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Lessons learned from studying syndromic autism spectrum disorders

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Syndromic autism spectrum disorders represent a group of childhood neurological conditions, typically associated with chromosomal abnormalities or mutations in a single gene. The discovery of their genetic causes has increased our understanding of the molecular pathways critical for normal cognitive and social development. Human studies have revealed that the brain is particularly sensitive to changes in dosage of various proteins from transcriptional and translational regulators to synaptic proteins. Investigations of these disorders in animals have shed light on previously unknown pathogenic mechanisms leading to the identification of potential targets for therapeutic intervention. The demonstration of reversibility of several phenotypes in adult mice is encouraging, and brings hope that with novel therapies, skills and functionality might improve in affected children and young adults. As new research reveals points of convergence between syndromic and nonsyndromic autism spectrum disorders, we believe there will be opportunities for shared therapeutics for this class of conditions.

The clinical problem

In 1943 the child psychiatrist Leo Kanner provided the first systematic description of 11 children with "autistic disturbances of affective contact" and an "anxiously obsessive desire for the maintenance of sameness"[1]. Today, we know that the causes for these postnatal conditions are heterogeneous, and together they are referred to as autism spectrum disorders (ASDs). ASDs are more often diagnosed in boys, with a male:female ratio of 4:1 (ref. 2). Comorbidities occur in more than 70% of ASD cases and include language deficits, epilepsy, intellectual disability, motor abnormalities, anxiety, and gastrointestinal problems. ASDs are among the most common developmental neuropsychiatric disorders and an issue of public concern, as the estimated prevalence in the United States for 2010 is 1 in 68 births[3] (a 119.4% increase from 2000) and the national annual cost of supporting children and adults with ASDs is assessed at $236–262 billion[4].

Genetics of ASDs

ASDs have been typically classified into syndromic and nonsyndromic, a distinction that is exclusively based on clinical criteria. The term "syndromic" refers to conditions in which ASD occurs in conjunction with additional phenotypes and/or dysmorphic features. For these conditions the etiology in most cases is known and can involve chromosomal abnormalities, submicroscopic copy number variations, and mutations in a single gene, such as in fragile X syndrome (FXS)[5], Rett syndrome (RTT)[6], *MECP2* duplication syndrome (MDS)[7], tuberous sclerosis complex (TSC)[8,9], and *PTEN* macrocephaly syndrome[10]. It should be clearly stated that syndromic forms of ASD are not simply ASDs whose genetic causes are known, but are different clinical entities, with different developmental trajectories from nonsyndromic ASD. The term "nonsyndromic" typically refers to 'classic autism' as it was described by Kanner, in which no additional symptoms are present. For most nonsyndromic ASD cases the etiology is unknown, and the term "idiopathic autism" has been used alternatively. We have very few insights into the cause of nonsyndromic ASD. However, cumulative evidence from several twin studies over more than 40 years, starting in the 1970s, provided unequivocal evidence that nonsyndromic ASD susceptibility has a genetic component[11]. This prompted many laboratories and consortia to track genomic loci associated with genetic risk for ASD by using genetic linkage analysis[12,13] and more recently to look for common variants that contribute to ASD risk using genome-wide association studies[14,15]. Clear progress in the genetics of syndromic ASD began in the 1990s with the identification of genes implicated in these conditions, including *FMR1*, *TSC1*, and *MECP2* (**Fig. 1**). These monogenic disorders are rare, and none of these genes can explain more than 1–2% of ASD cases, but together they are estimated to represent ~5% of ASD cases. In the early 2000s, array comparative genomic hybridization technology allowed the identification of an enrichment of copy number variants, such as duplications and deletions in 15q11–13, 16p11.2, 22q11.2, and 7q11.23, in both syndromic and nonsyndromic ASD patients (refs. 16,17). Recently, whole-exome sequencing of families from the Simons Simplex Collection enabled the identification of about 120 new candidate genes with rare *de novo* single-base pair mutations associated with ASD[18–21].

Monogenic disorders

We will now discuss four examples of monogenic disorders associated with high penetrance of ASD (the so-called syndromic ASDs) and highlight their potential contribution to understanding the molecular mechanisms underlying the neurobiology of ASD in general. A comparison of clinical features across the most common syndromic ASDs with classic autism can be found in **Table 1**.

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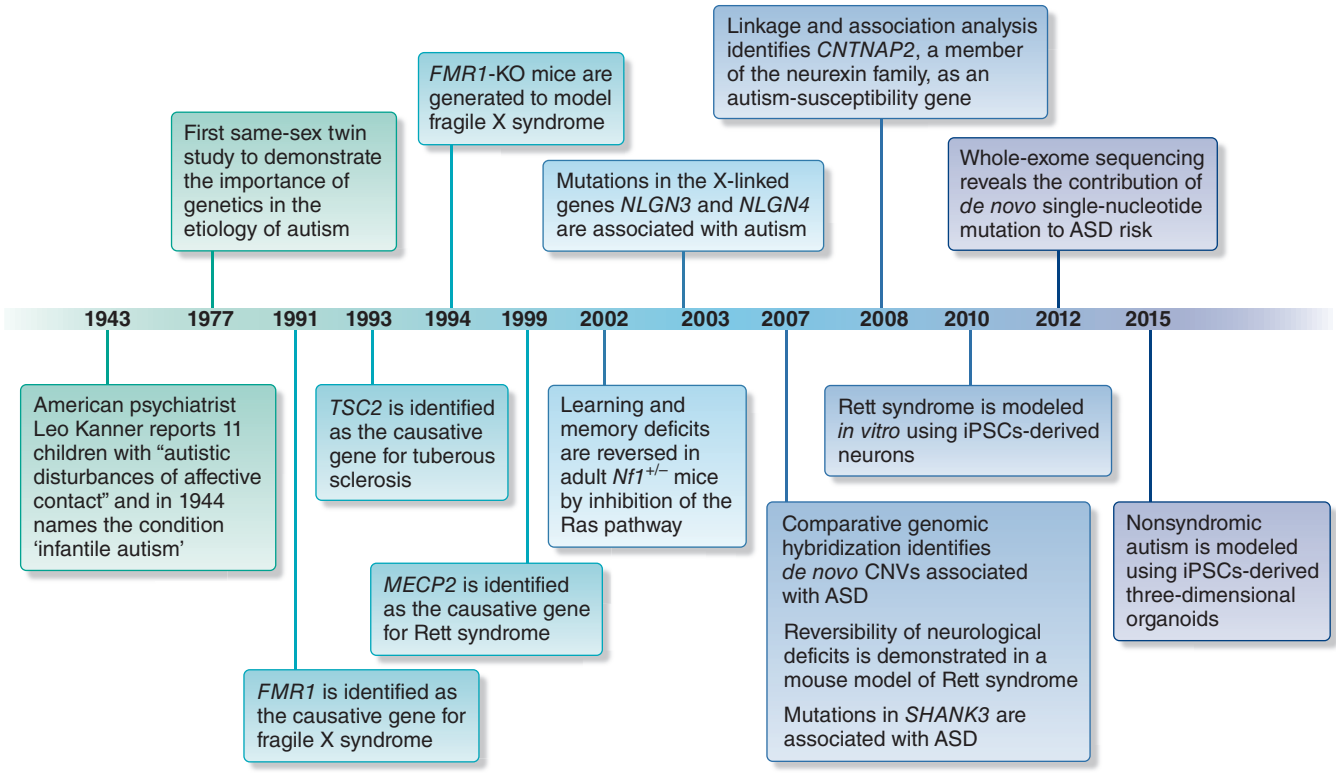

