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Advancing the understanding of autism disease mechanisms through genetics

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Progress in understanding the genetic etiology of autism spectrum disorders (ASD) has fueled remarkable advances in our understanding of its potential neurobiological mechanisms. Yet, at the same time, these findings highlight extraordinary causal diversity and complexity at many levels ranging from molecules to circuits and emphasize the gaps in our current knowledge. Here we review current understanding of the genetic architecture of ASD and integrate genetic evidence, neuropathology and studies in model systems with how they inform mechanistic models of ASD pathophysiology. Despite the challenges, these advances provide a solid foundation for the development of rational, targeted molecular therapies.

Over the last few decades, technological and methodological advances in genetics and genomics have permitted the identification of mutations that are involved in thousands of rare Mendelian conditions and in the etiology of more common, complex diseases^{1–7} (http://www.ebi.ac.uk/ gwas/). In this regard, genetic findings have played a major role in neurodevelopmental disorders, such as ASD, for which many contributing genes have been identified (**Fig. 1**), providing a platform for unraveling the causal chain of events that result in ASD.

The challenges in understanding ASD are many, ranging from defining ASD's heritable genetic components and understanding ASD risk more completely in individuals to determining whether the probable hundreds of different genetic forms of ASD might converge into a tractable set of targetable pathways for treatment^{8,9}. Additionally, given ASD's clinical and genetic heterogeneity, it is perhaps not surprising that no common macroscopic or microscopic neuropathology is recognized and that no specific brain region or cell type is uniquely implicated^{9–11} (**Fig. 2**). Nevertheless, the identification of veritable genetic risk factors provides a solid mechanistic grounding on which to base therapeutic development. Here we start by reviewing the clinical features of the syndrome and providing a broad overview of genetic findings. We then describe how mouse and *in vitro* human stem cell–based models can advance mechanistic understanding. Finally, we highlight the

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evidence for the most prevalent neurobiological models that attempt to bring together diverse genetic findings, molecular pathways and model systems to develop an evidence-based theoretical framework for understanding ASD.

Clinical overview

The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (ref. 12) defines ASD by deficits in two core domains-social interaction and communication, and repetitive, restrictive behaviors-with onset during early development. ASD unifies three previously separate but highly related diagnoses: autistic disorder, Asperger's disorder and pervasive developmental disorder-not otherwise specified (PDD-NOS). Although prevalence estimates in the early 1990s were on the order of 1 in 1,000, they have consistently increased and are presently 1 in 68 children under 8 years of age in the USA¹³. There are substantial differences in ASD prevalence between the sexes (1 in 42 for males; 1 in 189 for females), suggesting mediation by as yet unknown biological factors^{14,15}. ASD also occurs with frequent comorbidities, such as motor deficits (hypotonia, apraxia or motor delay), sleep abnormalities, gastrointestinal disturbances and epilepsy¹⁶. Sensory hyper- or hyposensitivity, previously listed as a frequent (~90%) comorbidity, is now integrated into the core diagnosis within the repetitive and restrictive domain. Another salient comorbidity is intellectual disability (ID), which is observed in ~35% of individuals with ASD and can markedly confound diagnostic instruments^{17,18}. These comorbidities also present challenges in disease modeling, as they can complicate assessment of core ASD behaviors in animal models and overlap with phenotypes observed in other neuropsychiatric disorders, such as schizophrenia, attention deficit-hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD).

Current therapeutic options are predominantly restricted to behavioral interventions, which can be highly successful in a subset of patients, and, as such, early intervention is warranted^{19,20}. The only FDAapproved drugs, risperidone, which is effective in treating aggressive and repetitive behaviors, and aripiprazole, which reduces irritability, are not directed at the core social deficits. We have few ways to prognosticate



Figure 1 Genetic architecture of autism spectrum disorders. (a) The inheritance patterns of syndromes with known genetic etiology and high incidence of autism, as well as that of genes recently identified to be associated with autism. The red stars indicate a causal allele and the red pie charts indicate a small proportion of risk. Most dominant disorders show *de novo* inheritance. Autosomal recessive, autosomal dominant and X-linked inheritance patterns best fit a major gene model, whereas a polygenic model is best represented by additive risk. (b) The types of genetic variation (left and middle) and the developmental disorders (right) associated with autism. Genes that have been associated with ASD are also indicated. (c) The penetrance of known syndromic mutations summarized from multiple studies. 95% binomial proportion confidence intervals, based on Wilson's score interval, are shown. (d) The percentage of individuals with ASD harboring known mutations, as well as the percentage of liability from different classes of mutations (taken from ref. 57). The percentage variance in liability measures the contribution of a particular variant or class of variants relative to the population variance in a theoretical variable called liability. Liability is a continuous and normally distributed latent variable that represents each individual's risk (both genetic and environmental) for developing a disease²⁶⁶. Notably, percentage variance in liability is directly dependent on the frequency of the variant, and it is inversely dependent on the frequency of the disease in the population. References for this figure are found in **Supplementary Table 1**.

and stratify patients for treatment at this point. We also do not have any clear biomarkers, although tracking eye movements²¹ and electroencephalogram (EEG) parameters²² have shown promise. The hope is that understanding the underlying genetic risk and neurobiologically anchored disease mechanisms in individual patients will fuel therapeutic development and patient selection for the most appropriate treatments.

Genetic architecture of ASD

We are just starting to elucidate the genetic architecture of ASD (**Box 1**). In **Figure 1**, we highlight the specific risk genes that have been identified, the types of mutations, the patterns of inheritance and the contribution of these genes to ASD. A key early insight into the genetic basis of ASD came from the recognition that dozens of rare medical genetic syndromes with diverse modes of inheritance have high penetrance (**Box 1**) for ASD^{23–26}. Each of these known syndromes are rare, and none are found in more than 1% of patients with ASD; however, collectively they are estimated to be found in ~5% of the total population of individuals with ASD (**Fig. 1**).

Family and twin studies show that ASD is highly heritable²⁷⁻³², and this has spurred genome-wide analyses of genetic variants using microarrays, whole-exome sequencing (WES) and, more recently, whole-genome sequencing (WGS) (Box 1). However, the most successful efforts in gene discovery so far have identified rare proteindisrupting genetic variants in the affected child that are not found in the healthy parents, which represent new, or de novo, copy-number variants (CNVs)³³⁻⁴¹ or single-base pair mutations (single-nucleotide variants; SNVs) that have arisen in the germline^{18,42-49} (Box 1). These studies have shown that there is an overall enrichment in 'likely gene-disrupting' mutations (LGDs; nonsense, frameshift and splice site mutations that often result in production of truncated proteins) in individuals with ASD as compared to their healthy relatives or to other unaffected individuals. Furthermore, these studies have identified individual rare de novo mutations that show strong evidence for their involvement in ASD, including mutations in chromodomain helicase DNA binding protein 8 (CHD8) and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), and a deletion or duplication of chromosome 16 (16p11.2) that encompass a ~600-kb region containing dozens of genes (Fig. 1 and Supplementary Table 1). Because the ASD-linked mutations are rare, comparing the difference in variant frequency at an individual gene in individuals with ASD versus the variant frequency in control subjects, which is a typical case-control design, while correcting for genome-wide comparisons of mutation rate has not yet reached statistical significance for the association of a mutation in individuals with ASD at any individual gene within current sample sizes. In general, current estimates of the significance of an association between a mutation and ASD are based on comparing the frequency of the observed mutations in patients to the expected rate at which null mutations would occur in that gene^{47,50,51}. This has resulted in somewhat of a moving target for genetic significance estimates, as significance depends on the number of variants found in cases and controls, as well as the overall number of cases and controls. WES is not only allowing the discovery of rare de novo heterozygous mutations, but it has also identified rare recessive mutations that are inherited in consanguineous families^{52–55}.

Genetic models. Several models have been presented to explain the observed familial aggregation patterns and recent genetic findings (**Fig. 1**). Polygenic risk models (**Box 1**) assume that there are many inherited variants contributing to ASD, each with a small effect that, in combination with environmental factors, result in an individual crossing a risk threshold to develop the disease^{56–59}. In contrast, major gene models (**Box 1**) assume that either one highly penetrant rare mutation or a limited number of moderately to highly penetrant mutations (oligogenic) are sufficient

to cause ASD^{60,61}. An important instance of the major gene model is a unified theory of ASD inheritance and occurrence^{62,63} that groups families into two types: low risk and high risk. In the more prevalent low-risk group, children develop ASD due to *de novo* mutations⁶². Female children of low-risk families with *de novo* mutations are 'protected' by an as yet unidentified mechanism and are less likely to develop ASD. In high-risk families, unaffected mothers transmit mutations that have reduced penetrance in females in a dominant manner to multiple, predominantly male, affected children. The polygenic and major gene models are not mutually exclusive, and both have support in the literature, but the extent of their contribution to ASD is a subject of debate^{57,63}.

Polygenic models are supported by multiple lines of evidence. Firstly, recurrence of ASD in families implies a strong inherited genetic component^{28,29}. Secondly, the first-degree relatives of children with ASD show related phenotypes more than in the general population, such as social and behavioral differences^{64–66}. Finally, heritability estimates using single-nucleotide polymorphism (SNP) (**Box 1**) data demonstrate that commonly inherited genetic variants (minor allele frequency > 0.05) or variants that are tagged by common genetic variants collectively explain a large proportion of the variance in susceptibility to $ASD^{57,67,68}$, as in many other common, complex disorders^{67,69}. However, a weakness of the polygenic model is that statistically significant and replicable commonly inherited variants have not yet been identified for ASD^{70} . This is probably due to the limited statistical power using current sample sizes and study designs^{6,71,72}.

The unified major gene model^{62,63} is supported by the significant increase in damaging *de novo* mutations found in subjects with ASD

BOX 1 Glossary of genetic terms

Genetic architecture: the relative contributions of different forms of genetic variation.

Penetrance: the proportion of mutation carriers who also are diagnosed with the disease or carry a given phenotype. **WES:** Whole-exome sequencing—reading only the genetic

sequence that encodes for proteins in an organism.

WGS: Whole-genome sequencing—reading the entirety of the genetic code of an organism.

De novo mutation: A mutation that is present in the offspring but that was not inherited from either parent.

SNV: Single-nucleotide variant—a rare (<1%) or common single-bp change in the genome.

CNV: Copy-number variation—deletion or duplication of large genomic regions leading to changes in the number of copies of the genetic elements encoded within those regions.

Polygenic model: a model that describes the genetic risk factors of a disease as many inherited variants, each of which contributes a small, additive risk for developing a disease.

Oligogenic model: a model that describes the genetic risk factors of a disease as a few variants, each of which contributes a large risk for developing a disease.

Major gene model: a model that describes the genetic risk factors of a disease as due to genetic variants, each of which contributes a large risk for developing a disease. One major gene mutation is typically considered sufficient to cause a disease in an individual. **SNP:** Single-nucleotide polymorphism—a single bp change that is common (>1%) in the population.

Simplex family: a family in which only one individual is affected with a disease.

Multiplex family: a family in which multiple individuals are affected with a disease.

a Molecular



as compared to their unaffected siblings^{44,49}. Further support for this model is seen in the phenomenon that there are more inherited SNVs that disrupt protein function in conserved genes transmitted from the mother to individuals with ASD than in unaffected siblings⁷³. However, all pathogenic CNVs considered as a group have not yet shown evidence for enriched maternal transmission to probands⁷⁴.

There are findings that are inconsistent with a major gene model. For example, if *de novo* mutations comprise the majority of genetic risk, then one expects monozygotic (MZ) twins (who share both germline de novo and inherited genetic risk factors) to be concordant for ASD more than twice as frequently as dizygotic (DZ) twins, who share on average only half of their inherited genetic risk factors. Evidence for this was demonstrated by early twin studies. However, larger, more recent studies show a DZ concordance rate that is higher than expected for de novo mutations that comprise the major contribution to ASD risk (reviewed in refs. 32,49). Similarly, if most cases of ASD were due to de novo mutations in the parental germline, then risk in relatives would be very low⁵⁰. However, familial risk implies a strong inherited genetic component, consistent with the polygenic model described above²⁹. Furthermore, estimates of the influence of de novo mutation on risk for ASD have been derived mostly from a sample of simplex families (Box 1), which may inflate the contribution of *de novo* mutations^{40,44,45,47,49}. Estimates of the heritability of ASD on the basis of the amount of common genetic variation (as measured from SNPs) shared in unrelated affected individuals^{57,67,68} demonstrate that the collective commonly inherited genetic variants can explain a large proportion of the variance in predisposition of a population to ASD, supporting the polygenic model. It is also recognized that some predictions of a major gene model have not yet been rigorously tested; the model predicts dominant maternal transmission of highly penetrant alleles, but estimating the contribution of dominance effects from family or population data is difficult to separate from the contribution of interaction between different loci^{29,75}.

After comparison of the currently available data for genetic association with ASD, the data fit a model in which the largest component of genetic risk derives from common genetic variants of an additive effect with a smaller, although clearly important, contribution from *de novo* and rare inherited variation⁵⁷ (**Fig. 1**). This is relevant because presumptions about genetic architecture have important implications for future study design, nosology and treatment. For example, if *de novo* mutations provide the major contribution to ASD risk, then we should focus genetic discovery efforts in simplex families with parents (and unaffected siblings when available). On the other hand, common variation is most efficiently detected with large case-control association studies, whereas heritable rare-variant detection is best served by studying larger multiplex families⁷⁶ (**Box 1**).

With regards to nosology, highly penetrant variants may be very useful for defining subtypes of ASD⁷⁷. For example, LGDs in *CHD8* and *DYRK1A* have been found in individuals with ID and ASD but as yet have never been observed in unaffected individuals, indicating high penetrance of these mutations^{43,44}. Patients with these rare mutations were then extensively phenotyped to identify distinct syndromic sub-types^{78–80}. In contrast, individual common variants indicate only a small risk for disease and cannot be used for diagnosis. It is possible that the aggregate risk from common variants can be used as a part of the classification tools for diagnosis and subtype definition within and across disorders after confounders, such as population structure, are properly taken into account^{81–85}.

