Mini-Symposium

Current Perspectives in Autism Spectrum Disorder: From Genes to Therapy

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Autism spectrum disorder (ASD) is a constellation of neurodevelopmental presentations with high heritability and both phenotypic and genetic heterogeneity. To date, mutations in hundreds of genes have been associated to varying degrees with increased ASD risk. A better understanding of the functions of these genes and whether they fit together in functional groups or impact similar neuronal circuits is needed to develop rational treatment strategies. We will review current areas of emphasis in ASD research, starting from human genetics and exploring how mouse models of human mutations have helped identify specific molecular pathways (protein synthesis and degradation, chromatin remodeling, intracellular signaling), which are linked to alterations in circuit function and cognitive/social behavior. We will conclude by discussing how we can leverage the findings on molecular and cellular alterations found in ASD to develop therapies for neurodevelopmental disorders.

Key words: **animal models; autism spectrum disorder; genetics; therapeutics**

Introduction

Independent studies have consistently demonstrated that autism spectrum disorder (ASD) has a strong genetic component (Abrahams and Geschwind, 2008; Sandin et al., 2014; Colvert et al., 2015; Geschwind and State, 2015). Despite this genetic contribution, finding high-confidence ASD risk genes has been extremely vexing until recently. The notable exception being the realization that individuals with mutations in genes leading to syndromes once considered independent: *FMR1* for Fragile X syndrome (FXS), *TSC1/2* for Tuberous Sclerosis Complex (TSC), *MECP2* for Rett syndrome (RTT), often met criteria for an ASD diagnosis. The major source of difficulty for gene discovery in ASD, and indeed other complex disorders, is the wide spectrum of genetic and phenotypic heterogeneity observed. The genetic architecture of ASD at a population level is so complex that risk is most likely conferred by rare mutations and common variants at hundreds of independent loci (Fig. 1). Dominant, recessive, oligogenic/polygenic, and gene × environment mechanisms all clearly play a role; however, their individual contributions in different ASD subpopulations are still to be fully elucidated.

Animal models for rare monogenic syndromes, such as FXS, TSC, and RTT, have been critical to study the pathophysiology of ASD and to discover that there are multiple neurobiological processes that, when perturbed, increase the risk of autism (Zoghbi and Bear, 2012). Here, we will review how advances in the human genetics of ASD and corresponding murine models are pointing to key cellular mechanisms, including protein synthesis and degradation, chromatin regulation, and disrupted activity within specific brain circuits. We will discuss the benefits and challenges of studying human genetic mutations using rodents, with additional focus on the skewed male/female ratio in ASD. We will conclude with examples of how mechanistic understanding in ASD from patients and animal models can help develop new strategies to identify therapeutic targets leading to clinical trials (Fig. 1).

The complex genetics of ASD

Recent advances, including genome-wide copy number arrays, massively parallel sequencing, new analysis paradigms, and innovative cohorts, have begun to unravel the genetic complexity in ASD. The transformative technology of whole-exome sequencing (WES) has identified novel high-confidence "idiopathic" ASD risk genes for arguably the first time. Starting in 2009, the technology to selectively sequence all of the protein-coding regions (exons) of the genome (i.e., the "exome") became widely avail-

Received July 13, 2016; revised Aug. 23, 2016; accepted Aug. 25, 2016.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.2335-16.2016