Figure 1 Timeline of key discoveries in the history of ASD research, with a focus on genetic advances. Since 1943, when classic autism was first clinically described by Kanner, the technological advances in genetics made it possible to identify mutations, involving single or multiple genes, that are directly associated or increase the risk for ASD. During these last eight decades, parallel efforts of scientists studying the genetics of ASD focusing on humans (twin studies, gene mapping, genome-wide association studies, etc.) and scientists using animal and cellular models allowed rapid progress in the elucidation of the genetic and cellular bases of ASD. The finding of mutations in single genes causing syndromic ASDs was critical for the generation of genetically modified animal models and, later on, for the demonstration that neurological deficits can be reversed, even after complete brain development. New research strategies, such as animals carrying human cells or genes, iPSCs, and cerebral organoids, in combination with clinical studies, will increase our confidence in potential therapies for human patients.

Tuberous sclerosis complex

TSC is an autosomal dominant genetic disorder affecting ~1 in 6,000 live births[22], characterized by benign tumors in the brain and other organs, epilepsy, cognitive impairment, and high penetrance of ASD[23]. TSC is caused by mutations in the *TSC1* or *TSC2* genes[24,25], which encode hamartin and tuberin, respectively. These two proteins dimerize and form a complex that negatively regulates the mammalian target of rapamycin (mTOR)[23] protein complex. The prevalence of ASD in TSC varies among studies, but it is estimated to range from 36% to 50%[26,27]. A recent study comparing the autistic symptoms profile of TSC ASD patients with those in toddlers with nonsyndromic ASD revealed that the social communication impairments and the repetitive behavior and restricted interests of children with TSC ASD are identical to those of children with nonsyndromic ASD, suggesting convergence and a potential common pathway for both disorders[28]. A direct role of mTOR in the psychiatric symptoms of TSC is supported by evidence in mouse studies, showing that treatment with rapamycin (a US Food and Drug Administration–approved mTOR inhibitor) rescues the physiological and behavioral deficits[29,30]. Dysfunction of the mTOR signaling pathway in neurons leads to the abnormal development of fundamental processes such as axonal and dendritic morphogenesis, synapse formation, and cell growth, abnormalities that have been proposed to contribute to the behavioral deficits seen in ASD[31].

Fragile X syndrome

First described by Martin and Bell[32] in 1943, FXS is considered the most frequent form of inherited intellectual disability in boys[33] and the most common monogenic cause of syndromic ASD[34]. The disorder is characterized by cognitive disability, delayed or absent speech, autistic features, attention deficit, hyperactivity, anxiety, a high incidence of epilepsy, and macroorchidism. FXS accounts for about 2% of all ASDs[35] and about 30–60% of males with FXS meet the diagnostic criteria for ASD[26,36] (FXS ASD). The similarity in the behavioral phenotype between FXS ASD and classic autism is controversial. Some studies have found that the phenotype of children with FXS ASD closely resembles that of children with classic autism[37,38], but others reported a distinct behavioral phenotype, including higher rate of repetitive behavior[39], less severe compulsive behavior[40], and lower impairment in particular social behaviors[41]. FXS is caused by an expansion of a CGG triplet repeat in the 5′ UTR of the *FMR1* gene[5], leading to hypermethylation of its promoter and a complete transcriptional inhibition of its gene product[42]. FMRP, encoded by the *FMR1* gene, is an RNA-binding protein that regulates the translation, stability, and transport of several mRNAs encoding proteins that are essential for synaptic plasticity and have been associated with increased risk of ASD, such as SHANK3, PSD-95, PTEN, CYFIP1, neuroligins and neurexins[43–45]. The current model for how *FMR1* mutations can lead to the ASD phenotype[34] suggests that the total absence of FMRP causes