Finally, the complexity of therapy development is probably proportional to the number of targetable biological pathways in the population, not the number of genetic insults identified. Although the polygenic model predicts a large number of loci to be associated with ASD, drug development may be simplified if convergent targetable pathways are identified. Conversely, if many different pathways are associated with ASD, then many different treatments may be necessary to alleviate the burden of disease across the population. Current evidence from known mutations does suggest significant convergence in the pathways in which the mutations are found, but the full extent of convergence will be better defined after further clarification of the genetic landscape^{8,18,86–89}. Notwithstanding these challenges, the identification of genetic variants provides a clear causal foothold into the underlying biology of ASD^{18,90}.

Neurobiological models and mechanisms of ASD

Genetic advances have fueled the generation and characterization of numerous mouse models of ASD (Table 1 and Supplementary Table 2), the major strengths and limitations of which are summarized in Box 2 (refs. 91,92). A variety of established assays are now considered standard assessments for ASD-related behavioral phenotypes⁹³ (Box 2). It is notable that the majority of mouse models for monogenic forms of ASD have social impairment or repetitive behaviors; however, these core features of ASD and additional comorbidities, such as motor dysfunction, hyperactivity and anxiety, vary widely (Table 1 and Supplementary Table 2). Despite their potential limitations (Box 2), mouse models have been valuable in translating genetic findings and have provided evidence for shared molecular pathways and phenotypes in ASD. Primate models or invertebrates have not yet been widely used to model ASD, but they are complementary to mouse models⁹². Nonhuman primates are expected to more closely model complex behavior and higher cortical functions, whereas zebrafish and invertebrates offer efficient, higher-throughput genetic manipulation^{92,94,95}.

In parallel, advances in stem cell biology in the past decade make it possible to generate and study human neurons and their development⁹². Differentiation into functional neurons that may model phenotypes of ASD has been shown to be possible from human embryonic stem cells (hESCs), human induced pluripotent stem cells (hiPSCs) and primary human neural progenitor cells (phNPCs) (**Table 2** and **Supplementary Table 3**), although limitations exist (**Box 3**). Together, human neural

Figure 2 Convergent neurobiological mechanisms in ASD. Normal brain development requires the generation and positioning of the correct number and type of cells, the growth and targeting of neuronal processes, and the formation of the precise number and type of synapses. (a) These events are regulated by molecular pathways in development. Genes within these pathways for which there is genetic evidence for a link to ASD¹⁸ (**Fig. 1**), including from our meta-analysis of SNVs and CNVs (compiled from refs. 43,44,73,74), are colored in gold. Chemical compounds that reverse behavioral or cellular ASD phenotypes in model systems are indicated in green font near their predicted site of action. (b) The cellular events leading to changes in the higher-order organization of the brain, including disruption of fetal cortical development and synaptic function. The cortical laminae are depicted from early fetal to neonatal stages (not to scale). The numbers indicate the molecular pathways important at each stage of development. (c) The widespread pathology¹⁰ and functional phenotypes observed in ASD, including altered brain growth trajectories, altered cortical cytoarchitecture (red triangles indicate excitatory upper layer neurons; green triangles are excitatory deep-layer neurons; blue triangles are interneurons; numbers indicate cortical layers; WM, white matter) and connectivity, may arise from combined deficits in neurogenesis, cell fate, neuronal migration and morphogenesis during fetal development and dysregulated synaptic function, possibly in combination with reactive microglia infiltration and astrocytosis. RG, radial glia; oRG, outer radial glia; IP, intermediate progenitor; MN, migrating neuron; EN, excitatory neuron; IN, interneuron; A, astrocyte; E/I, excitatory or inhibitory neuron; U/D, upper-layer neuron. MPEP, 2-methyl-6-(phenylethynyl)-pyridine; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; DCS, p-cycloserine; IGF1, insulin-like growth factor 1. VZ, ventricular zone; ISVZ, inner subvent

Table 1 Mouse models of ASD

		Social	Social	Repetitive		Molecular, cellular and	
Molecular function	Mouse model	interaction	cation	behavior	Other symptoms	circuit phenotypes	Treatment
Multiple	dup 15q11- q13 (refs. 267,268)	Impaired	↓Calls	Behavioral inflex- ibility	NA	Altered serotonergic signaling, †spine dynamics	NA
Transcriptional	Tbr1 HT	Impaired	Impaired	Behavioral	CTA defects, learning	Axonal projection defects in amygdala	DCS (adult) ¹⁷⁰
regulator			SIFP	Inflexibility	deficits	↓NMDAR function	clioquinol (A) ²⁶⁹
Translational regulator	Fmr1 K0 ²⁵⁵	Impaired	↓Calls	Hand flapping	PPI of startle, audiogenic seizure, learning deficits	↑mGluR function, immature protrusion	MPEP (A) ^{233,237}
					Learning impairment	PI3K signaling, ↑spine density, impaired AMPAR-mediated synaptic plasticity	5-HT and DA compound (A) ^{270,271}
					Audiogenic seizure	Hypersensitivity to ERK1/2 pathway activation, †protein synthesis	SL327 (A) ²⁷²
			↓Calls		NA	↑Fetal or early postnatal GABA and Cl ⁻ , abnormal EEG	Bumetanide, oxyto- cin (P13-15) ²⁵¹
	<i>Tsc1</i> HT, <i>Tsc1</i> Cb KO	Impaired	↑Calls	Grooming, behav- ioral inflexibility	Ataxia	Cerebellar deficits	Rapamycin (P7) ¹⁸³
	<i>Tsc2</i> HT	Impaired	↑Calls	Increased marble burying	Lethality, learning deficits	Brain enlargement, hyperactive mTOR signaling, autophagy deficiency	Rapamycin (A) ^{144,240}
	Pten cKO	Impaired	NA	NA	Learning deficits, seizure, anxiety	Macrocephaly, cellular hypertrophy, PI3K pathway hyperactivation	Rapamycin (4–6 weeks) ^{114,184}
Neuron-glia interaction, K ⁺ channel clustering	<i>Cntnap2</i> K0	Impaired	↓Calls	Grooming	Seizure, hyperactivity	↓Interneuron number, abnormal neuronal migration	Risperidone (A) ¹⁰⁹
		Impaired		Grooming	Hyperactivity	↓Oxytocin neurons	Oxytocin (P7-P21; A) ²⁴⁹
Na ⁺ channel	<i>Scn1a</i> KO	Impaired	NA	Grooming	Seizure, learning impairment	↓GABAergic interneuron firing	Clonazepam (A) ²³⁹
Synaptic adhesion molecule	<i>Nrxn1a</i> K0 ^{273,274}	Impaired, aggression in males	NA	Grooming	Anxiety, sensory-gating deficits, motor learning	↓Glutamatergic trans. and synaptic density	NA
	<i>Nlgn3</i> R451C Kl ^{275,276}	Impaired	↑Calls	NA	Enhanced learning	Context-dependent impaired glutama- tergic and GABAergic trans	NA
	NIgn3 KO ²⁷⁷	Impaired	↓Calls	Normal behav- ioral flexibility, Stereotyped motor routine	Hyperactivity	↓Brain volume, cerebellar deficit ↓GABAergic trans. in D1-MSN in NAc	NA NLGN3 expression in D1-MSN ²¹¹
	Nlgn4 KO ²⁷⁸	↓Impaired, aggression	↓Calls	Normal	NA	↓Brain volume	NA
Synaptic scaffold- ing molecule	<i>Shank2</i> exon7 KO ²⁷⁹	Impaired	↓Calls, pattern change	Grooming	Hyperactivity, anxiety	↑NMDAR function, ↑LTP	NA
	Shank2 exons 6–7	Impaired	↓Calls	Jumping	Hyperactivity, anxiety	${\downarrow} \text{NMDAR}$ function, ${\downarrow} \text{LTP}$ and LTD	CDPPB, DCS (A) ²³²
	КО						clioquinol (A) ²⁶⁹
	<i>Shank3B</i> KO ²¹⁰	Impaired	NA	Grooming	Anxiety	Striatal dysfunction	NA
	<i>Shank3</i> exons 4–9 KO ²⁸⁰	Impaired	Pattern change	Grooming	Learning deficits	↓Activity-dependent AMPAR distribu- tion and LTP	NA
	<i>Shank3</i> HT ²⁸¹	Impaired	↓Calls	NA	Motor coordination	↓Glutamatergic trans. by presynaptic mechanism, ↓LTP	IGF1 (P13–P28) ¹⁷⁷
	<i>Shank3</i> +/∆C (ref. 282)	Impaired	NA	Grooming	NA	↓NMDAR function, Rac1, PAK, cofilin signaling defects,	TAT–p-cofilin peptide (A)
		Impaired		Grooming		F-actin dysregulation in PFC	CA-Rac1 (A)

Selected mouse models of ASD organized by the molecular function of the mutated gene. Phenotypes rescued by tested therapeutic or gene re-expression strategies are in bold, whereas non-rescued phenotypes are italicized. Non-bold or non-italicized phenotypes were not tested in rescue experiments. Genetic evidence for each modeled variant in ASD, if available, can be found in the listed reference. Up and down arrows signify an increase or decrease in the measured phenotype, respectively. STFP, social transmission of food preference; CTA, conditioned taste aversion; PPI, prepulse inhibition; A, adult; DCS, p-cycloserine; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; PI3K, phosphoinositide 3-kinase; 5-HT, 5-hydroxytryptamine (secrotonin); DA, dopamine; SL327, α -[amino[(4-aminophenyl)) thio]methylene]-2-(trifluoromethyl)benzeneacetonitrile; P, postnatal day; EEG, electroencephalogram; D1-MSN, D1-dopamine receptor–expressing medium spiny neuron; NAc, nucleus accumbens; LTP, long-term potentiation; LTD, long-term depression; trans., transmission; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl) benzamide; IGF1, insulin-like growth factor 1; TAT-p-cofilin, HIV TAT domain conjugated with phospho-cofilin; CA-Rac1, constitutively active Rac1; PFC, prefrontal cortex; KO, knockout; cKO, conditional knockout; KI, knock-in; HT, heterozygous.

stem cell models suggest that abnormalities in neurogenesis, cell fate, neuronal morphogenesis and synaptic function contribute to the pathogenesis of ASD (Table 2 and Supplementary Table 3). Transcriptomic studies in these models point to dysregulation in specific molecular processes that may be driving pathogenesis, including chromatin modifications, RNA-splicing, Wnt signaling and Ca²⁺ signaling⁹⁶⁻⁹⁹. A small number of drugs, including insulin-like growth factor 1 (IGF1) and roscovitine, have been used to reverse phenotypes associated with Rett syndrome, Timothy syndrome and Phelan-McDermid syndrome (PMDS) in hiPSC models^{99–102} (Table 2). Although promising, human in vitro studies using neurons derived from stem cells have focused on syndromic ASD variants, and thus, modeling of idiopathic ASD¹⁰³ and ASD-associated de novo variants will be crucial to obtain a comprehensive picture of phenotypic overlap and potential, convergent disease mechanisms. A further challenge to in vitro studies is that it is not currently certain what the most relevant cellular and physiological ASD phenotypes are that need to be modeled in vitro.

Several molecular or cellular mechanisms of ASD pathophysiology have multiple lines of supporting evidence from studies in humans or model systems (**Table 3**). Most of these mechanisms are individually quite broad and considerable work is needed to refine these models to targetable molecular pathways. Furthermore, these mechanisms are not entirely distinct. Indeed, the same genes or molecular pathways contribute to several of these processes at different points during development (**Fig. 2**), and it is not always clear how early developmental dysfunction relates to later events. An important caveat is that we only have a limited knowledge of the specific genetic contributions to autism susceptibility, and study designs to date have presumably identified the low-hanging fruit or specific types of mutations (for example, *de novo* mutations with large effects), which may paint a skewed picture of the underlying biology. With these factors in mind we discuss the evidence for each, in turn, below.

Altered fetal cortical development. Both human genetic evidence and post-mortem studies indicate that ASD can be caused by dysregulation of fetal cortical development^{10,87–89}. Neuropathological studies, albeit with small cohorts (fewer than 36 individuals per study), have identified a number of cortical abnormalities in individuals with ASD that may be caused by errors in cortical development-including decreased neuron size, increased neuron number, ectopic cells, misoriented pyramidal neurons, irregular lamination, reduction in white matter tracks and dendritic abnormalities¹⁰ (Fig. 2). A few small studies consistently report narrower and more densely packed cortical minicolumns (a basic processing unit of cortical circuits)¹⁰⁴. Recently, patches of cortical cells lacking specific laminar markers were observed in a large proportion of ASD cases within a small cohort¹⁰⁵, although the neurodevelopmental process that this abnormality corresponds to is unknown. Furthermore, smaller brain size at birth followed by overgrowth during childhood is a widely reported phenotype for individuals with ASD¹⁰⁶, although its anatomical or cellular basis also remains undefined.

Studies in model systems have established a role for genetic mutations associated with major syndromic forms of ASD—including mutations in fragile X mental retardation 1 (*FMR1*), tuberous sclerosis 1 and 2 (*TSC1* and *TSC2*), phosphatase and tensin homolog (*PTEN*), contactin associated protein-like 2 (*CNTNAP2*) and chromodomain helicase

BOX 2 Modeling ASD with mice

Mouse models provide an experimental platform for studying ASD at multiple levels, including molecular, cellular, circuit and behavioral analyses, and offer one of the few systems in which behavioral abnormalities and reversal by potential therapeutics can be tested before translating them to humans. A variety of established assays are now considered standard assessments for ASD-related behavioral phenotypes in mouse models⁹³. The juvenile social interaction and three-chamber test, in which the time spent with a conspecific versus a novel object is measured, are widely used in both juvenile and adult mice to assess social interaction and social novelty recognition and is presumed to test behavioral correlates of social deficits in humans. The ultrasonic vocalization (USV) test measures the frequency and properties of vocal communication in multiple settings, including separation of pups from dams or adult males interacting with estrous females. Motor stereotypies, such as repetitive grooming, jumping and digging, are assessed by the marble-burying assay or home-cage behavioral analysis. Human correlates for restricted patterns of behavior and perseverance are tested by the alternating T-maze and reversal learning in the Morris water maze.