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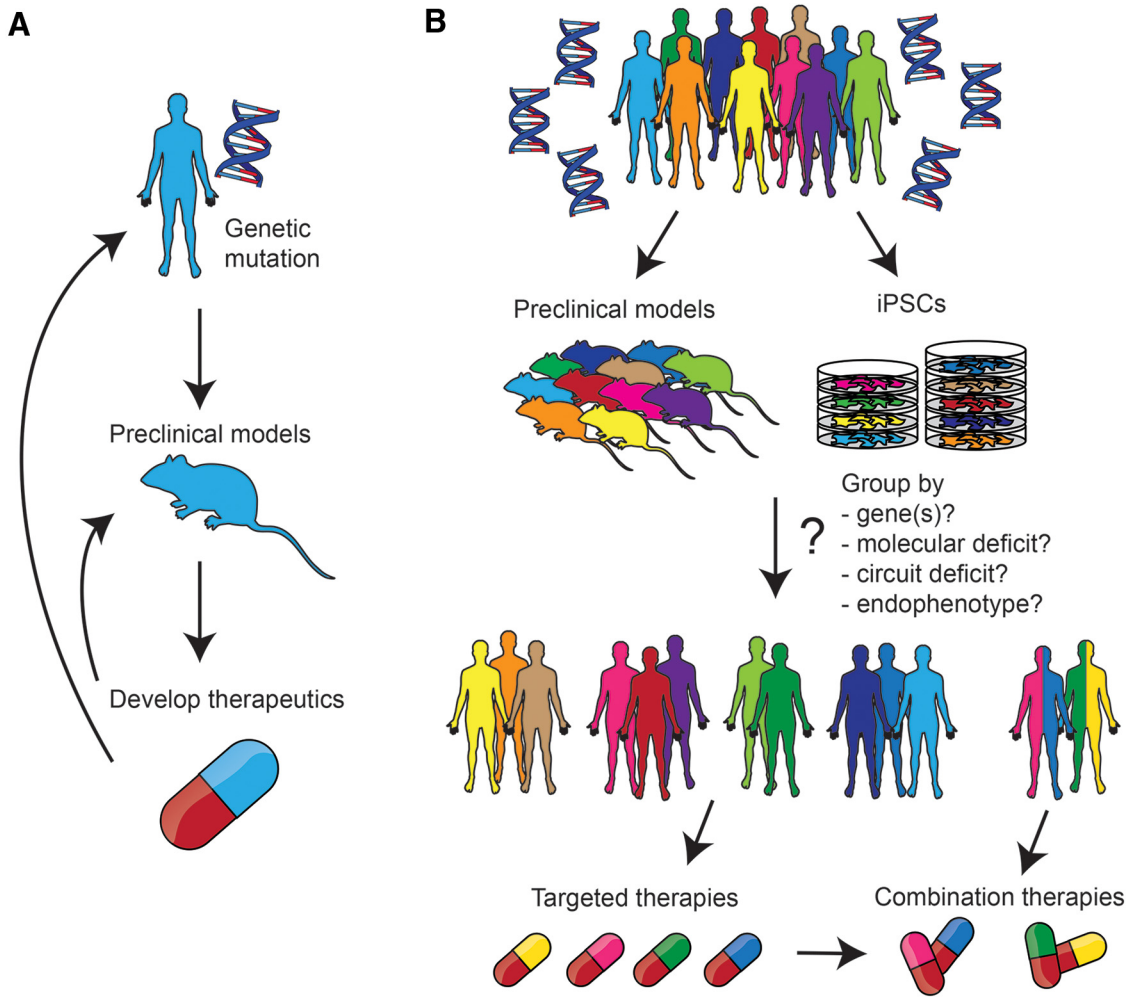

Figure 1. The complex path from gene identification to therapy development in ASD. Although the ideal path from gene identification to therapy development via animal models appears linear (***A***), genetic and phenotypic heterogeneity prevents therapeutic advances in ASD and other complex disorders. Possible therapies will need to be customized based on the specific underlying molecular changes for subcategories that are still in the process of being defined (***B***). Combination approaches (e.g., multiple compounds targeting different mechanisms or endophenotypes) may also be necessary.

able. This allowed for unbiased genome-wide discovery of coding variants or mutations contributing to a disorder's risk at single-base resolution. Several groups began piloting WES in different neurodevelopmental/psychiatric disorders using a trio (father, mother, affected child/individual) or other family design, specifically in families with no previous family history of the disorder, also called simplex or sporadic families (Vissers et al., 2010; Girard et al., 2011; O'Roak et al., 2011; Xu et al., 2011). The working hypothesis of these studies was that, in some fraction of these simplex families, there may be a new or "*de novo*" mutation (not present in either parent) that coappeared with the disorder in the affected individual. These studies showed the feasibility of this approach to detect the ~1 true expected *de novo* mutation and a large fraction of "possible" candidate gene mutations (Veltman and Brunner, 2012).