Table 1 Clinical features across ASDs

Syndrome (OMIM number)	Primary gene affected	Estimated autism prevalence	Verbal	ID	Behavior	Motor	Dysmorphic features	Other associated conditions
Fragile X (300624)	*FMR1*	30–60% (males only)[26,36]	Delayed	Moderate	Little social interaction, aversion to touch, poor eye contact	Hyperactivity, stereotypical movements, weakness of connective tissue	Large head, long face, prominent forehead and chin, protruding ears	Developmental delay, abnormal behavior, macroorchidism at puberty
Rett (312750)	*MECP2*	61% (females only)[26]	Limited or absent	Moderate to severe	Expressionless face, lack of eye contact early on, social anxiety	Stereotypical hand movements, progressive scoliosis, ataxia, apraxia	Normal	Regression, microcephaly, hyperventilation, epilepsy
MECP2 duplication (300260)	*MECP2*	>90% (males only)[62,63]	Limited or absent	Severe to profound	Gaze avoidance, limited facial expression, atypical socialization	Hypotonia, progressive spasticity, developmental delay	Brachycephaly, large ears, midface hypoplasia, depressed nasal bridge	Respiratory infections, epilepsy, GI dysfunction, premature death
Angelman (105830)	*UBE3A*	34%[26]	Limited or absent	Severe to profound	Excessive laughter and smiling, decreased eye gaze	Ataxia, hypermotoric behavior, jerky movements, scoliosis, developmental delay	Brachycephaly, wide mouth, protruding tongue, prominent chin	Microcephaly, epilepsy
Tuberous sclerosis complex (191100, 613254)	*TSC1* or *TSC2*	36–50%[26,27]	Absent to normal	Mild to severe in ~50%	ADHD, impulsivity, hyperactivity, social impairment, anxiety	Normal	None	Epilepsy, benign tumors in multiple tissues, lung and kidney dysfunction, cortical tubers
Phelan-McDermid (606232)	*SHANK3*	75%[149]	Absent or delayed	Moderate to profound	Impulsivity, social anxiety, biting, obsessive chewing	Hypotonia, psychomotor delay	Long eyelashes, prominent ears, pointed chin, elongated head, deep-set eyes, dysplastic nails	Epilepsy, kidney dysfunction, cardiac anomalies
Timothy (601005)	*CACNA1C*	60%[150]	Severely delayed	Mild to moderate	Shyness, social avoidance	Developmental delay	Flattened nasal bridge, small teeth, low-set ears, small upper jaw, thin upper lip	Congenital heart malformations, cardiac arrhythmia, syndactyly, weakened immune system, premature death
Neurofibromatosis type 1 (162200)	*NF1*	18%[26]	Delayed	Mild cognitive disabilities	ADHD, social anxiety, depression, aggressive behavior	Hyperactivity, scoliosis, pseudoarthrosis	None	Multiple benign neurofibromas, abnormal skin pigmentation, macrocephaly, epilepsy
Idiopathic ASD (209850)	—	100%	Delayed, or absent	Mild to severe in ~50%	Impaired social interaction, restricted or repetitive behavior	Typically normal but some have coordination problems	None	Anxiety, GI problems, sleep disorder, macrocephaly in some, sometimes seizures

ID, intellectual disability; GI, gastrointestinal.

upregulation of metabotropic glutamate receptor 5 (mGluR5) signaling (the so-called 'mGluR theory of FXS')[46], a parallel inhibition of the $GABA_A$ receptor pathway[47], and dysregulation of proteins related to synaptic plasticity, together leading to the excitation/inhibition imbalance and abnormal connectivity typical of ASD.

Rett syndrome

RTT is a progressive neurological disorder usually caused by loss-of-function mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene[6]. RTT affects ~1 in 10,000 female live births and is characterized by 6–18 months of normal development followed by rapid regression, autistic features, loss of purposeful hand use and language skills, cognitive deficits, motor impairments, breathing abnormalities, seizures, and acquired microcephaly[48–50]. The wide interest in RTT increased in 1999 when it became the first sporadic ASD with a defined genetic etiology[6]. Classic autism and RTT share many common features. Both classic autism and RTT appear after a period of normal development, both disorders affect social behavior and speech acquisition, and in both cases patients display stereotypic behaviors[51]. However, clear differences exist as well. In contrast to classic autism, the autistic features in RTT are transient, as they appear during the regression period but improve by school age (except for the language deficits and stereotyped behaviors). Moreover, whereas RTT is characterized by postnatal deceleration of head growth (microcephaly), classic autism is frequently associated with postnatal acceleration of head growth (macrocephaly)[51,52]. At the cellular level, in both RTT and classic autism the pathological deficit involves abnormal dendritic synaptic function[53,54], suggesting the possibility of common molecular and cellular mechanisms. The fact that the autistic features in RTT typically manifest