One serious challenge in neuropsychiatric disease is that the circuitry underlying social behaviors in most model systems or their human parallels are unknown. Moreover, many relevant phenotypes in humans are assessed via patient report, whereas internal states can only be inferred from outward behavior in animal models. Although these comorbidities are also observed in individuals with ASD, they confound interpretation. Sociability can be impaired by sensory dysfunction, fear and anxiety, learning deficits and abnormal locomotor activity, and similar cross-modal effects can influence repetitive behavior. Therefore, it is important to perform a full battery of behavioral tests, rather than to evaluate only core ASD-associated behaviors. Rigorous assessment of construct validity (whether a model recapitulates the genetic variant or mechanism of disease as in individuals with ASD) and convergent validity (whether application of more than one test in a given domain yields a high correlation among tasks) is critical to interpreting animal model behavioral data⁹¹.

Advantages	Limitations
 Homologs exist for most human genes. Reasonable degree of conservation of some relevant circuits (for example, cortico-striatal²⁸⁶). 	1. Evolutionary distance, which manifests in many ways, including limited knowledge of the circuitry of social behaviors and its human counterparts, as well as the inability to model poorly conserved regulatory elements.
2. Practical, genetically tractable and cost-effective. Multiple ASD models displaying core phenotypes are available.	2. Certain behavioral phenotypes in neuropsychiatric disease are internal states, as assessed by patient report or not measurable in mice (language); however, some are inferred in mouse models on the basis of outward behaviors.
3. Well-established phenotypic batteries for core ASD phenotypes.	3. Assessment of core ASD phenotypes can be confounded by common comor- bidities, including sensory dysfunction, learning deficits, locomotor dysfunction, fear and anxiety. Multiple tests are required for meaningful interpretation.
4. Uniquely accessible to multiple levels of experimentation: molecular, cellular, circuit and behavioral.	4. Invertebrate and other vertebrate models (for example, zebrafish) permit higher-throughput studies.

Advantages and limitations of mouse models

Table 2 Human in vitro models of ASD

Syndrome	Kev gene	Modeled mutation	Cell model	Brain region or cell type	Cell maturity	Cellular phenotype(s)	Suggested mechanism	Rescue or treatment
ASD ⁹⁸	CHD8	CHD8 loss of function (shRNA)	iPSC	Not defined	NPC	Dysregulated expression of genes related to neuronal development (RNA-seq)	CHD8 regulates different sets of genes associated with ASD by direct and indirect mechanisms	NA
PMDS ¹⁰²	SHANK3	22q11.3	iPSC	Forebrain neurons	3 weeks, syn- apses, electrical activity (APs, sEPSC, sIPSCs)	↓neuron production, = resting membrane potential, capacitance and APs, ↑input resistance, ↓amplitude and frequency of sEPSCs, ↓AMPA and NMDA EPSC amplitude, = IPSC ampli- tude and frequency, ↓AMPA and NMDA receptors. (WB), ↓currents from focal AMPA or NMDA application, but not GABA, ↓excitatory synapse density, ↓SHANK3 expression	Reduced SHANK3 expression leads to excitatory synapse dysfunction. IGF1 promotes synapse maturation	SHANK3 expression: sEPSC amplitude and frequency, evoked AMPA (all cells) and AMPA + NMDA (partial) EPSC; IGF1: excitatory synapse density, sEPSC amplitude and fre- quency, evoked EPSC amplitude (AMPA or NMDA), focal AMPA application (partial), focal NMDA appli- cation (full), input resistance
Rett ¹⁰⁰	MECP2	T158M, Q244X, R306C, 1155del32; <i>MECP2</i> - specific shRNA, <i>MECP2</i> expression	iPSC	Not defined	NPC–8 weeks, synapses, dendritic spines, electrical activity (APs, sEPSCs, sIPSCs)	= cell death, = GABAergic neuron number, = cell cycle progression, ↓soma size, ↓dendritic spine density, ↓synapse number, ↓activity (Ca ²⁺ imaging), ↓sEPSC and sIPSC frequency and amplitude	Dysregulation in MeCP2 expression	MECP2 expression: synapse number. IGF1: synapse number. Gentamycin: MeCP2 levels and synapse number
Timothy ⁹⁹	CACNA1C	1216G->A	iPSC	Cortical neu- rons (lower 85% /upper 15%)	NPC-45 d, electrical activity (APs)	 NPC proliferation, = neuron generation, = NPC migration, AP amplitude or threshold, resting membrane potential, input resistance or capaci- tance, wider AP at midpoint, ↑depolarization-induced Ca²⁺ rise, ↓in lower-layer neurons, ↑in upper-layer neurons, altered callosal/subcortical projection neuron production, ↑in TH⁺ neurons and catechol- amine secretion 	Decreased CACNA1C inactivation leads to Ca ²⁺ signaling dysregula- tion, dysregulated gene expression and increased catecholamine synthesis in a subpopulation of cortical neurons	Roscovitine: propor- tion of TH ⁺ neurons
Non-syndromic ¹⁰³	Unknown	NA	iPSC 3D organoid	Dorsal forebrain neurons	Rosette–50 d, synapses, electrical activity (APs, sEPSCs)	 = neuron generation, = AP threshold; upregulation of neural cell fate, axon guid- ance, synaptic and GABAergic genes, downregulation of non-neuronal genes, ↓ cell cycle length, ↑MAP2 density, ↑inhibitory synapse density, ↑GABAergic progenitor and neu- ron production 	Altered cell fate leading to increased production of GABAergic progenitors and neurons caused by FOXG1 upregulation	FOXG1-specific shRNA: DLX1, DLX2 and GAD1 expres- sion, GABAergic pro- genitor and neuron production

Selected human *in vitro* syndromic and idiopathic models of ASD point to dysregulation in gene expression, neurogenesis and cell fate, and synaptic function in ASD. Rescued phenotypes are listed under the specific treatment or genetic manipulation. Upward and downward arrows signify an increase or decrease in the measured phenotype, respectively. Equal sign signifies no change in that phenotype. iPSC, induced pluripotent stem cells; NPC neural progenitor cell; PMDS, Phelan-McDermid syndrome; AP, action potential; EPSC, excitatory postsynaptic current; IPSC, inhibitory postsynaptic current; sEPSC, spontaneous excitatory postsynaptic current; sIPSC, spontaneous inhibitory postsynaptic current; IGF1, insulin-like growth factor 1; WB, western blot. References for each model are included in the first column.

DNA binding protein 7 (*CHD7*)—in fetal brain development^{107–111}. A number of these syndromes are caused by loss-of-function variants in multiple genes that may converge in the mechanistic target of rapamycin (mTOR) pathway, which regulates cell proliferation, growth and neuronal morphogenesis (**Fig. 2**)^{112,113}. Indeed, *PTEN* mutations cause forebrain macrocephaly in both mice and humans, consistent with defects in corticogenesis^{51,114}.

Similarly, ASD-associated genetic variants are enriched in genes involved in the Wnt pathway¹¹⁵ (**Fig. 2**), a regulator of the balance between radial glia self-renewal and neuronal differentiation, as well as

dorsoventral patterning in the brain^{116,117}. In mice, modulation of the Wnt pathway results in altered cortical neuronal production and ASD-like social deficits^{118–121}. Moreover, many of the ASD-related rare *de novo* mutations that are predicted to disrupt gene function are in genes also thought to regulate or have cross-talk with canonical Wnt signaling and that are involved in chromatin modification and regulation of gene expression, such as *CHD8*, T-box brain 1 (*TBR1*), and members of the Brg1-associated factors (BAF) and mixed-lineage leukemia (MLL) complexes^{122–129} (**Fig. 2**). Recent work indicates that these genes are highly coexpressed in the human fetal brain during the period of neu-

rogenesis (4–24 weeks after conception)^{88,130} and are expressed in both neural progenitors and newly born neurons¹³¹, again implicating altered fetal cortical development, as mutations in these genes are expected to affect cortical development.

The CNS function of the majority of these chromatin-modifying genes and transcriptional regulators is mostly unknown, with the exception of the BAF complex, a multi-subunit chromatin-remodeling complex that regulates neurogenesis and neuronal morphogenesis¹²⁷. The ASD candidate gene SMARCC2, which encodes a subunit of the SWI/ SNF chromatin-remodeling complex, was shown to regulate cortical thickness by modulating neurogenesis¹³². In addition, TBR1 has a role in cortical deep layer neuron generation, and mice lacking *Tbr1* have impaired callosal and thalamocortical axon projections¹²⁸. Knockdown of CHD8 expression in human neural progenitors causes downregulation of genes governing cell adhesion, neuronal differentiation and axon guidance, the Wnt pathway and genes related to chromatin modification that are enriched in *de novo* ASD variants^{98,133}. Moreover, individuals with LGD mutations in CHD8 have macrocephaly, consistent with its reported function as a regulator of Wnt signaling, which is known to regulate brain size via regulation of neurogenesis^{80,134}.

Synaptic dysfunction. Mutations in genes encoding excitatory and inhibitory synaptic cell-adhesion molecules (including neurexins^{43,73,74,135,136} and neuroligins¹³⁷), excitatory synaptic scaffolding molecules (including the SH3 and multiple ankyrin-repeat domain (SHANK) proteins^{43,74,138,139}), the excitatory glutamatergic receptor GRIN2B^{43,44,73} and inhibitory GABAergic receptor (GABAR) subunits^{43,73,140}, and exonic deletions in the gene encoding the inhibitory synaptic scaffolding molecule gephyrin (*GPHN*)^{43,141}, are associated with ASD in multiple unbiased and targeted sequencing studies. Neurotransmitter release regulators including the synaptotagmins^{43,45,47} and synapsins^{43,142,143} also harbor mutations, but so far there is less statistical support for their involvement in ASD.

Genetic evidence for synaptic dysfunction is also supported by neuropathological studies, providing suggestive evidence of increased spine density^{144,145}, abnormalities in inhibitory function (such as reduced GABARs in the cortex and hippocampus¹⁴⁶⁻¹⁴⁹), abnormal mRNA expression of glutamate decarboxylase (GAD1 and GAD2) in the cortex and cerebellum^{150–153}, and downregulation of interneuron markers (such as parvalbumin (PVALB) and somatostatin (SST)) in post-mortem brains⁸⁹. Impaired glutamatergic and GABAergic transmission, as has been reported in several mouse models of ASD, can result in ASDlike behaviors that can be alleviated by modulators of AMPA receptor (AMPAR), NMDA receptor (NMDAR) and GABA_AR (Table 1). Similarly, human neurons derived from individuals with PMDS, a syndromic form of ASD associated with deletions of SHANK3, have deficits in excitatory (AMPAR- and NMDAR-mediated) transmission¹⁰². Together, these studies provide evidence that dysregulation in synaptogenesis and synaptic transmission¹⁵⁴ has a role in ASD (Fig. 2 and Tables 1 and 2).

The observation of defects in both glutamatergic and GABAergic synaptic function has led to the hypothesis that alterations in the excitatory/inhibitory (E/I) balance contribute to ASD. Consistent with this, mouse models with altered synaptic transmission or plasticity (activitydependent changes in synaptic strength usually related to learning and memory^{155,156}) that are outside the normal range in either direction exhibit social dysfunction. Indeed, directly increasing the E/I ratio in the medial prefrontal cortex (mPFC) of the brain, using optogenetic stimulation, led to impaired social interactions in mice¹⁵⁷.

However, E/I imbalance is a broad concept that is frequently observed in a wide variety of brain disorders including epilepsy, Alzheimer's disease and schizophrenia^{158–160}; therefore, the contribution of E/I imbalance to ASD pathophysiology requires considerable refinement. Identifying spatiotemporal dynamics of synaptic dysfunction in multiple ASD model systems may help to delineate this question—for example,

BOX 3 Human in vitro models of ASD

The need for human models of brain development and function is supported by a growing list of differences between rodents and humans^{212,287}, a poor track record in drug development⁹² and the scarcity of human tissue, especially representing the period of disease onset¹⁰. Human neural stem cells overcome the species barrier, provide a high-throughput experimental platform for drug discovery and phenotypic screening, and could potentially be used in isogenic cell-based therapies. To gain meaningful insights into disease mechanisms, the differentiation of neural progenitors into specific neuronal subtypes of sufficient maturity is necessary^{116,288}; however, cell identity and maturity have not been consistently assessed in human neural stem cell models of ASD (**Table 2** and **Supplementary Table 3**), with all but a few studies using markers to rigorously define the populations of the cells under study^{99,101–103,289,290}. Human stem cell–derived neurons are immature, approaching fetal stages of development even after prolonged *in vitro* culture, thus limiting their potential to model synaptogenesis and synaptic function^{130,291–294}. Unbiased genome-wide frameworks can be applied¹³⁰ to measure the extent to which *in vitro* models recapitulate *in vivo* development and to assess their neuroanatomical identity and maturity. Thus, this and other studies²⁹⁵ point to the validity of using neural stem cells to model early stages of human corticogenesis that are predicted to be dysregulated in ASD^{88,296}. Advances in 3D organoid culture systems, organotypic slice culture and cell engraftment into rodent models may provide avenues for studying cortical lamination, circuit connectivity and more mature stages, including synaptic function^{290,297–300}.

