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Slivers of the spectrum

Large-scale genomic studies are reinvigorating interest in a small group of molecularly defined autism-associated disorders and spurring renewed interest in genetic therapies.

Malory Branca

This year will mark milestones for several first-in-human trials of molecularly targeted agents for autism-spectrum disorders (ASDs), including gene therapies and antisense oligonucleotides (ASOs; Table 1). These follow several recent announcements of programs in ASD from major biopharmaceutical companies, including Novartis, Roche, Biogen, Ionis, PTC Therapeutics, Sarepta Therapeutics and Amicus Therapeutics. Last year, Novartis announced a deal for \$75 million up front—worth potentially \$720 million with milestones—for rights to Sangamo Therapeutics' gene-regulating platform based on zinc-finger proteins (ZFPs). And

in March, investors jumped in with a \$40 million investment in a new gene-therapy company, Jaguar Therapeutics (founded by former AveXis executives), which is taking forward a program for treating one of the specific genetic causes of autism (undisclosed).

Until relatively recently, the biology of autism was thought to be too poorly understood, and the disease too heterogeneous, for it to be a focus for molecularly targeted drug development. Now, the deal with Sangamo puts Novartis into the thick of what has become an increasingly competitive race to the clinic for ASD driven by genomics-based discovery.

Hundreds of genes have been associated with autism to date, and confidence is growing in the penetrance of several of them, thanks to a boom in large-scale efforts to create large genomic databases and cutting-edge laboratory tools.

But the long road from genome association studies to therapies is just beginning, and with an indication as heterogeneous as autism, both in phenotype and in genotype, there are challenges at every step—from getting a proper diagnosis, to identifying a disease-modifying agent that can reach the affected areas of the brain, to validating biomarkers of treatment outcome to designing a clinical trial to assess them.

Table 1 | Gene-targeted therapies for ASD conditions in clinical development

Company	Disease	Modality	Clinical stage	Delivery route
GeneTx Biotherapeutics	Angelman's syndrome	2'-MOE ASO	Phase 1/2	Intrathecal
Roche	Angelman's syndrome	LNA ASO	Phase 1	Intrathecal
Novartis	Rett's syndrome	MECP2/AAV-9 gene therapy	Phase 1	Lumbar intrathecal

Table 2 | Selected autism-spectrum disorder data collections

Project	Content
Autism Genome Project Consortium (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000267.v2.p2)	50 centers from North America and Europe; 4,415 subjects
National Database for Autism Research (https://catalog.data.gov/dataset/national-database-for-autism-research-ndar)	Genomic, imaging, laboratory, clinical and behavioral data sources
Autism Speaks MSSNG (https://research.mssng.org)	Whole-genome sequencing of 10,000 individuals from families in the Autism Genetic Research Exchange repository
SFARI Gene (https://gene.sfari.org/)	Content extracted from peer-reviewed literature in a set of modules: human gene module (annotated list of genes), copy-number variant module, animal models, protein interaction, gene scoring
AutDB Autism Informatics Portal (http://autism.mindspec.org/autdb/Welcome.do)	Human genes, animal models, protein interaction copy-number variant, gene scoring modules

On the rise

The World Health Organization describes autism as “a range of conditions characterized by some degree of impaired communication and language, and a narrow range of interests and activities that are both unique to the individual and carried out repetitively.” It estimates that one in 160 children worldwide has an ASD. Their prevalence is growing¹, prompting some to describe this as an ‘epidemic’, although the extent to which better diagnosis and reporting is driving the increase remains unclear.

The search for genes associated with ASD has been underway for several decades. But most identified to date affect only a small fraction of patients, and clinical presentations can vary widely, with symptoms manifesting in myriad ways. Some patients may show severe cognitive impairment or an inability to speak; others appear almost unaffected, except under certain circumstances. As a result, a major challenge in developing genomics-driven treatments for ASD is sorting through all the data on genes and phenotypes to find the targets with the greatest phenotypic consequence.

