CI, 0.085-0.21) and 1.6% ($\beta = 0.11$, 95%CI, 0.089-0.15) of the variance, respectively, at age ~68/~70 years. The variance marginally declines across the five waves. This work demonstrates that frailty PGS can predict frailty in two independent cohorts, particularly at early ages (~68/~70) when the target sample (GWAS) is most similar to the independent samples (LBC1936 and ELSA). These results suggest a need to investigate the genetic mechanisms of frailty further, especially as frailty progresses and worsens at older ages.

JF is a PhD student at the Advanced Care Research Centre (funded by Legal and General). The funder had no role in the conduct of the study.

Conflict of Interest: None declared

P25.065.A Utilising a CTG18.1 expansion-negative Fuchs endothelial corneal dystrophy cohort to identify novel genetic risk loci

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Background/Objectives: Expansions (≥50) of a CTG repeat in TCF4 (termed CTG18.1) are the typical genetic determinant of Fuchs endothelial corneal dystrophy (FECD). However, genetic risk factors in cases without CTG18.1 expansions are largely unknown. Here, to identify novel genetic risk loci, we conducted the first genome-wide association study (GWAS) in a genetically refined FECD cohort stratified by CTG18.1 expansion status matched to UK Biobank (UKBB) controls.

Methods: Blood-derived DNA samples obtained from FECD patients (n = 1,033) were genotyped for CTG18.1 length using standard PCR-based methods and expansion status was classified as expanded (repeat-length≥50) or non-expanded (repeat-length<50). DNA samples were genotyped on an Affymetrix UKBB Axiom Array. Initially, 20,000 UKBB controls were selected and matched for sex, age, and ethnicity, excluding participants with significant corneal disease. Following joint case/control calling, individuals/SNPs were filtered based on missingness and concordance. A subset of controls was selected iteratively based on 20 principal components (genomic inflation>1.05). The joint calling process was repeated accordingly. Subsequently, controls were further filtered by kinship and predicted ethnicity. Imputation was then performed before a final GWAS.

Results: Out of a cohort of 793 European FECD cases, 137 were CTG18.1 expansion-negative. The control-selection pipeline from UKBB yielded 5,000 ideal controls. Both groups were included in the GWAS to identify novel genetic risk factors of FECD.

Conclusion: Our pipeline has enabled the first stratified GWAS of a genetically refined cohort of FECD individuals for novel genetic risk factor discovery.

Grant References: Moorfields Eye Charity PhD studentship GR001395

Conflict of Interest: None declared

P25.066.B Deep analysis of GWAS data for multiple sclerosis at the MHC locus reveals differentially associated combinations suggesting different underlying pathogenesis mechanisms

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Multiple sclerosis (MS) GWAS have confirmed 13 MS alleles in 7 *MHC* genes : *HLA-A*02:01– HLA-B*38:01/*44:02/*55:01–LTA-H51P–HLA-DRB1*03:01/*08:01/*13:03/*15:01–HLA-DQA1*01:01–HLA-DQB1*03:01/*03:02–DPB1*03:01*. Our objective was to search for differentially MS-associated *MHC*-combinations of MS-genotypes.

We analysed WTCCC GWAS data for 11,376 MS cases and 18,872 controls, using principal component analysis (PCA) for genetic homogeneity selection. *HLA* alleles were imputed using the HIBAG R package or inferred with proxy SNPs (*rs2229092, rs9273912* and *rs9277565*). We searched for MS-associated genotype combinations in 20% of the data, to test nominal combinations for replication in the remaining 80%. Confirmed combinations were investigated in the global sample (100%), searching for non-overlapping odds ratios (OR) 95% confidence intervals (CI) (N.O.-combinations).

Following PCA analysis, we retained 9,024 MS and 13,923 controls. Analysing 20% of the data, we observed 776 different genotype combinations, of which 55 were MS-associated ($P < 0.05$). 35 combinations were replicated (Bonferroni $P < 0.05/55$), representing 36.6% of MS-patients, with OR from 2.21 to 12.67 (CI[1.57-52.46]). Of these, 22 combinations (27.6% of MS-patients) have 1 to 6 N.O.-combination(s) sharing one genotype. For 8 combinations sharing *HLA-DRB1*15:01/X (X*: non-MS allele) genotype, including *LTA-C/A-HLA-DRB1*15:01/X-HLA-DQB1*03:02/X-XX4* (4 genes *X/X*) (OR 12.67 CI[4.08-52.46]) and *HLA-A*02:01/X-HLA-DRB1*15:01/X-XX5* (OR 2.21 CI[1.59-3.06]). There are 12 *HLA-A*02:01/X* (9 N.O.-combinations), 1 *HLA-B*44:02*, 6 *LTA-C/A*, 2 *HLA-DQA1*01:01*, 8 *HLA-DQB1*03:01/X*, 6 *HLA-DQB1*03:02/X* (2 N.O.-combinations) and 17 *HLA-DPB1-T/C* (7 N.O.-combinations) combinations (some combinations shared).

