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Overview of Mouse Models of Autism Spectrum Disorders

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Abstract

This overview covers many well-characterized mouse models of autism spectrum disorders (ASDs). As there are numerous mouse models of varying design and description in the literature, the models presented here represent examples of genetically engineered models where human genetic evidence supports a causative relationship between the targeted mutation and the behavioral phenotype of the mouse models. As the diagnosis of ASDs is primarily based on behavioral evaluations in humans, we highlight the murine behavioral analogs to human autistic behaviors in different models. However, because the characteristic behaviors of ASDs in humans are in the domains of social interaction, communication, and restricted interests, the translational value of various behavioral assays used in rodents remains a subject of debate. Using genetically engineered mouse models with good construct and face validity will provide a platform for investigating underlying pathophysiological mechanisms and discovering potential therapeutic interventions which will hopefully translate into effective treatments for ASDs. We also illustrate several significant challenges in the field of modeling ASDs in rodents due to the considerable clinical and molecular heterogeneity associated with ASDs in humans.

Keywords

autism spectrum disorders; ASDs; mouse models; genetically engineered; behavior; therapeutic interventions

Introduction

Autism spectrum disorders (ASDs) are a range of neurodevelopmental disorders defined in *The Diagnostic and Statistical Manual of Mental Disorders* (5th ed; *DSM-5*; American Psychiatric (American Psychiatric Association, 2013) as persistent difficulties in both social communication/interaction and repetitive/restrictive patterns of behavior presenting early in

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**Due to the limited space for references, we apologize for many papers that are not included in this overview.

childhood and causing significant functional impairment. The clinical presentations and severity of symptoms are quite heterogeneous. Comorbidities including intellectual disability (ID), movement disorders, and seizures are common. As the incidence of ASDs has climbed to 1-in-68 children and the costs of providing support to affected individuals is estimated at $35–85 billion per year in the United States alone, development of effective therapies has become an essential public health priority(Autism Developmental Disabilities Monitoring Network, 2014; Ganz, 2007). However, the diverse and often unknown etiologies of ASDs combined with limited experimental techniques available in human research have emphasized the need for valid and well-characterized animal models in order to further elucidate disease mechanisms and identify opportunities for intervention.

Twin and family studies indicate that genetic heritability contributes as much as 80–90% to the occurrence of ASDs, although the roles of environment and development are not well understood in the manifestation of symptoms (Miles, 2011). While the cause of ASDs has not been identified for the majority of patients, 20–30% of them have known genetic causes (see Figure 1) including ~5% with cytogenetically visible chromosomal abnormalities by traditional karyotyping technique, ~10–20% with sub-microscopic copy number variations (CNVs) detected by array based comparative genomic hybridization, and ~5% due to single-gene mutations (Miles, 2011). Caglayan (2010) provides a review of the known genetics of ASDs, but with the application of new genome technology in clinical and research settings, many more ASD genes have been discovered with more to come in the near future.

The considerable clinical and molecular heterogeneity of ASDs have posed a great challenge to studying autism in animal models. Ideally, a valid model for autism is required to meet both “construct validity” (the molecular defect mimics that seen in human ASD) and “face validity” (the phenotypes are analogous to human clinical presentation). The finding of causative genes or chromosomal defects in a small subset of ASD patients provides the opportunity to make genetically engineered mouse models with good construct validity. There is no reliable neuropathological hallmark found in ASD brains that reflect the underlying pathophysiology. Given that the current diagnostic criteria are purely behavioral in ASDs, valid mouse models should recapitulate analogous behavioral abnormalities relevant to the ASD diagnosis.

