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Advancing the understanding of autism disease mechanisms through genetics

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Abstract

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

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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 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 USA[13]. 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 epilepsy[16]. 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–hyper-activity 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 FDA-approved 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 and stratify patients for treatment at this point. We also do not have any clear biomarkers, although tracking eye movements[21] and electroencephalogram (EEG) parameters[22] 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).

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.

Family and twin studies show that ASD is highly heritable[27–32], 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 protein- disrupting 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* mutations[62]. 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 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 siblings[73]. However, all pathogenic CNVs considered as a group have not yet shown evidence for enriched maternal transmission to probands[74].

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 low[50]. However, familial risk implies a strong inherited genetic component, consistent with the polygenic model described above[29]. 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 variation[57] (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 families[76] (Box 1).

With regards to nosology, highly penetrant variants may be very useful for defining subtypes of ASD[77]. 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 subtypes[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 phenotypes[93] (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, hyper-activity 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 models[92]. 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].

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 models[93]. 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 data[91].

Advantages and limitations of mouse models

	Advantages		Limitations
1	Homologs exist for most human genes. Reasonable degree of conservation of some relevant circuits (for example, cortico-striatal[286]).	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 comorbidities, 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.

In parallel, advances in stem cell biology in the past decade make it possible to generate and study human neurons and their development[92]. 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 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 Ca^{2+} signaling[96–99]. 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 ASD[103] 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*.

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 development[92] and the scarcity of human tissue, especially representing the period of disease onset[10]. 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 applied[130] 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 studies[295] 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

	Advantages		Limitations
1	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 potential to properly model later developmental stages.
3	High experimental tractability, including genetic manipulation, neuronal physiology and the potential for high-throughput assays for phenotypic screening and drug discovery.	3	Neuronal migration, cortical lamination, projection patterns and circuit-level 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 *in vitro* system, *in vivo* connectivity

Advantages and limitations of human *in vitro* models

Advantages	Limitations
	and external milieu are not preserved; thus findings may not precisely translate to *in vivo* biology.

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 abnormalities[10] (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 cohort[105], 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 ASD[106], 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 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 fore-brain 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 pathway[115] (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 neurogenesis (4–24 weeks after conception)[88,130] and are expressed in both neural progenitors and newly born neurons[131], 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 morphogenesis[127]. The ASD candidate gene *SMARCC2*, which encodes a subunit of the SWI/SNF chromatin-remodeling complex, was shown to regulate cortical thickness by modulating neurogenesis[132]. In addition, TBR1 has a role in cortical deep layer neuron generation, and mice lacking *Tbr1* have impaired callosal and thalamocortical axon projections[128]. 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 neuroligins[137]), 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 hippocampus[146–149]), 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 brains[89]. Impaired glutamatergic and

GABAergic transmission, as has been reported in several mouse models of ASD, can result in ASD-like 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) transmission[102]. Together, these studies provide evidence that dysregulation in synaptogenesis and synaptic transmission[154] 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 (activity-dependent 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 mice[157].

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, 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* models[103] (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 activity- dependent 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 activity-induced transcription factor myocyte enhancer factor 2C (*MEF2C*)[168]; abnormal imprinting and microdeletion of MEF2-regulated ubiquitin-protein ligase E3A (*UBE3A*) (which causes Angelman syndrome[169]) 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 cytoskeleton- associated protein,

Arc[172], and brain-derived neurotrophic factor, BDNF[173]), Mecp2 (such as BDNF[174]) and Tbr1 (such as Grin2b[171]) 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 *Mecp2*$^{y/-}$ (refs. 175,176) and *Shank3*$^{+/-}$ mice[177], as well as synaptic defects in iPSCs derived from subjects with PMDS[102], 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 pathway[113], support dysregulation of neuronal translation in individuals with ASD[179]. Other ASD risk loci, including *FMR1*, which encodes fragile X mental retardation protein (FMRP) that also regulates neuronal translation[180], and dup15q11-q13, which contains the FMRP interactor and translational repressor cytoplasmic FMR1-interacting 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 Fmrp[188], 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 mGluR- mediated LTD[189], 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 elimination[192], and Mef2 has a crucial role in mGluR5-mediated 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 rapamycin[144].

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 pathogenesis[88]. 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 frontal-striatal 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 corticostriatal 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 examined[204]; 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 mice[183], 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 identified[205]. In fact, developmental injury in cerebellar circuitry may increase ASD risk 36-fold, whereas adult injury does not lead to social dysfunction[206], 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*$^{+/-}$ mice[170], 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 humans[209]. Mice lacking *Shank3b* showed striatal dysfunction, including striatal hypertrophy, and reduced corticostriatal excitatory synaptic transmission along with repetitive behavior[210]. Mice lacking *Nlgn3* display stereotyped motor routines that are dependent on inhibitory transmission by D1-dopamine receptor–expressing medium spiny neuron (D1-MSNs) in the nucleus accumbens[211].