22 differentially MS-associated *MHC* genotype-combinations have up to 6 N.O.-combination(s) sharing one genotype. Those combinations, contributing differently to MS pathogenesis, could lead to the discovery of the *HLA*-associated diseases mechanisms.

Conflict of Interest: None declared

P25.068.D Unravelling genetic interactions affecting quantitative traits in the UK Biobank

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Background/Objectives: Many mutations have been associated with human phenotypes, enabling disease risk prediction through polygenic scores (PGSs). Because genetic influences on traits are

expected to operate through biological networks, identifying interactions between mutations would aid biological understanding. However, this task is complicated by the vast search space of variant combinations, and the importance of interactions remains uncertain.

Methods: To improve power, we created a test for interactions between a SNP and *groups* of variants, e.g. within a PGS, and to refine identified groups of interacting markers. In realistic simulations, our method avoids false positives and is well-powered to identify interacting networks.

Results: We identified 144 interactions involving 52/97 traits within the UK Biobank. Interaction signals at *FTO*, *HFE*, *HLA-C*, *LDLR*, or *TCF7L2* imply association signals at these loci in fact involve modulatory effects on distant loci. Our approach correctly identified a known interaction between *ABO* and *FUT2* affecting alkaline phosphatase and further, novel, genes revealing a wider network: *FUT6*, *PIGC*, *ASGR2*, and *ZNF678*.

An interaction signal for eosinophil count links a noncoding *IL33* variant and an *ALOX15* missense mutation. Interestingly, an independent recent functional study in mice identified *ALOX15* as reducing *IL33*-induced airway eosinophilic inflammation, implicated in asthma. We infer additional interactions involving distinct Alzheimer's-associated *APOE* coding mutations, for distinct traits (lipoprotein(a) and the liver function marker alanine aminotransferase), supportive of proposed connections among Alzheimer's disease, lipids, and liver function.

Conclusion: Our results demonstrate the power of widely-available GWAS data to uncover key "core" genes interacting with other trait-influencing loci.

Conflict of Interest: None declared

P25.071.C Haplotype based expression Quantitative Trait Loci mapping

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Background: The main focus of expression quantitative traits loci (eQTLs) studies is on the effects of individual genetic variants nearby genes, but they do not adequately identify whether multiple variants act synergistically as part of haplotypes or whether the effects of individual genetic variants depend on the genetic background.

Method: To address this, we utilized a Hidden Markov Model (HMM) to cluster observed haplotypes into a small set of "founder" haplotypes and provided per-individual dosages for these that vary continuously along the genome based on the underlying linkage disequilibrium structure. We conducted linear regressions to test the resulting dosages for association with the expression of nearby genes within a 1Mb range. Finally, we used a Likelihood Ratio Test (LRT) to identify genes for which the underlying haplotypic structure better explains the variation in expression levels compared to genotypes alone.

Results: We used RNA-seq and genotype data from 358 individuals in the Geuvadis study to validate our clustering approach and found that our clustering captured blocks of linkage disequilibrium that closely match those defined in the HapMap project. Our association tests found evidence that approximately 15% of the eQTLs reported in the Geuvadis study could be better explained by the haplotypic structure.

Conclusion: In conclusion, we believe that our method is well-suited to determine whether an eQTL signal is of genotypic or haplotypic origin, and it is a valuable addition to the toolbox for fine-mapping eQTL effects and genome-wide association studies.

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Conflict of Interest: None declared

P25.072.D Interaction between genetic and non-genetic risk factors in pediatric Multiple Sclerosis (Pedigree): the role of gut microbiota

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Background: Multiple Sclerosis (MS) is a chronic inflammatory multifactorial disease that occurs between 20 and 40 years of age. We focused on MS with onset before 18 years (pediatric MS-PedMS) which offers a unique opportunity to gain clinical and biological data in proximity to the actual disease onset, lifestyle and environmental information.