Advantages and limitations of human in vitro models

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Limitations

$1. \ \mbox{Genomic conservation not an issue; allows for modeling of coding and non-coding variants. }$	1. Culture variability, heterogeneity and reproducibility issues arising from multiple sources, including culture methodology and differences in lines and clones used.		
2. One of the few living systems in which human brain development can be			
studied.	2. Cultures to date produce immature fetal-like neurons, limiting their potenti		
3. High experimental tractability, including genetic manipulation, neuronal	to properly model later developmental stages.		
physiology and the potential for high-throughput assays for phenotypic screening and drug discovery.	 Neuronal migration, cortical lamination, projection patterns and circuit-leve organization are difficult to model in 2D cultures. Tissue engineering and 3D organoid cultures will enable the study of some of these phenotypes. 		
4. Neurons derived directly from affected individuals (iPSCs) can be used to			
model disease without knowing the causal or contributory genetic variants.	4. So far, mostly limited to syndromic ASD or small cohorts of idiopathic ASD.		
	5. As a fundamentally <i>in vitro</i> system, <i>in vivo</i> connectivity and external milieu are not preserved; thus findings may not precisely translate to <i>in vivo</i> biology.		

whether there is a critical period for an E/I imbalance that mediates ASD-associated behavior, or whether the E/I imbalance in ASD is circuit specific. Furthermore, an E/I imbalance may arise not only from changes in synaptic physiology but also from altered cell fate that can lead to abnormal proportions of inhibitory and excitatory cells, as evidenced by recent findings in human *in vitro* models¹⁰³ (**Table 2** and **Fig. 2**). This further highlights how early developmental abnormalities may have repercussions later on. It is also important to note that E/I imbalance studies have mainly been carried out in animal models, hence a detailed evaluation of when, where and how an E/I shift contributes to the ASD phenotypes in humans is warranted^{161–163}.

Activity-dependent transcription and translation. In neurons, gene transcription and protein translation are dynamically regulated by neuronal activity, creating spatially or contextually restricted gene expression within subcellular compartments^{164,165}. Disruptions in activitydependent transcriptional regulators or their targets are associated with ASD. These include mutations in methyl-CpG-binding protein 2 (MeCP2)¹⁶⁶ and calcium channel, voltage-dependent L-type, alpha 1C subunit (CACNA1C)¹⁶⁷; de novo mutations in the neuronal activityinduced transcription factor myocyte enhancer factor 2C (MEF2C)¹⁶⁸; abnormal imprinting and microdeletion of MEF2-regulated ubiquitinprotein ligase E3A (UBE3A) (which causes Angelman syndrome¹⁶⁹) or duplication (dup) 15q11-q13 syndrome (which also encompasses UBE3A); and de novo mutations in TBR1, whose product is required for activity-dependent Grin2b expression^{170,171}. Studies with iPSCs derived from individuals with Timothy syndrome demonstrate that CACNA1C regulates a network of genes involved in synaptic function^{96,99}. Moreover, targets of Mef2 (such as activity-regulated cytoskeletonassociated protein, Arc172, and brain-derived neurotrophic factor, BDNF¹⁷³), Mecp2 (such as BDNF¹⁷⁴) and Tbr1 (such as Grin2b¹⁷¹) have established roles in synaptic transmission and plasticity, thus providing a point of mechanistic convergence between distinct genetic etiologies of ASD (Fig. 2). IGF1 treatment rescued core ASD behaviors that are present in untreated Mecp2y/- (refs. 175,176) and Shank3+/mice¹⁷⁷, as well as synaptic defects in iPSCs derived from subjects with PMDS¹⁰², presumably via cross-talk with activity-dependent signaling pathways^{175,178} (Fig. 2).

Mutations in TSC1 and TSC2, which encode canonical components of the mTOR pathway¹¹³, support dysregulation of neuronal translation in individuals with ASD¹⁷⁹. Other ASD risk loci, including FMR1, which encodes fragile X mental retardation protein (FMRP) that also regulates neuronal translation¹⁸⁰, and dup15q11-q13, which contains the FMRP interactor and translational repressor cytoplasmic FMR1interacting protein 1 (CYFIP1)^{181,182}, also suggest convergence on neuronal translational regulation (Figs. 1 and 2). Consistent with this, mice with disruptions in mTOR signaling or translation initiation have core ASD behaviors^{144,183–187} (Table 1), possibly through the modulation of translation of neuroligin 2 (Nlgn2), neurexin 1 (Nrxn1) and Shank3 through Fmrp¹⁸⁸, a point of molecular convergence between synaptic function and translational regulation. Further, metabotropic glutamate receptor (mGluR) activation modulates FMRP-mediated translational inhibition and FMRP modulates AMPAR trafficking and mGluRmediated LTD¹⁸⁹, emphasizing the cross-talk between synaptic plasticity and translational control.

Activity-dependent transcription and translation also regulate synaptic pruning and stability^{190,191}. Increased dendritic spine density in the temporal lobe of individuals with ASD has been reported, although the cohorts have been very small^{144,145}. Mef2 and Fmr1 cooperatively modulate synapse elimination¹⁹², and Mef2 has a crucial role in mGluR5mediated synapse elimination by stimulating the expression and dendritic translation of the Mef2 target gene *Arc*¹⁹³. Spine-elimination deficits were also observed in *Tsc2*^{+/-} mice, which were restored by treatment of the mice with the mTOR inhibitor rapamycin¹⁴⁴.

Many genes known to be associated with an increased risk for ASD (which we refer to as ASD risk genes) are also predicted to be transcriptionally co-regulated by MEF2A, MEF2C and SATB homeobox 1 (SATB1), and to be translationally regulated via FMRP, further implicating activity-dependent gene regulation as a potential convergent mechanism in ASD pathogenesis⁸⁸. How changes in synapse dynamics that are under the control of ASD risk genes lead to the specific behavioral deficits observed in model systems and humans with ASD is a critical area of investigation. One can speculate that even small changes in synaptic function and timing will preferentially disrupt the connectivity of higher-order association areas that mediate social behavior, which include the frontal-parietal, frontal-temporal and frontalstriatal circuits^{11,194}. Identification of the spatiotemporal dynamics of transcriptional and translational regulation and the subsequent changes in micro- and macro-circuit connectivity will be necessary to link synaptic dysfunction to complex behavioral traits in individuals with ASD.

Altered neural circuitry. Human cognitive neuroscience and neuroimaging in ASD have been extensively reviewed elsewhere^{195–197}. Neuroimaging and neuropathology studies in humans suggest that changes occur both in resting state network activity and alterations in macro-circuit connectivity within the cortex and in cortico-striatal circuits^{198–203}. The only study using systematic imaging phenotyping in ASD mouse models highlighted the parieto-temporal lobe, the cerebellar cortex, the frontal lobe, the hypothalamus and the striatum as the most affected regions, which were shared across many of the 26 models examined²⁰⁴; however, not all mouse models showed structural phenotypes, including mice lacking *Cntnap2*, which had normal gross anatomy.

The brain circuitry underlying social behavior in mice is not yet well defined. In addition to frontal circuits, cerebellar function has also been implicated in social behavior. Cell type-specific knockout of Tsc1 in cerebellar Purkinje cells was sufficient to elicit core ASD-like behavior in mice¹⁸³, providing experimental evidence that cerebellar dysfunction can lead to ASD-like social deficits in mice. Systemic analysis of cerebellar function in five ASD mouse models has identified defects in cerebellum-dependent learning, although the functional implication of cerebellar dysfunction in core ASD behaviors remains to be identified²⁰⁵. In fact, developmental injury in cerebellar circuitry may increase ASD risk 36-fold, whereas adult injury does not lead to social dysfunction²⁰⁶, suggesting that the cerebellum may not be the direct neural correlate of social behavior, but that instead cerebellar injury during early development may lead to a cascade of long-term deficits in cerebellar-associated targets, leading to the core behavioral deficits observed in ASD. Therefore, comprehensive mapping of developmental circuit formation will be essential to finding the neural correlates of social behavior.

The amygdala is another candidate region that may be affected in ASD because of its role in modulating fear and social behavior^{202,207,208}. *Tbr1*^{+/-} mice have defective amygdala axonal projections and neuronal activation. Notably, direct infusion of D-cycloserine, a partial agonist of NMDAR, to basolateral amygdala restored social deficits of *Tbr1*^{+/-} mice¹⁷⁰, even though Tbr1 has an established role in deep-layer neuron generation and cortical lamination.

In contrast to social behavior, the neuroanatomical substrate for repetitive behavior is better understood in both mice and humans. Several lines of evidence suggest that striatal dysfunction is a neural substrate for repetitive behavior and motor routine learning in mice and in humans²⁰⁹. Mice lacking *Shank3b* showed striatal dysfunction, including striatal hypertrophy, and reduced cortico-striatal excitatory synaptic transmission along with repetitive behavior²¹⁰. Mice lacking *Nlgn3* display stereotyped motor routines that are dependent on inhibi-

tory transmission by D1-dopamine receptor–expressing medium spiny neuron (D1-MSNs) in the nucleus accumbens²¹¹.

How these mouse phenotypes relate to human circuits is not well understood, and because many of the implicated brain regions in humans, such as the frontal and temporal lobes, have undergone massive changes during primate evolution²¹², additional comparative studies, which may involve primate models in addition to mouse models, are needed to relate neuroanatomical traits to the candidate brain circuits implicated in ASD pathogenesis. Moreover, given the developmental stage–related manifestations of ASD, the temporal trajectory of neural circuitry abnormalities and the developmental disconnectivity in ASD warrants study. Recent advances in connectomics^{213,214} and optogenet-ics^{215,216} may help in delineating the functional neural correlates for core ASD-associated behaviors, thus providing therapeutic opportunities.

Brain region and mechanism	Supporting evidence	Caveats and limitations	Treatment potential	
Neocortex				
Brain overgrowth	• Multiple studies with large cohorts measur- ing brain size (MRI) and head circumference	 Small effect size (2 mm) Potential bias in measurements 	• Limited. Targeting key pathways potentially risks broad developmental problems	
	\bullet Mutations in genes controlling neurogenesis and growth (Fig. 2)	Incomplete understanding of biological mechanism. Evidence for both white and gray matter origin	• Rapamycin treatment reversed macrocephaly in a mouse model ¹⁸⁴	
Altered cortical cytoar- chitecture (neuron size,	• Cumulative evidence from >12 neuropatho- logical studies	• Small cohorts. No systematic assessment of the same brain regions and phenotypes	• Limited. Targeting key pathways potentially risks broad developmental problems	
number, positioning and/or orientation)	• Mutations in genes controlling neurogenesis, growth and neuronal migration (Fig. 2)	Multiple phenotypes; none unique to ASD		
	• Modeling in mice consistent with observed phenotypes (Table 1)			
Neuronal morphogenesis	White matter reduction in neuropathological studies. Narrow minicolumns and altered con- nectivity in cortical circuits	Small cohorts. Limited number of studies	• Limited. Targeting key pathways will prob- ably lead to broad developmental problems	
	• Mutations in genes controlling axon growth or guidance and dendrite arborization (Fig. 2)		• Rapamycin successfully used in rescue experiments in mice ^{184,240}	
Synaptogenesis	• Increased layer-specific dendritic spine den- sity in frontal (L2), parietal (L2) and temporal	 Only two studies with small cohorts^{144,145} Unclear mechanism: both increase and 	 Promising. Phenotypic reversal possible in postnatal periods 	
	 Iobes (L2, L5)^{144,145} Mutations in genes converge in pathways 	decrease in synapse density reported in mouse models (Table 1)	• IGF1 successfully used in rescue experi- ments in hiPSC ^{100,102}	
	regulating synaptogenesis (Fig. 2)Increased spines and upregulated spine		• PI3K antagonists rescue FXS-associated increased spine density in mice ²⁷¹	
	dynamics in some mouse models ^{192,193,283}			
Synaptic dysfunction E/I imbalance	• Decreased GABA receptor density and altered GAD1 and GAD2 levels. Functional	Poorly documented in neuropathological studies. Small cohorts	Promising. Phenotypic reversal possible in postnatal periods	
	imaging studies identify local hyperconnectiv- ity and decreased long-range connections	Unclear mechanism: both increase and decrease in excitatory synaptic function	• IGF1 rescues phenotypes in mouse models and hiPSC ^{100,102,177}	
	 Mutations in genes converge in pathways regulating synaptic function (Fig. 2) 	reported with and without concomitant inhibitory compensation. Multiple molecular	• Positive allosteric modulators for GABA _A receptor ²³⁹ , mGluR5 antagonists and agonists ^{230,232,233} , NMDAR partial agonist ^{170,232} , and blockers of NKCC1 cation-chloride cotransporter ²⁵¹ restored behavioral deficits in mice	
	• Mouse models support disruption in E/I balance leads to ASD phenotypes (Table 1). Increasing E/I in prefrontal cortex using optogenetics leads to social deficits ¹⁵⁷	mechanisms leading to synaptic dystunction, including altered translation, Ca ²⁺ signaling and activity-dependent transcription (Fig. 2)		
Cerebellum				
Purkinje cell (PC) loss and dysfunction	Reported decrease in PC size and number. Motor coordination problems in ASD	 Small cohorts. Limited number of studies. Gliosis observed in most 	• Promising. Postnatal cerebellar development increases therapeutic potential	
	• PC-specific ablation of ASD risk gene <i>Tsc1</i> in mice recapitulates core ASD phenotypes and PC degeneration ¹⁸³	Global gene expression profiles between cerebellums of control subjects and those with ASD very similar ⁸⁹	Rapamycin successfully used in rescue experiments in mice ¹⁸³	
	• Developmental cerebellar injury increases ASD risk ²⁰⁶	• Limited knowledge of the role of the cerebel- lum in ASD behavioral domains		
Widespread				
Neuron-glia signaling	 Reported increased microglia infiltration and astrogliosis in multiple brain regions (neuropa- thology and PET imaging) 	Small cohorts. Limited number of studies Lack of genetic evidence suggests a reactive role	 Untested, but promising Microglia- and astrocyte-specific rescue experiments in Rett mouse models rescues disease phenotypes^{284,285} 	
	Post-mortem transcriptome identifies increased microglial and immune signa- ture ^{89,223}	Limited characterization in ASD mouse models		
	• Role of microglia and astrocytes in regulat- ing synapse formation, function and pruning. Disrupted neuron-microglia signaling in mice			

Neuropathological and neuroimaging findings discussed here were recently reviewed¹⁰. See the section titled 'Neurobiological models and mechanisms of ASD' for additional references and detailed information on the genetic evidence and the function of specific genes involved in each biological process. hiPSC, human induced pluripotent stem cells; MRI, magnetic resonance imaging; PET, positron emission tomography; FXS, fragile X syndrome; IGF1, insulin-like growth factor 1; PC, Purkinje cell.

is the presence of activated microglia and astrocytosis in multiple brain regions (Fig. 2). Neuropathological and positron emission tomography (PET) imaging studies have identified microglial infiltration and activation in the frontal, prefrontal, cingulate, frontoinsular and visual cortices and in the cerebellum²¹⁷⁻²²⁰. Astrocytosis has been observed in the frontal, parietal, cingulate and temporal cortices and in the cerebellum^{220–222}. Alongside these observations, transcriptomic studies in post-mortem brains consistently find that genes enriched in activated microglia and astrocytes are upregulated in the cortex of brains from individuals with idiopathic ASD, and to a lesser extent in the cerebellum^{89,223}. No studies have identified causal genetic variants associated with ASD in microglia- or astrocyte-specific genes, which combined with transcriptomic and genetic evidence suggests that this process is most likely a reaction or a secondary process coupled to underlying synaptic dysfunction⁸⁹. However, the lack of primary genetic evidence for association with ASD does not reduce the value of these microglial or astrocyte pathways as potential therapeutic windows to explore. Notably, knockdown of chemokine (C-X3-C motif) receptor 1 (CX3CR1), which is not mutated in individuals with ASD, leads to a reduction in microglia, deficits in synaptic pruning and ASD-like behavioral and functional connectivity defects^{224,225}. Thus, synaptic dysfunction in individuals with ASD could also arise from dysregulated synaptic pruning and homeostasis that is promoted by a vicious cycle of microglial and astrocyte upregulation. Because astrocytes and microglia regulate synaptic development and pruning²²⁶⁻²²⁸, this may provide another opportunity for therapeutic development. Mouse models have not been rigorously assessed for microglial activation, but given the observations in human brain, this should be done.

