REVIEW SUMMARY

NEURODEVELOPMENT

Genes, circuits, and precision therapies for autism and related neurodevelopmental disorders

Mustafa Sahin* and Mriganka Sur*

BACKGROUND: Neurodevelopmental disorders are caused by abnormalities in the developing brain. Such abnormalities can occur as a result of germline or somatic mutations or because of epigenetic or environmental factors. These disorders affect a large number of children in the developed world, as well as the developing world. The societal cost of neurodevelopmental disorders is immense, making the pursuit of treatments for individuals with neurodevelopmental disorders a top unmet medical need.

ADVANCES: Research in the genetics of neurodevelopmental disorders such as autism suggests that several hundred genes are likely involved as risk factors for these disorders. This heterogeneity presents both a challenge and an opportunity for researchers. Although the exact identity of many of the genes remains to be discovered, functional analysis of genes underlying several single-gene disorders has yielded considerable progress. Most genes identified to date appear to encode proteins that serve certain conserved pathways: protein synthesis, transcriptional or epigenetic regulation, and synaptic signaling. Genetic syndromes such as fragile X syndrome, Rett syndrome, and tuberous sclerosis complex provide insights into the molecular pathways commonly affected in autism spectrum disorder (ASD). Understanding the basic biology of these diseases has led to mechanism-based treatment designs.

These genetic disorders, once thought to be irreversible, are now the subject of trailblazing new clinical trials for neurodevelopmental disorders. On the basis of research in genetic mouse models, it is hypothesized that different genetic disorders will respond to different therapies, such as mammalian target of rapamycin inhibitors (tuberous sclerosis and PTEN hamartoma tumor syndrome), metabotropic glutamate receptor 5 antagonists (fragile X and 16p11.2 deletion), and insulin-like growth factor 1 (Rett and Phelan-McDermid syndromes). It is not yet clear whether such trials will result in approval of the drugs for these specific conditions. Subsets of nonsyndromic autism patients may also benefit from one of these therapies, but further investigation will be required to provide the tools and methods to stratify the individuals with nonsyndromic autism into treatment groups.

A remaining hurdle is the lack of precise understanding about the brain regions and neuronal circuits underlying autism. Studies in mouse models of autism suggest abnormalities in specific brain regions, as well as in certain cell types. Excitatory and inhibitory neurons in the neocortex, as well as subcortical structures such as basal ganglia and cerebellum, have been implicated. Astrocytes and microglia also play roles in ASD. Further studies will be required to provide definitive evidence that similar brain regions, cell types, and circuits are relevant to autism symptoms in the human brain.

OUTLOOK: The next generation of research in neurodevelopmental disorders must address the neural circuitry underlying behavioral symptoms and comorbidities, the cell types in these circuits, and common signaling pathways that link diverse genes. Early attempts at treating neurodevelopmental disorders have yielded mixed results, underscoring the necessity of choosing the right cohort of patients to treat, developing more sensitive and dynamic outcome measures, using cogent biomarkers, and utilizing technologies such as stem cell–derived neurons to predict response to treatment.

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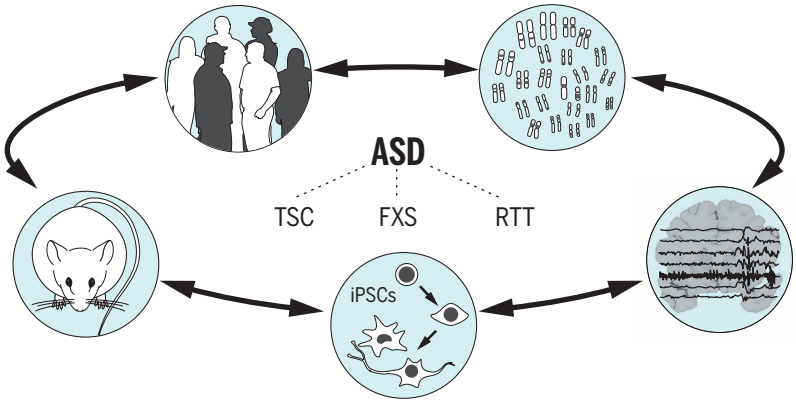
Biomarkers can be helpful in predicting subjects most likely to respond, confirm target engagement, and detect early signals of efficacy. Given that ASD represents circuit dysfunction, biomarkers that allow us to analyze autism-related circuit function are likely to be most relevant. Especially, translatable biomarkers that can be used in both mouse models and human subjects, such as electroencephalography, magnetic resonance imaging, visual or auditory evoked potentials, and eye-blink conditioning, can be particularly powerful.

One potential new tool to identity those who are likely to respond is induced pluripotent stem cell (iPSC)–derived neurons. This technology allows the possibility of testing the effects of a compound on a patient's neurons before it is given to the patient. Modeling the effects of mutations in iPSC-derived neurons can be informative about the molecular and cellular defects underlying autism.

Only when we can leverage the heterogeneity of neurodevelopmental disorders into precision medicine will the mechanism-based therapeutics for these disorders start to unlock success. ■



Translational research and clinical trials in ASD. Translational studies in ASD have gained momentum from genetically defined causes such as fragile X syndrome (FXS), Rett syndrome (RTT), and tuberous sclerosis complex (TSC). The patients with these disorders are phenotyped in detail by means of advanced imaging and electrophysiology studies, with the aim of identifying potential biomarkers. There are cell-based models (both rodent and human) as well as mouse models of these syndromes, enabling preclinical trials. Together, these efforts have led to clinical trials in some of these disorders. It is important to remember that the discovery cycle will likely take more than one round to achieve safe and effective therapies for these disorders.