Adding to this challenge is the fact that because it is a neurodevelopmental disorder, it is not clear when ASD actually manifests, or when might be the optimal time to begin treatment. Even studying its onset

is challenging. “Brains are hard to access in general, and to properly study autism we should be looking at them during fetal development and onward,” says Stephan Sanders, associate professor of psychiatry at the Weill Institute for Neurosciences at the University of California, San Francisco.

But two things are helping accelerate the search for penetrant variants: access to an increasing number of databases devoted specifically to autism that are collating large sets of variants (Table 2) and the introduction of animal model systems and technologies for exploring the underlying biology associated with particular targets.

Genetic underpinnings

Although it has long been suspected that autism has a genetic component, researchers and clinicians today are more comfortable calling it a genetic disease. “In the 1970s and 80s [clinicians] started to realize this was a genetic disorder, largely based on small twin studies,” explains Sven Sandin, an epidemiologist focused on the etiology of autism and related neurodevelopmental disorders at Mount Sinai, in New York. Although it was a good start, “the problem with those studies was they were small and made a lot of assumptions; for example, that each twin had been raised together and treated similarly,” he says.

Sandin was recently involved in a large, collaborative study that followed more than

2 million children from birth to 15 years from five countries—Denmark, Finland, Sweden, Israel and Australia—between 1998 and 2012. Just over 22,000 of them developed ASD during the study period². By studying detailed medical histories of these children, the researchers determined that about 80% of the risk of developing autism is due to genetics. “People have long wondered about certain environmental influences, such as vaccines or the mother’s exposure to things such as pesticides,” Sandin says. “But our study was the largest of its kind and it strongly supported the fact that autism is mainly due to genetics.”

Reverse genetics and drug targets

Over 1,000 genes have been associated with ASD³, but researchers have narrowed these down to roughly 100 that appear to play a substantive role in autism as well as other neurodevelopmental disorders. In the largest genetic study of ASD to date, the Autism Sequencing Consortium, a multi-institutional team, performed whole-exome sequencing of over 35,000 individuals (almost 12,000 with ASD) and identified 102 genes that confer risk for ASD. Although some had been identified previously, 30 genes were ‘novel’ (i.e., not implicated in any previous study of de novo or rare variants⁴; Fig. 1). Through gene ontology analysis and literature review, the consortium found that most of these genes play roles in regulating gene expression, neuronal communication or the cytoskeleton.

Sanders, a consortium member, is drilling down on those genes with the tightest associations (the smallest *P* values, which when plotted as a negative log of the *P* value show up at the top of the Manhattan plot in Fig. 1). Those, Sanders reasons, should be the most common mutations and might have the most penetrative effects. Through functional analyses of numerous missense mutations found in autistic patients of one such gene, *SCN2A* (encoding sodium voltage-gated channel alpha subunit 2), Sanders’ group showed that all the variants found in autistic children blocked or dampened neuronal excitability, suggesting that this could contribute to their autism⁵. Variants in the same gene show up in patients with epilepsy, but performing the

