HISTORY OF GENETIC DISEASE

Down syndrome and genetics — a case of linked histories

David Patterson and Alberto C. S. Costa

Down syndrome, the most common genetic cause of intellectual disabilities, was first described in 1866, during an era of great change in our understanding of genetics and evolution. Because of its importance, the history of research on Down syndrome parallels the history of human genetics. In many instances, research on Down syndrome has inspired progress in human genetics. In this article, we describe the interplay between advances in the understanding of genetics and the understanding of Down syndrome from its initial description to the present, and on the basis of this historical perspective, speculate briefly about the future of research on Down syndrome.

We have known since the late 1950s that Down syndrome, which occurs in about 1 in 800 live births[1], is the result of the trisomy of chromosome 21 (Ts21)[2]. However, the molecular factors that underlie specific phenotypic components of this chromosomal disorder remain elusive. Over the past 15 years, progress in the quantitative description of specific traits that are associated with Down syndrome, the development of viable Down syndrome mouse models and our progressively more sophisticated knowledge of the human and mouse genomes have brought renewed excitement to this field (TIMELINE).

To appreciate the pace of progress in the quality of life of individuals with Down syndrome, we simply need to consider that a little over 30 years ago the median age of death of a person born with Down syndrome was 1 year[3], most people with Down syndrome were institutionalized and many physicians and scientists believed that people with Down syndrome would never achieve most of the typical developmental milestones. Now, almost all children with Down syndrome in the developed world are raised by their parents and attend schools in inclusive or semi-inclusive settings. Also, largely owing to advances in medical care and attitude changes, the median age of death in this population has increased to 49 years (REF. 3), and the life expectancy of a 1-year-old person with Down syndrome is more than 60 years and is likely to improve[4]. As their life expectancy continues to increase, however, the intellectual disabilities and neurodegenerative disorders faced by people with Down syndrome become an ever more important personal and societal problem. Current trends indicate that, until our understanding of the mechanisms that underlie chromosomal non-disjunction advances to the point that we can effectively prevent this crucial causal event in the production of Ts21, the number of individuals with Down syndrome in the population is likely to increase[4].

The general clinical aspects of Down syndrome have been reviewed thoroughly elsewhere[5,6] and are not discussed here. We focus primarily on Down syndrome as a genetic disorder that affects the brain, which has both neurodevelopmental and neurodegenerative components.

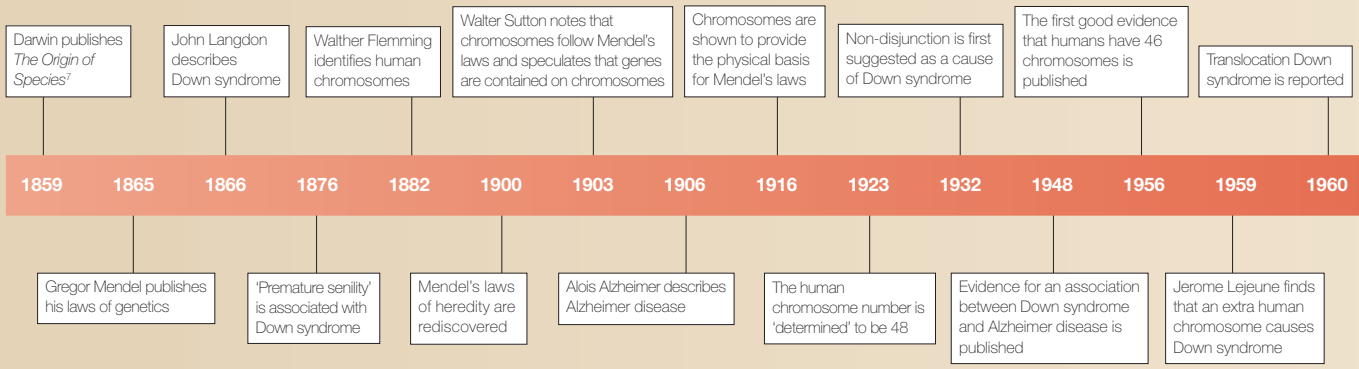
The early history of Down syndrome

Research on Down syndrome is closely intertwined with the history of genetics. In 1859, Charles Darwin published the *Origin of Species*, in which he laid out the theory of evolution[7]. Darwin's classical work dealt almost exclusively with animals, but it contributed to the debate about the origins and evolution of humans. John Langdon Down provided Darwin with access to a subject for his second seminal work, *The Descent of Man*[8], so it is clear that he was aware of the intellectual ferment about evolution and genetics.

In 1865, Gregor Mendel published his laws of genetics[9]. It is in this context that Down published *Observations on an Ethnic Classification of Idiots*; his description of Down syndrome[10]. Down felt that Down syndrome represented a racial "retrogression" caused by "instances of degeneracy arising from tuberculosis in the parents." He grouped people with Down syndrome as having "Mongol" features, and this is where the inappropriate term 'Mongol', referring to people with Down syndrome, originated. Down discussed the philosophical implications of this terminology in terms of whether humans are grouped into immutable races or whether "…races are merely varieties of the human family having a common origin." He closed with the statement "I cannot but think that the observations which I have recorded are indications that the differences in races are not specific but variable. These examples of the result of degeneracy among mankind, appear to me to furnish some arguments in favour of the unity of the human species."

In what is arguably the best biographic account on John Langdon Down, Owen Conor Ward[11] wrote "He was a man of his time, with great insight compared to many of his contemporaries, but with some of the inevitable limitations that are to be expected when so much of the knowledge and the means of acquiring knowledge — which we now take for granted — were simply not available." Ward's account includes two irresistible pieces of trivia: Down's son, Reginald

Timeline | **Genetics and Down syndrome**



Langdon Down, was the first to describe the classical pattern of palmar creases in people with Down syndrome; and Reginald Langdon Down's son — also named John Langdon Down — had Down syndrome.

In the several decades after the publication of Down's pioneering work, the proposed causes of Down syndrome included foetal HYPERTHYROIDISM (see Glossary) or another endocrine dysfunction, syphilis, environmental insult during pregnancy, social position, alcoholism, consanguinity, race and uterine exhaustion[12]. Interestingly, the association between Down syndrome and premature cognitive decline was recognized as early as 1876 (REF. 13), more than 30 years before Alois Alzheimer described the neuropathological–neuropsychiatric entity that now bears his name[14], and more than 70 years before the association of Alzheimer disease with Down syndrome was published[15].

In 1961, as solid epidemiological and cytogenetic information about Down syndrome became available, a prestigious group of 20 biomedical researchers signed a letter that was published in *The Lancet*[16], calling on their colleagues to stop using the term 'mongolism' and its derivatives to describe people with Down syndrome and proposed, among others, the term "trisomy 21 anomaly". At the 1965 World Health Assembly, the delegation for the Mongolian People's Republic informally approached the Director General of the World Health Organization (WHO) with the request that the terms Mongol and mongolism be avoided[17]. Although such objectionable terms disappeared from WHO publications after this request, they continued to appear in the scientific literature throughout the early 1970s, until slowly they fell into disuse owing in part to continued pressure from parent groups and prominent Japanese and Chinese scientists.

Chromosomes and Down syndrome

The role of chromosomal trisomy in Down syndrome emerged slowly. In 1882, Walther Flemming described chromosomal behaviour during cell division[18]. In 1900, Mendel's laws were rediscovered, and in 1903, Walter Sutton noted that chromosomes follow Mendel's laws and speculated that genes might be contained on chromosomes[19]. In 1915, Thomas H. Morgan and colleagues published a synthesis of years of work entitled *The Mechanism of Mendelian Heredity*[20], which made an almost incontrovertible case that genes are located on chromosomes. By 1920, the concept that chromosomes carry genes was widely accepted.

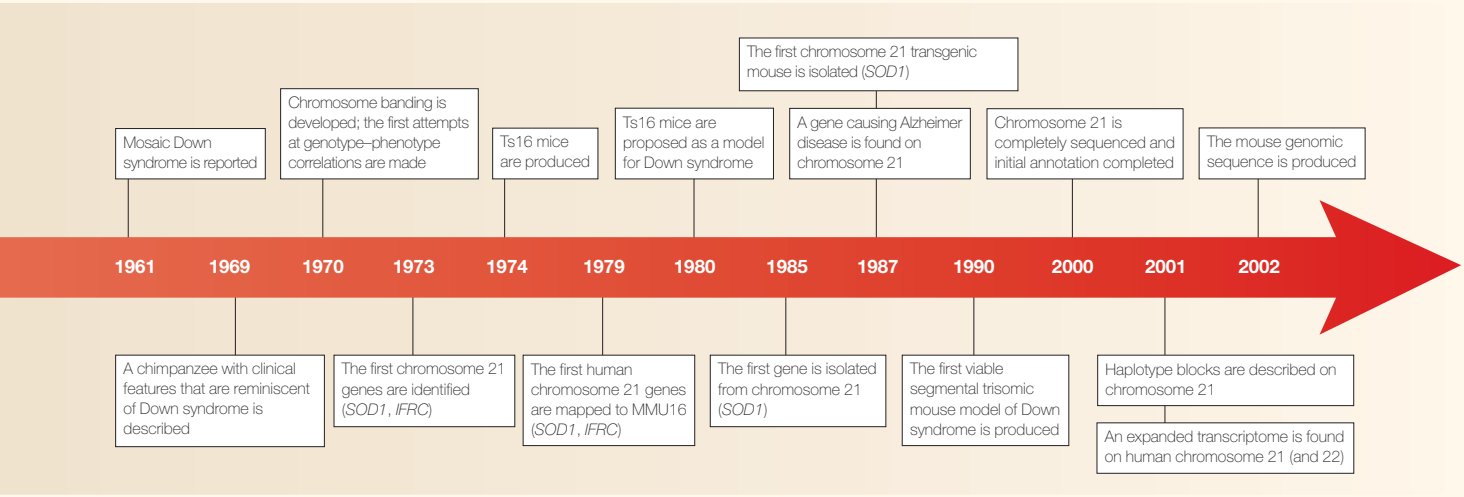
In 1921, Theophilus S. Painter presented evidence that humans have 45 to 48 chromosomes[21]. By 1923, he had concluded that the correct number of chromosomes was 48 based on the analysis of tissue from only two individuals — inmates of the Texas State Insane Asylum[22]. In 1932, Charles B. Davenport speculated that chromosomal irregularities might cause intellectual disabilities, including Down syndrome. He obtained a tissue sample from an individual with Down syndrome and sent it to Painter, who found no obvious chromosomal irregularities[23]. In that same year, Petrus J. Waardenburg speculated that non-disjunction that leads to trisomy or monosomy might be a cause of Down syndrome[24]. It was more than 25 years before the correct number of chromosomes in humans was determined and Waardenburg's hypothesis validated.