Dysregulated neuron-glia signaling and neuroinflammation. Another

consistently reported observation in brains from individuals with ASD

Therapeutic strategies

Currently, the following major molecular pathways have been primarily targeted in model systems to evaluate novel therapeutic strategies. The E/I imbalance hypothesis highlights glutamatergic and GABAergic receptor modulators as potential therapeutic strategies; roscovitine, mGluR5 antagonists and agonists, NMDAR agonists and GABA_AR agonists have shown varying degrees of preclinical efficacy in mouse models, including the alleviation of social deficits or repetitive behavior (Table 1 and Supplementary Table 2)^{170,229–239}. Translational inhibition by eukaryotic translation initiation factor (eIF) 4E and eIF4G interaction inhibitor (4EGI-1) and rapamycin have been effective in alleviating behavioral and neuronal phenotypes in models with perturbations in the mTOR pathway^{144,183–186,240–242}. Transcriptional modulation through phosphatidylinositol 3-kinase (PI3K) and Ras signaling by IGF1 and BDNF also showed efficacy by rescuing physiological and behavioral abnormalities in some models, including in Mecp2-null mice175-177,243,244. Treatment with clenbuterol and fingolimod also alleviated behavioral deficits in Mecp2-null mice by increasing levels of BDNF and IGF1 (refs. 245,246). Treatment with oxytocin, a neuropeptide involved in the modulation of various aspects of social behavior^{247,248}, ameliorated ASD-like social deficits in several mouse models^{249–251} and has been implicated in improving information transfer by modulating inhibitory transmission²⁵². In addition to these genetic models, striking evidence for a role of the gut microbiome in brain development and function has been demonstrated. Oral treatment of mice with Bacteroides fragilis restored social behavioral abnormalities in maternal immune activation (MIA) models²⁵³, but the direct relevance to various forms of human ASD has not yet been established.

The lack of consistency in experimental findings between different laboratories and the genetic background effects of different mouse strains are important issues that should be addressed to increase the therapeutic utility of animal models^{254–256}. Even when mouse models suggest a potential mechanism or therapeutic avenue, caution should be exercised in translating animal studies to humans. The efficacy of mGluR5 antagonists and oxytocin, which showed promise in mouse models, is uncertain following clinical trials in humans²⁵⁷⁻²⁶⁰. It is unknown whether these failures are due to inadequate trial design and outcome measures, the lack of appropriate target engagement or simply choosing the wrong target²⁶¹. It should be noted that the outcome measurements used in clinical trials are often not the same as those in animal models. This emphasizes the importance of understanding the factors that lead to variable results. Social behavior is particularly vexing in this regard, as its environmental context and the state of the subject are so important, both in humans and in model organisms. In addition to genetic background effects, how the animals are housed and treated, when they are tested and who the examiner is can have profound effects on the outcome²⁶². The development of objective scoring systems, as well as the identification of measurable biomarkers and endophenotypes (for example, EEG, magnetic resonance imaging (MRI) scans and molecular profiles) will help in more accurate cross-species assessment of the therapeutic effects.

Conclusions and future directions

Genetic evidence frames ASD not as a single disease but as a number of etiologically distinct conditions with diverse pathophysiological mechanisms that lead to similar behavioral manifestations. This is supported by mouse models, which demonstrate that multiple mechanisms can lead to parallel outward social deficits. At the same time, the convergence of risk variants in molecular pathways and the identification of common transcriptomic signatures in brains from subjects with ASD argue for an unexpected degree of convergence at the molecular and cellular levels. Genetic findings now provide a firm causal foundation on which to understand the relationship of molecular pathways with cellular and circuit dysfunction, and ultimately with behavior. Furthermore, although little is known about the mechanisms of increased male prevalence, genetic models provide a route for identifying and testing the role of potential female protective factors, which could provide new therapeutic opportunities^{14,263}. Finally, evidence for several models based on maternal environmental factors, such as infection and the gut microbiome, are also growing²⁵³, but their definitive causal role in ASD and their relationship to genetic risk factors warrant further definition.

Work in animal models and in human cell lines supports some of the observations in post-mortem human brains and in clinical studies; yet we are far from parsimonious explanations. Microglial activation is variably observed in brains from individuals with ASD, along with a deficit in synaptic pruning. Conversely, a mouse model with a reduction in microglia displays deficits in synaptic pruning²²⁵. Understanding the role of glial cells in synaptic homeostasis and their adaptive or maladaptive roles in brains from subjects with ASD are thus important goals with significant therapeutic implications.

Interdisciplinary approaches combining genetics, functional genomics, experimental modeling and ultimately their integration into cohesive biological models, in line with those that are developing in cancer, may help drive therapeutic innovations²⁶⁴. The road ahead necessitates advances in each of these approaches. We must gauge where to put our resources for genetic discovery. Lessons from other common neuropsychiatric disorders suggest that large cohorts (>50,000 subjects) are needed to identify predicted common variants⁶. The success of identifying *de novo* variants should not occlude the efforts of identifying inherited variants. Shared resources, such as single-cell transcriptomes and expression quantitative trait loci (eQTL) studies at relevant epochs, will provide critical support to molecular pathway analysis that can bridge genetics, animal model and human studies.

Translating mechanistic understanding that is derived from model systems faces many challenges. One of them is understanding the optimal timing for treatment²⁴⁹. Model systems based on neurodevelopmental syndrome genes demonstrate the ability to reverse certain deficits in adults, providing important hope that treatment long after birth can be efficacious. Yet, given the developmental roles of many ASD risk genes, we must acknowledge that there may be critical periods for certain treatment modalities^{249,265}. Another important avenue is developing human biomarkers that are robust and, optimally, have parallels in animal models. These are challenging problems to be faced with, but they underscore the extraordinary recent progress in defining both the causes and mechanisms of ASD and a number of plausible routes toward developing more effective treatments.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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- Altshuler, D., Daly, M.J. & Lander, E.S. Genetic mapping in human disease. Science 322, 881–888 (2008).
- Gilissen, C. *et al.* Genome sequencing identifies major causes of severe intellectual disability. *Nature* **511**, 344–347 (2014).
- Lee, H. *et al.* Clinical exome sequencing for genetic identification of rare Mendelian disorders. *J. Am. Med. Assoc.* **312**, 1880–1887 (2014).
- Psychiatric GWAS Consortium Bipolar Disorder Working Group. Large-scale genomewide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat. Genet. 43, 977–983 (2011).
- Lambert, J.C. *et al.* Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat. Genet.* 45, 1452–1458 (2013).
- Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511, 421–427 (2014).
- Hindorff, L.A. *et al.* Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc. Natl. Acad. Sci. USA* 106, 9362–9367 (2009).
- Geschwind, D.H. Autism: many genes, common pathways? *Cell* **135**, 391–395 (2008).
 Geschwind, D.H. Genetics of autism spectrum disorders. *Trends Cogn. Sci.* **15**,
- 409–416 (2011).
 10. Chen, J.A., Peñagarikano, O., Belgard, T.G., Swarup, V. & Geschwind, D.H. The emerging picture of autism spectrum disorder: genetics and pathology. *Annu. Rev. Pathol.* 10, 111–144 (2015).
- Geschwind, D.H. & Levitt, P. Autism spectrum disorders: developmental disconnection syndromes. *Curr. Opin. Neurobiol.* **17**, 103–111 (2007).
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5* 5th edn. (American Psychiatric Association, Arlington, Viginia, USA, 2013).
- Autism and Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators. Prevalence of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2010. MMWR Surveill. Summ. 63, 1–21 (2014).
- Werling, D.M. & Geschwind, D.H. Understanding sex bias in autism spectrum disorder. Proc. Natl. Acad. Sci. USA 110, 4868–4869 (2013).
- Robinson, E.B., Lichtenstein, P., Anckarsäter, H., Happé, F. & Ronald, A. Examining and interpreting the female protective effect against autistic behavior. *Proc. Natl. Acad. Sci. USA* 110, 5258–5262 (2013).
- 16. Geschwind, D.H. Advances in autism. Annu. Rev. Med. 60, 367–380 (2009).
- Volkmar, F.R. & McPartland, J.C. From Kanner to DSM-5: autism as an evolving diagnostic concept. Annu. Rev. Clin. Psychol. 10, 193–212 (2014).
- Geschwind, D.H. & State, M.W. Gene hunting in autism spectrum disorder: on the path to precision medicine. *Lancet Neurol.* 14, 1109–1120 (2015).
- Rogers, S.J. *et al.* Autism treatment in the first year of life: a pilot study of infant start, a parent-implemented intervention for symptomatic infants. *J. Autism Dev. Disord.* 44, 2981–2995 (2014).

- Kasari, C., Shire, S., Factor, R. & McCracken, C. Psychosocial treatments for individuals with autism spectrum disorder across the lifespan: new developments and underlying mechanisms. *Curr. Psychiatry Rep.* 16, 512 (2014).
- Jones, W. & Klin, A. Attention to eyes is present but in decline in 2- to 6-month-old infants later diagnosed with autism. *Nature* 504, 427–431 (2013).
- Jeste, S.S., Frohlich, J. & Loo, S.K. Electrophysiological biomarkers of diagnosis and outcome in neurodevelopmental disorders. *Curr. Opin. Neurol.* 28, 110–116 (2015).
- Zafeiriou, D.I., Ververi, A., Dafoulis, V., Kalyva, E. & Vargiami, E. Autism spectrum disorders: the quest for genetic syndromes. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 162B, 327–366 (2013).
- 24. Miles, J.H. Autism spectrum disorders—a genetics review. *Genet. Med.* **13**, 278–294 (2011).
- Betancur, C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. *Brain Res.* 1380, 42–77 (2011).
- Gillberg, C. & Coleman, M. The Biology of the Autistic Syndromes (Mac Keith Press, London, 1992).
- Folstein, S. & Rutter, M. Genetic influences and infantile autism. *Nature* 265, 726–728 (1977).
- Hallmayer, J. *et al.* Genetic heritability and shared environmental factors among twin pairs with autism. *Arch. Gen. Psychiatry* 69, 1095–1102 (2011).
- 29. Sandin, S. et al. The familial risk of autism. J. Am. Med. Assoc. 311, 1770–1777 (2014).
- Bailey, A. et al. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol. Med. 25, 63–77 (1995).
- Lichtenstein, P., Carlström, E., Råstam, M., Gillberg, C. & Anckarsäter, H. The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. *Am. J. Psychiatry* 167, 1357–1363 (2010).
- Ronald, A. & Hoekstra, R.A. Autism spectrum disorders and autistic traits: a decade of new twin studies. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 156B, 255–274 (2011).
- Sebat, J. et al. Strong association of de novo copy-number mutations with autism. Science 316, 445–449 (2007).
- Szatmari, P. et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat. Genet. 39, 319–328 (2007).
- Marshall, C.R. *et al.* Structural variation of chromosomes in autism spectrum disorder. *Am. J. Hum. Genet.* 82, 477–488 (2008).
- Bucan, M. *et al.* Genome-wide analyses of exonic copy-number variants in a familybased study point to novel autism susceptibility genes. *PLoS Genet.* 5, e1000536 (2009).
- Glessner, J.T. *et al.* Autism genome-wide copy-number variation reveals ubiquitin and neuronal genes. *Nature* **459**, 569–573 (2009).
- Itsara, A. et al. De novo rates and selection of large copy-number variation. Genome Res. 20, 1469–1481 (2010).
- Pinto, D. *et al.* Functional impact of global, rare copy-number variation in autism spectrum disorders. *Nature* 466, 368–372 (2010).
- Levy, D. et al. Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron 70, 886–897 (2011).
- Griswold, A.J. *et al.* Evaluation of copy-number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. *Hum. Mol. Genet.* 21, 3513–3523 (2012).
- 42. Neale, B.M. *et al.* Patterns and rates of exonic *de novo* mutations in autism spectrum disorders. *Nature* **485**, 242–245 (2012).
- De Rubeis, S. et al. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515, 209–215 (2014).
- Iossifov, I. *et al.* The contribution of *de novo* coding mutations to autism spectrum disorder. *Nature* 515, 216–221 (2014).
- lossifov, I. *et al. De novo* gene disruptions in children on the autistic spectrum. *Neuron* 74, 285–299 (2012).
- O'Roak, B.J. et al. Sporadic autism exomes reveal a highly interconnected protein network of *de novo* mutations. *Nature* 485, 246–250 (2012).
- Sanders, S.J. et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485, 237–241 (2012).
- Gilman, S.R. *et al.* Rare *de novo* variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. *Neuron* 70, 898–907 (2011).
- Sanders, S.J. *et al.* Multiple recurrent *de novo* CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. *Neuron* 70, 863–885 (2011).
- Gratten, J., Visscher, P.M., Mowry, B.J. & Wray, N.R. Interpreting the role of *de novo* protein-coding mutations in neuropsychiatric disease. *Nat. Genet.* 45, 234–238 (2013).
- O'Roak, B.J. et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science 338, 1619–1622 (2012).
- Morrow, E.M. *et al.* Identifying autism loci and genes by tracing recent shared ancestry. *Science* **321**, 218–223 (2008).
- Strauss, K.A. *et al.* Recessive symptomatic focal epilepsy and mutant contactinassociated protein-like 2. *N. Engl. J. Med.* 354, 1370–1377 (2006).
- Lim, E.T. *et al.* Rare complete knockouts in humans: population distribution and significant role in autism spectrum disorders. *Neuron* 77, 235–242 (2013).
- 55. Yu, T.W. *et al.* Using whole-exome sequencing to identify inherited causes of autism. *Neuron* **77**, 259–273 (2013).
- Lee, S.H. *et al.* Estimating the proportion of variation in susceptibility to schizophrenia, captured by common SNPs. *Nat. Genet.* 44, 247–250 (2012).