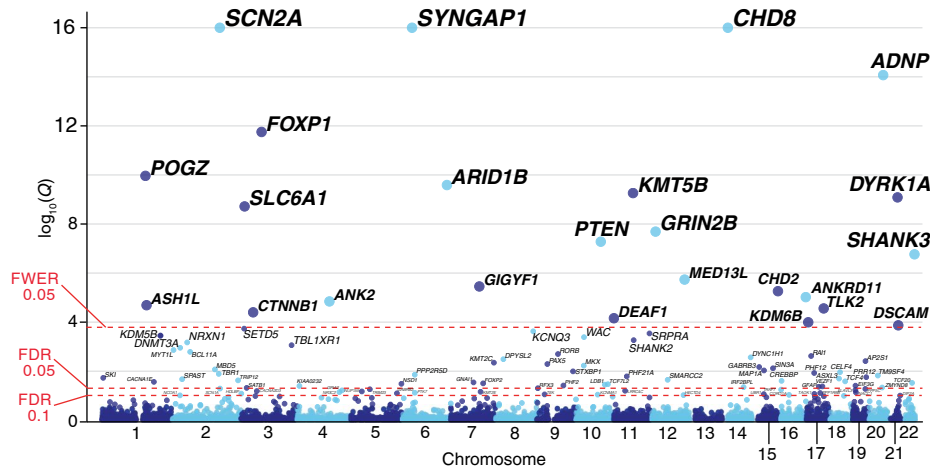


Fig. 1 | Autosomal genes associated with ASD. In whole-exome sequencing, 102 autosomal genes passed the more stringent false discovery rate (y axis) threshold of 0.1 or less; 26 passed the threshold of 0.05 or less. Position along the chromosomes is shown on the x axis. Reprinted with permission from ref. 4, Elsevier.

same functional analysis indicated that the variants had the opposite effect—they enhanced excitability. This demonstrates the importance of functional studies in determining whether a variant causes a loss or a gain of function, and suggests that caution is required in developing therapies for these conditions, as a substantial proportion of autistic kids (20%) have seizures.

Another ASD-associated gene, *CHD8*, was the subject of study at the University of Washington in Seattle; this gene was chosen since disruptive mutations in it had previously been associated with ASD. Group leader Evan Eichler described this study as “almost reverse genetics.” The study involved targeted sequencing of *CHD8* in just over 6,100 children with autism or developmental-delay phenotypes, which netted 15 independent variants not found in either 2,289 unaffected siblings or 6,503 controls. Many of the children with these mutations had similar appearances, sleep disturbances and gastrointestinal disorders, which are common among children with ASD in general. Using a zebrafish model, the researchers were able to validate the gene’s function in neurological development. Disrupting *CHD8* in the fish gave them larger heads, wider-set eyes, constipation and difficulty in digesting food. In fish, the researchers found, variants lead to an increase in neurons in the brain but a decrease of neural receptors in the gut⁶.

In a study in a zebra-finch model, a group from Texas Southwest Medical Center found that knocking out the gene *FOXP1* (implicated in ASD as linked to language and intellectual disability) prevents young

male birds from learning songs from adults⁷. These model system studies may illustrate how to make that important link from genotype to phenotype, but Eichler warns that they appear much simpler than they are. “That [*CHD8* study] was one gene out of 500 prospects,” he points out. Though strong associations with ASD have already been made to more than a dozen genes, dozens more need further study. These studies also illustrate the importance of taking what Eichler calls a “genotype first” approach to ASD. “Although it required targeted resequencing of 6,176 patients with autism and developmental delay to recover 15 patients with severe truncating mutations, the clinical re-contact and detailed characterization of this small subset was critical,” he wrote⁶.

These studies and others are providing valuable insights into ASD etiology and mechanisms that could potentially inform therapeutic approaches, but this is complicated by the heterogeneity of clinical presentation and by a growing appreciation that only a fraction of cases of ASD can be explained by variation in an individual gene and thus are likely to be amenable to molecularly targeted therapies. Sanders holds out hope that a common pathway to autism will eventually be discovered (Fig. 2). “Just like in a heart attack, you’ve got cholesterol, smoking, blood pressure, diabetes as risk factors, but they all converge on a plaque in the coronary artery,” he says. “One of my concerns is that we need to develop hundreds of different therapeutics, one per gene, but this would still only help the 15% of individuals with autism who have a known genetic disorder.”

Autism diagnostics

With an estimated million pediatric and adolescent patients between the ages of 5 and 17 in the United States alone, even a segmented autism market is attractive, particularly if it serves a range of subtypes. But getting a firm diagnosis is challenging because many neurodevelopmental disorders, such as intellectual disability, epilepsy and speech disorders, have overlapping or similar symptoms. Genetic testing holds the key to proper diagnosis, even before therapies can be considered.