In 1956, Joe H. Tjio and Albert Levan analysed 261 chromosome spreads from 4 unselected aborted foetuses and concluded that humans have only 46 chromosomes[25]. They noted that the number 48 was so ingrained that researchers who found 46 chromosomes in their samples suspended their work because they could not find the missing 2 chromosomes. This finding was not accepted easily. For example, Masuo Kodani reported that humans could have three different chromosome numbers; 46, 47 and 48 (REF. 26). In 1958, Tjio and Theodore T. Puck published an analysis of several hundred metaphase spreads from five tissues and seven individuals that confirmed that the correct chromosome number in humans is 46 (REF. 27). Within a year, Jerome Lejeune showed that Down syndrome is caused by trisomy of HSA21 (REF. 2). Almost simultaneously, Patricia Jacobs and colleagues published a manuscript[28] that confirmed Lejeune's findings. In rapid succession, several studies reporting on Down syndrome owing to chromosomal translocations that involve HSA21 and mosaicism for Ts21 were published[29–31].

Within a decade, these cytogenetic discoveries led to the technical feasibility of prenatal testing for Down syndrome based on the karyotype of metaphase spreads from cell samples that are derived from foetuses. Clearly, the chief purpose of these tests is to allow prospective parents the option of pregnancy termination, although there are other reasons; for example, to allow planning for the birth of a child with Down syndrome (see BOX 1).

Genotype–phenotype correlations

In the 1960s, numerous examples of so-called tandem translocations between two HSA21s that result in trisomy for various regions of HSA21 were reported. In 1970, Caspersson and colleagues introduced chromosome banding to human cytogenetics, so that individual chromosomes could be subdivided and specific regions or bands could be identified[32]. In that same year, Caspersson *et al.*[33] analysed blood samples from several



individuals with Down syndrome and an individual without Down syndrome but with trisomy for part of HSA21 — but not the distal part of the long arm — and stated that "the genetic material, which in triplicate gives Down syndrome, is on the distal part on the long arm of chromosome 21." Soon, other investigators also proposed that trisomy of the distal third of HSA21, band 21q22, could cause Down syndrome, and that the severity of the syndrome might depend on the extent of the trisomic region[34,35]. These were the initial attempts at genotype–phenotype correlations for Down syndrome.

Other researchers extended the concept of genotype–phenotype correlation in Down syndrome. By comparison of many individuals with partial Ts21, a Down syndrome critical region was defined[36,37]. In an important modification of the original concept, Korenberg and colleagues developed the hypothesis that specific aspects of the phenotype of Down syndrome could be associated with specific regions of HSA21 (for example, congenital heart disease), whereas others (for example, intellectual disabilities) could not[38,39].

Origins of the phenotypic features

There are two principal hypotheses for how Ts21 causes Down syndrome. Both are based on the assumption that if a gene is present in three copies rather than two the level of expression of that gene will be elevated — in the simplest case, by 50%. The gene dosage-effect hypothesis states that elevated expression of specific trisomic genes directly leads to specific features of Down syndrome. The amplified developmental instability hypothesis states that, in general, the most important cause of the array of phenotypic features that are associated with Down syndrome does not actually involve direct contributions of specific genes on HSA21, but rather that elevated activity of sets of genes, regardless of their identity, will lead to a decrease in genetic stability or homeostasis[40,41]. Therefore, the larger the number of trisomic genes, the more susceptible the foetus will be to developmental abnormalities. These two hypotheses are not mutually exclusive. The chief proponents of the amplified genomic instability hypothesis readily admit that there might be some phenotypes of Down syndrome that are influenced to a large extent by specific trisomic genes.

The amplified genetic instability hypothesis seeks to explain two observations — the fact that phenotypic commonalities occur in

Box 1 | **Prenatal diagnosis and screening**

It is beyond the scope of this article to attempt to review the ethical and legal issues surrounding the termination of pregnancies that involve the possibility of a foetus with Ts21. We can point out, however, that this debate continues to this day. A sobering reminder of this fact is that recent evidence indicates that an increasing number of prospective parents are deciding not to avail themselves of prenatal diagnosis, even when at risk for birth of a child with Down syndrome[132] (because of a positive screening test or advanced maternal age). This trend is likely to accelerate as progress in research and medical care continues to improve the outcome for people with Down syndrome.

Prenatal diagnosis by amniocentesis has been offered since the early 1970s. By the mid 1980s, prenatal diagnosis using chorionic villus sampling also has become an option[133]. In fact, most prenatal diagnosis is offered when there is the possibility of the birth of a child with Down syndrome. Generally, because these procedures are not without risk, they are only routinely offered to pregnant women that are older than thirty-five.

Because of the frequency of Down syndrome and the risk from invasive testing, researchers have attempted to devise non-invasive screening procedures to detect a foetus with Down syndrome. The possibility of using biochemical markers in maternal blood for screening became a reality in 1984, when Merkatz *et al.* reported an association between low maternal serum α-foetoprotein and foetal chromosomal abnormalities[134]. Since that time, further biochemical markers (up to six) have been added to screening tests for Down syndrome to increase specificity and to reduce false positive and false negative results[135]. Also, in 1992, the use of ultrasound to detect FOETAL NUCHAL TRANSLUCENCY to screen for chromosomal aneuploidies was reported[136]. The biochemical screens have been combined with ultrasound in an attempt to improve the success of screening. In addition, detection of foetal cells in maternal blood has also been examined as a possible method of non-invasive screening for Down syndrome[137]. All these tests and combinations thereof have significant false positive rates and do not detect all cases of Down syndrome.

It seems imperative that more bioethical debate occurs before non-invasive prenatal diagnostic techniques for Down syndrome are developed and widely implemented. Issues to be considered include, but should not be limited to, the possibility that such tests devalue the humanity of people with Down syndrome and that they might increase legal or other forms of discrimination against people with Down syndrome (see for example, REF. 138).

many ANEUPLOID states; and that phenotypic traits of Down syndrome occur in euploid people, but at much lower frequencies. Some features of Down syndrome are seen in other aneuploidies, but some are not. Other aneuploid conditions do not result in the appearance of the neuropathological signs of Alzheimer disease or an increased risk of Alzheimer disease. It was this unique increased risk of Alzheimer disease-like pathology and Alzheimer disease-like dementia in people with Down syndrome that led to the hypothesis that a gene on HSA21 must be involved in Alzheimer disease[42] and to the demonstration that mutations in a gene that encodes the amyloid precursor protein, *APP*, cause early-onset Alzheimer disease[43]. Although it can be argued that this apparent uniqueness of Alzheimer disease-like pathology in adults with Down syndrome is simply a consequence of the increased longevity of these individuals compared with people with other autosomal aneuploid conditions, several other distinctive features also are observed. For example, MYELODYSPLASTIC SYNDROME and ACUTE MYELOID LEUKAEMIA (see also Online links), which are associated with Down syndrome, have unique clinical characteristics[44] and are rarely, if ever, seen in other aneuploid conditions. The most frequent forms of cardiac malformation that are seen in people with Down syndrome are atrioventricular canal defects, whereas the most common heart defects in trisomy 13, 18 and 22 are ventricular septal defects[5,6,45]. Also, DUODENAL ATRESIA, which is not characteristic of other aneuploidies, affects 2–5% of infants with Down syndrome, whereas 20–30% of all children with duodenal atresia have Down syndrome[5,6].

Mapping and sequencing of HSA21

Somatic-cell hybrid mapping. The finding that fusion between somatic rodent and human cells often leads to hybrid cells that retain one or a small number of human chromosomes had a major impact on mapping of HSA21. In 1973, the first two genes to be mapped to HSA21 were mapped using this approach — a gene for an interferon receptor (*IFNAR1*) and the gene for indophenol oxidase B, now known as cytosolic superoxide dismutase (*SOD1*)[46]. The hybrid cell line that is described in this report and its derivatives are still used today.

In 1977, Moore *et al.*[47] mapped the gene for the purine biosynthetic enzyme phosphoribosylglycineamide synthase (*GARS*) to HSA21 using hybrids between human cells and Chinese hamster ovary (CHO) cells that were defective in the *GARS* gene and required purines for growth. In this way, HSA21 or fragments thereof (as long as they contained *GARS*), could be selectively retained in somatic-cell hybrids. Using this approach, a large number of somatic-cell hybrids that retain various fragments of HSA21 were created and rapidly used by many laboratories to regionally map genes on HSA21. In 1990, a set of four hybrids was adopted by the HSA21 research community as a generally available HSA21 somatic-cell hybrid mapping panel[48]. By 1995, this panel contained 27 well-characterized somatic-cell hybrids[49,50].

Radiation hybrid mapping. In 1975, Goss and Harris published an elegant paper describing the use of somatic-cell hybrids to determine the relative physical linkage and order of genetic markers along chromosomes[51]. This method involved irradiating human cells, immediately fusing them with CHO cells that lack hypoxanthine phosphoribosyltransferase (HPRT) activity, isolating a large number of hybrid cells that contain the human HPRT selectable marker and then measuring how often other markers on the X chromosome co-segregated with the HPRT marker (which is known to be on the X chromosome).

In 1990, a general radiation hybrid mapping method was described[52]. First, a CHO/human somatic-cell hybrid that contains HSA21 was irradiated and then fused with an *HPRT*-deficient CHO cell. Second, HPRT positive clones, which must contain the functional *HPRT* gene from the irradiated hybrid, were selected. Third, the presence and co-segregation of HSA21 markers were assessed and analysed statistically to determine the distances between DNA markers and the order of markers. This method has become a mainstay of human genome mapping.