These early proof-of-concept studies emboldened several groups to expand these efforts with four large WES studies published in 2012 on >900 combined independent families (Iossifov et al., 2012; Neale et al., 2012; O'Roak et al., 2012a; Sanders et al., 2012), and culminating in 2014 with two large-scale studies, including >4000 affected children (De Rubeis et al., 2014; Iossifov et al., 2014). Data from the family-based Simons Simplex Collection suggests that ~30% of all probands have a *de novo* mutation of major effect that contributes to their diagnosis, which may be up to 50% of girls (Iossifov et al., 2014). Estimates suggest that the *de novo* events in these genes can substantially increase the risk for ASD (odds ratio: ≥20) (De Rubeis et al., 2014). Furthermore, based on WES and targeted sequencing approaches, 49 different genes no longer merely represent "candidate" ASD genes, but are now mid- to high-confidence genes based on their recurrent disruption by *de novo* mutations in unrelated probands (O'Roak et al., 2012b, 2014; De Rubeis et al., 2014; Iossifov et al., 2014). Genes with *de novo* mutations also show strong enrichment for fragile X mental retardation protein targets, chromatin modifiers (e.g., *CHD8*, *CHD2*, *ARID1B*), embryonically expressed genes (e.g., *TBR1*, *DYRK1A, PTEN*), and nominal enrichment for postsynaptic density proteins (e.g., *GRIN2B*, *GABRB3*, *SHANK3*). Additionally, networks constructed using these high-confidence ASD risk genes as seeds reveal a converging molecular biology that includes translational control and chromatin regulation as key players disrupted in ASD (O'Roak et al., 2012a; Parikshak et al., 2013; Willsey et al., 2013; Hormozdiari et al., 2015).

In addition to rare *de novo* mutations, recent WES studies have also identified a role for rare inherited variants in ASD risk; including maternally transmitted predicted loss-of-function (LoF) variants (<10%) and recessive/hemizygous LoF variants

(~5%) (Chahrour et al., 2012; Novarino et al., 2012; Lim et al., 2013; Yu et al., 2013; Krumm et al., 2015). The overall impact of inherited variants on ASD risk will likely be higher when taking into account missense variants, whose possible impact is currently difficult to quantify. Furthermore, it is possible that recessive mutations might contribute to ASD susceptibility and phenotype penetrance as either protective or risk-conferring alleles. Studies focusing on consanguineous and nonconsanguineous families have shown that rare recessive ASD mutations have as much or more heterogeneity in molecular mechanisms as shown by *de novo* mutations. Examples of ASD genes with recessive LoF mutations identified in families with autism, intellectual disability (ID), and epilepsy include: *CNTNAP2* (Strauss et al., 2006), *SLC9A9/NHE9* (Morrow et al., 2008), *BCKDK* (Novarino et al., 2012), and *CC2D1A* (Manzini et al., 2014). Some recessive ASD mutations are hypomorphic alleles (retaining partial activity) of genes whose complete inactivation causes severe neurological syndromes (Yu et al., 2013). *UBE3B* was identified as a candidate ASD gene (Chahrour et al., 2012) and was subsequently associated with a syndrome of ID and microcephaly (Basel-Vanagaite et al., 2012). Hypomorphic missense variants in genes encoding *AMT*, *PEX7*, and *VPS13B* were identified in consanguineous families with ASD. However, complete LoF of these genes will lead, respectively, to nonketotic hyperglycinemia, rhizomelic chondrodysplasia punctata, and Cohen syndrome (Yu et al., 2013).

While large-scale sequencing efforts to date have contributed to our understanding of the complex architecture of ASD genetics, multiple challenges remain. As hundreds of additional genes may be mutated in only a handful of cases, larger and larger cohorts need to be studied. One such effort, Simons Foundation Powering Autism Research for Knowledge (SPARK), aims at partnering with families, clinicians, researchers, and community organizations to build a cohort of 50,000 individuals with ASD over the next 3 years. We still have incomplete understanding of the role of different types of variants/mutations and their impact in individuals and different subpopulations of ASD (e.g., females and older adults with ASD). Moreover, our understanding of how multiple variants might act in concert in a single individual and the risk from noncoding variation and gene × environment interactions are still in their infancy. The next emerging phase in the field is the development of high-throughput functional validation screens to assess the impact of identified variants on protein function and phenotype development. Functional validation is especially important for missense variants that are currently largely ignored unless they occur in known disease-causing genes. Several bioinformatic tools that predict the deleteriousness of genomic variants have been developed (e.g., SIFT, PolyPhen-2, and CADD among others) (Kumar et al., 2009; Adzhubei et al., 2010, 2013; Kircher et al., 2014). Despite the value of these tools in prioritizing variants from large sequencing data, functional validation remains essential to test the biological impact of identified variants.