But creating diagnostics itself is challenging, as the past few years has seen a surge in gene discovery. Amanda Lindy, director of neurogenetics at GeneDx, a diagnostics developer that was spun out of the US National Institutes of Health in 2001, says that they scour the literature for new variants, as well as conducting their own discovery with patients coming to them seeking a diagnosis.

And as the population of patients diagnosed with ASD has grown substantially in recent years, so too have the number and kind of diagnostics that are available. Stephen Scherer, a medical geneticist at The Hospital for Sick Children in Toronto, has done a systematic analysis of marketed ASD diagnostics and finds that they are all over the map, with respect to both the number and identity of the variants being queried. In a 2018 survey of commercial ASD tests, the number of variants tested by a product ranged from 26 to 2,562. Current genetic testing usually starts out with microarrays, which capture 7% (mainly copy-number variants), and if nothing comes up, continues with exome sequencing, which turns up another 7% of non-overlapping variants, generally smaller changes such as point mutations or small deletions, according to Scherer. “The two technologies are mostly complementary,” he says. Although some companies report exomes, many actually sequence the whole genome, as it is more efficient and quicker to get the data, he says.

Ambry Genetics offers broad testing for neurological diseases with a panel of about 200 genes that they say covers over 60% of patients identified as having a genetic cause for a neurodevelopmental disorder, including developmental delay, intellectual disability and/or ASDs. But they also offer more focused diagnostics; for ASD, they test for a few dozen genes. “There is definite interest to make more specific panels,” says Kelly Radtke, manager of rare disease scientists at Ambry. Smaller panels focus on genes thought to be causative of, rather than just associated with, ASD.

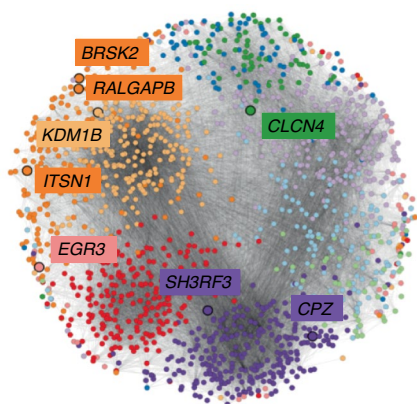


Fig. 2 | Network analysis of ASD-associated genes. Eight tightly connected clusters with distinct biological functions were identified by assigning variants discovered in over 400 families with ASD. Reprinted with permission from ref. ²³, Springer Nature.

For the autism panel, “We included genes that were seen only in patients that had isolated neurological signs such as delayed development or behavioral disorders, but not multisystemic genetic syndromes that have many additional, often very identifiable, symptoms,” says Radtke.

But there are reasons to cover many genes. As tests don’t come cheap, and not all payers are on board with paying for extensive assessments, expanding the number of variants covered in one test increases the chances of finding something. “The companies couldn’t justify the cost for a small panel that might capture only a percent or two of autism. Thus, they throw everything in, for more than just autism—which is why some panels have a thousand, or more, variants—but also variants for neuronal development delays and autism-associated genes that are shared with some Mendelian diseases,” says Scherer.

GeneDx’s Autism/ID Xpanded panel, for example, includes over 2,300 genes, ~400 of which have known pathogenic variants (as opposed to just associations). “We’ve analyzed over 10,000 patients using that gene list. The number of patients we test via this panel increases daily, as does the number of genes from which we’ve reported pathogenic variants,” says Lindy. Although gene lists quickly become outdated—it’s been said that the day an ASD gene sequencing panel is made, it is already out of date—“phenotypically driven exome-based panels are faster and easier to update, making them the least out-of-date panels on the market,” she says.

And with greater attention to ASD, parents and caregivers are increasingly

looking to genetic testing for answers. Testing for autism has skyrocketed, says Lindy. “[Our] test orders for the Autism Xpanded panel have grown exponentially, not linearly, compared with other types of testing.”