Linkage mapping. Genetic linkage mapping of HSA21 allowed the ordering of markers along the chromosome and revealed insights about the possible roles of meiotic recombination, which might be important in understanding the non-disjunction events that lead to Down syndrome[53]. For example, it is now clear that meiotic recombination varies in level and position along HSA21. Analysis of polymorphic markers was also important in establishing that the vast majority of extra HSA21s in Down syndrome are maternally derived[54]. As DNA clones that contain the markers used for linkage became available, the linkage map was rapidly integrated with the cytogenetic, somatic-cell hybrid and physical maps of the chromosome. This integration allowed independent validation of each map.

Physical mapping. The initial physical map of HSA21 was the cytogenetic map, which still presents advantages for relating chromosome anomalies to the DNA sequence[55]. Other maps were aligned with the cytogenetic map, to the resolution limits of the light microscope. However, production of a physical map of HSA21 in terms of base pairs of DNA required further technical advances; in particular, methods for separating large DNA fragments by size on gels, the use of restriction enzymes that cut human DNA at rare intervals and methods of cloning large DNA fragments (on the order of hundreds of kilobases of DNA). Developed between 1984 and 1992, these methods were rapidly applied to mapping HSA21 (REFS 56–64).

A crucial step in both the mapping and sequencing of HSA21, and even the entire human genome, was the development of methods to clone large DNA fragments. The first successful method involved the creation of YACs[58], which allowed cloning of DNA fragments that are larger than 1 million base pairs into yeast cells. These could then be analysed for DNA markers and assembled into contiguous clones that cover large regions of the genome. HSA21q was one of the first two chromosome arms for which a contiguous set of overlapping YAC clones was constructed[60].

YACs were a major technical breakthrough but they had a tendency to rearrange and to contain DNA from different genomic regions, thereby producing chimaeric clones. It turned out to be extremely difficult to produce a minimal set of YACs that covered HSA21q with no gaps[50,65]. Fortunately, methods of cloning slightly smaller DNA fragments in bacteria, up to a few hundred kilobases, were soon developed[62,64], thereby avoiding problems of rearrangement and chimaerism.

In 1990, the HSA21 community created the Chromosome 21 Joint Yeast Artificial Chromosome Effort[48]; an effort by which libraries of large-insert DNA clones were screened for specific clones from all researchers working on HSA21. In 3 years, more than 1,200 large-insert clones were made available to more than 70 laboratories throughout the world.

Sequencing. Building on their long history of collaboration, the HSA21 community discussed (in 1994) and officially established (in 1996) a consortium to sequence HSA21 (REF. 66). The actual DNA sequence of HSA21q was obtained primarily by laboratories in Japan and Germany, but it built on the work of the entire HSA21 research community. The publication of the DNA sequence

of HSA21 represented the culmination of the mapping effort[67]. This publication integrated the physical maps, linkage maps and somatic-cell hybrid maps, and reported the initial annotation of the gene content of HSA21q and several other physical features of HSA21, such as CpG ISLAND content, repeat sequence content and the distribution of transcripts. It represents a seminal achievement in Down syndrome research and provides the basis for studies of gene structure, expression and function, and guides the production of mouse models for Down syndrome, analysis of methylation patterns[68] and the investigation of novel RNA transcripts from HSA21 (REF. 69).

Analysis of the HSA21 DNA sequence confirmed several speculations about HSA21. For example, many researchers had speculated that one reason Ts21 is compatible with life is that HSA21 might be relatively gene-poor. On the basis of the apparently inordinate importance of 21q22, it was speculated that this band might be gene-rich relative to other areas of the chromosome. Both these speculations were proved to be true[67]. The publication of the mouse genomic sequence allowed comparison of the SYNTENIC REGIONS of the mouse genome with HSA21 (REF. 70), which enhanced the continued annotation of HSA21 and is of great importance for evaluating the suitability of mouse models of Down syndrome[71].

In 2001, Patil *et al.*[72] used the sequence of HSA21 and somatic-cell genetic techniques to examine the distribution of SNPs on HSA21 from different individuals. This approach revealed blocks of limited haplotype diversity. By the use of somatic-cell hybrid technology to separate individual HSA21s, they were able to eliminate ambiguity resulting from the presence of two HSA21s in human euploid cells. This was the first study to define HAPLOTYPE BLOCKS for an entire chromosome. This finding has potentially profound implications for comprehensive WHOLE-GENOME ASSOCIATION STUDIES designed to map loci that are relevant to disease because, once the blocks are defined, the number of SNPs that need to be examined can be markedly reduced.

The transcriptome and proteome

The transcriptome. Measuring mRNA levels is the most direct method of assessing alterations in gene expression in Down syndrome. Mao *et al.*[73] carried out such an analysis on human foetal Ts21 material. The results indicate a global upregulation in the expression of genes that map to HSA21, but not of other genes in the genome. Microarray analysis of Ts21 and Ts13 cell lines indicate a subtle elevation in the expression of trisomic genes compared with diploid cell lines[74]. In contrast to the findings of Mao *et al.*[73], this approach provided evidence for widespread, more extreme dysregulation of genes that are not on the trisomic chromosome. The authors reported consistent differences in the genes that are dysregulated in trisomic cells. It is not only important to determine whether HSA21 genes are expressed according to gene dosage in Down syndrome, but also to establish the temporal and spatial expression patterns of these genes. Work to address this issue is underway[75,76].

In an intriguing set of experiments, microarray technology has been used to examine the transcriptome of HSA21 and HSA22 (REFS 69,77). This work provides evidence that the transcriptome of HSA21 is much larger than previously thought, with perhaps only 31.4% of the transcribed nucleotides encoding well-annotated genes[69]. The nature of the additional transcripts is not fully understood at present. This observation might lead to a reassessment of the number of genes that are present on the HSA21 and perhaps a broadening of our concept of what constitutes a gene.

The proteome. In 1975, O'Farrell described TWO-DIMENSIONAL GEL ELECTROPHORESIS (2DGE)[78]. 2DGE was applied rapidly to the analysis of Down syndrome[79–83], but the general conclusions were rather disappointing. With the exception of SOD1, no proteins could be identified. Proteins either increased or decreased owing to the presence of an extra HSA21, but, at that point in history, precise quantification was almost impossible. No studies were done on tissue samples from individuals with Down syndrome, and studies on cell cultures might have limited their relevance to protein patterns *in vivo*[84]. Probably for these reasons, these studies were not pursued for the most part.

Many of these difficulties have now been overcome by technical advances such as the use of MASS SPECTROMETRY and database searching to identify proteins. The complexity of the proteome requires further protein fractionation methods, and progress is being made in this area. Mouse models might serve as useful alternatives to overcome the difficult problem of obtaining appropriate human samples. Quantification issues might be amenable to new experimental approaches, for example DIFFERENCE GEL ELECTROPHORESIS (DIGE)[85].

The use of mouse models

Cognitive impairment is one of the most difficult and yet one of the most necessary features of Down syndrome to address experimentally. It is imperative to link cognition with neurobiology and neurophysiology. This will require the use of model organisms. The most useful models so far have been mice[86]. Nevertheless, mice are not humans and, for each new Down syndrome-like phenotype studied, it will be necessary to determine the applicability of results from mouse models to the human situation.

For an animal model to be most useful to study Down syndrome, two features are necessary. First, the phenotype of the animal model must possess features that are relevant to Down syndrome. Second, the animal model should be trisomic for a gene or genes that are present on HSA21. Until 1969, there was no evidence that these features occurred in any non-human species. In that year, a chimpanzee was described that had many phenotypic features that are associated with Down syndrome[87]. Examination of the chromosomes of this chimpanzee showed that it was trisomic for a small ACROCENTRIC CHROMOSOME reminiscent of HSA21, chimpanzee chromosome 22. This was an early indication that mammalian autosomes might be at least partially conserved, and that phenotypes that are reminiscent of Down syndrome could be observed in other species. The speculation that these two chromosomes were similar was confirmed when the DNA sequence of chimpanzee chromosome 22 was analysed[88]. These analyses have been extended to other hominoids and allow conclusions to be made about the evolution of the hominoids and some inferences about the unexpected complexity of genomic changes after speciation[88,89]. Importantly, the conservation of chromosomal regions can be extended to other mammals, including the mouse, as described below.

Trisomy mouse models. In the 1970s, Alfred Gropp and collaborators developed mouse strains that allow the generation of trisomy for any mouse chromosome[90]. In 1978, Charles Epstein[91] speculated that "For studying human trisomy 21, it would be useful to have a mouse model of aneuploidy for a chromosome or chromosome segment homologous to that part of human chromosome 21 that gives rise to Down syndrome. Once the human chromosome 21 loci, such as superoxide dismutase-1, the antiviral genes, and glycinamide ribonucleotide synthase, are mapped on the mouse genome, it will be possible to study systematically the consequences of aneuploidy on the functions of these loci." The first gene located on HSA21 was mapped to mouse chromosome 16 (MMU16) in 1979 (REF. 92). By 1980, on the basis of phenotypic

similarities between mouse Ts16 and Down syndrome and synteny between parts of HSA21 and MMU16, mouse Ts16 was identified as a potential model for Down syndrome[93,94]. Ts16 mice have many features that are reminiscent of Down syndrome and have been used profitably to study aspects of Down syndrome. However, the model has several inherent disadvantages. MMU16 has many genes in addition to those present on HSA21 and also does not contain all the genes on HSA21. Furthermore, Ts16 mice usually die at birth, so they are not suitable for many studies that are relevant to Down syndrome[95].

The Ts65Dn mouse. The most robust and widely used mouse model of Down syndrome, the Ts65Dn mouse, was produced by Muriel Davisson's group[96,97]. The Ts65Dn mouse contains an extra chromosome, spanning most of the region of MMU16 that is homologous to HSA21, translocated to a MMU17 centromere. It possesses many physical, behavioural and neurological features that are reminiscent of those seen in people with Down syndrome, including CRANIOFACIAL DYSMORPHOGENESIS, age-related loss of CHOLINERGIC MARKERS in the basal forebrain, and notable learning and memory deficits[98–105] (TABLE 1). The trisomic region extends from *Mrp139* to *Znf295* and contains roughly 136 genes that are orthologous to human genes on HSA21 (REF. 106).