Methods: An Italian multicentric study group was realized aiming to enroll pediatric MS patients, matched healthy controls, MS adults with a disease onset <18 years in order to analyze genetic, epigenetic, transcriptomic, environmental factors, their interactions, and gut microbial composition. In a case-control experiment, we performed 16S sequencing, primary analyses by SmartSeq-MicrobAT software, and more complex and comparative analyses by MicrobiomeAnalyst and Qiime2 pipeline that produced analogous outcomes.

Results: We analyzed 87 PedMS and 55 HC. Preliminary results show a similar composition at phylum level between groups, with *Firmicutes* (44%) and *Baciteroidetes* (33%) being the most abundant phyla. The heat tree method revealed a significant different abundance ($p < 0.05$) at species level: *unclassified_Blautia*, *unclassified_Lachnospiraceae*, *Butyrate_producing_bacterium_L2_12* and *unclassified_Firmicutes* were enriched in PedMS,

while *Bacteroides sp. Smarlab_3302996*, *Odoribacter* and *Unclassified_Rikenellaceae* were decreased.

Conclusion: We profiled gut microbial composition of PedMS patients and HC. We found significant abundance differences at species level, also confirmed in literature, but considering alpha- and beta-diversity indexes the two groups appeared very similar. Further analyses will include an integration with host-genetic data (GWAS-SNP) and other host-omics, together with clinical and environmental information to explore gene-environment interaction that could lead to a pediatric disease onset.

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P25.074.B Meta-analysis identifies novel loci associated with reading ability

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Background/ Objectives: Reading is a fundamental skill central to educational development. The genetic architecture of individual differences in reading ability is polygenic, with twin-based heritability estimates of 30-80%. The largest genome-wide associate study to-date identified one significant region, indicating larger samples are required.

Methods: To boost sample size and increase power, we utilise a multi-trait analysis (MTAG) across GWAS summary statistics from phenotypes related to word-reading ability in GenLang (N = 33,959; Eising et al., 2022) and dyslexia (N = 113,887; Doust et al., 2022) (rg = -0.71, standard error (SE) = 0.047). Variant and gene-based analyses were performed in FUMA to reveal novel biological pathways associated with reading ability, and we examine the significant SNPs for evidence of selection in ancient human populations using CLUES (Stern et al., 2019) and PALM (Stern et al., 2021).

Results: Multi-trait analysis of word-reading and dyslexia summary statistics resulted in an equivalent GWAS sample size of 117,497. Forty-one regions were significantly associated with reading ability, including 11 novel loci, 29 previously reported in the dyslexia GWAS, and one in the GenLang word-reading study. Gene-set analysis revealed enrichment for genes involved in neuronal differentiation and oncogenic senescence pathways. Evolutionary analysis identified no evidence of polygenic selection acting on reading ability in recent human history (<15,000ya).

Conclusions: Multi-trait association analysis utilises phenotypically related GWAS to increase power, revealing novel loci associated with reading ability. These results expand our understanding of the biological basis of reading.

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Conflict of Interest: Hayley Mountford: None declared, Pierre Fontanillas 23andMe, Else Eising: None declared, Catherine Doust: None declared, Gokberk Alagoz: None declared, Tim Bates: None declared, Nick Martin: None declared, Simon Fisher: None declared, Michelle Luciano: None declared

P25.076.D Using genetics to explore patterns of change in fetal growth trajectory in Born in Bradford

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Introduction: Fetal growth is a key indicator of antenatal health. Poor fetal growth is a predictor of adverse pregnancy outcomes, e.g. stillbirth and neonatal death. Genome-wide association studies (GWAS) have often used birthweight as a proxy for fetal growth. The single GWAS (N = 1849) explored genetic determinants of ultrasound-estimated fetal weight (EFW) at three time points during pregnancy, and found maternal rs746039 (*ITPR1*) reached genome-wide significance at 27 gestational weeks +6 days. Neither approach provides information on between-person variations in growth during different time of pregnancy.

Materials and Methods: We conducted GWAS of change in fetal growth in Born in Bradford – a UK birth cohort using a two-step approach stratified by ethnicity. First, fetal growth trajectories were modelled based on repeated EFW and offspring birthweight from medical records, using factorial polynomial and linear spline models. Consistent with both models, a linear growth period between 23-36 gestational weeks was identified, and the predicted-EFW change (i.e. growth rate, grams/week) was extracted for each participant from the linear spline model. Second, we conducted GWAS of this growth rate using Plink.