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REVIEW

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Genes, circuits, and precision therapies for autism and related neurodevelopmental disorders

Mustafa Sahin[1]* and Mriganka Sur[2]*

Research in the genetics of neurodevelopmental disorders such as autism suggests that several hundred genes are likely risk factors for these disorders. This heterogeneity presents a challenge and an opportunity at the same time. Although the exact identity of many of the genes remains to be discovered, genes identified to date encode proteins that play roles in certain conserved pathways: protein synthesis, transcriptional and epigenetic regulation, and synaptic signaling. The next generation of research in neurodevelopmental disorders must address the neural circuitry underlying the behavioral symptoms and comorbidities, the cell types playing critical roles in these circuits, and common intercellular signaling pathways that link diverse genes. Results from clinical trials have been mixed so far. Only when we can leverage the heterogeneity of neurodevelopmental disorders into precision medicine will the mechanism-based therapeutics for these disorders start to unlock success.

Neurodevelopmental disorders include a wide range of conditions such as epilepsy, intellectual disability, and autism spectrum disorder (ASD). Patients with ASD exhibit early-childhood onset of symptoms, first described over 60 years ago (*1*), that persist throughout life and produce notable impairments in social, communicative, cognitive, and behavioral functioning (*2*). According to the U.S. Centers for Disease Control, ASD affects 1 in 68 children and 1 in 42 boys. ASD is a major public health problem that leads to considerable disability and disrupts families, resulting in a total annual societal cost of ~$35 billion in the United States alone (*3*).

ASD diagnosis comprises a constellation of behavioral symptoms, as defined by a group of experts (DSM-5), and requires persistent deficits in social communication and interaction across multiple contexts, as well as restricted, repetitive patterns of behavior, interests, and activities. A key characteristic in ASD is its heterogeneity. Patients with ASD present with wide variation and levels of impairment with different comorbidities, and the expression of these symptoms can change over time. Heterogeneity has been a huge obstacle in ASD research, but in recent years, researchers have started to take advantage of the heterogeneity of ASD. Rather than focusing on "pure autism" (autism not confounded by intellectual disability) (*4*, *5*), research has now opened up to examining genetic disorders with high penetrance of ASD, such as fragile X syndrome (FXS), Rett syndrome (RTT), and tuberous sclerosis complex (TSC), which have now come to the forefront of translational efforts to find treatments for subsets of mechanism-based classification of ASD (*6*). Complementary to this effort is the National Institute of Mental Health (NIMH) initiative to define psychiatric disorders according to mechanistic descriptions of symptom clusters rather than symptom inventories, also known as research domain criteria (*7*, *8*). In ASD, the etiology seems to vary according to the individual's genome and interaction with his or her environment. Genetic heterogeneity and overlap with other neuropsychiatric disorders make it difficult to find a unique risk factor for ASD. Improved understanding and classification of ASD-based domains and levels of analysis could improve precision and treatment efficacy.

Here, we review research on neurodevelopmental disorders that spans genes, molecules, cells, and circuits, as well as the whole individual and environment. We discuss current efforts and obstacles in clinical trials and offer recommendations for the future that lead toward precision medicine.

Genes

The genetic component of ASD susceptibility is evidenced by twin studies that demonstrated higher concordance of ASD among monozygotic than dizygotic twins and has benefited from modern genome scanning initiatives to yield many new genes worthy of further study. Genome analysis revealed the association of copy-number variants (such as 15q11-13, 16p11.2, and 22q11.2) and single-nucleotide variants with ASD. Some of these variants are de novo (not found in either parent) and thus easier to deem as causal. Variants that are not de novo or sequencing variants that are not obviously deleterious are harder to evaluate. Several studies have used whole-exome sequencing to reveal a number of ASD susceptibility genes, such as *CHD8*, *GRIN2B*, and *SCN2A*. These studies estimate that 400 to 1000 genes are involved in ASD susceptibility (*9*). The vast majority of ASD susceptibility genes have not yet been identified and will require much larger cohorts for adequate statistical power, as was necessary for schizophrenia (*10*). Germline mutations are not the only contributor to brain disorders; somatic mutations that affect a subset of brain neurons can cause epilepsy, brain malformations, and quite possibly ASD (*11*). Somatic mosaicism affecting the brain will confound the genetic analysis of cohorts, which are almost always based on bulk DNA derived from the blood and intended to represent the inherited genome.

Along with larger cohort sizes, identifying many of the remaining hundreds of ASD susceptibility genes will require thoughtful and innovative study designs. One approach is to study families with consanguinity to reduce inherited variation and help identify rare recessive variants (*12*, *13*). Another approach is to study groups that are relatively protected from ASD. Because ASD is much more common among males than females, focusing on families with a history of severe autism among women appears to enrich for highly penetrant rare variants (*14*).

The estimated heritability of ASD is 0.7 to 0.8, which, while relatively high, leaves room for noninherited factors, including de novo mutations and epigenetic and environmental factors, leading to a complex risk architecture. Environmental influences such as perinatal injury and maternal infection could play an important role in the context of a susceptible genetic background and contribute to the development of ASD. For instance, premature infants with isolated cerebellar hemorrhage have a 30-fold higher incidence of ASDs compared to the general population (*15*, *16*). Other epidemiological studies have implicated activation of the maternal immune system during gestation as a contributor to the development of various neuropsychiatric disorders (*17*–*19*) and more specifically in the development of autism (*19*–*21*). Maternal immune activation leads to region-specific changes in brain cytokines (*22*) and neuropathological changes that can be detected even in nonhuman primates (*23*). Interestingly, maternal immune activation is implicated in the exacerbation of syndromic forms of ASD. For example, maternal immune activation has been shown to intensify social behavior deficits observed in *Tsc2*+/− mutant mice (*24*). Finally, the relationship between the gut microbiome and neurodevelopmental symptoms has attracted attention (*25*). Autism and accompanying gastrointestinal symptoms are associated with distinct gut microbial compositions (*26*). Furthermore, probiotic treatment can improve both the metabolite abnormalities and the behavioral deficits in

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a maternal immune activation mouse model, supporting a connection between gut microbiome and autism (27). Further studies are needed to test how robust these initial observations are and what cellular mechanisms mediate them.