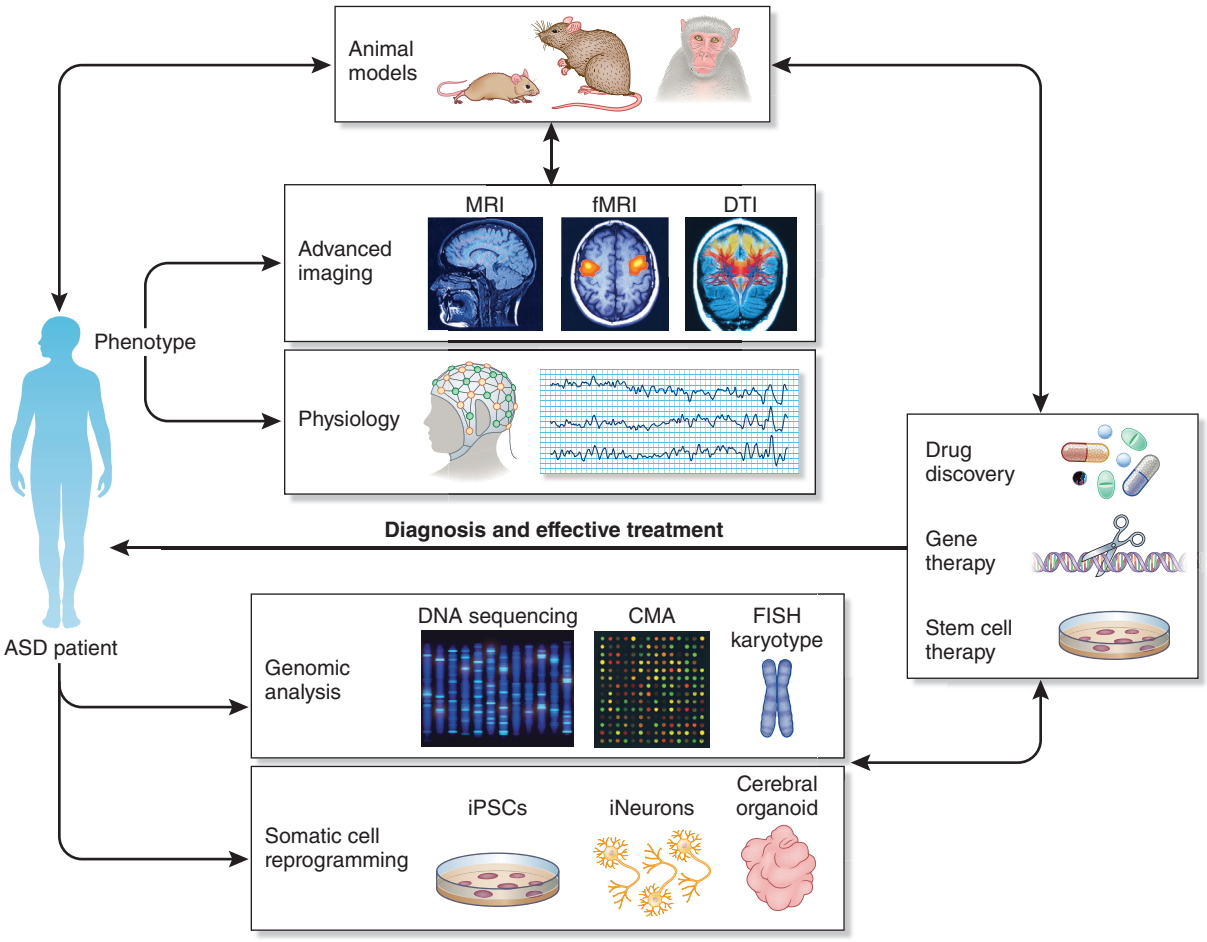

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Figure 2 Animal and cellular models for ASDs. ASD research today combines animal models, cells and organoids derived from patients, and cutting-edge technology to diagnose and characterize human disease. This conglomerate of models at different evolutionary and organizational levels allows scientists to see the whole picture, with the ultimate goal of developing effective diagnosis and treatment for human patients. The discovery of ASD-associated genes is the starting point for the generation of most transgenic animal models, but it is also possible to predict the existence of a human syndrome from findings in animal models as well, as it was the case with *MECP2* duplication syndrome. Potential therapies are proposed from insights obtained in preclinical and clinical data, and they can be tested in animal and cellular models until approved for human use. The constant and reciprocal sharing of information between preclinical and clinical laboratories is therefore essential to advancing therapeutic innovations. MRI, magnetic resonance imaging; fMRI, functional MRI; DTI, diffusion tensor imaging; CMA, chromosomal microarray; FISH, fluorescence *in situ* hybridization; iNeurons, induced neurons derived from iPSCs.

following a period of normal growth[48], the discovery that several deficits can be reversed upon reactivation of MeCP2 expression in adult *Mecp2* knockout mice[55], and the finding that RTT-like symptoms can be induced by inactivation of MeCP2 in the adult mouse brain[56] together argue that ASDs may be caused by impaired maintenance of neural circuits, rather than defective embryonic and perinatal development.

MECP2 duplication syndrome

In 1999 Herbert Lubs described five related boys with hypotonia, recurrent respiratory infections, profound intellectual disability, seizures, and premature death. The putative causative gene was mapped to distal Xq28 and the five clinical cases were proposed as a new X-linked neurological disorder[57]. The first clue that doubling MeCP2 levels in the correct spatial and temporal distribution causes a progressive neurological syndrome came from mice[58]. Shortly after, the first human cases of submicroscopic microduplications of Xq28 containing *MECP2* were detected[7,59–61] and MDS was established as a clinically recognizable disorder. Two studies have concluded that ASD is 100% penetrant in MDS patients and that increased dosage of *MECP2* contributes to the defining features of ASD[62,63]. More than 1,000 patients have been reported so far, and it has been estimated that MDS might explain about 1% of X-linked intellectual disability cases[60,64]. Using genetic manipulation and a pharmacological approach (antisense oligonucleotides), we recently demonstrated that restoration of MeCP2 to its normal level can largely reverse the phenotype of adult symptomatic mice, including social behavior dysfunction and seizures[65]. These results are encouraging and serve as a proof of concept for potential therapeutic strategies in other duplication disorders with high ASD incidence, such as Potocki–Lupski syndrome[66], 15q11–13 duplication syndrome[67], Williams-Beuren region (7q11–23) duplication syndrome[16], and Down syndrome[68].

In vivo systems to study ASD: strengths and limitations

The rapid increase in ASD prevalence and the immense societal cost of supporting ASD patients led to a rapid rise in the use of animals for preclinical and translational ASD studies (**Fig. 2**). Traditionally, the ideal animal model should have face, construct, and predictive validity (**Box 1**). The latter two are especially difficult to address for classic

autism, as the etiology is often unknown and no specific pharmacological treatments are available for this condition. As animal models of ASD, or other neuropsychiatric disorders, are not possible, animals should be best considered as systems to understand particular features of the disorder, such as impaired social communication, or used to study the biological impact of disease-specific mutations.

Rodents to study syndromic ASD

The laboratory mouse, *Mus musculus*, is a social animal that displays a rich and complex social behavior repertoire, including reciprocal interactions, social dominance, communal nesting, social thermoregulation, sexual behavior, and parental care[69,70] (for gold-standard and expanded mouse behavioral tests for ASD, see **Supplementary Table 1**). In the past decade the focus has been on modeling highly penetrant monogenic human disorders such as RTT and FXS for which the genetic cause is clearly known[71]. Interrogating features relevant to ASD in mice has many advantages. First, 95–98% of mouse genes have an equivalent in the human genome. Second, the ability to manipulate the mouse genome to selectively activate or repress genes in specific tissues or cell populations allows neuroscientists to ask precise questions about the potential involvement of different genes and neural circuits in ASD pathology. However, attempting to understand ASD features in mice has also some limitations. The mouse brain is about 3,000 times smaller than the human brain, and the prefrontal cortex, which is potentially impaired in ASD, is very rudimentary in comparison to the human counterpart. In addition, many ASD features, such as language deficits and impairments in face recognition and theory of mind, are unique to humans and are likely to be impossible to model in mice.