Results: We identified an association of maternal rs80322602 [G] (*PHLPP1*) with the growth rate (1.26 grams/week per allele, 95% confidence interval: 0.82-1.71, $P = 2 \times 10^{-8}$) among 2529 European mothers. No common variants achieved genome-wide significance among 2664 South Asian mothers, or GWAS of fetal genotype.

Conclusion: This study applied a novel approach to conduct GWAS of repeated measures and identified a novel locus. Eligible cohorts in Early Growth Genetics Consortium will be included to improve statistical power.

Conflict of Interest: Qian Yang: None declared, gemma clayton: None declared, Tom Bond: None declared, Deborah Lawlor Medtronic LTD and Roche Diagnostics

P25.077.A Fine-mapping, signal-colocalization and quantitative trait locus causal analysis of the chr17q12-21 asthma locus, implicates basal lymphocytes and eosinophil levels

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Background: Asthma is a heterogeneous disease, with certain forms related to age-of-onset (AO), characterized by the activation of white blood cell (WBC) subsets. While specific biological mechanisms explaining the top signal at one of the most highly replicated asthma-associated loci, the chr17q12-21 pediatric locus have been explored, the role of WBC subsets have not been investigated in a comprehensive evaluation across all independent asthma signals within the locus.

Methods: We analyzed chr17q12-21(37.2-38.8Mb, hg19) across five populations: European (EUR); African (AFR); East-Asian (EAS); South-Asian (SAS) from the UK Biobank (UKB) and East-Asian from the Biobank Japan (BBJ). We identified independent signals (GCTA-COJO) and tested for colocalization in all asthma-WBC trait-pair combinations (COLOC-SuSiE). Finally, we conducted statistical fine-mapping (SuSiE) and tested for evidence of mediation of asthma signals through WBC traits (Regmedint).

Results: We identified a union of 8 conditionally independent asthma signals (*S1-S8*) across AO-related strata in the UKB-EUR ($R^2 < 0.5$). We prioritized *S4* and *S8* with the most compelling evidence linking asthma and WBC traits. *S4* was found to colocalize with multiple WBC traits (UKB-EUR) and causally united asthma with lymphocyte count among UKB-EUR, UKB-SAS and BBJ (H4 PP > 0.6). Multi-causal variant colocalization with lymphocyte count was identified for *S4* and *S8* (UKB-EUR). Evidence of mediation through eosinophil count was found for 27.5% of the total effect of *S8* on asthma (UKB-EUR).

Conclusion: Our results coupled with functional validation from quantitative trait locus causal analysis suggest basal WBC levels may be causally related to certain asthma association signals.

Conflict of Interest: Chief Ben-Eghan Full time, Alex Diaz-Papkovich Full time, Markus Münter Full-time, Chikashi Terao Full-time, Principal Investigator, Simon Gravel Full-time, Principal Investigator, Mark Lathrop Full-time, Principal Investigator, Scientific Director of the McGill Genome Centre, Audrey Grant Full-time, Principal Investigator

P25.078.B Beyond Genome wide association studies (GWAS) in multiple sclerosis (MS): fine mapping and functional analysis in MS susceptibility regions containing drug target genes

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Background/Aim: Multiple Sclerosis (MS) is an autoimmune multifactorial disease affecting central nervous system.

Large-scale Genome Wide Association Studies (GWAS) detected 200 MS risk loci. Linkage disequilibrium (LD) represents a limitation of GWAS in pinpointing the likely causative variant among the large number of associated SNPs, thus fine-mapping studies are required.

Methods: Functionally informed fine-mapping was performed using Paintor and CaviarBF on 36 known MS regions showing a significant replication in an Italian cohort with genotypes imputed against HRC panel (5903 individuals, 4259 MS, 1644 HC), and overlapping with at least one drug target gene (Drug-Gene Interaction database v4.2 (DGIdb). We used GWAVA, CADD and FINSURF scores for variant annotation and Open Targets Genetics tool for variant-to-gene mapping.

For 5 regions, we functionally analysed all the SNPs in LD (r2>0.75) with the lead SNP using Massively Parallel Reporter Assay (MPRA), a high-throughput in vitro screening method able to test thousands of sequences for their putative transcription regulation role, and MpraLM tool.

Results: For 11 regions, Paintor and CaviarBF prioritized at least 1 SNP with evidence of causality (posterior inclusion probability, PIP >0.75), and 5 of these SNPs target a drug target gene. For 4 regions, MPRA analyses identified at least one SNP influencing gene expression of a drug target gene, with a statistically significant effect.