Molecular and cellular pathways

Every identified ASD susceptibility gene sheds new light on the cellular mechanisms underlying ASD. Many of the genes implicated in ASD converge onto a few major signaling pathways: transcriptional control and chromatin remodeling, protein synthesis and cellular metabolism, and synapse development and function (6, 28–30). Although many of these cellular processes are shared between neurons and nonneuronal cells, they appear to play roles particularly relevant to ASD in the brain (Fig. 1).

Transcriptional control and chromatin remodeling

Several ASD genes influence transcription (31, 32), including those that are highly penetrant such as Rett syndrome. MeCP2 (methyl CpG binding protein 2), which underlies Rett syndrome, is a molecular multitasker that regulates gene expression by interacting with chromatin remodeling, transcription, and splicing (33). Initial findings suggested that MeCP2 binds to methylated CpG sites in the promoters of genes and associates with chromatin silencing complexes to repress gene expression (34–36). However, subsequent studies have demonstrated that MeCP2 also interacts with chromatin and transcriptional activators to activate gene expression (37, 38). MeCP2 also mediates microRNA-mediated posttranscriptional control of gene expression (39, 40), as does FMRP (fragile X mental retardation protein) (41).

Signaling pathways and protein synthesis

One cellular process that has been implicated in multiple studies is that of mRNA synthesis and protein translation (28). Two key pathways of protein synthesis that contribute to synaptic function are the PI3K/mTOR (phosphatidylinositol 3-kinase/mammalian target of rapamycin) pathway and the Ras-MAPK (mitogen-activated protein kinase) pathway. These pathways have been linked to neurodevelopmental disorders and to synaptic dysfunction. Owing to the availability of specific and Food and Drug Administration (FDA)–approved inhibitors, the mTOR pathway has been well characterized. TSC and PTEN (phosphatase and tensin homolog) hamartoma tumor syndrome (PHTS) are two paradigmatic "mTORopathies" such that loss of TSC1, TSC2, or PTEN function leads to activation of mTOR kinase activity and high incidence of intellectual disability, seizures, and ASD (42). Other mutations in this pathway that present with ASD include the *neurofibromin 1* (*NF1*) gene that results in neurofibromatosis type I. *NF1* encodes a guanosine triphosphatase–activating protein that suppresses the activity of the proto-oncogene *Ras* and also alters mTOR activity.

Dysregulation of protein synthesis is a prominent feature of several other neurodevelopmental disorders, such as FXS (43). FMRP is an mRNA binding protein that regulates the translation of mRNAs and is silenced in FXS, resulting in