Modeling features of ASD using the laboratory rodent

Well-controlled *in vivo* studies in a tractable model organism with a high degree of genetic conservation relative to humans have been instrumental to our current understanding of ASD pathogenesis, with the most useful mouse models having high construct and face validities (Crawley, 2007). Although forging definitive links between genetic alterations and behavioral impairments is challenging, validated neurobehavioral tests for rodents provide an opportunity to gain insight into how specific genetic mutations impact the core behavioral features of ASD. Social-communication deficits and restricted, repetitive behaviors can be tested in ASD mouse models using well-established assays (for review, see Silverman et al., 2010; Kazdoba et al., 2016). Conservative analyses through reductionist strategies, such as the "endo-phenotype" approach to study simplified components of complex neuropsychiatric and neurodevelopmental disorders are critical to avoid anthropomorphization of rodent behavior (Gottesman and Gould, 2003).

Interestingly, converging lines of evidence from genetically modified mice, especially models of monogenic disorders and disease-causing copy number variants, such as Phelan-McDermid syndrome (PMDS) (Jiang and Ehlers, 2013), TSC (Sundberg and Sahin, 2015), and RTT (Chahrour and Zoghbi, 2007), support the hypothesis of a shared underlying pathophysiology of ASD involving alterations in cellular properties that result in abnormalities in neural network activity and behavioral deficits (Zoghbi, 2003). If models of different genetic mutations show similar alterations, these findings may broadly influence current perspectives on the generalizability of therapeutic interventions for ASD with seemingly disparate genetic etiologies. It may be possible to encourage "repurposing" of existing compounds and interventions shown to have therapeutic benefit in one category of ASD (e.g., rapamycin and rapalogues for TSC) (Curatolo et al., 2015) and deep brain stimulation for RTT (Hao et al., 2015a), to either other clinical indications or other ASD categories.

As with many disease models, the laboratory mouse has limitations. It remains debatable that behavioral phenotypes in the mouse, including those modeling ASD-like features, accurately depict the human condition (face validity). There is an increasing need to identify behavioral phenotypes that are robust and disease-relevant leading to reliable preclinical outcome measures (Landis et al., 2012). Relying on the laboratory mouse alone may also contribute to difficulties in translating findings in the mouse to the clinic. For example, the mouse model of FXS displays behavioral phenotypes that are opposite of what may have been predicted based on observed features in FXS patients (Peier et al., 2000; Spencer et al., 2011), and initial clinical trials targeting mGluR5 signaling in FXS did not result in phenotypic reversal as seen in the mouse model (Dölen et al., 2007; Berry-Kravis et al., 2016). However, genetic ASD rat models (Hamilton et al., 2014; Patterson et al., 2016; Veeraragavan et al., 2016) may serve as complementary mammalian rodent tools, and comparative studies in both mouse and rat would likely strengthen the predictive validity of potential preclinical outcome measures. In addition, multiple other vertebrate and invertebrate model organisms have been used to recapitulate molecular, cellular, and/or behavioral phenotypes linked to ASD (for review, see McCammon and Sive, 2015). Among these, the zebrafish is emerging as a model for rapidly testing the pathogenicity of human genetic variants (Deciphering Developmental Disorders, 2015; Turner et al., 2015) and for drug screening (Hoffman et al., 2016). In general, whether each ASD animal model replicates the human disease and whether they are appropriate for the scientific question being asked must be carefully assessed when considering translation to the clinic. Finally, a combination of both genetic and environmental factors, including gene × environment interactions, must be considered to reconcile the relatively high heritability with the rising prevalence estimates of ASD (Sandin et al., 2014).