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 evolution[212], 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 optogenetics[215,216] may help in delineating the functional neural correlates for core ASD-associated behaviors, thus providing therapeutic opportunities.

Dysregulated neuron-glia signaling and neuroinflammation

Another consistently reported observation in brains from individuals with ASD 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 cerebellum[217–220]. 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 dysfunction[89]. 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 pruning[226–228], 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.

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 mice[175–177,243,244]. 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 transmission[252]. 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) models[253], 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 humans[257–260]. 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 target[261]. 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 outcome[262]. 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

growing[253], 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 pruning[225]. 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 innovations[264]. 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 variants[6]. 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 treatment[249]. 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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Supplementary Material

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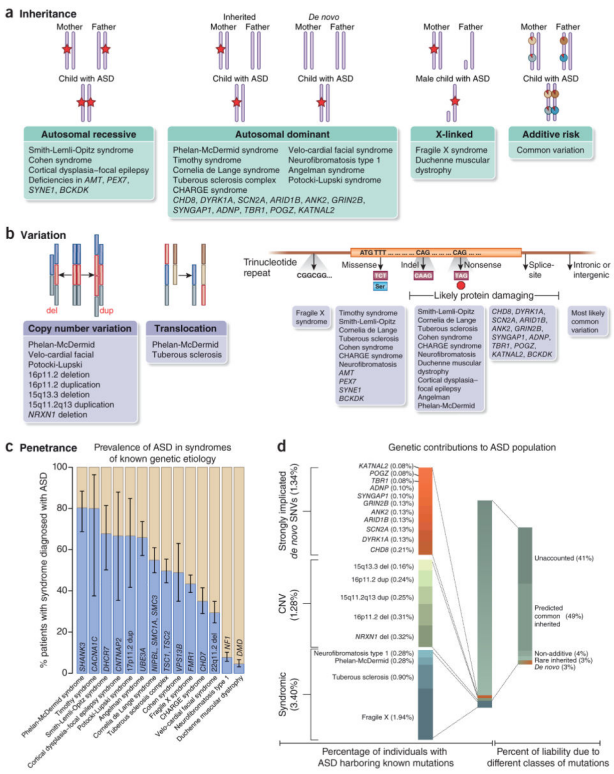

Figure 1.

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 disease[266]. Notably, percentage variance in liability is directly dependent on the frequency of the variant and the effect size 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.

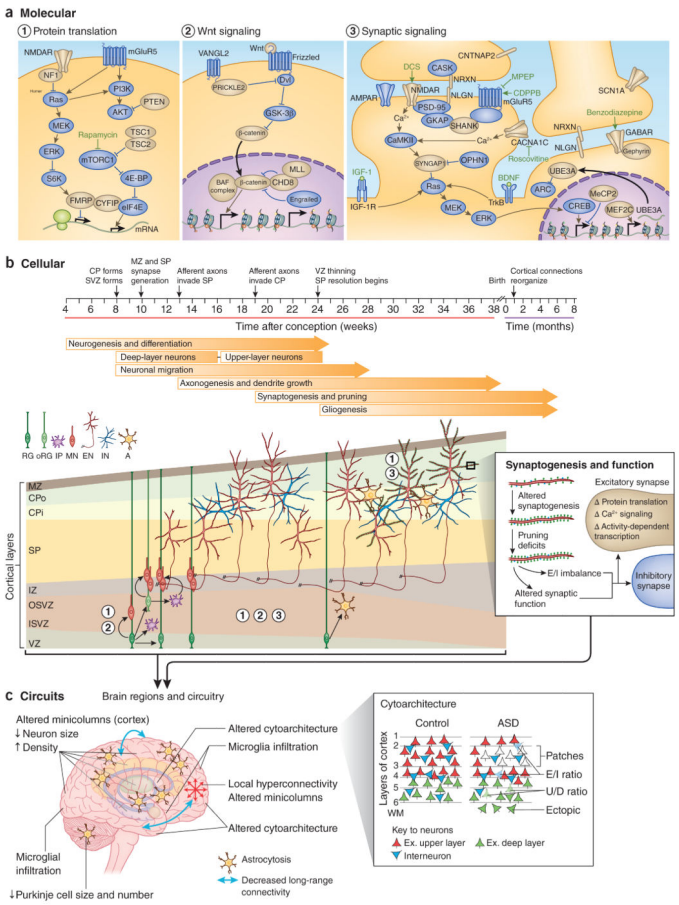

Figure 2.