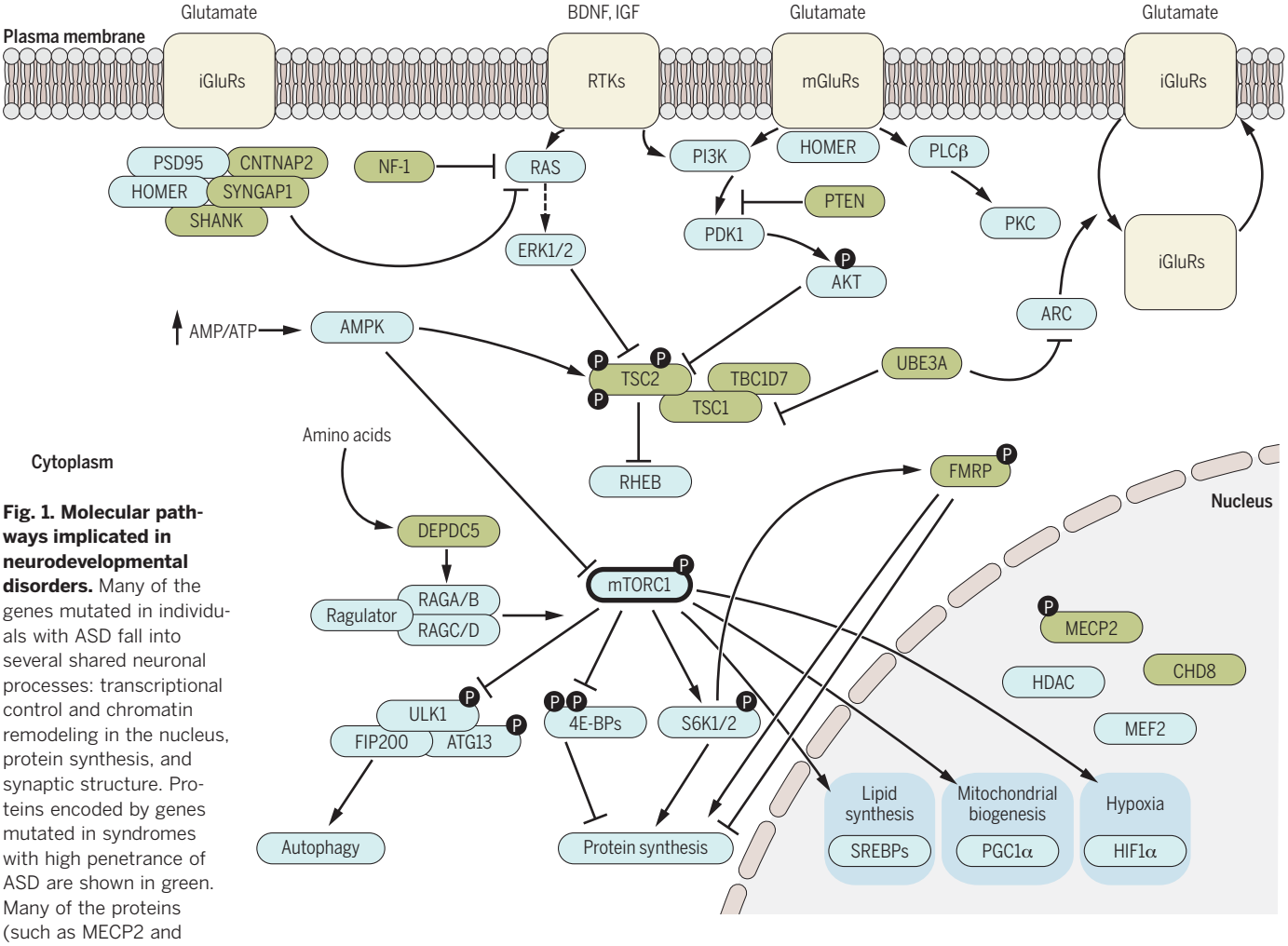

Fig. 1. Molecular pathways implicated in neurodevelopmental disorders. Many of the genes mutated in individuals with ASD fall into several shared neuronal processes: transcriptional control and chromatin remodeling in the nucleus, protein synthesis, and synaptic structure. Proteins encoded by genes mutated in syndromes with high penetrance of ASD are shown in green. Many of the proteins (such as MECP2 and FMRP) have multiple functions and interactions in the cell but are represented with the dominant functional role for the sake of clarity. Abbreviations not found in text include the following: RTKs, receptor tyrosine kinases; iGluRs, metabotropic glutamate receptors; PGC-1α, peroxisome proliferator–activated receptor gamma coactivator 1-alpha); SREBP, sterol regulatory element–binding proteins; HIF1α, hypoxia-inducible factor 1 alpha; ULK1, unc-51–like kinase 1; ARC, activity-regulated cytoskeleton-associated protein; UBE3A, ubiquitin protein ligase E3A.

aberrant protein synthesis from key transcripts implicated in synaptic plasticity (*44*). Likewise, while MeCP2 influences expression of several hundred genes (*37*), levels of BDNF (brain-derived neurotrophic factor) and IGF1 (insulin-like growth factor 1) are reduced in *Mecp2* mutant mice, along with other molecules that cause both the PI3K/mTOR and ERK/MAPK pathways to be down-regulated (*37*, *45*–*49*). Treatment with recombinant human IGF1 up-regulates these pathways in mice and induced pluripotent stem cell (iPSC)–derived human neurons (*50*, *51*) and ameliorates symptoms in mice (*48*). Preliminary results in human trials also appear promising (*52*). It is important to remember that PI3K/mTOR and ERK/MAPK pathways regulate a large number of cellular processes, including transcription, autophagy, metabolism, and organelle biogenesis and maintenance. The role of each of these cellular processes in the pathogenesis and therapeutics of ASD remains to be determined.

Disruptions of signaling pathways can change scaffolding of proteins at synapses. Such changes may cause neurodevelopmental disorders. PSD95 (postsynaptic density protein 95) anchors *N*-methyl-D-aspartate (NMDA) and AMPA receptors at glutamatergic synapses. PSD95 expression is influenced by PI3K signaling; its levels, as well as excitatory synaptic transmission, are reduced in *Mecp2* mutant mice (*48*) and rescued by IGF1 application. Similarly, *SHANK3*, which lies in the 22q13.3 deletion region associated with Phelan-McDermid syndrome (PMS), encodes a synaptic protein that regulates other protein partners, such as PSD95; up-regulation of the PI3K pathway by IGF1 rescues synaptic deficits in iPSC-derived human patient neurons (*53*) and *Shank3* mutant mice (*54*), at least partly by up-regulating PSD95.

Molecular convergence of pathways implicated by human genetics of ASD is apparent in studies of the *Fmr1* knockout mouse. First, among the mRNA binding partners of FMRP are postsynaptic proteins such as SHANK3 and signaling proteins such as TSC2 and PTEN. Second, a number of studies in *Fmr1* knockout mice indicate that interfering with protein synthesis in different ways can normalize the phenotype of the knockout mice. Knockout of S6 kinase (*55*), Cebp (*56*), and PI3K (*57*, *58*) is sufficient to ameliorate aspects of the *Fmr1* knockout pathogenesis, raising the possibility of having multiple potential targets for intervening with loss of FMRP. Dysregulation of metabotropic glutamate receptor (mGluR) and aberrant mGluR-dependent long-term depression (LTD) have been reported first in FXS mouse models (*59*, *60*) and in several other ASD animal models, including *Nlgn3* (*neuroligin 3*) knockout and 16p11.2 knockout (*61*, *62*). Although we do not yet know if murine hippocampal LTD models the human ASD brain function, these findings raise the possibility that convergent cellular and molecular pathway targets exist in subsets of ASDs.