Ultimately, the goal is to find genes associated with features specific to autism, with particular attributes such as social behavior, anxiety or intellectual disability. Scherer is skeptical that a single gene could cause something like social behavior. “But we won’t know until we look,” he says. And there is reason for optimism. Lindy says that with some ASD-associated phenotypes, it has been possible to find genes that cause a large proportion of cases. With epilepsy, for example, they found that 24.8% of positive cases (proband with a causative variant) had a pathogenic variant in the *SCN1A* gene and 13.2% had a one in the *KCNQ2* gene⁸.

Gene therapies for monogenic disorders

The most likely first targets for gene-targeted treatments are highly penetrant monogenic conditions with ASD features. Fragile X syndrome, a frequent form of intellectual disability associated with autism, can be traced to expanded repeats in the promoter region of the gene (*FMR1*) encoding fragile X mental retardation protein (FMRP; a RNA-binding protein with a vital role in synaptogenesis and synaptic plasticity that influences various aspects of mRNA metabolism and biology); Rett’s syndrome, which affects 10,000 in the United States and shares many attributes of autism (including loss of speech and repetitive behaviors) is due to mutations in the gene for methylcytosine binding protein 2 (*MECP2*); tuberous sclerosis, which leads to autism in as many as half of those affected, is caused by mutations in two genes encoding the proteins tuberous sclerosis 1 and 2 (*TSC1* and *TSC2*); and Angelman’s syndrome, affecting around 15,000 people in the United States, arises through a deletion of the maternal copy of the ubiquitin ligase E3A gene (*UBE3A*, duplications of which are also associated with autism).

Preclinical work has suggested several possible avenues for targeting genes associated with autism. The first is supplementation of function via viral gene therapy. Adeno-associated viruses (AAVs) are gaining wide acceptance as an effective tool for delivering genes to the brain, in particular AAV serotype 9 (AAV-9), which is uniquely capable of crossing the blood–brain barrier (BBB).

In a mouse model of fragile X, encouraging results have been obtained using AAV-9–mediated *FMR1* gene therapy,

resulting in partial or complete correction of defects in the mice⁹. But it is in Rett’s syndrome that the technology has advanced furthest.

Novartis, following its acquisition of AveXis, is moving AVXS-201, an AAV-9 gene therapy targeting *MECP2*, into the clinic for this rare neurodevelopmental disorder that almost exclusively affects girls. Preclinical work in mouse and primate models showed that the gene therapy is capable of restoring *MECP2* expression in the brain¹⁰. The company—which had to rerun many of the preclinical experiments after an internal investigation in 2019 found that AveXis researchers had committed data manipulation in preparing the package for another AAV-9 gene therapy, Zolgensma (onasemnogene abeparvovec)—now plans to submit an investigational new drug (IND) application at the end of 2021.

Another company exploring AAV gene therapy for Rett’s and Angelman’s syndromes is Sarepta Therapeutics. Sarepta is collaborating with StrideBio on AAV programs to deliver *UBE3A*, *SCN1A* or *MECP2*. StrideBio, which was founded by the University of Florida’s Mavis Agbandje-McKenna and Duke University’s Aravind Asokan, seeks to exploit high-resolution cryo-electron microscopy data on the AAV capsid to engineer vectors¹¹ that can evade neutralizing antibodies and have increased tissue tropism, vector potency and manufacturability. Sarepta has also established a research partnership on Rett’s syndrome (*MECP2*) with University of Massachusetts investigators Guangping Gao, Miguel Sena Esteves and Michael Green, as well as a collaboration focusing on a library of novel, human-derived AAV capsids.

Finally, PTC Therapeutics is developing AGILAS, a hippocampus-delivered AAV-9 *UBE3A* gene therapy to treat Angelman’s syndrome, which was granted orphan status by the US Food and Drug Administration (FDA) in 2015. The company is exploring several other AAV serotypes, and the IND filing for GT-AS is currently delayed because of COVID-19 but is expected later this year. Amicus Therapeutics has also provided the laboratory of James M. Wilson, director of the Gene Therapy Program and the Orphan Disease Center at the University of Pennsylvania, with \$50 million in funding to optimize the group’s AAV-9 vector for delivery to the brain¹². The program is focusing on finding human analogs of a glycosylphosphatidylinositol-anchored lymphocyte antigen 6 complex locus A protein found in the BBB of mice to facilitate ingress into brain tissue.