Great progress has been made in developing murine behavioral assays of social interaction, vocalizations, and repetitive/restrictive behaviors which are thoroughly reviewed elsewhere (Silverman et al., 2010b). Table 1 describes examples of commonly used behavioral tests for ASD-related and frequent comorbid behaviors. Many mouse models of known pathogenic mutations for human ASDs (i.e. with good construct validity) demonstrate abnormal behaviors which correlate to the pathological human behaviors in ASDs. However, caution is still warranted when evaluating ASD model mice, as there are few tests which have direct analogs between humans and mice. Future efforts should focus on refining existing behavioral paradigms and developing new ones with attention to reproducibility across cohorts of mice and between laboratories, sensitivity to quantifying subtle behavioral differences, and translational utility from pre-clinical models to human research. Likewise, the heterogeneity and spectrum of behavioral impairments seen in human patients likely have origins outside of a given pathogenic mutation and thus are difficult to capture in a

single-gene mouse model. Future efforts to study multiple genes as well as gene and environment interactions are warranted.

There have been many mouse models of ASDs involving environmental and pharmacological manipulations, possible naturally occurring mutations in inbred strains, and genetically engineered models targeting candidate human ASD genes. Efforts have been made to generate a comprehensive database of animal models of ASDs, with details about all levels of molecular, electrophysiological, and behavioral phenotypes (Kumar et al., 2011). While all of these categories have models which exemplify the analog murine behavioral abnormalities across the ASD domains, inclusion criteria for the scope of this overview were defined as models with good construct validity, i.e. multiple lines of human genetic evidence for the causality of the candidate mutation, as well as good face validity across murine ASD-like behavioral tests and associated comorbidities. Particular attention is drawn to models which have been used for testing therapeutic interventions, including genetic and pharmacological manipulations, as these hypotheses warrant further pre-clinical testing such that progress towards targeted treatments for patients may be made. These represent ideal opportunities for targeted animal models which can result in better understanding of disease mechanisms and directed therapeutic research without the confounds of genetic, developmental, and environmental diversity found in humans.

This overview covers a range of mouse models for genetic defects including (i) autism associated with defined genetic syndromes due to mutations in a single gene such as fragile X or Rett syndromes, (ii) non-syndromic autism associated with pathological mutations in single genes, such as the *Neuroligin* or *SHANK* family genes, and (iii) CNVs associated with autism such as 15q11-q13 or 16p11.2. Table 2 summarizes the key features of the ASD-like phenotype, comorbidities, therapeutic interventions and source for the models discussed in this review. One non-genetic model of an inbred strain with face validity across the three ASD-related behavioral domains is included due to its extensive characterization and use in pre-clinical testing, although many other examples of inbred, environmental, or exposure based models exist and are reviewed elsewhere (Moy and Nadler, 2008). When considering which mouse model to select, there are additional criteria to consider beyond how well the genetic construct recapitulates the human mutations and how many ASD-related behavioral domains are affected and to what degree. These include reproducibility of phenotypes across genetic backgrounds, laboratories, and testing conditions, as well as sensitivity to therapeutic interventions. While this information is not available for many of the more recently created models, we highlight those mutant mice which have withstood such rigorous evaluation and may hold great utility for mechanistic and interventional research. For all genetically engineered models, analyses from molecular, electrophysiological, and behavioral aspects were usually conducted by variable methods. Due to the limited scope of this overview, and considering the diagnosis of ASDs in humans is currently behavioral, we will primarily focus on reviewing and comparing the evidence from behavioral analyses. For readers interested in other aspects of these models, select reviews are available for some models (Chung et al., 2012; Zoghbi and Bear, 2012).

Single-Gene Syndromic Models

Fragile X Syndrome

Genetics—Fragile X Syndrome (FXS), the most common monogenic intellectual disability (ID) syndrome in males, is caused by CGG triplet repeat expansion in the 5′ untranslated (UTR) region of the *FMR1* gene on the X chromosome. Clinically, FXS is characterized by ID, epilepsy, hyperactivity, dysmorphologies, and macroorchidism (Bhakar et al., 2012). In addition, 25–50% of FXS patients meet full ASD diagnostic criteria with many more demonstrating some degree of autistic behavior, and FXS patients account for up to 5% of the known causes of ASD cases. *FMR1* mutations are the most frequent single-gene cause of ASD, primarily in males, but females can also be affected due to skewed patterns of X chromosome inactivation (Caglayan, 2010).