Brain regions and neural circuits

Molecular pathways in brain cells affect the function of neurons and synapses, and hence neuronal connectivity and circuits, to modify brain function. However, we lack insight about the brain regions and neuronal circuits underlying ASD. We do not yet know whether one cell type or circuit is crucial for the behavioral deficits observed in ASD patients. It is likely that different gene mutations perturb the neural circuitry underlying social interactions and repetitive behaviors at different nodes, resulting in a complicated matrix of genes, brain regions, and behavioral correlates (Fig. 2).

Histopathological and imaging-based evidence that implicates specific brain regions and circuits underlying ASD is limited. The pathological studies are hampered by small sample size, and thus there is an urgent need for systematic and widespread collection of pathological specimens from those affected with a wide range of ASD. Imaging studies have been performed mostly on those with high-functioning ASD because patients must be able to tolerate and comply with magnetic resonance imaging (MRI) protocols. Thus, although the functional MRI studies performed to date implicate certain areas of the brain in the "high functioning" ASD group, it is not clear whether the same circuits are involved in those who have more severe cognitive deficits. It is also possible that differences between ASD and control groups identified in such studies do not represent the aberrant circuits that are causally related to the behavioral abnormalities, but instead represent the activation of other brain regions that compensate for the neural circuitry abnormalities. Clinical protocols that enable MRI studies in ASD patients with intellectual disability and those who are younger will enhance our understanding of ASD and its associated neural circuitry. Such studies performed in individuals with genetically identified subsets of ASD may also shed light on genotype-phenotype correlations (*63*, *64*).

In addition to functional MRI, complementary techniques to investigate neuronal connectivity such as structural MRI, diffusion tensor imaging tractography, near-infrared spectroscopy, magnetoencephalography, and EEG can contribute to our understanding of brain connectivity at different time scales and with different spatial resolutions. It is likely that we will need to corroborate the findings from one modality with that from others to determine the most robust connectivity abnormalities in ASD. Some of these techniques may be more amenable for individuals at different ages and at different functional levels.

Studies in genetic mouse models of ASD suggest abnormalities in specific brain regions, as well as in certain cell types. Two studies analyzed the coexpression patterns of a number of ASD genes in the human brain (*65*, *66*). One study found enrichment in mid-gestation layer 5/6 cortical projection neurons, and the other found enrichment in superficial cortical layers and glutamatergic projection neurons. Although the exact layers of the cortex involved were different in the two analyses, the fact that cortical projection neurons were indicated in both studies is potentially important.

In addition to cortical projection neurons, there is increasing evidence for the role of other neuronal subtypes in the pathogenesis of ASD. Multiple mouse models of ASD display reduction in parvalbumin (PV)–cell density in the neocortex (*67*). PV knockout mice display behavioral phenotypes with relevance to the core symptoms present in human ASD patients (*68*). In contrast, other groups have reported a selective increase in PV-immunopositive interneurons in the CA1 and CA3 subfields and calretinin-immunopositive neurons in CA1 in patients with ASD (*69*). Loss of PTEN in mice results in a preferential loss of a different subtype of GABAergic (γ-aminobutyric acid–releasing) neurons, somatostatin (SST) interneurons (*70*). Interneuron-specific deletions of ASD-related genes result in neurodevelopmental deficits in

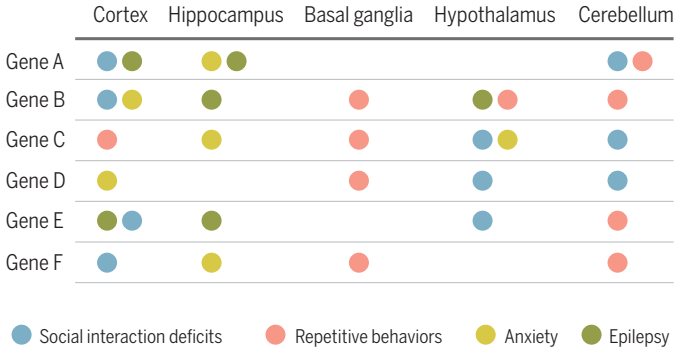

Fig. 2. Hypothetical matrix of genetic mutations and brain regions mapping onto behavioral profiles. The approach to focus on mechanistic descriptions of symptom clusters rather than symptom inventories requires an understanding of the neural circuit(s) underlying these behavioral symptoms. One way to examine the neural circuits in animal models is to probe the relationship between a gene's function in a certain brain region and the behavioral deficits in the animal. Use of conditional knockout mice has started to provide such information in certain genetic diseases such as TSC and RTT (*93*, *118*). This matrix represents a hypothetical framework, which needs to be populated by future experimentation. One concrete example of this approach is currently in effect in epilepsy. Absence seizures are thought to arise from voltage-gated calcium channel dysfunction in the thalamus and respond best to ethosuximide treatment. In contrast, complex partial seizures occur as a result of increased excitation or decreased inhibition and thus respond to glutamate antagonists or GABAergic agonists. Such delineation of genetic, cellular, and circuit defects also may prove helpful in treating behavioral deficits associated with ASD with better precision.

mice. For example, loss of MeCP2 from GABAergic interneurons leads to autistic-like repetitive movements, seizures, and deficits in auditory event–related potentials (*71*, *72*). Deficits in inhibitory neurotransmission, along with altered balance of excitation and inhibition (*73*), have been consistently observed in cortical and hippocampal neurons and circuits in diverse mouse models (*74*–*77*). In addition, the reversal potential of GABA may not mature fully when specific ASD genes are mutated, causing GABA to be depolarizing rather than hyperpolarizing (*78*). Consistent with these findings, a propensity for seizures is a major phenotype of ASDs. Taken together, these findings make a compelling case for dysregulation of inhibition as having a major role in neurodevelopmental disorders. More generally, cell type–specific and brain region–specific deletion of ASD genes is crucial for dissecting the circuit pathophysiology of ASD and in tying it to distinct symptom domains.