Emerging molecular defects in ASD: protein synthesis and degradation

One of the clusters of genes that when mutated give rise to forms of syndromic autism are the ones encoding for proteins involved

in regulating protein synthesis, such as *FMR1*, *TSC1/2*, and *PTEN*. Deletions or LoF mutations in these genes lead to alteration of the translational control machinery ultimately resulting in exaggerated protein synthesis in the brain. Accordingly, studies using animal models showed increased protein synthesis in the brain, such as the investigations on mice with deletion of *Fmr1* (Qin et al., 2005; Dölen et al., 2007). Importantly, enhanced protein synthesis was also demonstrated in lymphoblastoid cells of patients affected by FXS (Gross and Bassell, 2012). Moreover, mice with genetic deletion of *Fmr1*, *Tsc1*, *Tsc2*, or *Pten* displayed behavioral impairments and synaptic alterations consistent with ASD (Kelleher and Bear, 2008; Bourgeron, 2009; Ebert and Greenberg, 2013; Santini and Klann, 2014). Finally, mice with genetic deletion of *Tsc1*, *Tsc2*, or *Pten* showed a normalization of the ASD-like behaviors after treatment with rapamycin (Ehninger et al., 2008; Meikle et al., 2008; Zhou et al., 2009; Tsai et al., 2012), a potent inhibitor of mTORC1, the macromolecular complex central to the regulation of translation in eukaryotes (Santini and Klann, 2011). Overall, these studies suggest a correlation between dysregulated protein synthesis and the occurrence of behavioral and synaptic defects consistent with ASD.

Novel mouse models of ASD engineered from rare genetic mutations discovered in patients (Yonan et al., 2003) were important to establish a causal relation between dysregulated translation and the appearance of ASD-like behavioral and synaptic alterations (Gkogkas et al., 2013; Santini et al., 2013). For instance, in one of these models, the cap-binding protein eIF4E was overexpressed in the brain (*eIF4E* transgenic mice), resulting in an increase in protein synthesis. The mice exhibited synaptic and behavioral impairments consistent with ASD, including the presence of repetitive behaviors and social defects. Moreover, when the effects of eIF4E overexpression were normalized by administering the specific drug inhibitor 4EGI-1, protein synthesis in the brain was reduced in concomitance with some of the behavioral and synaptic defects (Santini et al., 2013). Remarkably, these results were confirmed by a parallel study that used mice with the genetic deletion of the eIF4E-binding protein 2 (4E-BP2), one of the repressor proteins of eIF4E, leading to similar biochemical, behavioral, and synaptic phenotypes (Gkogkas et al., 2013). These studies indicate causality between dysregulation of protein synthesis in the brain and certain ASD phenotypes.

In parallel to protein synthesis, multiple protein degradation genes are mutated in ASD, showing how tight regulation of protein expression is critical for brain function. The proteasome pathway is a highly conserved mechanism of targeted protein degradation that relies on ubiquitination. Ubiquitination is a post-translational modification that involves conjugating a ubiquitin moiety to target proteins through sequential steps mediated by several enzymes. The specificity of the process is largely determined by the E3 ligases, which catalyze the last step of transferring the ubiquitin to substrate proteins (Berndsen and Wolberger, 2014). In addition to its traditional role of targeting proteins for degradation, protein ubiquitination also plays a key part in brain development, through the regulation of neurogenesis, gliogenesis, neuronal migration, and neurite and synapse formation (Kawabe and Brose, 2011). Mutations in several ubiquitin ligases result in ASD and ID, including *UBE3A*, the gene affected in Angelman syndrome; *HUWE1*, which is mutated in syndromic X-linked ID; and *UBE3C*, which has recently been associated with autism risk (O'Roak et al., 2012a). Haploinsufficiency of the deubiquitinating enzyme *USP7* (ubiquitin specific peptidase 7) has been recently associated with ASD (Hao et al., 2015b). Interestingly, USP7 functions in concert with MAGEL2 to regulate endosomal protein recycling. Duplications spanning *USP7* have also been identified in ASD (Sanders et al., 2011).

Although important clues to the biochemical pathways underlying ASD have evolved from these studies, it still remains to be delineated whether this knowledge can be directly translated to human patients. Few of the proteins whose synthesis is dysregulated in animal models of syndromic ASD have been identified. Multiple studies focused on matrix metalloproteinase 9, which is overexpressed in FXS and ASD (Gkogkas et al., 2014; Sidhu et al., 2014), but more targets must be characterized to understand which ones could be translatable to patients as diagnostic biomarkers or therapeutic ends.