***Fmr1* mutant mice—**The *Fmr1* mutant mouse was produced in 1994 by targeting coding exon 5 using gene targeting method in embryonic stem (ES) cells (The Dutch-Belgian Fragile X Consortium, 1994). The most commonly used mice are male mutants *(Fmr1*−/Y) in the FVB background (JAX 004624), although *Fmr1* mice backcrossed to C57BL/6J, the most commonly used inbred strain for neurobehavioral research, are also available (JAX 003025). Ever since the *Fmr1* knockout (KO) mice were reported, they have been extensively studied to dissect the molecular pathogenesis of ID and ASD. It should be noted that the *Fmr1* mutant mouse has the same molecular consequence for the expression of the *Fmr1* gene, i.e. loss of its encoded fragile X mental retardation protein (Fmrp), as that caused by triplet repeat expansion in humans. However, the molecular nature of the mutation mechanism is different between human and mouse. *Fmr1* KO mice were produced by deleting coding exon 5, while in humans, the triplet repeat mutation causes the loss of expression of FMRP due to methylation of the promoter region of *FMR1*. Male *Fmr1* mutant mice have demonstrated ASD-related and comorbid behaviors including abnormal sociability and social recognition as well as hyperactivity and impaired learning and memory, although there have been many conflicting reports (Moy and Nadler, 2008; Silverman et al., 2010b). Recently, Spencer et al. (2011) demonstrated that genetic background (such as congenic C57BL/6J versus individual hybrids of C57BL/6J with each of A/J, DBA/2J, FVB/NJ, 129S1/SvImJ, and CD-1) impacts the expression of all three ASD-related domains. No two genetic backgrounds had the same phenotypes, and only one out of six tested backgrounds (C57BL/6J x DBA/2J hybrid) showed abnormalities in all three of the ASD domains: sociability, USVs, and repetitive behaviors. Of note, some groups have identified specific ASD-related phenotypes which can be ameliorated by pharmacological and genetic interventions targeting excessive metabotropic glutamate receptor (mGluR)-mediated protein synthesis, hypothesized to be an important pathophysiological mechanism (Bhakar et al., 2012). These include abnormal social interaction, compulsive marble burying, and comorbidities such as increased seizures and abnormal motor learning on the rotarod. In addition, environmental enrichment has been shown to ameliorate anxiety-like behavior and aberrant habituation responses in *Fmr1* mutant mice, and it remains to be seen whether this intervention can impact more ASD-relevant behaviors (Restivo et al., 2005).

Assessment—Despite the presence of inconsistent findings in some reports, *Fmr1* mice, which mimic both the genetic causes as well as many of the neurobehavioral features of FXS, have been a valid and popular mouse model to dissect a monogenic form of human ASD. Perhaps more so than any other known genetic cause of ASD, the underlying pathophysiologic mechanism of dysregulated protein synthesis downstream of aberrant mGluR-mediated signaling in synapses has been characterized and ameliorated in the *Fmr1* mouse. This body of research has translated into multiple drug candidates currently under testing in human clinical trials, with the promise of targeted therapeutic development on the horizon. Given the wide use of this model, reproducibility of key phenotypes, and sensitivity to a range of mGluR-modulating therapeutic interventions, we recommend continued research into understanding the correlation between the synaptic, cellular, and circuit defects and the behavioral phenotypes in these mutant mice in order to discover new therapies and refine existing drug targets. In addition, the mGluR hypothesis should be examined in other mouse models of ASDs in order to identify disparate genetic causes with shared underlying pathophysiology which may prove similarly sensitive to pharmacological treatments.