Connections between basal ganglia and cortex may underlie certain aspects of ASD. *Neuroligin1* knockout mice exhibit ASD-like repetitive behaviors and abnormal corticostriatal synapses (*79*). *Neuroligin3* mutants have similar abnormalities, but the defect appears to be due to a selective synaptic impairment in the nucleus accumbens/ventral striatum (*80*). SHANK3 is expressed in the basal ganglia, and *Shank3* knockout mice exhibit repetitive grooming behavior, abnormal social interactions, and changes at corticostriatal synapses (*81*).

The cerebellum is implicated in the pathogenesis of ASD via histopathology, imaging, and epidemiological studies of injury. First, neuropathological studies demonstrate loss of cerebellar Purkinje cells in individuals diagnosed with ASD versus typically developing controls (*82*–*85*). Second, imaging studies of patients diagnosed with ASD indicate gray and white matter abnormalities in the cerebellum, dating to early childhood (*86*–*90*). Premature infants with isolated cerebellar hemorrhage have a higher incidence of ASDs, suggesting that cerebellar dysfunction early in life contributes to the pathogenesis of autism (*15*, *16*). The developmental vulnerability of this circuit is further illustrated by study of genetic syndromes associated with ASD. Positron emission tomography studies in pediatric TSC patients with ASD demonstrate hypermetabolism in the cerebellar nuclei—the output of the cerebellar cortex—in TSC patients with ASD but not in TSC patients without ASD (*91*). The selective loss of *Tsc1* or *Tsc2* genes in the output cells of the cerebellum, the Purkinje neurons, appears to be sufficient to lead to an autistic-like phenotype in the two mouse models of TSC (*92*, *93*). These findings suggest that abnormal cerebellar function contributes to ASD.

Non-neuronal cells in the brain such as astrocytes and microglia have also been implicated in the pathogenesis of neurodevelopmental disorders (*94*). Astrocyte processes extend into excitatory synapses, and they influence synaptic development (*95*) and synaptic transmission via uptake of glutamate (*96*), as well as by calcium-mediated alterations in synaptic function and plasticity (*97*, *98*). ASD genes such as *Fmr1* and *Mecp2* are now known to influence astrocyte function (*99*). Astrocytes express mGluRs, providing a pathway for mGluR signaling to influence fragile X pathophysiology (*100*). Astrocyte-specific restoration of MeCP2 in *Mecp2* mutant mice restores function (*101*). Microglia also shape neuronal development and plasticity, and modulate synaptic transmission in the adult brain, via cytokine and chemokine release, as well as phagocytosis (*102*, *103*). Transplantation of wild-type microglia has been reported as reversing symptoms in a mouse model of Rett syndrome, though the interpretation of these findings remains controversial (*104*, *105*).

Treatments

Despite the many discoveries in basic neuroscience and human genetics, FDA-approved drugs for ASD patients are limited to risperidone (a dopamine antagonist) and aripiprazole (a dopamine agonist), which are both aimed at treating irritability and not the core features of ASD. Given the large number of genes that potentially confer ASD risk, the genetic heterogeneity of ASD presents a substantial obstacle to development of one-size-fits-all therapies. One can imagine several scenarios. It would be ideal to have one treatment for all causes of ASD. This seems rather unlikely; ASD is not one disease, and some genetic causes of ASD appear to have diametrically opposite manifestations at the synaptic level (*106*). It is also equally unlikely that different interventions can be developed for every genetic cause of ASD. So, the most realistic (and hopeful) scenario is that there will be a convergence upon a few molecular and circuit pathways that can be targeted by a limited number of interventions. The current

Table 1. Clinical trials for genetically defined neurodevelopmental disorders. The table lists the trials listed for genetic syndromes highly associated with ASD according to a search on the clinicaltrials.gov website on 30 July 2015. Only actively recruiting or recently completed trails are included. The final column lists primary or secondary end points with relevance to neurodevelopment.

Disorder	Study drug	ASD/neurocognitive outcomes
Tuberous sclerosis	Everolimus (RAD001)	ASD, memory, language skills, cognition, general executive function outcomes, behavioral changes, frequency or reduction of epileptiform events, reduced mTOR signaling
Rett syndrome	rhIGF-1	Behavior, cognition, cortical function, motor function
	Fingolimod (FTY720)	Slow regression of motor or language skills
	Dextromethorphan	Seizures, behavioral problems, cognition
	Glatiramer acetate	Epileptic activity, general behavior
	NNZ-2566	EEG, behavior, autonomic function
Fragile X syndrome	Acamprosate	Inattention or hyperactivity, social impairment, behavior, cognition
	NNZ-2566	Behavior, global and functional outcome measures
	Ganaxolone	Behavior, anxiety, attention, cognition
	Metadoxine (MG01CI)	Attention-deficit hyperactivity disorder
	Epigallocatechin-3-gallate (EGCG)	Improve intellectual disability, learning, memory, language
Angelman syndrome	Minocycline	Motor development, behavior, cognition, language
Down syndrome	RG1662	Cognition, behavior
	Low-dose nicotine	Cognitive improvement
	Donepezil (E2020)	Activities of daily living
	Thyroid hormone and folinic acid	Psychomotor development
Phelan-McDermid syndrome	rhIGF-1	Behavior, language, motor skills

focus is on the genetic syndromes with high penetrance of ASD symptoms, often caused by single-gene mutations (Table 1). That mouse models of many of the syndromes associated with ASD respond positively to treatment, even in adulthood (*107*–*109*), has further bolstered optimism about the utility of pharmacological treatments in these disorders.