Other segmental trisomy mouse models. Other segmental trisomy mouse models are available, but are still not as well characterized. The Ts1Cje mouse, produced in the laboratory of Charles Epstein, is a translocation between mouse chromosomes 12 and 16. The MMU16 translocation breakpoint is within *Sod1*, inactivating this gene. Therefore, this mouse is trisomic for the segment from the disrupted *Sod1* to *Znf295* (REF. 107). Ts1Cje mice contain ~97 genes with HSA21 orthologues. By judicious breeding of Ts1Cje and Ts65Dn mice, it is possible to generate mice that are trisomic for the region between *Mrp139* and the disrupted *Sod1*, which contains roughly 39 genes[108]. Comparison of the phenotypes of these mice is an extremely active area of research. Already, it is clear that trisomy of different regions of MMU16 leads to notable differences in phenotypes[104,107,108].

Segmental trisomy mice have been used to examine changes in the transcriptome owing to trisomy with mixed results. In a microarray analysis of Ts65Dn mouse cerebellum, Saran *et al.*[109] reported that they could detect alterations in HSA21 orthologues, as expected, but that changes in individual genes were subtle. They also reported alteration in the expression of thousands of genes in the cerebella of Ts65Dn mice, indicating global destabilization of transcription. Lyle *et al.*[110] used quantitative REAL-TIME PCR (RT-PCR) to show that in Ts65Dn mice, only ~33% of trisomic genes were 1.5-fold overexpressed, probably owing to gene dosage. Kahlem and colleagues[106] used cDNA array analysis and quantitative RT-PCR and obtained similar results. Amano *et al.*[111] found that in Ts1Cje mouse brains, expression levels of most of the genes in the trisomic region were elevated ~1.5-fold, whereas expression of genes on other chromosomes was largely unchanged.

Transgenic mice. Several transgenic mice that express from one or a few genes to entire segments of HSA21 (YAC transgenic mice) have been produced. As expected, none of these recapitulate the entire phenotype of Down syndrome, but many have features that are reminiscent of aspects of the phenotype. Transgenic mice containing human *SOD1* were the first mice produced to contain a gene encoded on HSA21 (REF. 112). These mice have some features that are similar to those seen in people with Down syndrome[113,114]. Many investigators have produced transgenic mice that express normal and mutant forms of *APP*, and have demonstrated learning and memory deficits in these mice, including performance decline with age. In a recent experiment, compound transgenic mice that express human *SOD1* and *APP* were created[115]. Working memory and long-term memory are severely impaired in these double transgenic mice; they have defects in APP processing, lipofuscin accumulation and mitochondrial anomalies. These findings

Table 1 | **Features in people with Down syndrome and Ts65Dn mice**

Feature	Down syndrome	Ts65Dn
Learning and memory deficits that potentially implicate abnormal hippocampal function	Yes	Yes
Learning and memory decline with age	Yes	Yes
Altered synaptic endocytosis	Yes	Yes
Degeneration of basal forebrain cholinergic neurons	Yes	Yes
Decreased TrkA receptors with increasing age	Yes	Yes
Decreased hippocampal volume	Yes	Yes
Decreased dendritic spines on cortical pyramidal cells	Yes	Yes
Hyperactivity	Some	Yes
Seizures	Some	Some
Stereotypical (repetitive) behaviours	Some	Yes
Difficulty in suppressing inappropriate behaviour	Some	Yes
Pain response	Altered	Altered
Decreased cerebellar volume	Yes	Yes
Decreased cerebellar granule cells	Yes	Yes
Gait abnormalities	Yes	Yes
Growth and development	Slow growth, short stature and obesity	Lag in growth and body weight
Craniofacial dysmorphogenesis	Yes	Yes
Haematological and/or immunological abnormalities	Yes	Yes
Male sterility	Yes	Yes
Pregnancy	Ovulation difficulties, shorter reproductive life	Small litters, shorter reproductive life
Ageing	Shorter life expectancy	Shorter life expectancy
Oxidative stress	Likely	Likely
Upregulation of trisomic gene transcription	Yes	Yes
Global transcription dysregulation	Yes?	Yes?
Brain myo-inositol levels	Elevated	Elevated
Vasoactive intestinal peptide anomalies	Yes	Yes
Altered plasma amino acids	Yes	Yes

Box 2 | **One-carbon and folate metabolisms**

In 1999, a provocative and controversial study was published suggesting that mutations in the methylenetetrahydrofolate reductase gene (*MTHFR*) that decrease the activity of the encoded enzyme might increase the risk of birth of a child with Down syndrome[139]. Many studies have been done to confirm or refute this claim, and there remains noteworthy disagreement[140]. One-carbon and folate metabolism might be altered in children with Down syndrome[141]. One intriguing study suggests that in families in which a neural tube defect had been observed there was an increase in the risk of a birth with Down syndrome and vice versa[142]. Given the known ability of folic acid supplementation before conception to decrease the risk of birth of children with neural tube defects, this indicates that altered folate metabolism might also have a role in conception or development of people with Down syndrome.

Most cases of Down syndrome arise from errors in maternal meiosis I, which occurs during female foetal development. The oocytes remain 'frozen' in meiosis I until ovulation[143]. It could be proposed that it is the nutritional status of the maternal grandmother that is important, and that no effect of folate supplementation would be seen until the second generation after supplementation[141]. In this regard, two studies suggest that there is a maternal grandmaternal-age effect in Down syndrome. That is, there is an increased incidence of births of children with Down syndrome as the maternal (but not paternal) grandmother's age increases[144,145]. This indicates that indeed the environment of the foetal development of the mother might be important for Down syndrome.

are intriguing in light of the increasing evidence for a link between mitochondrial dysfunction, oxidative stress, APP processing and Down syndrome[116].

Other genes that cause phenotypes that are relevant to Down syndrome when overexpressed in transgenic mice include *Pfkl*[117], *Dyrk1a*[118], *Sim2* (REF. 119), *S100β*[120] and *Ets2* (REF. 121).

Ideally, transgenic mouse models of Down syndrome would contain a single extra copy of the relevant gene and regulatory elements that are similar enough to wild-type genes to assure typical spatio-temporal patterns of expression. These conditions are rarely achieved. Also, most transgenic mice contain human transgenes, and these might not be appropriately expressed in mice. Nonetheless, it is remarkable that these individual transgenes, and sometimes combinations of transgenes, have phenotypes that are reminiscent of Down syndrome. Construction of more transgenic mice seems well justified.

Future research

The ultimate goal of research on Down syndrome should be to improve the lives of people with Down syndrome and their families. Much remains to be done to reach this goal. For the most rapid progress to occur, it will require the integration of numerous disciplines, such as cognitive neuroscience, neurology, psychiatry, mouse and human genetics, bioinformatics and computer modelling of the regulation of metabolic and/or signalling pathways. The importance of quantitatively defining more specific traits that are associated with Down syndrome cannot be overemphasized. As has previously been said[122], "being able to measure accurately the outcome of an experiment is half of the battle." Poorly defined phenotypes will necessarily lead to poorly defined genotype–phenotype correlations.

In recent years, significant progress has been made on the precise definition of specific neuropsychological deficits that are associated with Down syndrome. For example, Pennington and collaborators[123] showed that in people with Down syndrome, hippocampal function seems to be disproportionally affected in the general context of their cognitive disabilities. Quantitative neuroimaging studies in children and adults with Down syndrome have offered morphometrical support to this idea[124,125]. Functional imaging studies and *in vivo* radioligand binding imaging by POSITRON EMISSION TOMOGRAPHY (PET) should provide us with key information to guide future studies at the molecular and cellular levels in animal models.

At the molecular level, the various global approaches to assess the transcriptome and the proteome offer new opportunities. We are now able to search for possible alterations in an unbiased way, and to use these approaches to assess hypotheses that involve entire biological systems. An attractive feature of this approach is that it can detect alterations in the expression of genes that are not on HSA21 in Down syndrome. Alterations in entire biological systems — for example, metabolic pathways, signalling, proteasome function, regulation of transcription, energy generation and mitochondrial function — or some unrecognized pathway might be found using these unbiased approaches.

The general distribution of the first aneuploid model of Down syndrome (the Ts65Dn mouse) at reasonable numbers started less than 10 years ago. Therefore, it is understandable that most of the publications on experiments using these animals have been phenomenological descriptions of the behavioural or neurological phenotype based mostly on standard tests developed for mutant mice. However, new studies that are designed to obtain a deeper understanding of the neurobiology of mouse models of Down syndrome are beginning to be published (see for example, REF. 126).

A particularly elegant set of experiments that exploit several mouse models of Down syndrome involved the analysis of early ENDOSOMAL abnormalities that are associated with Alzheimer disease[102]. These abnormalities are considered by some investigators to be one of the earliest detectable abnormalities seen in Alzheimer disease and are also seen in the Ts65Dn mouse. Interestingly, Ts1Cje mice do not show these abnormalities, which indicates that one or more of the genes located in the Ts65Dn trisomic segment but not in the Ts1Cje segment might be necessary to produce them. Cataldo *et al.*[102] showed that

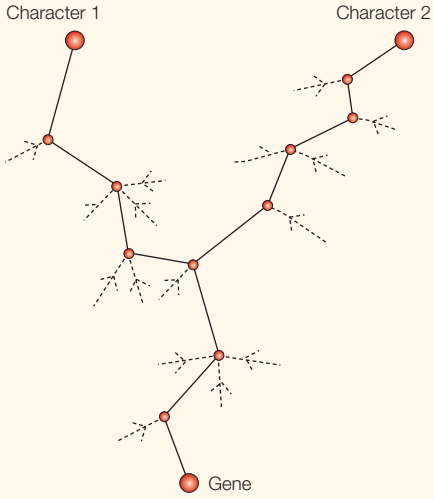


Figure 1 | **Systems biology or the study of "complex webs of biochemical processes".** Nobel Laureate Herman Muller, in a Harvey Lecture delivered in 1948 (REF. 146), stated that "The great majority of characters which a gene affects are not immediate products of it but are end-results of complex webs of biochemical processes, in which a given thread or chain of reactions can, theoretically, be picked out that has its root in the gene in question, and leads from the latter to the character through a considerable series of events (see Figure 7)." The figure he mentions, which is reproduced here, is similar to many metabolic and signalling pathway figures seen in today's scientific literature. Modified from the Harvey Society Lecture series

reduction of *App* from three copies to two in Ts65Dn mice eliminated the endosomal anomaly. However, elevated expression of *App* alone in transgenic mice does not produce endosomal anomaly, which shows that trisomy of *App* is necessary, but not sufficient, for the phenotypic expression of endosomal abnormality.