- Gaugler, T. et al. Most genetic risk for autism resides with common variation. Nat. Genet. 46, 881–885 (2014).
- Bulik-Sullivan, B.K. et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat. Genet. 47, 291–295 (2015).
- Wray, N.R. & Visscher, P.M. Quantitative genetics of disease traits. J. Anim. Breed. Genet. 132, 198–203 (2015).
- O'Roak, B.J. et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nat. Genet. 43, 585–589 (2011).
- Schaaf, C.P. *et al.* Oligogenic heterozygosity in individuals with high-functioning autism spectrum disorders. *Hum. Mol. Genet.* 20, 3366–3375 (2011).
- Zhao, X. et al. A unified genetic theory for sporadic and inherited autism. Proc. Natl. Acad. Sci. USA 104, 12831–12836 (2007).
- Ronemus, M., Iossifov, I., Levy, D. & Wigler, M. The role of *de novo* mutations in the genetics of autism spectrum disorders. *Nat. Rev. Genet.* 15, 133–141 (2014).
- Virkud, Y.V., Todd, R.D., Abbacchi, A.M., Zhang, Y. & Constantino, J.N. Familial aggregation of quantitative autistic traits in multiplex versus simplex autism. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **150B**, 328–334 (2009).
- Lyall, K. *et al.* Parental social responsiveness and risk of autism spectrum disorder in offspring. *JAMA Psychiatry* 71, 936–942 (2014).
- Lowe, J.K., Werling, D.M., Constantino, J.N., Cantor, R.M. & Geschwind, D.H. Social responsiveness, an autism endophenotype: genome-wide significant linkage to two regions on chromosome 8. *Am. J. Psychiatry* **172**, 266–275 (2015).
- Lee, S.H. *et al.* Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat. Genet.* 45, 984–994 (2013).
- Klei, L. *et al.* Common genetic variants, acting additively, are a major source of risk for autism. *Mol. Autism* 3, 9 (2012).
- Maier, R. *et al.* Joint analysis of psychiatric disorders increases accuracy of risk prediction for schizophrenia, bipolar disorder and major depressive disorder. *Am. J. Hum. Genet.* **96**, 283–294 (2015).
- Anney, R. *et al.* Individual common variants exert weak effects on the risk for autism spectrum disorders. *Hum. Mol. Genet.* 21, 4781–4792 (2012).
- Gratten, J., Wray, N.R., Keller, M.C. & Visscher, P.M. Large-scale genomics unveils the genetic architecture of psychiatric disorders. *Nat. Neurosci.* 17, 782–790 (2014).
- Geschwind, D.H. & Flint, J. Genetics and genomics of psychiatric disease. *Science* 349, 1489–1494 (2015).
- Krumm, N. *et al.* Excess of rare, inherited truncating mutations in autism. *Nat. Genet.* 47, 582–588 (2015).
- Pinto, D. *et al.* Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. *Am. J. Hum. Genet.* 94, 677–694 (2014).
- Vinkhuyzen, A.A., Wray, N.R., Yang, J., Goddard, M.E. & Visscher, P.M. Estimation and partition of heritability in human populations using whole-genome analysis methods. *Annu. Rev. Genet.* 47, 75–95 (2013).
- Sham, P.C. & Purcell, S.M. Statistical power and significance testing in large-scale genetic studies. *Nat. Rev. Genet.* 15, 335–346 (2014).
- Stessman, H.A., Bernier, R. & Eichler, E.E. A genotype-first approach to defining the subtypes of a complex disease. *Cell* 156, 872–877 (2014).
- Ji, J. et al. DYRK1A haploinsufficiency causes a new recognizable syndrome with microcephaly, intellectual disability, speech impairment and distinct facies. Eur. J. Hum. Genet. 23, 1473–1481 (2015).
- Bronicki, L.M. *et al.* Ten new cases further delineate the syndromic intellectual disability phenotype caused by mutations in *DYRK1A. Eur. J. Hum. Genet.* 23, 1482–1487 (2015).
- Bernier, R. *et al.* Disruptive *CHD8* mutations define a subtype of autism early in development. *Cell* **158**, 263–276 (2014).
- Jablensky, A. Schizophrenia or schizophrenias? The challenge of genetic parsing of a complex disorder. Am. J. Psychiatry 172, 105–107 (2015).
- Skafidas, E. *et al.* Predicting the diagnosis of autism spectrum disorder using gene pathway analysis. *Mol. Psychiatry* **19**, 504–510 (2014).
- Arnedo, J. *et al.* Uncovering the hidden risk architecture of the schizophrenias: confirmation in three independent genome-wide association studies. *Am. J. Psychiatry* 172, 139–153 (2015).
- 84. Robinson, E.B. *et al.* Response to 'Predicting the diagnosis of autism spectrum disorder using gene pathway analysis'. *Mol. Psychiatry* **19**, 859–861 (2014).
- Belgard, T.G., Jankovic, I., Lowe, J.K. & Geschwind, D.H. Population structure confounds autism genetic classifier. *Mol. Psychiatry* 19, 405–407 (2014).
- Noh, H.J. *et al.* Network topologies and convergent etiologies arising from deletions and duplications observed in individuals with autism. *PLoS Genet.* 9, e1003523 (2013).
- Willsey, A.J. et al. Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. Cell 155, 997–1007 (2013).
- Parikshak, N.N. *et al.* Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. *Cell* **155**, 1008–1021 (2013).
- Voineagu, İ. *et al.* Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474, 380–384 (2011).
- Abrahams, B.S. & Geschwind, D.H. Advances in autism genetics: on the threshold of a new neurobiology. *Nat. Rev. Genet.* 9, 341–355 (2008).
- Pankevich, D.E., Wizemann, T.M. & Altevogt, B.M. Improving the Utility and Translation of Animal Models for Nervous System Disorders: Workshop Summary (The National Academies Press, Washington, D.C., 2013).
- Dolmetsch, R. & Geschwind, D.H. The human brain in a dish: the promise of iPSCderived neurons. *Cell* 145, 831–834 (2011).
- Silverman, J.L., Yang, M., Lord, C. & Crawley, J.N. Behavioral phenotyping assays for mouse models of autism. *Nat. Rev. Neurosci.* 11, 490–502 (2010).

- Watson, K.K. & Platt, M.L. Of mice and monkeys: using nonhuman primate models to bridge mouse- and human-based investigations of autism spectrum disorders. *J. Neurodev. Disord.* 4, 21 (2012).
- McCammon, J.M. & Sive, H. Addressing the genetics of human mental health disorders in model organisms. Annu. Rev. Genomics Hum. Genet. 16, 173–197 (2015).
- 96. Tian, Y. *et al.* Alteration in basal and depolarization-induced transcriptional network in iPSC-derived neurons from Timothy syndrome. *Genome Med.* **6**, 75 (2014).
- Fogel, B.L. *et al. RBFOX1* regulates both splicing and transcriptional networks in human neuronal development. *Hum. Mol. Genet.* 21, 4171–4186 (2012).
- Sugathan, A. *et al. CHD8* regulates neurodevelopmental pathways associated with autism spectrum disorder in neural progenitors. *Proc. Natl. Acad. Sci. USA* 111, E4468–E4477 (2014).
- Paşca, S.P. et al. Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat. Med. 17, 1657–1662 (2011).
- Marchetto, M.C. *et al.* A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* **143**, 527–539 (2010).
- Li, Y. et al. Global transcriptional and translational repression in human embryonic stem cell-derived Rett syndrome neurons. Cell Stem Cell 13, 446–458 (2013).
- Shcheglovitov, A. *et al.* SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. *Nature* **503**, 267–271 (2013).
- Mariani, J. *et al. FOXG1*-dependent dysregulation of GABA-glutamate neuron differentiation in autism spectrum disorders. *Cell* **162**, 375–390 (2015).
- Casanova, M.F. et al. Minicolumnar abnormalities in autism. Acta Neuropathol. 112, 287–303 (2006).
- Stoner, R. *et al.* Patches of disorganization in the neocortex of children with autism. *N. Engl. J. Med.* **370**, 1209–1219 (2014).
- Redcay, E. & Courchesne, E. When is the brain enlarged in autism? A meta-analysis of all brain size reports. *Biol. Psychiatry* 58, 1–9 (2005).
- Magri, L. *et al.* Sustained activation of mTOR pathway in embryonic neural stem cells leads to development of tuberous sclerosis complex–associated lesions. *Cell Stem Cell* 9, 447–462 (2011).
- La Fata, G. *et al.* FMRP regulates multipolar to bipolar transition affecting neuronal migration and cortical circuitry. *Nat. Neurosci.* **17**, 1693–1700 (2014).
- Peñagarikano, O. *et al.* Absence of *Cntnap2* leads to epilepsy, neuronal migration abnormalities and core autism-related deficits. *Cell* 147, 235–246 (2011).
- Feng, W. et al. The chromatin remodeler CHD7 regulates adult neurogenesis via activation of SoxC transcription factors. Cell Stem Cell 13, 62–72 (2013).
- Zhou, J. *et al. Tsc1*-mutant neural stem and progenitor cells exhibit migration deficits and give rise to subependymal lesions in the lateral ventricle. *Genes Dev.* 25, 1595–1600 (2011).
- Sarbassov, D.D., Ali, S.M. & Sabatini, D.M. Growing roles for the mTOR pathway. *Curr. Opin. Cell Biol.* 17, 596–603 (2005).
- 113. Lipton, J.O. & Sahin, M. The neurology of mTOR. Neuron 84, 275-291 (2014).
- Kwon, C.H. *et al. Pten* regulates neuronal arborization and social interaction in mice. *Neuron* 50, 377–388 (2006).
- Krumm, N., O'Roak, B.J., Shendure, J. & Eichler, E.E. A *de novo* convergence of autism genetics and molecular neuroscience. *Trends Neurosci.* 37, 95–105 (2014).
 Hansen, D.V., Rubenstein, J.L. & Kriegstein, A.R. Deriving excitatory neurons of the
- neocortex from pluripotent stem cells. *Neuron* **70**, 645–660 (2011).
- Tiberi, L., Vanderhaeghen, P. & van den Ameele, J. Cortical neurogenesis and morphogens: diversity of cues, sources and functions. *Curr. Opin. Cell Biol.* 24, 269–276 (2012).
- Lijam, N. *et al.* Social interaction and sensorimotor-gating abnormalities in mice lacking *Dvl1. Cell* **90**, 895–905 (1997).
- Sowers, L.P. et al. Disruption of the noncanonical Wnt gene PRICKLE2 leads to autism-like behaviors with evidence for hippocampal synaptic dysfunction. *Mol.* Psychiatry 18, 1077–1089 (2013).
- Fang, W.Q. et al. Overproduction of upper-layer neurons in the neocortex leads to autism-like features in mice. Cell Reports 9, 1635–1643 (2014).
- Long, J.M., LaPorte, P., Paylor, R. & Wynshaw-Boris, A. Expanded characterization of the social-interaction abnormalities in mice lacking *Dvl1. Genes Brain Behav.* 3, 51–62 (2004).
- Bedogni, F. *et al. Tbr1* regulates regional and laminar identity of postmitotic neurons in developing neocortex. *Proc. Natl. Acad. Sci. USA* **107**, 13129–13134 (2010).
- 123. Sakamoto, I. *et al.* A novel β -catenin–binding protein inhibits β -catenin–dependent Tcf activation and axis formation. *J. Biol. Chem.* **275**, 32871–32878 (2000).
- Schuettengruber, B., Martinez, A.M., Iovino, N. & Cavalli, G. Trithorax group proteins: switching genes on and keeping them active. *Nat. Rev. Mol. Cell Biol.* 12, 799–814 (2011).
- 125. Nozawa, R.S. *et al.* Human POGZ modulates dissociation of HP1-α from mitotic chromosome arms through Aurora B activation. *Nat. Cell Biol.* **12**, 719–727 (2010).
- Chen, T. & Dent, S.Y. Chromatin modifiers and remodelers: regulators of cellular differentiation. *Nat. Rev. Genet.* 15, 93–106 (2014).
- Ronan, J.L., Wu, W. & Crabtree, G.R. From neural development to cognition: unexpected roles for chromatin. *Nat. Rev. Genet.* 14, 347–359 (2013).
- Hevner, R.F. *et al.* Tbr1 regulates differentiation of the preplate and layer 6. *Neuron* 29, 353–366 (2001).
- Sierra, J., Yoshida, T., Joazeiro, C.A. & Jones, K.A. The APC tumor suppressor counteracts β-catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev.* 20, 586–600 (2006).
- Stein, J.L. *et al.* A quantitative framework to evaluate modeling of cortical development by neural stem cells. *Neuron* 83, 69–86 (2014).