One of the first attempts at testing mechanism-based therapies in ASD was performed in FXS. The mGluR theory of fragile X predicted that many symptoms of FXS are due to exaggerated responses to activation of mGluRs. This was demonstrated to be true in many animal models of FXS (*110*). Nonetheless, two mGluR antagonists (one made by Roche and another by Novartis) failed to show efficacy in phase 2 trials (*111*). These negative results highlight the difficulties associated with clinical trials in neurodevelopmental disorders: Did the drugs engage their targets in the central nervous system? Were the end points chosen dynamic within the duration of the trial? Was the placebo effect too large? Was the right group of patients (e.g., patients at an appropriate stage of symptoms, or a subset with a particular genotype) chosen for enrollment?

Another important issue raised by these studies is how to best utilize animal models for developing therapies. Physiological and behavioral analyses in mice have been crucial for advancing our understanding of circuitry underlying social interactions and repetitive behaviors. However, a "good" mouse model needs to have both construct and face validity (*112*). More important, the circuit that is being analyzed needs to have some direct relevance to outcome measures in humans. Only then can the pharmacological interventions that modulate that circuit be translated effectively from mice to humans.

It is surprising that relatively few pharmacokinetic and pharmacodynamic (PK/PD) relationships are tested in preclinical studies in mouse models of neurodevelopmental disorders. Although the pharmacokinetics will not be the same for a compound in mice and humans, understanding how much of the target needs to be engaged and over what period of time it must be engaged to achieve efficacy is crucial to interpret the preclinical data correctly and translate the findings to clinical studies. To identify the correct target, more detailed preclinical studies will be necessary going forward. In terms of early-stage clinical trials, many interventions look promising in open-label studies but fail to show efficacy when compared to placebo. Thus, more placebo-controlled phase 2 trials will be needed.

Biomarkers can be crucial for predicting subjects most likely to respond to a test drug, confirming target engagement, and detecting early signals of efficacy. Finding biomarkers that will segregate similarly diagnosed ASD patients into subsets of biologically more homogeneous populations is a critical feature of good clinical trial design. A "stratification biomarker" can be a biochemical measure from patient samples, a structural feature of a human imaging study, or a functional feature of an imaging or electrophysiological study. Aside from stratification, biomarkers can also be helpful in early diagnosis and assessing phenotype and severity, as well as measuring PK/PD in drug studies. Given that ASD represents circuit dysfunction, biomarkers that allow us to examine ASD-related circuits are likely to be most relevant. Especially, translatable biomarkers that can be used in both mouse models and human subjects can be particularly powerful [e.g., EEG, MRI, visual or auditory evoked potentials, or eye-blink conditioning (*64*, *113*–*115*)]. Similarly,