More aneuploid mouse models of Down syndrome might prove extremely useful. All the segmental trisomy mice that currently exist are trisomic for regions of MMU16. Because MMU16 does not contain all the genes that are present on HSA21, mice containing segments of MMU16 will never represent a complete model of Down syndrome. Therefore, mice that are trisomic for MMU10 and MMU17 (which contain ~22 and ~53 HSA21 gene orthologues, respectively[71]) might provide key information about Down syndrome-like features that are not present in Ts65Dn mice (for example, cardiac malformations). In addition, two laboratories have taken the direct approach of introducing HSA21 into mouse embryonic stem cells to produce transchromosomal mice; that is, mice containing a HSA21 (REFS 127,128).

Conclusions

The history of research about Down syndrome is inextricably linked, both on a conceptual and technical basis, with the history of genetics. For example, it was impossible to think productively about the cause of Down syndrome until the presence and nature of chromosomes was known. Both the increased developmental instability and gene dosage hypotheses on Down syndrome depended on this conceptual breakthrough. The concept that certain regions of HSA21 might be particularly important in Down syndrome — the genotype–phenotype correlation — required the technical advances associated with the development of chromosomal banding methods.

On the other hand, many advances in genetics, especially human genetics, were pioneered by studies of HSA21, and the interest in this chromosome was, and continues to be, driven by its causal role in Down syndrome. For example, somatic-cell hybrid mapping, radiation hybrid mapping, the chromosome-wide discovery of haplotype blocks, the discovery of the unexpected complexity and size of the transcriptome, and the concept of synteny of mammalian autosomes, were all pioneered by studies of this chromosome. The first purposeful construction of a segmental trisomy mouse model of human aneuploidy — the Ts65Dn mouse — was carried out as a means to study Down syndrome.

Techniques such as large-DNA insert cloning and pulsed-field gel electrophoresis were applied to HSA21 as soon as they were developed, and often refinements in techniques were discovered during the course of these experiments. For example, the extensive nature of the difficulties in using YACs for building physical maps was largely determined by the application of this technology to HSA21.

Some long-lasting incorrect findings were the result of the technical limitations of their time. For example, the incorrect "determination" of the number of human chromosomes was largely due to technical issues. Therefore, the incorrect determination of 48 chromosomes became entrenched scientific dogma for more than 30 years. The lesson from this, which is certainly not new, is that one needs to be willing to challenge concepts and 'facts'. It can take decades not only to correct such errors, but for some knowledge to become accepted.

Some guidelines to future research are already becoming clear from consideration of the history of research about Down syndrome. For example, it seems unlikely that a Down syndrome critical region exists as was originally conceived. In a particularly telling experiment, Olson *et al.*[129] recently showed that in mice the Down syndrome critical region is not sufficient and for the most part not necessary to produce the

Glossary

ACROCENTRIC CHROMOSOME
A chromosome in which the centromere is near to one end, as in human chromosomes 13–15, 21, 22 and Y. All mouse chromosomes are acrocentric.

ACUTE MYELOID LEUKAEMIA
A cancerous overproduction of immature myeloid white blood cells (blast cells).

ANEUPLOID
Having an abnormal number of chromosomes that are not an exact multiple of the haploid number. The antonym is euploid.

CHOLINERGIC MARKERS
These are biochemical or immunological markers for cholinergic neurons (presynaptic neurons that produce acetylcholine). Although it remains controversial, it has been suggested that loss of function of basal forebrain cholinergic neurons might be linked to neurodegenerative changes in the cerebral cortex of individuals with Alzheimer disease and Down syndrome (the so-called cholinergic hypothesis).

CpG ISLAND
A genomic region of about one kilobase that has more than 50% C+G content.

CRANIOFACIAL DYSMORPHOGENESIS
Abnormal development of the bones of the skull, including the facial bones.

DIFFERENCE GEL ELECTROPHORESIS
A technique whereby two or more protein samples are labelled with different fluorescent dyes so that they can be mixed together, co-separated and visualized on a single 2D gel.

DUODENAL ATRESIA
A condition in which the duodenum (the first part of the small bowel) has not developed properly and does not allow the passage of stomach contents.

ENDOSOME
A vesicle formed by invagination of the plasma membrane.

FOETAL NUCHAL TRANSLUCENCY
The appearance on ultrasound examination of a subcutaneous collection of fluid behind the foetal neck.

HAPLOTYPE BLOCKS
The apparent haplotypic structure of the recombining portions of the genome, in which sets of consecutive co-inherited alleles are separated by short boundaries. There is debate about the origins of haplotype blocks and whether the boundaries correspond to recombination hotspots.

HYPERTHYROIDISM
An abnormality of the thyroid gland in which secretion of the thyroid hormone is increased and not regulated properly.

MASS SPECTROMETRY
A technique in which molecules are ionized, the ions are separated in the gaseous state and the ratio of mass to charge is determined to derive structural information. This technique requires only a small amount of sample.

MYELODYSPLASTIC SYNDROME
A condition in which the bone marrow cannot produce blood cells effectively; many of the blood cells that are formed are defective, which results in low blood cell counts.

POSITRON EMISSION TOMOGRAPHY
(PET). Imaging of the emission of positrons from the brain after a small amount of radioactive isotopes have been injected into the blood stream to routinely and quantitatively measure metabolic, biochemical and functional activity in living tissue.

REAL-TIME PCR
A technique designed to detect and quantify sequence-specific PCR products as they accumulate in 'real-time' during the PCR amplification process.

SYNTENIC REGIONS
A genomic region that is collinear in the order of genes (or of other DNA sequences) in a chromosomal region of two species.

TWO-DIMENSIONAL GEL ELECTROPHORESIS
A gel electrophoresis method in which a protein sample is separated by isoelectric point in one dimension and by size in a second, perpendicular dimension.

WHOLE-GENOME ASSOCIATION STUDIES
A set of methods that are used to correlate polymorphisms in genotype to polymorphisms in phenotype in populations on a genome-wide scale.

craniofacial phenotype of Ts65Dn mice. Still, vital single genes that have a notable effect on the phenotype, such as *App*, are emerging. Given that the gene products from HSA21 participate in biological systems that are integrated with proteins encoded on other chromosomes, the identification of the systems that affect the phenotype will be crucial (see for example, REF. 130). Such systems might directly involve genes on HSA21 or other biological pathways that are perturbed indirectly by trisomy. This added complexity should be looked on as an opportunity to expand possible therapeutic targets (for example, see BOX 2). Clearly, this is a 'systems biology' approach to Down syndrome. This approach, although it is perhaps now more amenable to experimental attack, is a concept that has been one of the central ideas of genetics for many years (FIG. 1).

Because of the unprecedented experimental and theoretical tools that are available today, it is not unreasonable to speculate that even the complicated cognitive disabilities that are associated with Down syndrome might be amenable to therapeutic interventions designed to help people with Down syndrome to maximize their potential. This might require multiple modes of intervention, which is not a concept unfamiliar to medicine. The realization of such a vision would probably have momentous bioethical implications. As Timothy Reynolds (Queen's Hospital, Staffordshire, UK) wrote recently[131] "The ethics of screening for cystic fibrosis are already being queried because the *CFTR* gene has been sequenced and a possible cure is expected. Who knows whether the human genome project will make trisomy 21 a treatable condition? We can only wait and see."

David Patterson and Alberto C. S. Costa are at the Eleanor Roosevelt Institute and the Department of Biological Sciences at the University of Denver and also at the Department of Psychiatry, University of Colorado Health Sciences Center, 1899 Gaylord Street, Denver, Colorado 80206, USA.