Rats are highly social animals and display some social behaviors that may be relevant to ASD but absent in mice, such as juvenile rough-and-tumble play[72]. The most widely used nongenetic manipulation in rats for potentially understanding features of ASD is that of pre- or postnatal exposure to valproic acid (VPA)[73], an anticonvulsant and mood stabilizer associated with increased risk of autism when used during pregnancy[74]. Rats exposed *in utero* to VPA show a marked decrease in social interaction[75,76] and increased stereotypic behavior[77,78]. Because the techniques to manipulate the genome were perfected in the mouse, the development of genetically altered rats to assess the impact of ASD risk genes has lagged behind that of mice. In recent years, however, the zinc-finger nuclease method allowed SAGE Labs to generate the first rat knockouts of ASD-related genes, including *Fmr1*, *Nlgn3*, *Nrxn1*, *Pten*, *Grm5*, *Mecp2*, *Met*, *Cntnap2*, *Cacna1c*, and *Rbfox1* (https://www.horizondiscovery.com/in-vivo-models/knockout). A few studies have started to phenotype these mutants[79–81], and engineered rats could complement mouse studies to increase the translatability of potential drugs for human clinical trials. It should be cautioned, however, that the biological impact of gene knockouts might not accurately reflect the pathogenic mechanisms resulting from a disease-associated gene variant.

Nonhuman primates to study syndromic and immune-driven ASD

Nonhuman primates have a well-developed prefrontal cortex comparable to that of humans and display a repertoire of behaviors that are likely more relevant to ASD research. One of the first attempts to model features of ASD in primates was based on the theory that exposure of the fetal brain to maternal autoantibodies can lead to ASD in the offspring[82]. Rhesus monkeys exposed prenatally to immunoglobulin G collected from mothers of children with ASD showed significantly increased stereotypies, hyperactivity, abnormal increased approaches to unfamiliar peers, and enlarged brain volumes[83,84]. More recently, transgenic monkeys overexpressing *MECP2* using a heterologous promoter were generated to understand MDS[85]. The animals exhibit increased repetitive behavior, increased stress responses, and an apparent deficit in social behavior. As the first attempt to generate a mutant primate to understand syndromic ASD, this study demonstrated the feasibility of using genetically modified primates as a model system. However, these results should be taken cautiously, as *MECP2* transgenic animals did not have disease-relevant levels or patterns of *MECP2* expression, showed normal interaction with wild-type animals, and did not mimic some human MDS symptoms, such as seizures and severe intellectual disability.

Box 1 Is your model relevant to the human disorder?

Traditionally, an ideal model of a human disorder should present three qualities:

1. Construct validity: the model is generated by mimicking the genetic, pharmacological, or environmental insult that causes the human disease.

2. Face validity: the model's phenotypes resemble the symptoms of the human disorder.

3. Predictive validity: the model and the human patients respond similarly to certain treatments.

It is of course not possible to perfectly model, in either cells or animals, human disorders, even if they possess all three qualities. For instance, if the etiology of a disorder is unknown (for example, idiopathic autism), it will be impossible for the model to have construct validity. If no treatment is yet available for the human patients, there will be no way to test the predictive validity of the model. And for most animal models, especially the ones modeling complex disorders, only some aspects of the human disease can be recapitulated. However, even if only some features of the disease can be reproduced, the animal or cellular model can still be of particular interest as long as scientists acknowledge their limitations and do not overstate the implications. A specific abnormal behavior, an altered cellular or tissue property, a disrupted genetic profile or a dysfunctional physiology all represent features of a disease that can be used to understand the altered molecular mechanisms of a specific disorder or to test potential treatments. In many cases, these phenotypes will depend on the background, the gender, or the age of the model organism. Therefore, when investigating a model system for the first time, we recommend performing a thorough characterization pertinent to a specific question, as the model can be very relevant to the human disorder, but only for certain phenotypes. Most importantly, the scientist should be aware of the limitations of the model and state a priori the goal and the hypothesis of the study.

In vitro systems to study ASD: potential and limitations

Since the initial discovery that human somatic cells can be reprogrammed to generate induced pluripotent stem cells (iPSCs), some features of CNS disorders have been modeled *in vitro* by direct differentiation of patient-derived iPSCs into neurons[86]. These studies clearly demonstrated that neurons derived from iPSCs can recapitulate the neuronal synaptic defects previously reported using post-mortem brain tissue and animals. Researchers have used iPSCs to understand monogenic ASDs such as RTT[87], MDS[88], FXS[89], Prader–Willi and

Angelman syndromes[90], Timothy syndrome[91], and Phelan-McDermid syndrome[92]. Recently, nonsyndromic ASD has been investigated using iPSCs from an individual who carries a *de novo* translocation that disrupts *TRPC6*, a gene encoding a Ca^{2+} channel involved in axon guidance, dendritic spine growth and synapse formation[93]. In a different study, three-dimensional telencephalic neural cultures (neural organoids or 'mini-brains') were generated from nonsyndromic ASD patients with no obvious genomic mutations but increased brain size (macrocephaly)[94].

There are some obvious advantages of using iPSC-derived neurons and mini-brains derived from patients. First, iPSCs can provide researchers with an unlimited source of stem cells to generate patient-specific neurons for basic research, drug discovery and toxicity screens. Second, iPSC-derived neurons provide an alternative strategy for ASDs caused by mutations that are difficult to engineer in mice, such as translocations, large duplication/deletions spanning multiple genes, and multiple risk alleles. Third, patient-derived iPSCs preserve the genetic background of the individuals, a feature that is especially important in complex diseases such as ASD. The limitations of the iPSC modeling approach include the early postnatal developmental stage represented, the high heterogeneity between iPSC clones, and the few available patients, which limits the ability to conduct well powered and properly controlled studies. Moreover, animal behavior is determined by interactions of different types of neurons functioning in a network, a condition that is still difficult to model *in vitro* with iPSCs. As long as the study design recognizes these limitations and focuses on surrogate outcomes, relevant mechanistic insights may be gleaned from iPSC-based studies.

Using syndromic ASD to understand the underlying mechanisms of ASDs

A prevailing theory regarding the pathophysiology of ASD postulates that the disorder results from a failure to maintain neuronal homeostasis, leading to weakened synaptic flexibility, circuit dysfunction and, ultimately, abnormal behavior[95]. This failure of neuronal adaptability can result from an unbalanced excitation/inhibition ratio[96], dysregulated transcriptional[97] or translational[98] control, impaired activity-dependent gene expression[99], or glial abnormalities[100]. Because of their monogenic etiology, syndromic forms of ASD have been the first to be studied in mice and have been essential in the elaboration of this theory. Indeed, one of the best examples of synaptic inflexibility is the neurophysiological phenotype of *Fmr1* knockout mice used for FXS. Loss of FMRP expression results in an excess of basal protein translation, leading to an exaggerated mGluR-dependent long-term depression in the hippocampus that is independent of protein synthesis and neuronal activity. Interestingly, many of the genes whose products are regulated by or interact with FMRP, such as *NLGN3*, *NLGN4*, and *CYFIP1* (ref. 101), are critical for synaptic maturation and plasticity. Surprisingly, *Nlgn3* knockout mice, used to study nonsyndromic ASD, exhibit deregulated mGluR-dependent long-term depression due to increased mGluR1α expression[102], suggesting a similar molecular pathway of synaptic dysfunction between fragile X and this form of nonsyndromic ASD.