PTEN Hamartoma Tumor Syndromes

Genetics—Mutations in the tumor-suppressor gene phosphatase and tensin homolog *(PTEN*) were initially associated with Cowden Syndrome and other related hamartomatous disorders such as Bannayan-Riley-Ruvalcaba and Proteus syndromes. These syndromes are characterized by hamartomas and macrocephaly, with variably penetrant neurobehavioral features such as ID and ASD. In addition, mutations in *PTEN* have also been reported in cases with primary presentation of ASD and significant macrocephaly (Butler et al., 2005; Goffin et al., 2001). Recently, mutations in *PTEN* have been associated with sporadic or non-syndromic cases of ASD by whole exome sequencing methods (O'Roak et al., 2012a; O'Roak et al., 2012b). It is estimated that anywhere from 1–17% of patients with ASD and macrocephaly harbor *PTEN* mutations from several studies with small sample sizes, although the total number of ASD patients with *PTEN* mutations is unclear, but likely quite small (Butler et al., 2005; Buxbaum et al., 2007).

***Pten* conditional knockout mice—**As conventional *Pten* homozygous mice are lethal, and heterozygotes develop tumors in a variety of tissues which impacts survival and performance on behavioral tests, conditional knockout (*Pten*-cKO) mice with loxP sites flanking exon 5 (JAX 006440) represent the best option to examine the ASD-related behaviors produced by *Pten* deficiency (Stiles et al., 2004). Kwon et al. (2006) conditionally deleted both copies of *Pten* in a subset of post-mitotic neurons and found that these mice recapitulated the macrocephaly seen in patients as well as ASD-related behavior including reduced sociability and preference for social novelty in the 3-chamber test, reduced interaction and impaired social recognition when investigating a juvenile conspecific, and impaired nest-building. Neither communication nor repetitive behavioral domains were tested. While motor learning using the rotarod and contextual and cued fear conditioning were normal, *Pten*-cKO demonstrated many other comorbid behaviors including spontaneous seizures, anxiety-like behavior in the open field and light/dark test, hyperactivity in novel environments, impaired learning and memory in the Morris water maze (MWM), and enhanced auditory startle, impaired prepulse inhibition (PPI), and

reactivity during handling all suggestive of altered sensitivity to sensory stimuli. A subsequent study found that treatment with rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), could rescue both the macrocephalic/hypertrophic and the behavioral phenotypes of *Pten*-cKO mice, including sociability and anxiety-like behavior (Zhou et al., 2009). A different group used the same Cre line to generate haploinsufficiency of *Pten* in post-mitotic neurons and again found that these mice also demonstrated altered sociability and social novelty preference in the 3-chamber test, as well as enhanced self-grooming, again validating the ASD-relevant phenotype of *Pten* mutants (Napoli et al., 2012).

Assessment—While *PTEN* mutations are probably rare, the human genetic evidence supports pathological mutations in this gene as causative for a subset of ASD cases. The *Pten* cKO mouse model exhibits several strengths, including robust and reproducible ASD-related and comorbid behaviors, and sensitivity to pharmacological interventions. Caveats include a lack of testing of communication-related and higher order repetitive behaviors and some weakness with regards to construct validity, as the cKO mice have deficiency of *Pten* in only a subset of post-mitotic neurons whereas humans with *PTEN* mutations are deficient in every cell throughout development. However, in addition to future research designed to address these areas, further work investigating the mechanisms responsible for ASD-related behavior in this model could lead to fruitful discoveries of targeted therapeutics for not only *PTEN*-related patients but perhaps a subset of ASDs that are known to have mild macrocephaly.

Rett Syndrome

Genetics—Rett syndrome (RTT) is a neurodevelopmental disorder predominantly affecting girls caused by mutations in the gene encoding methyl-CpG binding protein 2 (*MECP2*) on the X chromosome. The key clinical features of Rett syndrome include ID, progressive motor impairment, seizures, breathing abnormalities, and shortened lifespan. RTT was considered a prototypical single-gene cause of ASD in the *DSM IV*, and remains the most common cause of ASDs in females. RTT patients demonstrate autistic behaviors including stereotypies, loss of language abilities, and social withdrawal (Moretti and Zoghbi, 2006; Neul, 2012).