Correspondence to D.P.
e-mail: dpatter2@du.edu

doi:10.1038/nrg1525

Published online 10 January 2005

1. Roizen, N. J. & Patterson, D. Down's syndrome. *Lancet* **361**, 1281–1289 (2003).
2. Lejeune, J. Le mongolism: premier exemple d'aberration autosomique humaine. *Ann. Genet.* **1**, 41–49 (1959) (in French).
3. Friedman, J. M. Racial disparities in median age at death of persons with Down syndrome — United States, 1968–1997. *MMWR Morb. Mortal Weekly Rep.* **50**, 463–465 (2001).
4. Bittles, A. H. & Glasson, E. J. Clinical, Social, and ethical implications of changing life expectancy in Down syndrome. *Dev. Med. Child Neurol.* **46**, 282–285 (2004).
5. Cohen, W. I. in *Down Syndrome: a Promising Future Together* (eds Hassold, T. J. & Patterson, D.) 15–36 (Wiley-Liss, New York, 1999).
6. Tolmie, J. L. in *Emery and Rimoin's Principles and Practices of Medical Genetics* (eds Rimoin, D. L. *et al.)* 1129–1183 (Churchill Livingstone, London; New York, 2002).
7. Darwin, C. *The Origin of Species* (John Murray, London, 1859).
8. Darwin, C. *The Descent of Man and Selection in Relation to Sex* (John Murray, London, 1871).
9. Mendel, G. Versuche über Pflanzen-Hybriden. *Verhand. naturfors. Vereines in Brunn* **4**, 3–47 (1865) (in German); English translation available online at <http://www.mendelweb.org/Mendel.html>.
10. Down, J. L. H. Observations on an ethnic classification of idiots. *Lond. Hosp. Rep.* **3**, 259–262 (1866); reprinted in *Ment. Retard*. **33**, 54–56 (1995).
11. Ward, O. C. *John Langdon Down, 1828–1896: a Caring Pioneer (Eponymists in Medicine)* (R. Soc. Med. Press, London, 1998).
12. Shuttleworth, G. E. Mongolian imbecility. *BMJ* **2**, 661–665 (1909).
13. Fraser, M. & Mitchell, A. Kalmyc idiocy. *J. Ment. Sci.* **22**,169–179 (1876).
14. Alzheimer, A., Stelzmann, R. A., Schnitzlein, H. N. & Murtagh, F. R. An English translation of Alzheimer's 1907 paper, "Über eine eigenartige Erkankung der Hirnrinde". *Clin Anat.* **8**, 429–431 (1995).
15. Jervis, G. A. Early senile dementia in mongoloid idiocy. *Am. J. Psychiatry* **105**, 102–106 (1948).
16. Allen, G. *et al.* "Mongolism". *Lancet* **1**, 775 (1961).
17. Howard-Jones, N. On the diagnostic term "Down's disease". *Med. Hist.* **23**, 102–104 (1979).
18. Flemming, W. Beitrage zur kenntnis der zelle und ihrer Lebenserscheinungen, Thiel II. *Archiv. Mikroskopische Anatomie* **18**, 151–259 (1880) (in German); English translation available in *J. Cell Biol.* **25**, 3–69 (1965).
19. Sutton, W. S. The chromosomes in heredity. *Biol. Bull.* **4**, 231–251 (1903).
20. Morgan, T. H., Sturtevant, A. H., Muller, H. J. & Bridges, C. B. *The Mechanism of Mendelian Heredity* (Constable Press, London, 1915).
21. Painter, T. The Y-chromosome in mammals. *Science* **53**, 503–504 (1921).
22. Painter, T. Studies in mammalian spermatogenesis. II. The spermatogenesis of man. *J. Exp. Zool.* **37**, 291–336 (1923).
23. Davenport, C. B. in *Sixth International Congress of Genetics* Vol. 1. (ed. Jones, D. F.) 135–140 (Brooklyn Botanic Garden, New York, 1932).
24. Waardenburg, P. J. *Das Menschliche Auge und seine Erbanlagen* (Martinus Nijhoff, The Hague, 1932) (in German).
25. Tjio, J. H. & Levan, A. The chromosome number of man. *Hereditas* **42**, 1–6 (1956).
26. Kodani, M. Three chromosome numbers in whites and Japanese. *Science* **127**, 1339–1340 (1958).
27. Tjio, J. H. & Puck, T. T. Genetics of somatic mammalian cells. II. Chromosomal constitution of cells in tissue culture. *J. Exp. Med.* **108**, 259–268 (1958).
28. Jacobs, P. A., Baikie, A. G, Court–Brown, W. M. & Strong, J. A. The somatic chromosomes in mongolism. *Lancet* **1**, 710 (1959).
29. Polani, P. E. *et al.* A mongol girl with 46 chromosomes. *Lancet* **1**, 313–324 (1960).
30. Carter, C. O., Hamerton, J. L., Polani, P. E., Gunalp, E. & Weller, S. D. Chromosome translocation as a cause of familial mongolism. *Lancet* **2**, 678–680 (1960).
31. Clarke, C. M., Edwards, J. H. & Smallpiece, V. 21-trisomy/normal mosaicism in an intelligent child with some Mongoloid characters. *Lancet* **18**, 1028–1030 (1961).
32. Caspersson, T., Zech, L., Johansson, C. & Modest, E. J. Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma* **30**, 215–227 (1970).
33. Caspersson, T., Hultén, M., Lindsten, J. & Zech, L. Distinction between extra G-like chromosomes by quinacrine mustard fluorescence analysis. *Exp. Cell Res.* **63**, 240–243 (1970).
34. Aula, P., Leisti, J. & von Koskull, H. Partial trisomy 21. *Clin. Genet.* **4**, 241–251 (1973).
35. Niebuhr, E. Down's syndrome: the possibility of a pathogenetic segment on chromosome no. 21. *Humangenetik* **21**, 99–101 (1974).
36. Korenberg, J. R. *et al.* Molecular definition of a region of chromosome 21 that causes features of the Down syndrome phenotype. *Am. J. Hum. Genet.* **47**, 236–246 (1990).
37. Delabar, J.-M. *et al.* Molecular mapping of twenty-four features of Down syndrome on chromosome 21. *Eur. J. Hum. Genet*. **1**, 114–124 (1993).
38. Korenberg, J. R. *et al.* Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc. Natl Acad. Sci. USA* **91**, 4997–5001 (1994).
39. Barlow, G. M. *et al.* Down syndrome congenital heart disease: a narrowed region and a candidate gene. *Genet. Med.* **3**, 91–101 (2001).
40. Shapiro, B. L. Down syndrome — a disruption of homeostasis. *Am. J. Med. Genet.* **14**, 241–269 (1983).
41. Pritchard, M. A. & Kola, I. The 'gene dosage effect' hypothesis versus the 'amplified developmental instability' hypothesis in Down syndrome. *J. Neural Transm.* **57**, 293–303 (1999).
42. St George-Hyslop, P. H. *et al.* The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science* **235**, 885–890 (1997).
43. Goate, A. *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704–706 (1991).
44. Hasle, H. Pattern of malignant disorders in individuals with Down's syndrome. *Lancet Oncol.* **2**, 429–436 (2001).
45. Bacino, C. A. *et al.* Clinical and molecular studies in full trisomy 22: further delineation of the phenotype and review of the literature. *Am. J. Med. Genet.* **56**, 359–365 (1995).
46. Tan, Y. H., Tischfield, J. & Ruddle, F. H. The linkage of genes for the human interferon-induced antiviral protein and indophenol oxidase-B traits to chromosome G-21. *J. Exp. Med.* **137**, 317–330 (1973).
47. Moore, E. M., Jones, C., Kao, F.-T. & Oates, D. C. Synteny between glycinamide ribonucleotide synthetase and superoxide dismutase (soluble). *Am. J. Hum. Genet.* **29**, 389–396 (1977).
48. Cox, D. R. & Shimizu, N. Report of the committee on the genetic constitution of chromosome 21. *Cytogenet. Cell Genet.* **55**, 235–244 (1990).
49. Graw, S. L. *et al.* Molecular analysis and breakpoint definition of a set of human chromosome 21 somatic cell hybrids. *Somat. Cell Mol. Genet.* **21**, 415–428 (1995).
50. Gardiner, K. *et al.* YAC analysis and minimal tiling path construction for chromosome 21q. *Somat. Cell Mol. Genet.* **21**, 399–414 (1995).
51. Goss S. J. & Harris H. New method for mapping genes in human chromosomes. *Nature* **255**, 680–684 (1975).
52. Cox, D. R., Burmeister, M., Price, E. R., Kim, S. & Myers, R. M. Radiation hybrid mapping: a somatic cell genetic method for constructing high-resolution maps of mammalian chromosomes. *Science* **259**, 245–250 (1990).
53. Lynn, A. *et al.* Patterns of meiotic recombination on the long arm of human chromosome 21. *Genome Res.* **10**, 1319–1332 (2000).
54. Hassold, T. & Sherman, S. Down syndrome: genetic recombination and the origin of the extra chromosome 21. *Clin. Genet.* **57**, 95–100 (2000).
55. Cheung, V. G. *et al.* Integration of cytogenetic landmarks into the draft sequence of the human genome. *Nature* **409**, 953–958 (2001).
56. Schwartz, D. C. & Cantor, C. R. Separation of yeast chromosome-sized DNA's by pulsed field gradient gel electrophoresis. *Cell* **37**, 67–75 (1984).
57. Gardiner, K., Laas, W. & Patterson, D. Fractionation of large mammalian DNA restriction fragments using vertical pulsed-field gradient gel electrophoresis. *Somat. Cell Mol. Genet.* **12**, 185–195 (1986).
58. Burke D. T., Carle, G. F. & Olson, M. V. Cloning of large segments of exogenous DNA into yeast by mean artificial chromosome vector. *Science* **236**, 806–812 (1987).
59. Gardiner, K. *et al.* Analysis of human chromosome 21: correlation of physical and cytogenetic maps; gene and CpG island distributions. *EMBO J.* **9**, 25–34 (1990).
60. Chumakov, I. *et al.* Continuum of overlapping clones spanning the entire human chromosome 21q. *Nature* **359**, 380–386 (1992).
61. Ichikawa, H. *et al.* Long-distance restriction mapping of the proximal long arm of human chromosome 21 with *Not* I linking clones. *Proc. Natl Acad. Sci. USA* **89**, 23–27 (1992).
62. Shizuya, H. *et al.* Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proc. Natl Acad. Sci. USA* **89**, 8794–8797 (1992).
63. Wang, D., Fang, H., Cantor, C. R. & Smith, C. L. A contiguous *Not* I restriction map of band q22.3 of human chromosome 21. *Proc. Natl Acad. Sci. USA* **89**, 3222–3226 (1992).