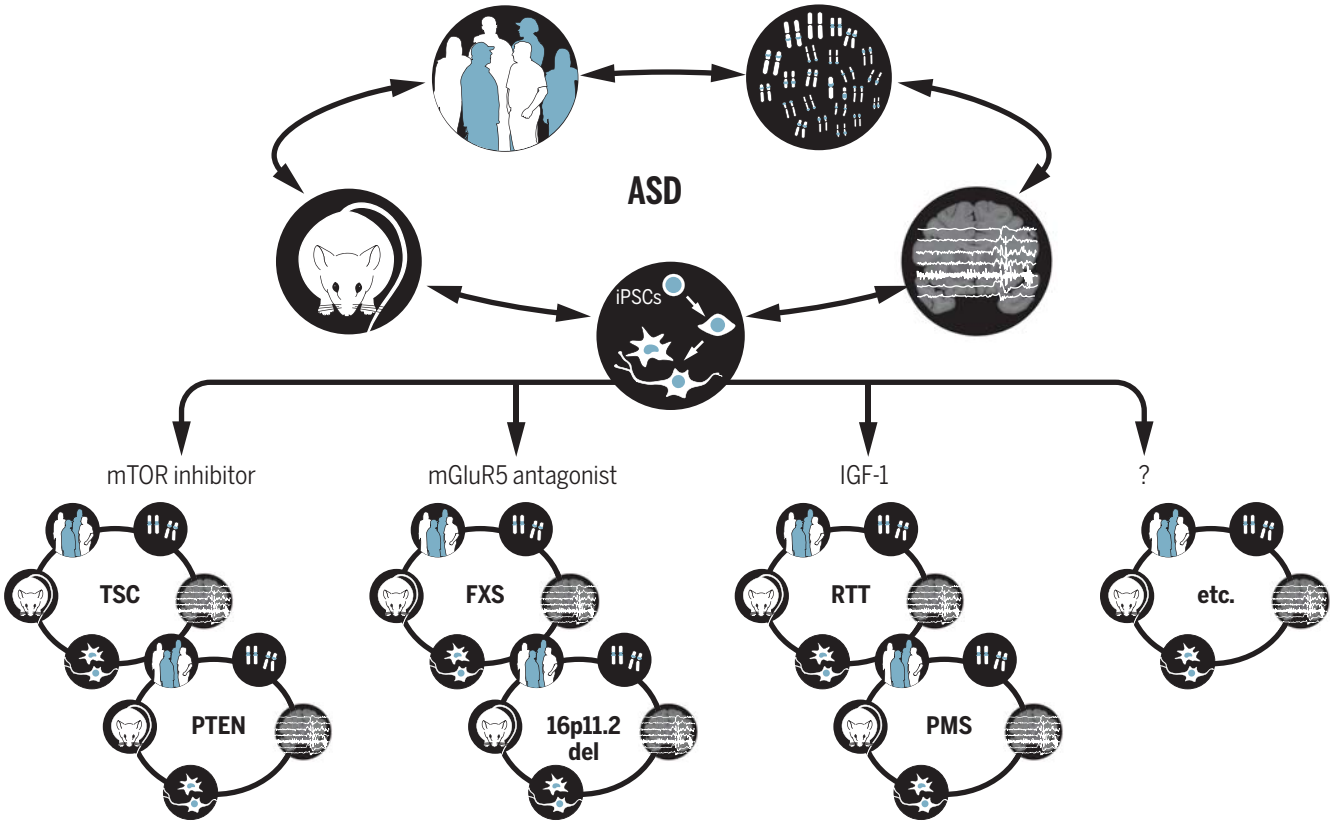

Fig. 3. Translational research and clinical trials in ASD. Translational studies in ASD have gained momentum from genetically defined causes such as FXS, TSC, and RTT. The patients with these disorders are phenotyped in detail by means of advanced imaging and electrophysiology studies, with the aim of identifying potential biomarkers. There are cell-based models (both rodent and human) as well as mouse models of these syndromes, enabling preclinical trials. Together, these efforts have led to clinical trials in some of these disorders. Based on the preclinical trials, the hypothesis is that different etiologies of ASD will respond to different therapies, such as mTOR inhibitors (TSC and PTEN), mGluR5 antagonists (FXS and 16p11.2 deletion), and IGF-1 (RTT and PMS). Subsets of nonsyndromic ASD patients may also benefit from one of these therapies, but further studies will be required to provide the tools and methods to stratify the individuals with nonsyndromic ASD into treatment groups. It is important to remember that the discovery cycle will likely take more than one round to achieve safe and effective therapies for these disorders.

outcome measures that are circuit-based may be more fruitful in detecting efficacy rather than measuring global functioning. A panel of relevant biomarkers, which together provide a unique profile of a patient, may be a crucial component of precision trial design in the future.

One of the most important questions about treatments is when mechanism-based treatments need to be initiated. Since the behavioral manifestations of ASD occur quite early in development, one may have to intervene before symptoms arise. Animal models of syndromic ASD indicate that restoring function well into adulthood can rescue some of the symptoms of the disease (*107*–*109*). It is not yet clear if the same is true in humans and what exactly the critical windows for treatment are. However, regardless of age at treatment onset, relevant biomarkers and efficacy measures would be important for establishing the effectiveness of treatment.

One potential new tool to identify subjects who are likely to respond to a test drug is iPSC-derived neurons. This technology allows the possibility of testing the effects of a compound on a patient's neurons first before giving it to the patient. Modeling the effects of mutations in iPSC-derived neurons can be informative about the molecular and cellular defects but is unlikely with the current technology to provide insights on the emergent dysfunctions at the level of neuronal circuits. Nonetheless, a preliminary testing of efficacy in a patient's iPSC-derived neurons should be a vital component of trial design for precision medicine.

Since many of the diseases being targeted in these initial trials are genetic, they may be amenable to gene therapy in theory. Gene therapy using viral vectors is undergoing a renaissance and may be particularly applicable to diseases that arise from loss of function of a particular gene such as *MECP2* or *CDKL5* (*cyclin-dependent kinase-like 5*). Aside from the delivery issues, one must pay close attention to the dosage effects, because many of the genes that result in an ASD-related phenotype also have deleterious effects when they are expressed at high dosage. Thus, expression of the exogenous genes may have to be regulated tightly in spatial and temporal terms, as well as levels of expression.

If we reach success in single genetically defined syndromes, there will be two new roadblocks in generalizing these findings to the larger ASD population. First, such an extension will require a comparative analysis of the different genetically defined causes of ASD to determine whether effective treatments in one may also be effective in another (Fig. 3). Such comparative understanding of the genetic etiologies underlying ASD is in its infancy (*106*). A second and more difficult hurdle will be applying these findings to the "idiopathic" genetically unknown or undefined ASD population. Currently, we do not have an analytical tool to determine if an individual with ASD would benefit from a treatment that is effective, for example, in TSC or in FXS. A marker to classify patients according to genetic, biochemical, or circuit abnormalities does not yet exist. However, even single-gene conditions involve multiple potential targets, and combination therapies are likely to be more effective than single drugs for single targets (*47*). The success of targeted pharmacological interventions would require integration of multiple kinds of data: knowledge of the genetic mutation and its signaling pathways and synaptic molecules, effectiveness of the therapy on neuronal and synaptic phenotypes in patient-derived neurons and non-neuronal cells in culture, and even analysis of transplanted human neurons in mice.

Although pharmacological treatments may normalize neuronal and synaptic abnormalities, cognitive function is still dependent on complex circuits and interaction of the individual with his or her environment. Thus, pharmacological treatments alone may not be sufficient to reach the optimal outcome without behavioral treatments. Behavioral interventions appear promising in mouse models (*116*, *117*) and could be combined with pharmacological interventions in future clinical trials. While simply correcting the synaptic abnormality with a pharmacological agent may not be sufficient to affect behavioral changes, it could accelerate the rate of learning and sociability in the setting of behavioral interventions. Although combining treatments adds complexity to the trial design, a few such trials are in the planning stages. Trials based on a mechanistic understanding of the disease, performed on a well-defined group of subjects, with evidence of target engagement and supportive biomarkers, are the most likely to succeed. Once such trials prove effective in the highly penetrant genetic syndromes, the next challenge will be to identify patients with idiopathic autism who may benefit from the same treatment. Such an approach will finally realize the notion of precision medicine for autism and related neurodevelopmental disorders.

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