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 ASD[18] (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 pathology[10] 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 or deep-layer neuron. MPEP, 2-methyl-6-(phenylethynyl)-pyridine; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; DCS, D-cycloserine; IGF1, insulin-like growth factor 1. VZ, ventricular zone; ISVZ, inner subventricular zone; OSVZ, outer subventricular zone; IZ, intermediate zone; SP, subplate; CPi, inner cortical plate; CPo, outer cortical plate; MZ, marginal zone.

Table 1

Mouse models of ASD

Molecular function	Mouse model	Social interaction	Social communi-cation	Repetitive behavior	Other symptoms	Molecular, cellular and circuit phenotypes	Treatment
Multiple	dup 15q11-q13 (refs. 267,268)	Impaired	↓Calls	Behavioral inflexibility	NA	Altered serotonergic signaling, ↑spine dynamics	NA
Transcriptional regulator	*Tbr1* HT	Impaired	Impaired STFP	Behavioral inflexibility	CTA defects, learning deficits	Axonal projection defects in amygdala ↓NMDAR function	DCS (adult)[170] clioquinol (A)[269]
Translational regulator	*Fmr1* KO[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, oxytocin (P13-15)[251]
	Tsc1 HT, *Tsc1*Cb KO	**Impaired**	↑Calls	Grooming, **behavioral inflexibility**	Ataxia	**Cerebellar deficits**	Rapamycin (P7)[183]
	Tsc2 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	*Cntnap2* KO	*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	*Scn1a* KO	**Impaired**	NA	Grooming	Seizure, **learning impairment**	**↓GABAergic interneuron firing**	Clonazepam (A)[239]
Synaptic adhesion molecule	*Nrxn1a* KO[273,274]	Impaired, ↑ aggression in males	NA	Grooming	Anxiety, sensory-gating deficits, motor learning	↓Glutamatergic trans. and synaptic density	NA
	Nlgn3 R451C KI[275,276]	Impaired	↑Calls	NA	Enhanced learning	Context-dependent impaired glutamatergic and GABAergic trans	NA
	Nlgn3 KO[277]	Impaired	↓Calls	Normal behavioral flexibility, **Stereotyped motor routine**	Hyperactivity	↓Brain volume, cerebellar deficit	NA
						↓GABAergic trans. in D1-MSN in NAc	NLGN3 expression in D1-MSN[211]
	Nlgn4 KO[278]	↓Impaired, aggression	↓Calls	Normal	NA	↓Brain volume	NA

Nat Med. Author manuscript; available in PMC 2016 October 20.

Molecular function	Mouse model	Social interaction	Social communi-cation	Repetitive behavior	Other symptoms	Molecular, cellular and circuit phenotypes	Treatment
Synaptic scaffolding molecule	*Shank2* exon7 KO[279]	Impaired	↓Calls, pattern change	Grooming	Hyperactivity, anxiety	↑NMDAR function, ↑LTP	NA
	Shank2 exons 6–7 KO	**Impaired**	↓Calls	*Jumping*	*Hyperactivity, anxiety*	**↓NMDAR function, ↓LTP and LTD**	CDPPB, DCS (A)[232] clioquinol (A)[269]
	Shank3B KO[210]	Impaired	NA	Grooming	Anxiety	Striatal dysfunction	NA
	Shank3 exons 4–9 KO[280]	Impaired	Pattern change	Grooming	Learning deficits	↓Activity-dependent AMPAR distribution and LTP	NA
	Shank3 HT[281]	Impaired	↓Calls	NA	**Motor coordination**	**↓Glutamatergic trans. by presynaptic mechanism**, ↓LTP	IGF1 (P13–P28)[177]
	$Shank3^{+/\Delta C}$ (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, D-cycloserine; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; PI3K, phosphoinositide 3-kinase; 5-HT, 5-hydroxytryptamine (serotonin); 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.

Nat Med. Author manuscript; available in PMC 2016 October 20.

Table 2

Human *in vitro* models of ASD

Syndrome	Key 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, synapses, 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 amplitude 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 frequency, evoked EPSC amplitude (AMPA or NMDA), focal AMPA application (partial), focal NMDA application (full), input resistance
Rett[100]	*MECP2*	T158M, Q244X, R306C, 1155del32; *MECP2*-specific shRNA, *MECP2* 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 neurons (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 capacitance, wider AP at midpoint, ↑depolarization-induced Ca^{2+} rise, ↓in	Decreased CACNA1C inactivation leads to Ca^{2+} signaling dysregulation, dysregulated gene expression and increased catecholamine synthesis in a subpopulation of cortical neurons	Roscovitine: proportion of TH^{+} neurons

Syndrome	Key gene	Modeled mutation	Cell model	Brain region or cell type	Cell maturity	Cellular phenotype(s)	Suggested mechanism	Rescue or treatment
						lower-layer neurons, ↑in upper-layer neurons, altered callosal/subcortical projection neuron production, ↑in TH^+ neurons and catecholamine secretion		
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 guidance, synaptic and GABAergic genes, downregulation of non-neuronal genes, ↓ cell cycle length, ↑MAP2 density, ↑inhibitory synapse density, ↑GABAergic progenitor and neuron production	Altered cell fate leading to increased production of GABAergic progenitors and neurons caused by FOXG1 upregulation	*FOXG1*-specific shRNA: *DLX1*, *DLX2* and *GAD1* expression, GABAergic progenitor 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.