- 131. Miller, J.A. *et al.* Transcriptional landscape of the prenatal human brain. *Nature* **508**, 199–206 (2014).
- Tuoc, T.C. *et al.* Chromatin regulation by BAF170 controls cerebral cortical size and thickness. *Dev. Cell* 25, 256–269 (2013).
- Cotney, J. *et al.* The autism-associated chromatin modifier CHD8 regulates other autism risk genes during human neurodevelopment. *Nat. Commun.* 6, 6404 (2015).
- Nishiyama, M., Skoultchi, A.I. & Nakayama, K.I. Histone H1 recruitment by CHD8 is essential for suppression of the Wnt-β-catenin signaling pathway. *Mol. Cell. Biol.* 32, 501–512 (2012).
- Vaags, A.K. et al. Rare deletions at the neurexin 3 locus in autism spectrum disorder. Am. J. Hum. Genet. 90, 133–141 (2012).
- 136. Gauthier, J. *et al.* Truncating mutations in *NRXN2* and *NRXN1* in autism spectrum disorders and schizophrenia. *Hum. Genet.* **130**, 563–573 (2011).
- Jamain, S. *et al.* Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat. Genet.* 34, 27–29 (2003).
- 138. Berkel, S. *et al.* Mutations in the *SHANK2* synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nat. Genet.* **42**, 489–491 (2010).
- Durand, C.M. *et al.* Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat. Genet.* 39, 25–27 (2007).
- Piton, A. *et al.* Analysis of the effects of rare variants on splicing identifies alterations in GABA_A receptor genes in autism spectrum disorder individuals. *Eur. J. Hum. Genet.* 21, 749–756 (2013).
- Lionel, A.C. *et al.* Rare exonic deletions implicate the synaptic organizer gephyrin (*GPHN*) in risk for autism, schizophrenia and seizures. *Hum. Mol. Genet.* 22, 2055–2066 (2013).
- 142. Fassio, A. *et al. SYN1* loss-of-function mutations in autism and partial epilepsy cause impaired synaptic function. *Hum. Mol. Genet.* **20**, 2297–2307 (2011).
- Corradi, A. *et al. SYN2* is an autism predisposing gene: loss-of-function mutations alter synaptic vesicle cycling and axon outgrowth. *Hum. Mol. Genet.* 23, 90–103 (2014).
- Tang, G. *et al.* Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron* 83, 1131–1143 (2014).
- Hutsler, J.J. & Zhang, H. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Res.* 1309, 83–94 (2010).
- Blatt, G.J. *et al.* Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. *J. Autism Dev. Disord.* **31**, 537–543 (2001).
- Oblak, A., Gibbs, T.T. & Blatt, G.J. Decreased GABA_A receptors and benzodiazepinebinding sites in the anterior cingulate cortex in autism. *Autism Res.* 2, 205–219 (2009).
- Oblak, A.L., Gibbs, T.T. & Blatt, G.J. Decreased GABA_B receptors in the cingulate cortex and fusiform gruss in autism. *J. Neurochem.* **114**, 1414–1423 (2010).
- 149. Fatemi, S.H. *et al.* mRNA and protein levels for GABA_A-α4, -α5, -β1 and GABA_BR1 receptors are altered in brains from subjects with autism. *J. Autism Dev. Disord.* **40**, 743–750 (2010).
- Yip, J., Soghomonian, J.J. & Blatt, G.J. Increased GAD67 mRNA expression in cerebellar interneurons in autism: implications for Purkinje cell dysfunction. *J. Neurosci. Res.* 86, 525–530 (2008).
- 151. Yip, J., Soghomonian, J.J. & Blatt, G.J. Decreased GAD65 mRNA levels in select subpopulations of neurons in the cerebellar dentate nuclei in autism: an *in situ* hybridization study. *Autism Res.* 2, 50–59 (2009).
- Yip, J., Soghomonian, J.J. & Blatt, G.J. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. *Acta Neuropathol.* 113, 559–568 (2007).
- 153. Fatemi, S.H. *et al.* Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biol. Psychiatry* 52, 805–810 (2002).
- 154. Südhof, T.C. Neuroligins and neurexins link synaptic function to cognitive disease. *Nature* **455**, 903–911 (2008).
- Citri, A. & Malenka, R.C. Synaptic plasticity: multiple forms, functions and mechanisms. *Neuropsychopharmacology* 33, 18–41 (2008).
- Mayford, M., Siegelbaum, S.A. & Kandel, E.R. Synapses and memory storage. Cold Spring Harb. Perspect. Biol. 4, a005751 (2012).
- Yizhar, O. *et al.* Neocortical excitation-inhibition balance in information processing and social dysfunction. *Nature* 477, 171–178 (2011).
- Palop, J.J. *et al.* Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* 55, 697–711 (2007).
- Fritschy, J.M. Epilepsy, E/I balance and GABA_A receptor plasticity. *Front. Mol. Neurosci.* 1, 5 (2008).
- Kehrer, C., Maziashvili, N., Dugladze, T. & Gloveli, T. Altered excitatory-inhibitory balance in the NMDA-hypofunction model of schizophrenia. *Front. Mol. Neurosci.* 1, 6 (2008).
- van de Lagemaat, L.N. et al. Age-related decreased inhibitory versus excitatory gene expression in the adult autistic brain. Front. Neurosci. 8, 394 (2014).
- Rojas, D.C., Singel, D., Steinmetz, S., Hepburn, S. & Brown, M.S. Decreased left perisylvian GABA concentration in children with autism and unaffected siblings. *Neuroimage* 86, 28–34 (2014).
- 163. Bejjani, A. *et al.* Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by ¹H MRS and ¹H MRSI. *PLoS One* 7, e38786 (2012).
- Greer, P.L. & Greenberg, M.E. From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. *Neuron* 59, 846– 860 (2008).

- Buffington, S.A., Huang, W. & Costa-Mattioli, M. Translational control in synaptic plasticity and cognitive dysfunction. *Annu. Rev. Neurosci.* 37, 17–38 (2014).
- 166. Amir, R.E. *et al.* Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG–binding protein 2. *Nat. Genet.* 23, 185–188 (1999).
- 167. Splawski, I. et al. Cav1.2 calcium channel dysfunction causes a multisystem disorder, including arrhythmia and autism. Cell 119, 19–31 (2004).
- Ebert, D.H. & Greenberg, M.E. Activity-dependent neuronal signaling and autism spectrum disorder. *Nature* 493, 327–337 (2013).
- Nicholls, R.D. & Knepper, J.L. Genome organization, function and imprinting in Prader-Willi and Angelman syndromes. *Annu. Rev. Genomics Hum. Genet.* 2, 153– 175 (2001).
- Huang, T.N. *et al. Tbr1* haploinsufficiency impairs amygdalar axonal projections and results in cognitive abnormality. *Nat. Neurosci.* 17, 240–247 (2014).
- 171. Chuang, H.C., Huang, T.N. & Hsueh, Y.P. Neuronal excitation upregulates *Tbr1*, a high-confidence risk gene of autism, mediating *Grin2b* expression in the adult brain. *Front. Cell. Neurosci.* 8, 280 (2014).
- Flavell, S.W. *et al.* Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science* **311**, 1008–1012 (2006).
- Chen, W.G. *et al.* Derepression of *BDNF* transcription involves calcium-dependent phosphorylation of MeCP2. *Science* **302**, 885–889 (2003).
- Zhou, Z. *et al.* Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth and spine maturation. Neuron 52, 255–269 (2006).
- 175. Castro, J. *et al.* Functional recovery with recombinant human IGF1 treatment in a mouse model of Rett Syndrome. *Proc. Natl. Acad. Sci. USA* **111**, 9941–9946 (2014).
- Tropea, D. *et al.* Partial reversal of Rett syndrome–like symptoms in MeCP2-mutant mice. *Proc. Natl. Acad. Sci. USA* **106**, 2029–2034 (2009).
- Bozdagi, O., Tavassoli, T. & Buxbaum, J.D. Insulin-like growth factor 1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay. *Mol. Autism* 4, 9 (2013).
- Subramaniam, S. *et al.* Insulin-like growth factor 1 inhibits extracellular signalregulated kinase to promote neuronal survival via the phosphatidylinositol 3-kinaseprotein kinase A-c-Raf pathway. *J. Neurosci.* 25, 2838–2852 (2005).
- Ehninger, D. & Silva, A.J. Rapamycin for treating tuberous sclerosis and autism spectrum disorders. *Trends Mol. Med.* 17, 78–87 (2011).
- Bassell, G.J. & Warren, S.T. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron* 60, 201–214 (2008).
- Napoli, I. *et al.* The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell* 134, 1042–1054 (2008).
- Nishimura, Y. *et al.* Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. *Hum. Mol. Genet.* 16, 1682–1698 (2007).
- Tsai, P.T. *et al.* Autistic-like behavior and cerebellar dysfunction in Purkinje cell *Tsc1*-mutant mice. *Nature* 488, 647–651 (2012).
- Zhou, J. *et al.* Pharmacological inhibition of mTORC1 suppresses anatomical, cellular and behavioral abnormalities in neural-specific *Pten*-knockout mice. *J. Neurosci.* 29, 1773–1783 (2009).
- Gkogkas, C.G. *et al.* Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature* 493, 371–377 (2013).
- Santini, E. *et al.* Exaggerated translation causes synaptic and behavioral aberrations associated with autism. *Nature* **493**, 411–415 (2013).
- Berg, J.M. *et al.* JAKMIP1, a novel regulator of neuronal translation, modulates synaptic function and autistic-like behaviors in mouse. *Neuron* 88, 1173–1191 (2015).
- Darnell, J.C. et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146, 247–261 (2011).
- Huber, K.M., Gallagher, S.M., Warren, S.T. & Bear, M.F. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl. Acad. Sci. USA* 99, 7746–7750 (2002).
- Cohen, S. & Greenberg, M.E. Communication between the synapse and the nucleus in neuronal development, plasticity and disease. *Annu. Rev. Cell Dev. Biol.* 24, 183–209 (2008).
- Hua, J.Y. & Smith, S.J. Neural activity and the dynamics of central nervous system development. *Nat. Neurosci.* 7, 327–332 (2004).
- 192. Tsai, N.P. et al. Multiple autism-linked genes mediate synapse elimination via proteasomal degradation of a synaptic scaffold PSD-95. Cell 151, 1581–1594 (2012).
- Wilkerson, J.R. et al. A role for dendritic mGluR5-mediated local translation of Arc (Arg 3.1) in MEF2-dependent synapse elimination. Cell Rep. 7, 1589–1600 (2014).
- Abrahams, B.S. & Geschwind, D.H. Connecting genes to brain in the autism spectrum disorders. Arch. Neurol. 67, 395–399 (2010).
- 195. Yerys, B.E. & Herrington, J.D. Multimodal imaging in autism: an early review of comprehensive neural circuit characterization. *Curr. Psychiatry Rep.* 16, 496 (2014).
- Luckhardt, C., Jarczok, T.A. & Bender, S. Elucidating the neurophysiological underpinnings of autism spectrum disorder: new developments. *J. Neural Transm.* 121, 1129–1144 (2014).
- Ecker, C., Bookheimer, S.Y. & Murphy, D.G. Neuroimaging in autism spectrum disorder: brain structure and function across the lifespan. *Lancet Neurol.* 14, 1121–1134 (2015).
- Minshew, N.J. & Williams, D.L. The new neurobiology of autism: cortex, connectivity and neuronal organization. Arch. Neurol. 64, 945–950 (2007).

- Zikopoulos, B. & Barbas, H. Changes in prefrontal axons may disrupt the network in autism. J. Neurosci. 30, 14595–14609 (2010).
- McPartland, J.C., Coffman, M. & Pelphrey, K.A. Recent advances in understanding the neural bases of autism spectrum disorder. *Curr. Opin. Pediatr.* 23, 628–632 (2011).
- Rudie, J.D. *et al.* Autism-associated promoter variant in *MET* impacts functional and structural brain networks. *Neuron* **75**, 904–915 (2012).
- Amaral, D.G., Schumann, C.M. & Nordahl, C.W. Neuroanatomy of autism. *Trends Neurosci.* 31, 137–145 (2008).
- Vissers, M.E., Cohen, M.X. & Geurts, H.M. Brain connectivity and high functioning autism: a promising path of research that needs refined models, methodological convergence and stronger behavioral links. *Neurosci. Biobehav. Rev.* 36, 604–625 (2012).
- Ellegood, J. et al. Clustering autism: using neuroanatomical differences in 26 mouse models to gain insight into the heterogeneity. Mol. Psychiatry 20, 118–125 (2015).
- Kloth, A.D. et al. Cerebellar associative sensory learning defects in five mouse autism models. eLife 4, e06085 (2015).
- Wang, S.S., Kloth, A.D. & Badura, A. The cerebellum, sensitive periods and autism. *Neuron* 83, 518–532 (2014).
- Amaral, D.G. The amygdala, social behavior and danger detection. Ann. NY Acad. Sci. 1000, 337–347 (2003).
- Baron-Cohen, S. et al. The amygdala theory of autism. Neurosci. Biobehav. Rev. 24, 355–364 (2000).
- Langen, M., Durston, S., Kas, M.J., van Engeland, H. & Staal, W.G. The neurobiology of repetitive behavior: ...and men. *Neurosci. Biobehav. Rev.* 35, 356–365 (2011).
- Peça, J. et al. Shank3-mutant mice display autistic-like behaviors and striatal dysfunction. Nature 472, 437–442 (2011).
- Rothwell, P.E. et al. Autism-associated neuroligin–3 mutations commonly impair striatal circuits to boost repetitive behaviors. Cell 158, 198–212 (2014).
- Geschwind, D.H. & Rakic, P. Cortical evolution: judge the brain by its cover. *Neuron* 80, 633–647 (2013).
- Kasthuri, N. *et al.* Saturated reconstruction of a volume of neocortex. *Cell* 162, 648–661 (2015).
- 214. Zingg, B. et al. Neural networks of the mouse neocortex. Cell **156**, 1096–1111 (2014).
- Gunaydin, L.A. *et al.* Natural neural projection dynamics underlying social behavior. *Cell* 157, 1535–1551 (2014).
- Marlin, B.J., Mitre, M., D'amour, J.A., Chao, M.V. & Froemke, R.C. Oxytocin enables maternal behavior by balancing cortical inhibition. *Nature* 520, 499–504 (2015).
- Morgan, J.T. *et al.* Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biol. Psychiatry* 68, 368–376 (2010).
 Suzuki, K. *et al.* Microglial activation in young adults with autism spectrum disorder.
- JAMA Psychiatry **70**, 49–58 (2013). 219. Tetreault, N.A. *et al.* Microglia in the cerebral cortex in autism. J. Autism Dev. Disord.
- 42, 2569–2584 (2012). 220. Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W. & Pardo, C.A.
- 220. vargas, D.L., Nascimbere, C., Krisman, C., Zimmerman, A.W. & Pardo, C.A. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann. Neurol.* 57, 67–81 (2005).
- Laurence, J.A. & Fatemi, S.H. Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. *Cerebellum* 4, 206–210 (2005).
- Lopez-Hurtado, E. & Prieto, J. A microscopic study of language-related cortex in autism. Am. J. Biochem. Biotechnol. 4, 130–145 (2008).
- Gupta, S. *et al.* Transcriptome analysis reveals dysregulation of innate immune response genes and neuronal activity–dependent genes in autism. *Nat. Commun.* 5, 5748 (2014).
- 224. Paolicelli, R.C. *et al.* Synaptic pruning by microglia is necessary for normal brain development. *Science* **333**, 1456–1458 (2011).
- Zhan, Y. *et al.* Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nat. Neurosci.* **17**, 400–406 (2014).
- Chung, W.S. *et al.* Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature* **504**, 394–400 (2013).
- Clarke, L.E. & Barres, B.A. Emerging roles of astrocytes in neural circuit development. Nat. Rev. Neurosci. 14, 311–321 (2013).
- Schafer, D.P. et al. Microglia sculpt postnatal neural circuits in an activity- and complement-dependent manner. Neuron 74, 691–705 (2012).
- 229. de Vrij, F.M. *et al.* Rescue of behavioral phenotype and neuronal protrusion morphology in *Fmr1* KO mice. *Neurobiol. Dis.* **31**, 127–132 (2008).
- Silverman, J.L., Tolu, S.S., Barkan, C.L. & Crawley, J.N. Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. *Neuropsychopharmacology* **35**, 976–989 (2010).
- Tian, D. et al. Contribution of mGluR5 to pathophysiology in a mouse model of human chromosome 16p11.2 microdeletion. Nat. Neurosci. 18, 182–184 (2015).
- Won, H. et al. Autistic-like social behavior in Shank2-mutant mice improved by restoring NMDA receptor function. Nature 486, 261–265 (2012).
- Yan, Q.J., Rammal, M., Tranfaglia, M. & Bauchwitz, R.P. Suppression of two major fragile X syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology* 49, 1053–1066 (2005).
- Benson, A.D., Burket, J.A. & Deutsch, S.I. Balb/c mice treated with D-cycloserine arouse increased social interest in conspecifics. *Brain Res. Bull.* 99, 95–99 (2013).
- Blundell, J. et al. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J. Neurosci. 30, 2115–2129 (2010).