Conclusion: These promising results need further validation with in silico and in vitro approaches, and have potential implication on the knowledge of disease mechanisms and novel drug target.

Grant References: 2019_R-Multi_033_Poster FISM 2022

Conflict of Interest: None declared

P25.080.D Improving the discovery of genomic loci associated with gestational duration through joint analysis of gestational duration and birth weight

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Background: Gestational duration is a critical factor in perinatal health. While previous genome-wide association studies (GWAS) have identified over twenty loci linked to gestational duration, the discovery of new loci has been hindered by the lack of available data on gestational duration relative to birth weight in large datasets such as UK Biobank (UKBB).

Methods: As gestational duration accounts for nearly 40% of the variance in birth weight, we developed a statistical method that uses birth weight as a surrogate for gestational duration, boosting the power of genomic discovery for gestational duration loci. We jointly analyzed the maternal and fetal GWAS summary results of gestational duration from 23andMe and the GWA summary results of birth weight from UKBB.

Results: Our joint analysis tripled the effective sample size for GWA analysis of gestational duration from 40,000 to 120,000 and identified 42 maternal loci associated with gestational duration. Notably, 27 of the maternal loci were replicated in a recent GWA meta-analysis. The newly discovered loci explained larger phenotypic variance and improved instrumental power in Mendelian randomization analysis. Furthermore, our method accurately estimated the maternal and fetal genetic effects on gestational duration and birth weight. Expression enrichment analysis of these loci showed that the maternal loci were enriched in endometrium, while the fetal loci were enriched in placenta.

Conclusion: This study demonstrates the advantage of joint GWA analysis in phenotypically correlated traits and provides

insights into the genetic mechanisms underlying gestational duration and birth weight.

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Conflict of Interest: Ge Zhang University of Cincinnati College of Medicine, full time, NIH/NICHD R01HD101669, Burroughs Wellcome Fund (Grant 10172896), the March of Dimes Prematurity Research Center Ohio Collaborative and the Bill & Melinda Gates Foundation., Huan Xu Cincinnati Children's Hospital Medical Center, full time, Jing Chen Cincinnati Children's Hospital Medical Center, full time, Pol Sole-Navais Department of Obstetrics and Gynecology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, full time, Jeffrey Murray: None declared, Rachel Freathy University of Exeter, full time, Wellcome Trust and Royal Society Sir Henry Dale Fellowship (WT104150), Wellcome Trust Senior Research Fellowship (WT220390), and NIH/NICHD R01HD101669., Bo Jacobsson University of Gothenburg, full time, The Swedish Research Council, Stockholm, Sweden (2015-02559), The Research Council of Norway, Oslo, Norway (FRIMEDBIO #547711, #273291), March of Dimes (#21-FY16-121), and NIH/NICHD R01HD101669., Louis Muglia Burroughs Wellcome Fund, full time

P25.081.A Rare protein-truncating variants identified in nutrient-sensing pathways contribute to healthy longevity in Bulgarian centenarians

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The molecular mechanisms governing cellular and organismal senescence are still poorly understood. The complex phenotypic trait of human longevity is expected to arise from an interplay between common genetic variants with small effect and rare variants with more significant contribution. We performed whole-genome sequencing on 16 centenarians from the Bulgarian population and bioinformatically analysed each genome individually in order to identify potential rare variants associated with longevity. NGS analysis was focused on gene ontology (GO) terms involved in different signalling pathways the modulation of which has been demonstrated to promote lifespan extension in model organisms. We identified extremely rare protein-truncating variants with expected loss of function in several important genes that play a role in different nutrient-sensing pathways such as insulin/insulin-like growth factor 1 signalling (*GPLD1*:p.Arg757Ter) and free fatty acid signalling (*FFAR3*:c.180_189del). Variants potentially downregulating mTOR-mediated signal transduction were also detected in some centenarians. Based on our findings we theorise that modulation of these evolutionarily conserved nutrient-sensing pathways could potentially mimic the effects of calorie restriction – a dietary regimen known to mediate pro-longevity effects in a wide range of species, leading to extended healthy lifespan in humans. Rare individual genetic variants that affect the regulation of certain signalling pathways identified in centenarian cohorts could contribute to elucidating the aetiology of age-associated physiological decline – a major risk factor for many diseases, and could also pave the way for the development of therapeutic agents that ameliorate the effects of ageing in the general population.

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