***Mecp2* mutant mice—**More than 10 different lines of mouse models targeting *Mecp2* have been made (Calfa et al., 2011; Katz et al., 2012). Similar to *MeCP2* mutations in humans, germline absence of *Mecp2* is embryonically lethal in mice, so many models have been made with disruption of *Mecp2* in various tissues, at various time points, or with various mutation strategies to produce a hypomorphic *Mecp2* mutant allele (Calfa et al., 2011). Several lines of *Mecp2* mice have been used more frequently and tested more rigorously for ASD-related phenotypes. Examples of *Mecp2* mutant mice which are publically available and demonstrate ASD-relevant behavioral phenotypes include: targeted deletion of exon 3 (*Mecp2*$^{tm1.1Jae}$ available from the Mutant Mouse Regional Resource Center, UC Davis #000415)(Chen et al., 2001), and a nonsense mutation generating a premature stop codon after amino acid 308 (*Mecp2*^{tm1Hzo}, JAX 005439)(Shahbazian et al., 2002). Others have made point mutations with good construct validity to human mutations, such as a missense mutation knock-in of T158A (*Mecp2*$^{tm1.1Joez}$, JAX 017741), but limited

testing of ASD-relevant phenotypes have been conducted (Goffin et al., 2012). These mutant mice were tested both in heterozygous females and hemizygous males, the former representing better construct validity but often having milder and delayed onset of phenotypes, and the latter with more severe and earlier onset of phenotypes due to the lack of a compensatory wild-type allele provided by the mosaicism that arises from X chromosome inactivation. Each of these lines recapitulates the gross motor dysfunctions and shortened lifespan that characterize the disease. However, for behaviors relevant to ASD, as reviewed in Katz et al. (2012), different lines of *Mecp2* mutant mice demonstrate variable phenotypes. We will highlight a few ASD-relevant but inconsistent features between two of the widely used lines. Social phenotypes range from decreased social interaction in *Mecp2*^{tm1Hzo} mice to enhanced sociability and preference for social novelty in *Mecp2*$^{tm1.1Jae}$ mice. These two models also have altered isolation USVs as pups, although *Mecp2*^{tm1Hzo} pups produce fewer isolation calls whereas the *Mecp2*$^{tm1.1Jae}$ pups produce more calls. Excessive and injurious self-grooming has been reported as an example of enhanced repetitive behaviors or motor stereotypies in the *Mecp2*^{tm1Hzo} mice. Like social and communication behavior, anxiety-like behavior has also been inconsistent across models with some lines, such as *Mecp2*$^{tm1.1Jae}$ or *Mecp2*$^{tm1.1Joez}$ mice, demonstrating less anxiety-like behavior, whereas others, such as the *Mecp2*^{tm1Hzo} mice, exhibit more anxious behavior. The reason for the significant variability related to ASD-like behavior is not immediately clear. While the molecular nature for the mutations in different lines of *Mecp2* mice could be a factor, different experimental conditions may also be considered as a cause for the phenotypic variability. In contrast, poor motor performance and cognitive impairment have been more reliable phenotypes across lines, with all three of the mutants highlighted in this overview demonstrating impaired motor skills on the rotarod or in tests of gait abnormalities, as well as impaired learning and memory in a variety of paradigms including novel object recognition and contextual fear conditioning. Numerous pharmacological interventions have been tried and are reviewed in Katz et al. (2012), although few have made it into large pre-clinical or clinical trials.

Assessment—Like FXS and the *Fmr1* mice, the multiple *Mecp2* lines and extensive and often inconsistent behavioral phenotypes make this particular animal model challenging to cover in this overview. The comprehensive and critical studies of these mice represent the level of investigation that all ASD mouse models should be submitted to prior to validating their use for identifying therapeutic targets and pre-clinical studies. The robust and reproducible behavioral impairments in motor coordination and learning and memory, as well as the sensitivity of these phenotypes to various pharmacological manipulations, strongly support the use of *Mecp2* mutant mice for continued pre-clinical research targeting improvement in these comorbid behaviors which greatly impair the lives of RTT patients. Based on the construct validity of the T158A knock-in model, it will be of great interest to determine whether these mice exhibit an ASD-like behavioral phenotype, and whether ASD-related phenotypes prove as reproducible and sensitive to intervention as the motor and learning phenotypes.