- Burket, J.A., Benson, A.D., Tang, A.H. & Deutsch, S.I. D-cycloserine improves sociability in the BTBR T⁺ Itpr3tf/J mouse model of autism spectrum disorders with altered Ras-Raf-ERK1/2 signaling. *Brain Res. Bull.* **96**, 62–70 (2013).
- 237. Auerbach, B.D., Osterweil, E.K. & Bear, M.F. Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature* **480**, 63–68 (2011).
- Han, S., Tai, C., Jones, C.J., Scheuer, T. & Catterall, W.A. Enhancement of inhibitory neurotransmission by GABA_A receptors having a2,3-subunits ameliorates behavioral deficits in a mouse model of autism. *Neuron* 81, 1282–1289 (2014).
- Han, S. *et al.* Autistic-like behaviour in *Scn1a^{+/-}* mice and rescue by enhanced GABA-mediated neurotransmission. *Nature* **489**, 385–390 (2012).
- Ehninger, D. et al. Reversal of learning deficits in a Tsc2^{+/-} mouse model of tuberous sclerosis. Nat. Med. 14, 843–848 (2008).
- Oguro-Ando, A. et al. Increased CYFIP1 dosage alters cellular and dendritic morphology and dysregulates mTOR. Mol. Psychiatry 20, 1069–1078 (2015).
- Ru, W., Peng, Y., Zhong, L. & Tang, S.J. A role of the mammalian target of rapamycin (mTOR) in glutamate-induced downregulation of tuberous sclerosis complex proteins 2 (TSC2). *J. Mol. Neurosci.* **47**, 340–345 (2012).
- Chang, Q., Khare, G., Dani, V., Nelson, S. & Jaenisch, R. The disease progression of *Mecp2*-mutant mice is affected by the level of *BDNF* expression. *Neuron* 49, 341–348 (2006).
- Kline, D.D., Ogier, M., Kunze, D.L. & Katz, D.M. Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in *Mecp2*-null mice. *J. Neurosci.* 30, 5303–5310 (2010).
- Mellios, N. *et al.* b2-adrenergic receptor agonist ameliorates phenotypes and corrects microRNA-mediated IGF1 deficits in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. USA* 111, 9947–9952 (2014).
- Deogracias, R. *et al.* Fingolimod, a sphingosine-1 phosphate receptor modulator, increases BDNF levels and improves symptoms of a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. USA* **109**, 14230–14235 (2012).
- Ross, H.E. & Young, L.J. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front. Neuroendocrinol.* **30**, 534–547 (2009).
- Insel, T.R. The challenge of translation in social neuroscience: a review of oxytocin, vasopressin and affiliative behavior. *Neuron* 65, 768–779 (2010).
- Peñagarikano, O. *et al.* Exogenous and evoked oxytocin restores social behavior in the *Cntnap2* mouse model of autism. *Sci. Transl. Med.* 7, 271ra8 (2015).
- Sala, M. et al. Pharmacologic rescue of impaired cognitive flexibility, social deficits, increased aggression and seizure susceptibility in oxytocin receptor–null mice: a neurobehavioral model of autism. *Biol. Psychiatry* 69, 875–882 (2011).
- Tyzio, R. et al. Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. Science 343, 675–679 (2014).
- Owen, S.F. *et al.* Oxytocin enhances hippocampal spike transmission by modulating fast-spiking interneurons. *Nature* 500, 458–462 (2013).
- Hsiao, E.Y. et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155, 1451–1463 (2013).
- Ey, E. *et al.* Absence of deficits in social behaviors and ultrasonic vocalizations in later generations of mice lacking neuroligin 4-like. *Genes Brain Behav.* **11**, 928–941 (2012).
- Bernardet, M. & Crusio, W.E. *Fmr1* KO mice as a possible model of autistic features. *ScientificWorldJournal* 6, 1164–1176 (2006).
- Chadman, K.K. et al. Minimal aberrant behavioral phenotypes of neuroligin-3^{R451C} knockin mice. Autism Res. 1, 147–158 (2008).
- Jacquemont, S. *et al.* Epigenetic modification of the *FMR1* gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci. Transl. Med.* 3, 64ra1 (2011).
- Tachibana, M. *et al.* Long-term administration of intranasal oxytocin is a safe and promising therapy for early adolescent boys with autism spectrum disorders. *J. Child Adolesc. Psychopharmacol.* 23, 123–127 (2013).
- Dadds, M.R. et al. Nasal oxytocin for social deficits in childhood autism: a randomized controlled trial. J. Autism Dev. Disord. 44, 521–531 (2014).
- Guastella, A.J. *et al.* The effects of a course of intranasal oxytocin on social behaviors in youth diagnosed with autism spectrum disorders: a randomized controlled trial. *J. Child Psychol. Psychiatry* 56, 444–452 (2015).
- Jeste, S.S. & Geschwind, D.H. Clinical trials for neurodevelopmental disorders: at a therapeutic frontier. *Sci. Transl. Med.* 8, 321fs1 (2016).
- Bailey, K.R., Rustay, N.R. & Crawley, J.N. Behavioral phenotyping of transgenic and knockout mice: practical concerns and potential pitfalls. *ILAR J.* 47, 124–131 (2006).
- Werling, D.M., Lowe, J.K., Luo, R., Cantor, R.M. & Geschwind, D.H. Replication of linkage at chromosome 20p13 and identification of suggestive sex-differential risk loci for autism spectrum disorder. *Mol. Autism* 5, 13 (2014).
- Cloughesy, T.F., Cavenee, W.K. & Mischel, P.S. Glioblastoma: from molecular pathology to targeted treatment. Annu. Rev. Pathol. 9, 1–25 (2014).
- Werker, J.F. & Hensch, T.K. Critical periods in speech perception: new directions. Annu. Rev. Psychol. 66, 173–196 (2015).
- Falconer, D.S. The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Ann. Hum. Genet.* 29, 51–76 (1965).
- Isshiki, M. *et al.* Enhanced synapse remodeling as a common phenotype in mouse models of autism. *Nat. Commun.* 5, 4742 (2014).
- Nakatani, J. et al. Abnormal behavior in a chromosome-engineered mouse model for human 15q11–13 duplication seen in autism. Cell 137, 1235–1246 (2009).
- Lee, E.J. *et al.* Trans-synaptic zinc mobilization improves social interaction in two mouse models of autism through NMDAR activation. *Nat. Commun.* 6, 7168 (2015).

- Lim, C.S. *et al.* Pharmacological rescue of Ras signaling, GluA1-dependent synaptic plasticity and learning deficits in a fragile X model. *Genes Dev.* 28, 273–289 (2014).
- 271. Gross, C. *et al.* Selective role of the catalytic PI3K subunit p110-β in impaired higher-order cognition in fragile X syndrome. *Cell Reports* 11, 681–688 (2015).
- 272. Osterweil, E.K., Krueger, D.D., Reinhold, K. & Bear, M.F. Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. *J. Neurosci.* **30**, 15616–15627 (2010).
- 273. Etherton, M.R., Blaiss, C.A., Powell, C.M. & Südhof, T.C. Mouse neurexin I alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. *Proc. Natl. Acad. Sci. USA* **106**, 17998–18003 (2009).
- 274. Grayton, H.M., Missler, M., Collier, D.A. & Fernandes, C. Altered social behaviors in neurexin-1α–knockout mice resemble core symptoms in neurodevelopmental disorders. *PLoS One* **8**, e67114 (2013).
- Tabuchi, K. *et al.* A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science* **318**, 71–76 (2007).
- Etherton, M. *et al.* Autism-linked neuroligin-3^{R451C} mutation differentially alters hippocampal and cortical synaptic function. *Proc. Natl. Acad. Sci. USA* **108**, 13764– 13769 (2011).
- 277. Baudouin, S.J. *et al.* Shared synaptic pathophysiology in syndromic and nonsyndromic rodent models of autism. *Science* **338**, 128–132 (2012).
- Jamain, S. *et al.* Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proc. Natl. Acad. Sci. USA* **105**, 1710–1715 (2008).
- Schmeisser, M.J. *et al.* Autistic-like behaviors and hyperactivity in mice lacking ProSAP1 (Shank2). *Nature* **486**, 256–260 (2012).
- Wang, X. et al. Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. Hum. Mol. Genet. 20, 3093–3108 (2011).
- Bozdagi, O. *et al.* Haploinsufficiency of the autism-associated *Shank3* gene leads to deficits in synaptic function, social interaction and social communication. *Mol. Autism* 1, 15 (2010).
- Duffney, L.J. *et al.* Autism-like deficits in *Shank3*-deficient mice are rescued by targeting actin regulators. *Cell Rep.* **11**, 1400–1413 (2015).
- Gdalyahu, A. *et al.* The autism-related protein contactin-associated protein-like 2 (CNTNAP2) stabilizes new spines: an *in vivo* mouse study. *PLoS One* **10**, e0125633 (2015).
- Derecki, N.C. *et al.* Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109 (2012).

- Lioy, D.T. *et al.* A role for glia in the progression of Rett's syndrome. *Nature* 475, 497–500 (2011).
- Langen, M., Kas, M.J., Staal, W.G., van Engeland, H. & Durston, S. The neurobiology of repetitive behavior: of mice.... *Neurosci. Biobehav. Rev.* 35, 345–355 (2011).
- Lui, J.H. *et al.* Radial glia require PDGFD–PDGFR-β signaling in human but not mouse neocortex. *Nature* 515, 264–268 (2014).
- Merkle, F.T. & Eggan, K. Modeling human disease with pluripotent stem cells: from genome association to function. *Cell Stem Cell* 12, 656–668 (2013).
- Griesi-Oliveira, K. *et al.* Modeling nonsyndromic autism and the impact of *TRPC6* disruption in human neurons. *Mol. Psychiatry* 20, 1350–1365 (2015).
- Ricciardi, S. *et al.* CDKL5 ensures excitatory synapse stability by reinforcing NGL-1– PSD95 interaction in the postsynaptic compartment and is impaired in patient iPSC-derived neurons. *Nat. Cell Biol.* 14, 911–923 (2012).
- Kadoshima, T. *et al.* Self-organization of axial polarity, inside-out layer pattern and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc. Natl. Acad. Sci. USA* 110, 20284–20289 (2013).
- Lepski, G. et al. Delayed functional maturation of human neuronal progenitor cells in vitro. Mol. Cell. Neurosci. 47, 36–44 (2011).
- 293. Mariani, J. *et al.* Modeling human cortical development *in vitro* using induced pluripotent stem cells. *Proc. Natl. Acad. Sci. USA* **109**, 12770–12775 (2012).
- Nicholas, C.R. *et al.* Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. *Cell Stem Cell* 12, 573–586 (2013).
- 295. van de Leemput, J. et al. CORTECON: a temporal transcriptome analysis of in vitro human cerebral cortex development from human embryonic stem cells. Neuron 83, 51–68 (2014).
- Ben-David, E. & Shifman, S. Combined analysis of exome sequencing points toward a major role for transcription regulation during brain development in autism. *Mol. Psychiatry* 18, 1054–1056 (2013).
- Pasca, A.M. *et al.* Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat. Methods* **12**, 671–678 (2015).
- Espuny-Camacho, I. *et al.* Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits *in vivo. Neuron* 77, 440–456 (2013).
- 299. Hansen, D.V., Lui, J.H., Parker, P.R. & Kriegstein, A.R. Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* **464**, 554–561 (2010).
- Lancaster, M.A. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379 (2013).