Mice engineered for the two reciprocal MeCP2 disorders, RTT and MDS, taught us about the importance of chromatin regulation in mature neurons and the sensitivity of the mature brain to mild changes in the expression of multifunctional regulatory genes such as *MECP2* (ref. 103). Several mutant mouse strains with different levels of MeCP2 have been generated over the years, and they result in several phenotypes reminiscent of the broad spectrum of symptoms and comorbidities among ASDs. These showed us that too much or too little of MeCP2 impairs neurological functions. At the two extremes are male *Mecp2*-null mice, with an onset of symptoms at the age of 3–4 weeks and premature death as early as 8 weeks[104,105], and male *MECP2*-TG3 mice created for the *MECP2* triplication syndrome, with an early severe phenotype and premature death at the age of 3 weeks[58]. In the middle there is a spectrum of mutants with milder phenotypes, including female mice heterozygous for the *Mecp2*-null allele[106], male mice expressing only 50% of wild-type MeCP2 (ref. 107), mice with hypomorphic alleles[108–110], and mice with mild overexpression of MeCP2 (refs. 58,111). Interestingly, loss-of-function *MECP2* mutations typically associated with RTT also can cause classic autism[112,113] (in individuals with favorable X chromosome inactivation patterns); decreased expression of MeCP2 has been found in the brains of patients with classic autism, Angelman syndrome, and Prader–Willi syndrome[114–116]; and elevated expression of MeCP2 has been found in leukocytes[117] and in different brain regions of individuals with classic autism[118]. These findings make it clear that MeCP2 expression must be carefully regulated and that therapeutic interventions that aim to normalize MeCP2 levels could potentially ameliorate symptom severity not only in RTT and MDS, but also in other ASDs.

Cell-specific studies in monogenic mutants and what they teach us about ASD

A key question in ASD research is how different cell types contribute to the symptoms and pathology of ASD. The advent of new genetic technologies, especially the Cre-*loxP* system, has enabled researchers to perform cell-type-specific loss- and gain-of-function studies to address these questions. For instance, mice with *Mecp2* deletion from forebrain glutamatergic neurons display abnormal motor coordination, have impaired social behavior, and avoid risk, but have normal locomotor activity and normal context-dependent fear conditioning learning[119]. Loss of MeCP2 expression from forebrain GABAergic neurons also induces a partial spectrum of behavioral phenotypes, but preserves normal respiratory function and lifespan[120]. Moreover, deleting *Mecp2* in either parvalbumin-positive or somatostatin-positive subtypes of inhibitory neurons results again in distinct, non-overlapping partial phenotypes[121]. However, only global deletion of *Mecp2* from all GABAergic neurons is able to recapitulate most of the features of RTT and *Mecp2*-null phenotypes, indicating that global GABAergic dysfunction is central to the pathogenesis of RTT[120]. Interestingly, abnormalities in the number and function of inhibitory cells has been described in several ASD mouse mutants[122,123], suggesting that disruption of inhibitory GABAergic signaling may be one common pathway underlying ASDs of different etiology. Recently, mouse mutants for TSC have been also generated using the Cre-*loxP* technology. Reduced cerebellar volume and loss of some Purkinje cells have been found in TSC patients[124,125], as well as in individuals with classic autism[126]. Deletion of either *Tsc1* or *Tsc2* in mouse cerebellar Purkinje cells results in their degeneration, together with abnormal social interaction and increase repetitive behavior[127,128], suggesting that circuits involving Purkinje cells contribute to the pathophysiology of ASD.

Testing reversibility in monogenic mutants: implications and relevance to interventions

ASDs have been historically considered irreversible, as it was believed that the anomalous neuronal connections formed during the prenatal developmental period are permanent and irreparable by the time a diagnosis is made, typically at 2–5 years of age. However, several studies using mutants to study monogenic ASDs have challenged this dogma. Neurofibromatosis type 1 (NF1) is a monogenic disorder characterized by learning disabilities and high prevalence of ASD. It is caused by mutations in *NF1*, a gene that encodes neurofibromin, a negative regulator of the Ras–Mapk signaling pathway. In 2002, Alcino Silva's group

demonstrated that the neurological deficits in a NF1 mouse mutant ($Nf1^{+/-}$) are due to hyperactivity of the Ras–Mapk signaling pathway. Remarkably, they found that the learning impairments in $Nf1^{+/-}$ mice can be rescued in adult symptomatic mice by genetic or pharmacological inhibition of the Ras pathway[129]. In 2007, Adrian Bird's group demonstrated the reversibility of neurological phenotypes after onset of symptoms in the *Mecp2*-null RTT mouse model[55]. These studies have inspired many others, and today reversibility of phenotypes in adulthood has been demonstrated for other monogenic ASD mutants, such as FXS[130], TSC[29], MDS[65], and Angelman syndrome[131]. Recently, Guoping Feng's group has shown that neuronal and behavioral function can be ameliorated in adult mice deficient in *Shank3*, the causative gene in Phelan-McDermid syndrome and implicated in about 1% of nonsyndromic ASD cases[132]. When *Shank3* expression was reactivated in adult *Shank3* knockout mice, some behavioral phenotypes where reversed (overgrooming and impaired social behavior), but mice continued to display hypoactivity, avoided risk, and had abnormal motor coordination and exploratory behavior. Interestingly, *Shank3* reactivation in young mice (postnatal day 20–21) led to even more behavioral improvement, and germline restoration of *Shank3* restored all behavioral phenotypes, suggesting that earlier intervention is critical for this specific disorder.