Apart from traditional AAV gene therapy, several companies are pursuing

Box 1 | From gene editing to gene regulation

Novartis's use of Sangamo's technology opens up a wide range of CNS conditions as potential targets, according to Gopi Shanker, director of psychiatry and an interim leader in the neuroscience division at Novartis Institutes for BioMedical Research. Some therapeutic interventions go beyond simple gene editing.

The company's most recent peer-reviewed study marries the DNA-binding specificity of ZFPs with the repressive activity of a TF to create an allele-selective transcriptional regulator²². The authors tested the activity of a set of ZFP-KRAB transcriptional repressor domain fusions designed against CAG repeats in the sense and antisense strands in fibroblasts and neurons derived from patients with Huntington's disease (HD). The highest-performing ZFP-KRAB fusions selectively repressed more than 99% of HD-causing alleles of the huntingtin gene (*HTT*) while preserving expression of more than 86% of normal

HTT alleles. According to the authors, lentivirally or AAV-6–delivered ZFP-TFs remained active in the neurons beyond 100 days in culture and for at least nine months in the mouse brain. In addition, three HD mouse models showed improvements in molecular, histopathological, electrophysiological and functional endpoints.

Another key to this particular collaboration is that Novartis claims to have some attractive targets in hand, although they are not saying what they are. "We are getting to the point where the most prevalent mutations are either already in our pipeline or are hugely competitive," said Ricardo Dolmetsch, head of neuroscience research at Novartis when the deal was announced. The type of genes Novartis and others seek are those that affect a number of symptoms associated with a condition such as autism, rather than targeting a single symptom, such as epilepsy or gastrointestinal tract problems.

other molecularly targeted approaches against ASDs, including ASO therapies, small molecules that promote readthrough of disease-associated nonsense mutations, and clustered regularly interspaced short palindromic repeat (CRISPR)-based gene editing.

Targeting RNAs

Using technology developed in the laboratory of Baylor University's Linyan Meng in collaboration with Ionis (then Isis) Pharmaceuticals¹³, the Foundation for Angelman Syndrome Therapeutics (FAST) has set up a subsidiary, GeneTx Biotherapeutics, that is developing GTX-102, a 2'-methoxyethyl (MOE) gapmer ASO that reactivates the paternal allele of *UBE3A* silenced by binding of the natural antisense transcript *UBE3A-AS*, curtailing transcription; the treatment thus restores readthrough of the downstream *UBE3A* gene in the opposite orientation.

In December 2020, interim data from a phase 1/2 trial (NCT04259281) in five patients presented at the FAST's annual Global Summit demonstrated that plasma levels of GTX-102 were dose proportional, with a mean change of +2.4 in the clinical global impression–improvement–Angelman's syndrome (CGI-I-AS) global score, and all patients showed improvements and stable seizure control. For four of the five treated patients, blinded electroencephalogram readings at baseline

and day 128 showed a decreased prevalence of abnormalities (epileptiform discharges) common in Angelman patients. The study is expected to resume in the coming months. Elsewhere, Basel, Switzerland–based Roche Pharmaceuticals is developing RO-7248824, a locked nucleic acid (LNA) ASO that also targets the paternal antisense *UBE3A-AS* RNA in Angelman patients. In June 2020, an open-label, multicenter, dose-escalation phase 1 study was initiated in Europe and the United States to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of RO-7248824 in patients under age 18 (NCT04428281)

In 2018, Biogen also signed a collaborative agreement with Ionis to develop a portfolio of 2'-MOE gapmer ASOs for various neurological disorders, paying Ionis \$375 million in upfront fees and \$500 million in equity plus a \$125 million cash premium. Among the diseases Biogen is considering focusing on for clinical development are fragile X, Angelman's and Rett's syndromes.