64. Shepherd, N. S. *et al.* Preparation and screening of an arrayed human genomic library generated with the P1 cloning system. *Proc. Natl Acad. Sci. USA* **91**, 2629–2633 (1994).
65. Korenberg, J. R. *et al.* A high-fidelity physical map of human chromosome 21q in yeast artificial chromosomes. *Genome Res.* **5**, 427–443 (1995).
66. Patterson, D. & Sakaki, Y. The international consortium to sequence human chromosome 21. *Genome Digest* **4**, 9–10 (1997).
67. Hattori, M. *et al.* The DNA sequence of human chromosome 21. *Nature* **405**, 311–319 (2000).
68. Yamada, Y. *et al.* A comprehensive analysis of allelic methylation status of CpG islands on human chromosome 21q. *Genome Res.* **14**, 247–266 (2004).
69. Kampa D. *et al.* Novel RNAs identified from an in-depth analysis of the transcriptome of human chromosomes 21 and 22. *Genome Res.* **14**, 331–342 (2004).
70. Waterston, R. H. *et al.* Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562 (2002).
71. Gardiner, K., Fortna, A., Bechtel, L. & Davisson, M. T. Mouse models of Down syndrome: how useful can they be: comparison of the gene content of human chromosome 21 with orthologous mouse genomic regions. *Gene* **318**, 137–147 (2003).
72. Patil, N. *et al.* Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21. *Science* **294**, 1719–1723 (2001).
73. Mao, R., Zielke, C. L., Zielke, H. R. & Pevsner, J. Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. *Genomics* **81**, 457–467 (2003).
74. FitzPatrick, D. R. *et al.* Transcriptome analysis of human autosomal trisomy. *Hum. Mol. Genet.* **11**, 3249–3256 (2002).
75. Reymond, A. *et al.* Human chromosome 21 gene expression atlas in the mouse. *Nature* **420**, 582–586 (2002).
76. Gitton, Y. *et al.* A gene expression map of human chromosome 21 orthologues in the mouse. *Nature* **429**, 586–590 (2002).
77. Kapranov, P. *et al.* Large-scale transcriptional activity in chromosomes 21 and 22. *Science* **296**, 916–919 (2002).
78. O'Farrell, P. H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**, 4007–4021 (1975).
79. Weil, J. & Epstein, C. J. The effect of trisomy 21 on the patterns of polypeptide synthesis in human fibroblasts. *Am. J. Hum. Genet.* **31**, 478–488 (1979).
80. Brown, W. T., Dutkowski, R. & Darlington, G. J. Localization and quantitation of human superoxide dismutase using computerized 2-D gel electrophoresis. *Biochem. Biophys. Res. Comm.* **102**, 675–681 (1981).
81. Klose, J., Zeindl, E. & Sperling, K. Analysis of protein patterns in two-dimensional gels of cultured human cells with trisomy 21. *Clin. Chem.* **28**, 987–992 (1982).
82. Patterson, D., Jones, C., Scoggin, C., Miller, Y. E. & Graw, S. Somatic cell approaches to Down's syndrome. *Ann. NY Acad. Sci.* **396**, 69–81 (1982).
83. Van Keuren, M. L., Goldman, D. & Merril, C. R. Protein variations associated with Down's syndrome, chromosome 21, and Alzheimer's disease. *Ann. N.Y. Acad. Sci.* **396**, 55–67 (1982).
84. Durr, E. *et al.* Direct proteomic mapping of the lung microvascular endothelial cell surface *in vivo* and in cell culture. *Nature Biotechnol.* **22**, 985–992 (1994).
85. Unlu, M., Morgan, M. E. & Minden, J. S. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* **18**, 2071–2077 (1997).
86. Davisson, M. T. & Costa, A. C. S. in *Advances in Neurochemistry* Vol. 9 (ed. Popko, B.) 297–327 (Kluwer Academic/Plenum, New York, 1999).
87. McClure, H. M., Belden, K. H., Pieper, W. A. & Jacobson, C. B. Autosomal trisomy in a chimpanzee: resemblance to Down's syndrome. *Science* **165**, 1010–1012 (1969).
88. Watanabe, H. *et al.* DNA sequence and comparative analysis of chimpanzee chromosome 22. *Nature* **429**, 382–388 (2004).
89. Shi, J. *et al.* Divergence of the genes on human chromosome 21 between human and other hominoids and variation of substitution rates among transcription units. *Proc. Natl Acad. Sci. USA* **100**, 8331–8336 (2003).
90. Gropp, A., Giers, D. & Kolbus, U. Trisomy in the fetal backcross progeny of male and female metacentric heterozygotes of the mouse. I. *Cytogenet. Cell Genet.* **13**, 511–535 (1974).
91. Epstein, C. J. in *Trisomy 21 (Down Syndrome) Research Perspectives* (eds de la Cruz, F. F. & Gerald, P. S.) 263–271 (Univ. Park Press, Baltimore, 1981).
92. Francke, U. & Taggart, R. T. Assignment of the gene for cytoplasmic superoxide dismutase (SOD1) to a region of chromosome 16 and of *Hprt* to a region of the X chromosome in the mouse. *Proc. Natl Acad. Sci. USA* **76**, 5230–5233 (1979).
93. Polani, P., E. & Adinolfi, M. Chromosome 21 of man, 22 of the great apes and 16 of the mouse. *Dev. Med. Child Neurol.* **22**, 223–233 (1980).
94. Miyabara, S., Gropp, A. & Winking, H. Trisomy 16 in the mouse fetus associated with generalized edema and cardiovascular and urinary tract anomalies. *Teratology* **25**, 369–380 (1982).
95. Gearhart, J. M., Davisson, M. & Oster-Granite, M. L. Autosomal aneuploidy in mice: generation and developmental consequences. *Brain Res. Bull.* **16**, 789–801 (1986).
96. Davisson, M. T., Schmidt, C. & Akeson E. C. in *Molecular Genetics of Chromosome 21 and Down Syndrome* (eds Patterson, D. & Epstein, C. J.) 263–280 (Wiley-Liss, New York, 1990).
97. Davisson, M. T. *et al.* in *The Phenotypic Mapping of Down Syndrome and Other Aneuploid Conditions* (ed. Epstein, C. J.) 117–133 (Wiley-Liss, New York, 1993).
98. Reeves, R. H. *et al.* A mouse model for Down syndrome exhibits learning and behaviour deficits. *Nature Genet.* **11**, 177–183 (1995).
99. Holtzman, D. M. *et al.* Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. *Proc. Natl Acad. Sci. USA* **93**, 13333–13338 (1996).
100. Hyde, L. A. & Crnic, L. S. Age-related deficits in context discrimination learning in Ts65Dn mice that model Down syndrome and Alzheimer's disease. *Behav. Neurosci.* **115**, 1239–1246 (2001).
101. Richtsmeier, J. T., Zumwalt, A., Carlson, E. J., Epstein, C. J. & Reeves, R. H. Craniofacial phenotypes in segmentally trisomic mouse models for Down syndrome. *Am. J. Med. Genet.* **107**, 317–324 (2002).
102. Cataldo, A. M. *et al. App* gene dosage modulates endosomal abnormalities of Alzheimer's disease in a segmental trisomy 16 mouse model of Down syndrome. *J. Neurosci.* **23**, 6788–6792 (2003).
103. Hampton, T. G., Stasko, M. R., Kale, A., Amende, I. & Costa, A. C. Gait dynamics in trisomic mice: quantitative neurological traits of Down syndrome. *Physiol. Behav.* **82**, 381–389 (2004).
104. Olson, L. E. *et al.* Down syndrome mouse models Ts65Dn, Ts1Cje, and Ms1Cje/Ts65SDn exhibit variable severity of cerebellar phenotypes. *Dev. Dyn.* **230**, 581–589 (2004).
105. Stasko, M. R. & Costa, A. C. S. Experimental parameters affecting the morris water maze performance of a mouse model of Down syndrome. *Behav. Brain Res.* **154**, 1–17 (2004).
106. Kahlem, P. *et al.* Transcript level alterations reflect gene dosage effects across multiple tissues in a mouse model of Down syndrome. *Genome Res.* **14**, 1258–1267 (2004).
107. Sago, H. *et al.* Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. *Proc. Natl Acad. Sci. USA* **95**, 6256–6261 (1998).
108. Sago, H. *et al.* Genetic dissection of region associated with behavioral abnormalities in mouse models for Down syndrome. *Pediatr. Res.* **48**, 606–613 (2000).
109. Saran, N. G., Pletcher, M. T., Natale, J. E., Cheng, Y. & Reeves, R. H. Global disruption of the cerebellar transcriptome in a Down syndrome mouse model. *Hum. Mol. Genet.* **12**, 2013–2019 (2003).
110. Lyle, R., Gehrig, C., Neergaard-Henrichsen, C., Deutsch, S. & Antonarakis, S. E. Gene expression from the aneuploid chromosome in a trisomy mouse model of Down syndrome. *Genome Res.* **14**, 1268–1274 (2004).
111. Amano, K. *et al.* Dosage-dependent over-expression of genes in the trisomic region of Ts1Cje mouse model for Down syndrome. *Hum. Mol. Genet.* **13**, 1333–1340 (2004).
112. Epstein, C. J. *et al.* Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in Down syndrome. *Proc. Natl Acad. Sci.* **84**, 8044–8088 (1987).
113. Avraham, K. B., Sugarman, H., Rotshenker, S. & Groner, Y. Down's syndrome: morphological remodeling and increased complexity in the neuromuscular junction of transgenic CuZn-superoxide dismutase mice. *J. Neurocytol.* **20**, 208–215 (1991).
114. Gahtan, E., Auerbach, J. M., Groner, Y. & Segal, M. Reversible impairment of long-term potentiation in transgenic Cu/Zn-SOD mice. *Eur. J. Neurosci.* **10**, 538–544 (1998).
115. Harris-Cerruti, C. *et al.* Functional and morphological alterations in compound transgenic mice overexpreszing Cu/Zn superoxide dismutaze and amyloid precursor protein. *Eur. J. Neurosci.* **19**, 1174–1190 (2004).
116. Busciglio J. *et al.* Altered metabolism of the amyloid β precursor protein is associated with mitochondrial dysfunction in Down's syndrome. *Neuron* **33**, 677–688 (2002).
117. Peled-Kamar, M., Degani, H., Bendel, P., Margalit, R. & Groner, Y. Altered brain glucose metabolism in transgenic-PFKL mice with elevated L-phosphofructokinase: *in vivo* NMR studies. *Brain Res.* **810**, 138–145 (1998).
118. Martinez de Lagrán, M. *et al.* Motor phenotypic alterations in TgDyrk1a transgenic mice implicate DYRK1A in Down syndrome motor dysfunction. *Neurobiol. Dis.* **15**, 132–142 (2004).
119. Chrast, R. *et al.* Mice trisomic for a bacterial artificial chromosome with the single-minded 2 gene (*Sim2*) show phenotypes similar to some of those present in the partial trisomy 16 mouse models of Down syndrome. *Hum. Mol. Genet.* **9**, 1853–1864 (2000).
120. Bell, K., Shokrian, D., Potenzieri, C. & Whitaker-Azmitia, P. M. Harm avoidance, anxiety, and response to novelty in the adolescent S-100β transgenic mouse: role of serotonin and relevance to Down syndrome. *Neuropsychopharmacology* **28**, 1810–1816 (2003).
121. Wolvetang, E. J. *et al.* Overexpression of the chromosome 21 transcription factor *Ets2* induces neuronal apoptosis. *Neurobiol. Dis.* **14**, 349–356 (2003).
122. Horton, J. C. in *Critical Thinking About Critical Periods* (eds Bailey, D. B., Bruer, J. T., Symons, F. J. & Lichtman, J. W.) (Paul H. Brooks, Baltimore, 2001).
123. Pennington, B. F., Moon, J., Edgin, J., Stedron, J. & Nadel, L. The neuropsychology of Down syndrome: evidence for hippocampal dysfunction. *Child. Dev.* **74**, 75–93 (2003).
124. Aylward, E. H. *et al.* MRI volumes of the hippocampus and amygdala in adults with Down's syndrome with and without dementia. *Am. J. Psychiatry* **156**, 564–568 (1999).
125. Pinter, J. D. *et al.* Amygdala and hippocampal volumes in children with Down syndrome: a high-resolution MRI study. *Neurology* **56**, 972–974 (2001).
126. Kleschevnikov, A. M. *et al.* Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. *J. Neurosci.* **15**, 8153–8160 (2004).
127. Hernandez, D., Mee, P. J., Martin, J. E., Tybulewicz, V. L. J. & Fisher, E. M. C. Transchromsomal mouse embryonic stem cell lines and chimeric mice that contain freely segregating segments of human chromosome 21. *Hum. Mol. Genet.* **8**, 923–933 (1999).
128. Shinohara, T. *et al.* Mice containing a human chromosome 21 model behavioral impairment and cardiac anomalies of Down's syndrome. *Hum. Mol. Genet.* **10**, 1163–1175 (2001).
129. Olson, L. E., Richtsmeier, J. T., Leszl, J. & Reeves, R. H. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. *Science* **306**, 687–690 (2004).
130. Gardiner, K., Davisson, M. T. & Crnic, L. S. Building protein interaction maps for Down's syndrome. *Brief. Funct. Genomic Proteomic* **3**, 142–156 (2004).
131. Reynolds, T. M. Correspondence in *J. Clin. Pathol.* **54**, 573–574 (2001).
132. Baker, D., Teklehaimanot, S., Hassan, R. & Guze, C. A look at a Hispanic and African American population in an urban prenatal diagnostic center: referral reasons, amniocentesis acceptance, and abnormalities detected. *Genet. Med.* **6**, 211–218 (2004).
133. Elias, S. *et al.* Chorionic villus sampling for first-trimester prenatal diagnosis: Northwestern University program. *Am. J. Obstet. Gynecol.* **152**, 204–213 (1985).
134. Merkatz, I. R., Nitowsky, H. M., Macri, J. N. & Johnson, W. E. . An association between low maternal serum α-fetoprotein and fetal chromosomal abnormalities. *Am. J. Obstet. Gynecol.* **148**, 886–894 (1984).
135. Christiansen, M. *et al.* Screening for Down's syndrome in early and late first and second trimester using six maternal serum markers. *Clin. Genet.* **65**, 11–16 (2004).
136. Nicolaides, K. H., Azar, G., Byrne, D., Mansur, C. & Marks, K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ* **304**, 867–869 (1992).
137. Bianchi, D. W. *et al.* Detection of fetal cells with 47,XY,+21 karyotype in maternal peripheral blood. *Hum. Genet.* **90**, 368–370 (1992).