Table 3

Evidence for distinct neurobiological mechanisms in ASD

Brain region and mechanism	Supporting evidence	Caveats and limitations	Treatment potential
Neocortex			
Brain overgrowth	• Multiple studies with large cohorts measuring brain size (MRI) and head circumference • Mutations in genes controlling neurogenesis and growth (Fig. 2)	• Small effect size (2 mm) • Potential bias in measurements • Incomplete understanding of biological mechanism. Evidence for both white and gray matter origin	• Limited. Targeting key pathways potentially risks broad developmental problems • Rapamycin treatment reversed macrocephaly in a mouse model[184]
Altered cortical cytoarchitecture (neuron size, number, positioning and/or orientation)	• Cumulative evidence from >12 neuropathological studies • Mutations in genes controlling neurogenesis, growth and neuronal migration (Fig. 2) • Modeling in mice consistent with observed phenotypes (Table 1)	• Small cohorts. No systematic assessment of the same brain regions and phenotypes • Multiple phenotypes; none unique to ASD	• Limited. Targeting key pathways potentially risks broad developmental problems
Neuronal morphogenesis	• White matter reduction in neuropathological studies. Narrow minicolumns and altered connectivity in cortical circuits • Mutations in genes controlling axon growth or guidance and dendrite arborization (Fig. 2)	• Small cohorts. Limited number of studies	• Limited. Targeting key pathways will probably lead to broad developmental problems • Rapamycin successfully used in rescue experiments in mice[184,240]
Synaptogenesis	• Increased layer-specific dendritic spine density in frontal (L2), parietal (L2) and temporal lobes (L2, L5)[144,145] • Mutations in genes converge in pathways regulating synaptogenesis (Fig. 2)	• Only two studies with small cohorts[144,145] • Unclear mechanism: both increase and decrease in synapse density reported in mouse models (Table 1)	• Promising. Phenotypic reversal possible in postnatal periods • IGF1 successfully used in rescue experiments in hiPSC[100,102] • PI3K antagonists rescue FXS-associated

Brain region and mechanism	Supporting evidence	Caveats and limitations	Treatment potential
	• Increased spines and upregulated spine dynamics in some mouse models[192,193,283]		increased spine density in mice[271]
Synaptic dysfunction E/I imbalance	• Decreased GABA receptor density and altered GAD1 and GAD2 levels. Functional imaging studies identify local hyperconnectivity and decreased long-range connections • Mutations in genes converge in pathways regulating synaptic function (Fig. 2) • 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]	• Poorly documented in neuropathological studies. Small cohorts • Unclear mechanism: both increase and decrease in excitatory synaptic function reported with and without concomitant inhibitory compensation. Multiple molecular mechanisms leading to synaptic dysfunction, including altered translation, Ca^{2+} signaling and activity-dependent transcription (Fig. 2)	• Promising. Phenotypic reversal possible in postnatal periods • IGF1 rescues phenotypes in mouse models and hiPSC[100,102,177] • 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
Cerebellum			
Purkinje cell (PC) loss and dysfunction	• Reported decrease in PC size and number. Motor coordination problems in ASD • PC-specific ablation of ASD risk gene *Tsc1* in mice recapitulates core ASD phenotypes and PC degeneration[183] • Developmental cerebellar injury increases ASD risk[206]	• Small cohorts. Limited number of studies. Gliosis observed in most • Global gene expression profiles between cerebellums of control subjects and those with ASD very similar[89] • Limited knowledge of the role of the cerebellum in ASD behavioral domains	• Promising. Postnatal cerebellar development increases therapeutic potential • Rapamycin successfully used in rescue experiments in mice[183]
Widespread			
Neuron-glia signaling	• Reported increased microglia infiltration and astrogliosis in multiple brain regions (neuropathology	• 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

Brain region and mechanism	Supporting evidence	Caveats and limitations	Treatment potential
	and PET imaging) • Post-mortem transcriptome identifies increased microglial and immune signature[89,223] • Role of microglia and astrocytes in regulating synapse formation, function and pruning. Disrupted neuron-microglia signaling in mice leads to social deficits[225]	• Limited characterization in ASD mouse models	models rescues disease phenotypes[284,285]

Neuropathological and neuroimaging findings discussed here were recently reviewed[10]. 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.