Taken together, these studies suggest that the neuroanatomy of the autistic brain may remain sufficiently intact that correction of the molecular dysfunction underlying the disorder could restore normal brain function. However, a word of caution is warranted here. Can these findings be extrapolated to other neurodevelopmental disorders, such as nonsyndromic ASD? The pathway to reversibility might not be as straightforward in classic autism as it is in monogenic disorders, as the etiology of nonsyndromic ASD might be more complex and sometimes multifactorial. Will these results in mice be paralleled by similar outcomes in humans? We are enthusiastic and expect that targeted therapies in humans will soon be able to improve symptoms such as motor dysfunction and seizures. However, it remains to be seen how challenging it will be to retrain and reverse behavioral and cognitive symptoms in patients who have grown to adulthood with one of these devastating disorders. Reversibility in adult mice underscores a maintenance function for the affected genes; however, it is possible that in humans early interventions before or during critical periods of brain development might result in better outcomes compared to treatment and retraining in adulthood. Therefore, in contemplating clinical trials, serious consideration should be given to the timing of testing interventions.

New therapeutic strategies for syndromic ASD

The available treatment for ASD is a combination of behavioral therapies[133,134] and the two drugs approved by the US Food and Drug Administration for ASD, the antipsychotics risperidone and aripiprazole, which ameliorate autism-associated irritability. The identification of the genes that cause syndromic forms of ASD and the generation of relevant mutants that recapitulate the core phenotypes led to the discovery of several molecular pathways involved in syndromic ASD and to the development of targeted treatments. For instance, pharmacological inhibition of the mTOR signaling pathway using rapamycin can rescue physiological, morphological, and behavioral deficits in mice that model protein translation disorders such as TSC[29,127], *PTEN* macrocephaly syndrome[135], and 15q11–13 duplication[136]. Ongoing clinical trials are investigating the efficacy and safety of rapamycin and its analogs for the treatment of neurological deficits in patients with TSC, including associated ASD (http://clinicaltrials.gov/: NCT01730209, NCT01713946, NCT02451696, NCT01929642, NCT01954693). Compounds that boost BDNF and IGF-1 levels improve neurological phenotypes and survival in a mouse engineered to model features of RTT[137–139]. Antagonists of mGluRs, agonists of $GABA_A$ and $GABA_B$ receptors, and repressors of MMP9 are among the pharmacotherapies proven to be successful in mutant mice modeling features of FXS[101,140]. Several finished and ongoing clinical trials are based on promising results in animals; however, the feasibility of translation is still uncertain[141].

New approaches for the treatment of ASDs have been recently proposed. Angelman syndrome is a monogenic disorder with high-associated ASD prevalence[26]. Most cases are caused by loss-of-function mutations in the maternal allele of the imprinted *UBE3A*, whereas the paternal normal allele is silenced by a long noncoding RNA, *UBE3A* antisense transcript (*UBE3A-ATS*). Antisense oligonucleotides (ASOs) targeting *Ube3a-ATS* have been used to correct cognitive deficits in a mouse engineered to model features of Angelman syndrome by unsilencing paternal Ube3a in mouse neurons and restoring normal UBE3A protein levels[131]. In the same vein, our laboratory has recently used ASOs to normalize MeCP2 levels and rescue the neurological deficits, including impaired social behavior and seizures, in mutant mice carrying an extra copy of *MECP2* (ref. 65). Gene therapy using adeno-associated virus (AAV) vectors is a promising way to replace a defective gene, and it has proven effective in both male[142] and female[143] mice carrying a null allele for *Mecp2*. The challenges for translation of both ASO and AAV strategies are in their safety (both must aim to restore the protein to normal levels), pharmacokinetics, correct delivery and distribution in the brain[144]. Deep brain stimulation targeted to the fornix has recently been shown to reverse hippocampus-dependent cognitive dysfunction in MeCP2 mutant female mice[145]. Because it is monitorable and reversible, deep brain stimulation could in the future become a promising strategy for the treatment of other ASDs and intellectual disability syndromes.

In spite of high heterogeneity in the genetics and the symptoms of ASDs, disrupted synaptic function appears to be a basis of ASD pathophysiology (**Fig. 3**). Indeed, several ASD-associated genes encode proteins that directly or indirectly affect synaptic function[146]. Impaired synaptic plasticity might lead to neuronal networks with reduced capacity to change their structure and function. Therefore, in addition to the targeted therapy approach for each specific condition as described above, several studies have investigated the possibility of ameliorating neurological deficits by enhancing synaptic plasticity in adult mice in a way that is independent of the disorder-specific etiology[147]. A juvenile-like state of enhanced plasticity can be induced in adult mice by several environmental and pharmacological manipulations, such as rearing in an enriched environment, food restriction, administration of the antidepressant fluoxetine, and disruption of the brain extracellular matrix (especially the perineuronal nets) by injection of the enzyme chondroitinase ABC[148]. The ability to temporally open a window of enhanced plasticity in the brain, in combination with behavioral therapy, offers a new strategy for treating a variety of neurodevelopmental disorders, including syndromic and nonsyndromic ASD.

Summary and concluding remarks

The progress made in understanding the genetics and the molecular mechanisms underlying the pathophysiology of syndromic ASDs has had a major impact on the field of neurogenetics and has provided many insights and potential therapies that might prove relevant to nonsyndromic ASD. Shared mechanisms between syndromic and nonsyndromic ASDs are being discovered using both animals[102] and human neurons[86], suggesting that some therapies found to be effective in syndromic ASD could potentially benefit the nonsyndromic ASD population. Effective translation of potential therapies strictly depends on the use of appropriate model systems, the choice of highly relevant behavioral assays, and the rigor in experimental design. It is also important to be aware of the strengths and