138. Glover, N. M. & Glover, S. J. Ethical and legal issues regarding selective abortion of fetuses with Down syndrome. *Ment. Retard.* **34**, 207–214 (1996).
139. James, S. J. *et al.* Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am. J. Clin. Nutr.* **70**, 495–501 (1999).
140. James, S. J. Maternal metabolic phenotype and risk of Down syndrome: beyond genetics. *Am. J. Med. Genet.* **127** (Part A), 1–4 (2004).
141. Pogribna, M. *et al.* Homocysteine metabolism in children with Down syndrome: *in vitro* modulation. *Am. J. Hum. Genet.* **69**, 88–95 (2001).
142. Barkai, G., Arbuzova, S., Berkenstadt, M., Heifetz, S. & Cuckle, H. Frequency of Down's syndrome and neural-tube defects in the same family. *Lancet* **361**, 1331–1335 (2003).
143. Hassold, T. & Hunt, P. To err (meiotically) is human: the genesis of human aneuploidy. *Nature Rev. Genet.* **2**, 280–291 (2001).
144. Aagesen, L., Grinsted, J. & Mikkelsen, M. Advanced grandmaternal age on the mother's side — a risk of giving rise to trisomy 21. *Ann. Hum. Genet.* **48**, 297–302 (1984).
145. Papp, Z., Varadi, E. & Szabo, Z. Grandmaternal age at birth of parents of children with trisomy. *Hum. Genet.* **39**, 221–224 (1977).
146. Muller, H. J. Precision of genetic adaptation. *Harvey Lect.* **43**, 165–229 (1948).

Acknowledgements
The authors would like to acknowledge the support of the US National Institutes of Health, the Jerome Legeune Foundation, the Bonfils–Stanton Foundation, the Boettcher Foundation, the Denver Foundation and the Alvin Itkin Foundation.

Competing interests statements
The authors declare no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
Entrez: http://www.ncbi.nih.gov/Entrez/
APP | *IFNAR1* | *SOD1* | *GARS* | HPRT | *Pfkl* | *Dyrk1a* | *Sim2* | *S100β* | *Ets2*
OMIM: http://www.ncbi.nlm.nih.gov/Omim/
Down syndrome | Alzheimer disease | acute myeloid leukaemia
Access to this interactive links box is free online.

OPINION

Neonatal screening by DNA microarray: spots and chips

Nancy S. Green and Kenneth A. Pass

Abstract | Newborn screening (NBS) is a public-health genetic screening programme aimed at early detection and treatment of pre-symptomatic children affected by specific disorders. It currently involves protein-based assays and PCR to confirm abnormal results. We propose that DNA microarray technology might be an improvement over protein assays in the first stage of NBS. This approach has important advantages, such as multiplex analysis, but also has disadvantages, which include a high initial cost and the analysis/storage of large data sets. Determining the optimal technology for NBS will require that technical, public health and ethical considerations are made for the collection and extent of analysis of paediatric genomic data, for privacy and for parental consent.

Reports in the medical literature of the myriad of applications of DNA microarray, or 'chip', technologies are increasingly common, conveying the power of this technology and its current and imminent uses in medicine[1,2]. Real, even realized, promises for the use of these technologies in medical research are accelerating. Perhaps most significantly, MICROARRAYS are already on the verge of clinical use, most prominently for treatment stratification of certain malignancies[3] and for pharmacogenetic assessment of commonly used medications (for example, the AmpliChip CYP450 test; see Online links box).

Newborn screening (NBS) is a public-health genetic screening programme that is aimed at early detection and treatment of pre-symptomatic children affected by specific disorders[4] (BOX 1). The World Health Organization defined the principles of NBS in 1968 (REF. 5) (BOX 2), as recently elaborated by the UK National Screening Committee (see Online links box). These disorders are nearly all autosomal recessive conditions, with only the few exceptions of CONGENITAL HYPOTHYROIDISM and infectious diseases. NBS is routinely performed in the United States, Australia, the United Kingdom and in most countries throughout Europe, Asia and South America. Over its 40-year history in the United States, NBS has evolved from screening for phenylketonuria (PKU) to universal screening by states for (at least) 4 to more than 30 different genetic disorders (see Online links box and TABLE 1). Assays for specific analytes are performed on protein that has been reconstituted from dried bloodspots on 'Guthrie' cards (named after Robert Guthrie's innovative use of filter paper for specimen collection). Population screening for each of the relatively rare disorders (which range in prevalence from 1 in 3,000 to >1 in 300,000 live births) must be highly sensitive to avoid missing affected infants, as well as sufficiently specific to minimize the need for follow-up of false positives.

Since 1963, NBS has expanded the list of disorders that it screens for by adding distinct analytical procedures — such as assays for specific enzymatic activity or proteins — for detecting each new disease marker. This analytical approach has limitations because of the varied performance levels of the assays, the additional costs of testing for individual disorders, and the availability of sufficient amounts of specimens. The recent introduction of TANDEM MASS SPECTROMETRY (MS/MS) to NBS now enables multiplexing procedures for simultaneous identification of more than 30 additional medical conditions from a single 3.2mm-punch of the Guthrie card[6]. However, even this sophisticated technology is limited, as many medical conditions in current NBS panels, such as galactosaemia and thyroid disorders, are not identifiable by MS/MS; they still need to be individually screened for by analyte-specific assays (TABLE 1).

If abnormalities are detected at this protein-assay stage, known as the 'first tier' test, it might be followed up by DNA analysis — the second tier. DNA for PCR amplification and detection of mutant alleles can be derived from bloodspots, and this test is being increasingly used for second-tier confirmation of abnormal results for cystic fibrosis (CF)[7], haemoglobinopathies[8] and ADRENAL HYPERPLASIA[9]. However, only a handful of disorders are confirmed in this way, and usually through analysis of a limited number of mutations[7,10]. The use of methods such as DNA microarrays in the first tier of NBS might allow for accurate detection of different missense, nonsense and deletion mutations in genes for a number of disorders, enabling comprehensive mutation analysis of selected genes. DNA microarrays that are currently in use for SNP analysis (see Affymetrix web site in the Online links box) could be used to efficiently screen for many mutations[11]. There are already examples of successful uses of microarray SNP analysis using small, preserved biological samples such cervico-vaginal Pap smear swabs[12], micro-dissected tumours[13,14] and newborn tissue[15]. Below we describe the advantages and the challenges of using DNA from bloodspot specimens in primary NBS using DNA chip technology.