Finally, PTC Therapeutics has initiated a phase 2 crossover study of its RNA-targeting small-molecule Translarna (ataluren), which was approved in Europe in 2016 for nonsense-mutation-mediated Duchenne's muscular dystrophy, to treat drug-resistant epilepsy in patients with nonsense mutations in the autism-related condition cyclin-dependent kinase–like 5 (CDKL5) deficiency disorder. There is controversy

surrounding Translarna's ability to cross the BBB; however, a proof-of-principle study indicated that at higher doses it can do so¹⁴.

Gene editing and binding

Most gene-editing companies have been pursuing therapies for diseases associated with tissues readily amenable to gene delivery—the eye, blood cells or the liver. As ASD disorders require delivery to the brain, direct injection of an AAV-9 vector into the cisterna magna of the brain is often the route of choice, with expression in ~10% of target neurons at best. Together with researchers at the University of Texas Health Science Center and the University of California, Berkeley, gene-therapy delivery startup GenEdit has explored the delivery of a single dose of CRISPR components via binding to oligonucleotide-conjugated gold nanoparticles, which are then coated with an endosome-disruptive polymer shell¹⁵. Injection into the striatum of fragile X syndrome model mice with low *Fmr1* expression largely rescued their behavioral deficits and display of exaggerated repetitive behaviors.

A team lead by Nissim Benvenisty of Hebrew University in Jerusalem and Dong-Wook Kim of Yonsei University College of Medicine in Seoul has also shown that editing works in human cells descended from individuals with fragile X syndrome. Using embryonic stem cells and induced pluripotent stem cells derived from disease sufferers, they were able to demonstrate CGG repeat correction that resulted in demethylation of the upstream CpG island of the *FMR1* promoter, leading to an open chromatin state and transcription initiation¹⁶.

The Novartis–Sangamo deal last July brings another gene-editing tool to bear: ZFP transcription factors (ZFP-TFs), which are not yet in any clinical trials (see Box 1). According to Gopi Shanker, director of psychiatry and an interim leader in the neuroscience division at the Novartis Institutes for Biomedical Research, the ZFP-TF platform complements the company's other AAV-9 gene-therapy programs. Novartis currently markets the AAV-9 gene therapy Zolgensma, approved in 2019 for spinal muscular atrophy, which was the basis for their \$8.7 billion acquisition of AveXis in 2018.

The platform also has an advantage: "There is a limit to the size of gene that you can package and deliver with AAVs," explains Shanker. "With ZFPs, we can use gene therapy to regulate the expression of the gene, turning it up or down, instead of replacing it or editing it." ZFPs can achieve this no matter what size of gene is being

targeted. The ZFP-TFs are packed into the AAV vector and delivered to the CNS, and then bind specifically to the target gene or allele without integrating into the genome.

Headwinds

Even though working on monogenic conditions increases researchers' confidence that a target will be disease modifying, drug development failures are inevitable. In 2014, Novartis discontinued mavoglurant (AFQ056), a small-molecule selective metabotropic glutamate receptor 5 (mGluR5) antagonist, after it failed in two 12-week, placebo-controlled phase 2b trials in adolescents and adult patients. Unlike most other therapies being tested in fragile X that deal with symptomology, mavoglurant inhibits activation of the mGluR5 receptor, thus reducing the synaptic defects that are the direct results of the absence of FMRP. Late last year, in a similar case of a targeted small-molecule therapy, Ovid Therapeutics suffered a devastating failure of its phase 3 trial in Angelman's syndrome for its small-molecule drug gaboxadol, an agonist for the δ -selective γ -aminobutyric acid A (GABA_A) receptor, which is deleted along with *UBA3*.