Understanding and modeling sex differences in ASD

From its initial description >70 years ago, autism has been predominantly diagnosed in boys (Kanner, 1943). ASD is four times more common in males than in females (Christensen et al., 2016); and when considering high-functioning individuals, the ratio increases to 8–10:1 male/female (Fombonne, 2005). Multiple theories have been put forth, suggesting, for example, that females are less susceptible to ASD due to a female protective effect (Robinson et al., 2013), or that increased fetal testosterone levels lead to an "extreme male brain," which is less social and more prone to repetitive behaviors (Baron-Cohen et al., 2011). Genetic, epigenetic, and hormonal explanations have been proposed, with none of these mechanisms being mutually exclusive (Baron-Cohen et al., 2011; Robinson et al., 2013; Werling and Geschwind, 2013). Genetic studies support the hypothesis of female protection indicating that females carry more severe mutations than males (Gilman et al., 2011; Levy et al., 2011) and analysis of the Simons Simplex Collection showed that females overlap genetically with the most severe males (Iossifov et al., 2014). Because of the smaller number of girls included in initial studies, it has often been difficult to define whether ASD presents differently in females. A large meta-analysis including ~4000 cases with 988 females showed that by 6 years of age girls show fewer stereotypical behaviors, despite having equal communication and social deficits (Van Wijngaarden-Cremers et al., 2014). In addition, females tend to display more compensatory behavioral changes, which could lead to underdiagnosis (Lai et al., 2011; Baldwin and Costley, 2016).

As described above, multiple animal models have been developed to study ASD using paradigms aimed at capturing behavioral deficits that resemble features of the human disease (for review, see Ey et al., 2011; Ogden et al., 2016). However, as behavior has traditionally been studied preferentially in males, females have often been excluded. Male and female littermates were paired for each genotype in a handful of studies showing no sex differences for mouse knock-outs of *Shank1* (Sungur et al., 2014), *Shank2* (Schmeisser et al., 2012), *Nlgn2* (Blundell et al., 2009), and *Scn8a* (McKinney et al., 2008). However, further analysis of specific behaviors revealed sexual dimorphism, such as a female-specific reduction in prepulse inhibition in the *Shank3* overexpression model of 22q13 duplication syndrome (Han et al., 2013), and a male-specific increase in novelty response in the *Nrxn1* heterozygous mouse (Laarakker et al., 2012).

Substantial sex differences were revealed in fully powered behavioral studies studying mice deficient for the GABA receptor subunit Gabrb3 (DeLorey et al., 2011) and the signaling scaffold Cc2d1a. *GABRB3* is located within the 15q11-q13 duplication region linked to ASD and imprinting of the same region is disrupted in Angelman syndrome (Cook et al., 1997). Not only do *Gabrb3* heterozygous mice display behavioral deficits differently

depending on the maternal or paternal origin of the mutated allele, but phenotypes are often found only in males. When females are also affected, they may not perform like males, as found in differential performance in motor learning on the rotarod (DeLorey et al., 2011). When cerebellar circuit function was studied in these mice, males and females displayed different circuit properties. Males increased mGluR1/5 activity in the cerebellar nuclei to counteract increased inhibition, whereas females already displayed higher levels of firing and did not need to upregulate mGluR1/5 (Mercer et al., 2016). In conditional knock-outs for the signaling scaffold *Cc2d1a*, which is mutated in ASD and ID (Manzini et al., 2014), males display an array of cognitive and social deficits in combination with anxiety and hyperactivity (Oaks et al., 2016b), whereas females only show mild cognitive deficits (Oaks et al., 2016a). In parallel, signaling changes are observed only in the male brain, despite equal removal of *Cc2d1a*.

These studies suggest that more careful analysis of sex differences in animal models could identify molecular and cellular mechanisms that could be used to understand sex differences in patients. To fully understand the mechanisms underlying ASD, it is of paramount importance that behavioral, physiological, and molecular differences are compared in males and females.

Translation of molecular insights into therapeutics

Mechanistic understanding of ASD can identify therapeutic targets and neural circuits suitable for development of quantitative translatable outcome measures. One such example is derived from careful studies of iPSC-derived neurons bearing patient mutations. Haploinsufficiency of *SHANK3* emerged as the primary pathogenic event in PMDS from studies of patient iPSC-derived neurons. Patient neurons with defects in synaptic transmission and membrane resistance were corrected by restoring *SHANK3* expression or by treating neurons with insulin-like growth factor 1 (Shcheglovitov et al., 2013). Pilot clinical trials for insulin-like growth factor 1 in PMDS patients have been promising (Kolevzon et al., 2014), but larger clinical trials are needed. The impending era of *in vitro* human neuron models of neurodevelopmental disorders will provide robust substrate for drug repurposing screens and opportunities for personalized drug screening.