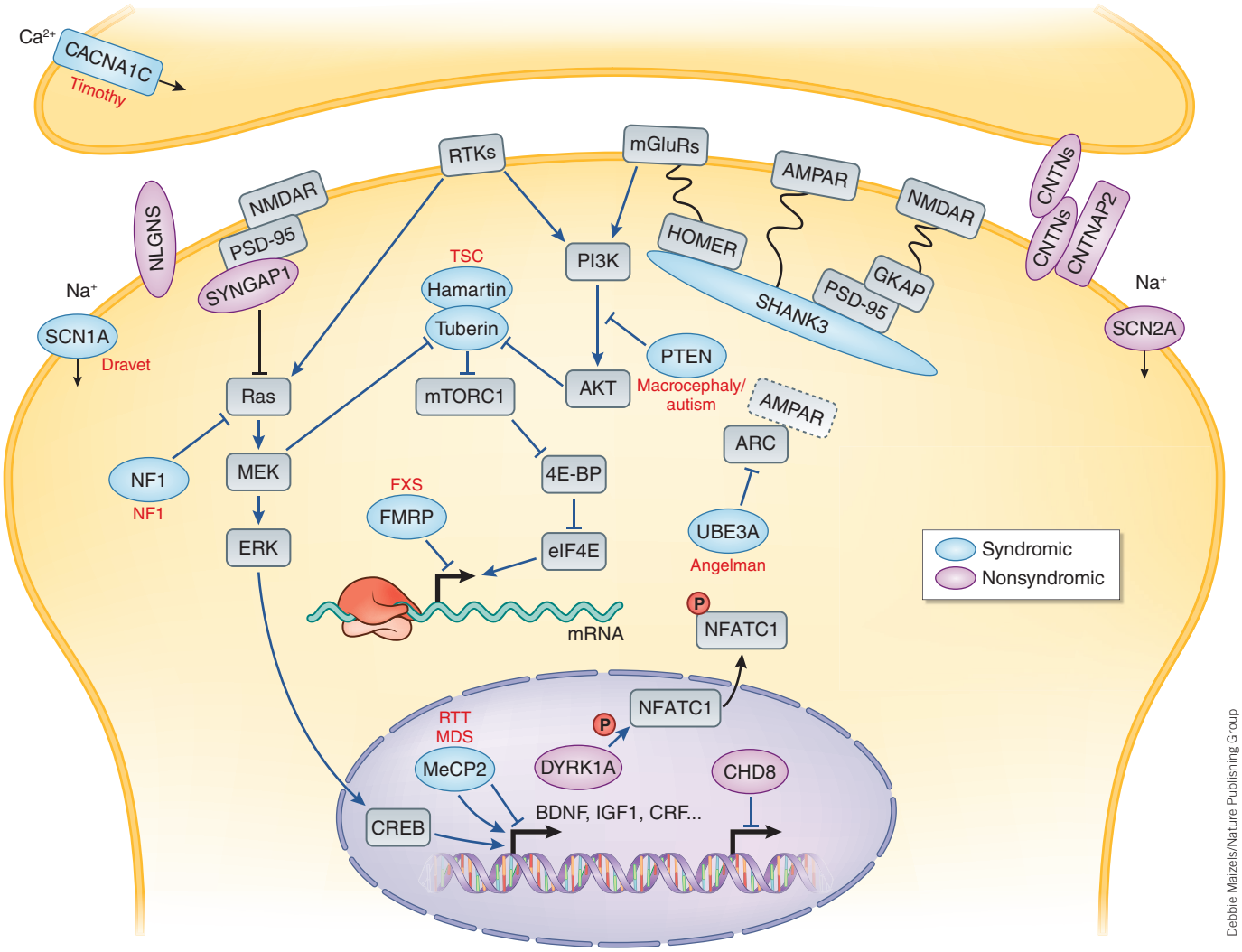

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Figure 3 Mutations in syndromic and nonsyndromic ASD. We describe the cellular location and the signaling pathways of proteins encoded by genes that cause the most common syndromic ASDs (blue), as well as by selected genes associated with increased risk of nonsyndromic ASD (purple). A common property of many of these genes is in their modulation of synaptic structure and function, a feature of neurons considered to be at the basis of ASD pathogenesis. NF1, neurofibromatosis type 1; FXS, fragile X syndrome; RTT, Rett syndrome; MDS, *MECP2* duplication syndrome; TSC, tuberous sclerosis complex; NLGNs, neuroligins; CNTNs, contactins; RTKs, receptor tyrosine kinases; mGluRs, metabotropic glutamate receptors; AMPAR, AMPA receptor; NMDAR, NMDA receptor; PSD-95, postsynaptic density protein 95; GKAP, guanylate kinase-associate protein; HOMER, homer protein homolog; PI3K, phosphoinositide kinase-3; PTEN, phosphatase and tensin homolog; SCN1A, sodium channel voltage gated type 1 α-subunit; CNTNAP2, contactin-associate protein-like 2; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; 4E-BP, eukaryotic translation initiation factor 4E-binding protein; eIF4E, eukaryotic translation initiation factor 4E; ARC, activity-regulated cytoskeleton-associated protein; NFATC1, nuclear factor of activated T cells; CREB, cAMP response element-binding protein; DYRK1A, dual specificity tyrosine-phosphorylation-regulated kinase 1A; CHD8, chromodomain helicase DNA-binding protein 8; CRF, corticotropin-releasing factor; CACNA1C, calcium voltage-gated channel subunit-α 1C; SYNGAP1, synaptic Ras GTPase activating protein 1.

limitations of the specific *in vivo* and *in vitro* models, in order to interpret the findings correctly. Moreover, we should be cautious with the inferences and extrapolations we make from animals to the human condition, as the evolutionary distance between the two is considerable. The study of iPSC-derived neurons and neural organoids from ASD patients can shorten this distance, and their use is increasing and becoming a promising tool for basic and translational research. More sophisticated approaches are expected to be incorporated in ASD research such as the generation of animals carrying human cells or genes, by either CNS transplantation of human induced neuronal progenitors or by complete replacement of a gene of interest with the human version. The main challenge, however, is to identify shared pathways between syndromic and nonsyndromic ASDs so that one day an effective and common therapy may be developed. The knowledge we are gaining from studying monogenic disorders and the cumulative evidence showing that neurological deficits can be reversed in adult symptomatic mouse mutants for ASD risk genes, provide hope that therapeutic options will be available in the near future to improve the symptoms in this diverse spectrum of autism disorders.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Editorial Summary

Autism spectrum disorders are highly heterogeneous and include both idiopathic and syndromic forms. Sztainberg and Zoghbi discuss insights gained from studying syndromic autism spectrum disorders and their potential contribution to our understanding of the molecular pathways critical for normal cognitive and social development, as well as the relevance to idiopathic autism.