Of course, small molecules are a tried and tested therapeutic modality. Gene therapies, by contrast, introduce risks all their own, beyond the expected attrition around a novel target. Although AAV vectors are considered generally safe—in part because they are thought to remain episomal (that is, to not integrate into the genome)—a long-term study of dogs treated for hemophilia A suggests that this may not always be true. A 10-year study of dogs given the gene for canine factor VIII in AAV vectors, led by Giang Nguyen of the University of Pennsylvania, found evidence for insertions of the vectors preferentially into genomic regions associated with cancer, raising the specter of long-term consequences.

Another safety concern with gene therapy that was anticipated in preclinical work is dorsal root ganglion (DRG) toxicity. The DRG is a cluster of neural cells on the outside of the spinal cord responsible for transmitting sensory messages. DRG toxicity was first seen in nonhuman primate studies using AAV vectors to deliver genes via the spinal cord fluid and intravenously, and it has also been observed in pigs^{12,17}. Those studies reported problems with axonal degeneration in some tracts of the spinal cord and peripheral nerves, thought to be due to transgene overexpression.

Novartis may have come up against this issue. The FDA is requesting a new clinical trial before the company can seek US approval of its experimental formulation of Zolgensma because of DRG mononuclear cell inflammation seen in animal tests by the therapy's original developer AveXis. The agency is seeking data to support findings from Novartis's STRONG trial, which tests a spinal injection version of Zolgensma in older children with SMA. (Zolgensma is currently approved as an intravenous application for children under 2 who have more serious disease.) However, Novartis will not be able to start the new trial in the United States until the FDA lifts the clinical hold on the study, which was imposed last year due to inflammatory responses seen in animal tests.

A meta-analysis published last summer suggests that DRG toxicity may be common in nonhuman primates, depending on the route of administration (more common when agents are administered intrathecally via cerebrospinal fluid than with intravenous injection), age and dose, but may not have clinical effects¹². The authors aggregated data from 33 preclinical studies involving more than 250 nonhuman primates and compared multiple factors, including routes of administration, capsid and transgene. According to a statement by Wilson, who was a coauthor of the study, "DRG pathology is almost universal after AAV vectors are delivered into the cerebral spinal fluid of nonhuman primates. However, none of the animals receiving a vector expressing a therapeutic transgene displayed any clinical signs."

Off-target effects are also a consideration with ASO and gene-editing therapies, despite their sequence specificity. As technologies for detecting off-target events mature, how serious an issue this is will become clearer, as will the options for mitigating it. Several recent studies have documented off-target effects of ASOs of various lengths by surveying the entire transcriptome of human cultured cells after treatment^{18,19}. And after the initial excitement over the ability to dial in a target with zinc fingers, reports of off-target cutting also surfaced²⁰, although researchers at Sangamo recently reported that they may be onto a solution²¹.

Cause for optimism?

Many questions remain about the best ways to find and vet targets for treating neurodevelopmental diseases such as ASD. Yet gene-therapy vectorology seems to be hitting its stride, which could help smooth

the way. Dozens of gene therapy clinical trials are underway, and several deals between big pharma and gene-therapy companies were announced in 2020: Bayer, Eli Lilly and UCB all joined Novartis with major investments in the field. This year will see the initiation of several clinical trials of molecularly targeted agents for ASDs.

But with thousands of underlying genetic lesions associated with ASDs, arising from sources ranging from simple single point mutations to deletions of chromosomal regions covering many genes, a range of molecular targeted therapies will be needed. At the moment, monogenic disorders are where commercial drug development is feasible. "One of my concerns is that we need to develop hundreds of different therapeutics, one per gene, but this would still only help the 15% of individuals with autism who have a known genetic disorder," says Sanders.

It will also take a major advance in diagnosing the various subgroups of ASD before a serious effort in gene-based approaches can be considered. As Scherer cautions, "Since autism is not a fatal disorder, taking an extreme decision to try it, at whatever stage, is a very complicated and tough one... The gene therapy discussion now should just be in the research realm being used as a method for generating models, figuring out ways to correct mutations." □

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