Challenges of working with iPSC-derived models have been documented previously (Paşca et al., 2014; McNeish et al., 2015; Nestor et al., 2016). Furthermore, cell-based assays do not easily allow circuit-based studies. A critical challenge to developing new treatments is identification of circuits underlying specific symptom domains and methods to monitor these circuits in clinical trials. Emerging data suggest that disruption of sensory processing by the cerebellum during a sensitive period will impair multisensory convergence of inputs onto Purkinje cells needed for the appropriate activity-dependent refinement of neocortical circuits recruited during social learning (Becker and Stoodley, 2013; Rogers et al., 2013; Wang et al., 2014). Consistent with this are findings that injury of cerebellum early in development is among the highest risk factors for developing ASD (Limperopoulos et al., 2014). Cerebellar circuitry is highly conserved across rodents and humans and can be monitored quantitatively and noninvasively in humans of all ages using eyeblink conditioning (Reeb-Sutherland et al., 2011). Impaired social approach behavior and eyeblink conditioning have both been observed in TSC mouse models of autism produced by selective disruption of TSC signaling in cerebellar Purkinje neurons during development (Kloth et al., 2015). The development of translatable outcome measures, such as eyeblink conditioning in clinical ASD patient populations (Oristaglio et al., 2013), will enable rational design of early-stage proof-of-concept clinical trials for therapeutic hypotheses that treat dysfunction of this circuit. Furthermore, genetic disorders associated with ASD represent a unique opportunity to leverage human genetics to develop and refine these outcome measures. Duchenne muscular dystrophy (DMD) is caused by inactivating mutations in the dystrophin gene (*DMD*), essential for maintenance of muscle fiber intensity (Monaco and Kunkel, 1988). However, several isoforms are highly expressed in the Purkinje cells of humans and rodents (Lidov et al., 1990, 1993), leading to a wide range of Purkinje cell deficits in excitability and plasticity (Anderson et al., 2003, 2004, 2005, 2010; Kueh et al., 2011; Snow et al., 2014). This is consistent with reports that ASD symptoms are comorbid with DMD in ~25% of patients (Wu et al., 2005; Hinton et al., 2007, 2009; Kohane et al., 2012; Banihani et al., 2015; Ricotti et al., 2016). Evidence of deficits in social behavior in mouse models of DMD (Alexander et al., 2016) provides a circuit-driven model for testing therapeutic hypotheses. These social deficits were reversed by inhibitors of cGMP-specific phosphodiesterase, consistent with the high concentrations of the phosphodiesterases PDE5A and PDE9A in Purkinje cells (Shimizu-Albergine et al., 2003; Kleiman et al., 2012). Thus, the DMD/ASD patient population may provide a valuable subset of ASD patients who exhibit symptoms stemming primarily from disturbances of cerebellar circuitry. Therapeutic approaches effective at treating ASD symptoms of DMD may provide a new paradigm for identification and treatment of other cerebellar-driven ASD symptoms.

In conclusion, the identification of multiple genetic risk factors for ASD and modeling of these genetic mutations in animal models, especially as it pertains to syndromes associated with ASD, has greatly increased our understanding of the pathophysiology of this spectrum of disorders. The more we discover how complex ASD is, the more we realize that we need to do more: partner with more families, develop more translatable models, and study their physiological, cellular, and molecular changes in more detail across more circuits. ASD is varied and autism subtypes likely exist with specific molecular or circuit alterations, which could differ between males and females. Translational efforts would benefit from standardized uses of rodent models (addressing both basic and translational questions), as well as cross-species comparisons to enhance the predictive validity of outcome measures. A critical challenge for translation is identification of endpoints to monitor the circuitry underpinning symptoms in preclinical models and patients. As new molecular targets are identified, there is great promise for repurposing existing drugs, many of which have already undergone safety studies. However, access to pharmacokinetic data and expertise needed to experimentally measure drug levels in animal models for follow-up studies is critical to conducting meaningful translational studies in academic settings and requires new infrastructure (Kleiman and Ehlers, 2016). Although many challenges remain, the tremendous recent advances make us hopeful as researchers for a brighter future on the horizon for individuals with ASD and their families.

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