Timothy Syndrome

Genetics—Timothy Syndrome (TS) is caused by a common *de novo* mutation of pGly406Arg in exon 8 of the *CACNA1C* gene encoding the L-type voltage gated calcium channel, CaV1.2. The pGly406Arg mutation is believed to be a gain-of-function mutation affecting the calcium channel's function. The mutation results in congenital heart disease, cardiac arrhythmias, dysmorphologies, and ID, with ASD affecting ~30–50% of TS patients (Splawski et al., 2011). The number of ASD patients with *CACNA1C* mutations is unknown, but likely quite rare. However, the finding of a mutation in a calcium channel in autism makes this direction particularly interesting regarding the neurobiology of ASDs.

***Cacna1c* mutant mice Ts2-neo mice—**Due to the strong evidence supporting a gain-of-function mutation in human TS, mutant mice with the common point mutation of pGly406Arg are most appropriate to model human TS. Unexpectedly, mutant mice carrying homozygous and heterozygous pGly406Arg are not viable. Interestingly, mice heterozygous for this point mutation but retaining the neomycin-selection cassette (Ts2-neo, JAX 019547) are viable, which presumably is due to reduced levels of the mutant protein via transcriptional interference. Ts2-neo heterozygous mice demonstrated ASD-like behavior including reduced sociability in an extended version (measured across 4 hours) of the 3-chamber test although performance in the standard 10-minute version of this test and the habituation-dishabituation test of social recognition were normal. Reduced duration of USVs but normal number, amplitude, and frequency were observed in Ts2-neo pups. In addition, enhanced self-injurious grooming, repetitive marble burying and inflexibility in reversal learning of the MWM were observed (Bader et al., 2011; Bett et al., 2012). As far as comorbidities, the Ts2-neo mice demonstrated normal spatial learning in the initial acquisition of the MWM, and enhanced associative learning and memory in cued and contextual fear conditioning. These mice also demonstrated reduced locomotion and approach to novelty, although they had normal activity in familiar environments (i.e. home cage) and normal anxiety levels in light/dark test and elevated zero maze (Bader et al., 2011; Bett et al., 2012).

Assessment—Although TS is quite rare, mutations in other ion channel proteins account for additional cases of ASDs (Schmunk and Gargus, 2013). The Ts2-neo mice recapitulate the point mutation responsible for human TS, as well as abnormal behaviors across all three ASD-relevant domains and several comorbid behaviors. Given this degree of construct and face validity and the direct impact of channel mutations on neuronal excitability, further research into how this putative gain-of-function mutation results in aberrant circuit and behavioral function is warranted. Targeted therapies to restore normal calcium-mediated signaling and thus regulate excitability represent an exciting possibility for this syndrome and other ASDs resulting from mutations in ion channels and associated proteins.

Tuberous Sclerosis Complex

Genetics—Tuberous sclerosis complex (TSC) is an autosomal dominant neurodevelopmental syndrome caused by mutations in either *TSC1* or *TSC2*, encoding the respective proteins hamartin and tuberin, which function as inhibitors of the mammalian target of rapamycin (mTOR) signaling cascade. TSC is characterized by the development of

astrocytomas and cortical tubers with variable penetrance of epilepsy, ID, and autism. Fifty percent of TSC patients carry an ASD diagnosis, and TSC mutations account for ~1–4% of ASD cases (Caglayan, 2010).

***Tsc1* heterozygous and conditional knockout mice—**Mouse mutants for tuberous sclerosis complex have been characterized extensively. At least two lines of *Tsc1* mutant mice have been made, one targeting exons 6–8 (available from Dr. Kobayashi, Juntendo University, Japan) and another targeting exons 5–7 (available from Dr. Cheadle, Cardiff University, United Kingdom) with similar results: homozygous *Tsc1* mutant mice are lethal while heterozygotes are viable. Although, they have not shown the pathognomonic tuber formation in the brain or seizures, these mice have demonstrated ASD-like behavior. *Tsc1* heterozygous mice exhibit reduced social interaction in pairs, which could be improved by treatment with rapamycin, and impaired nest building (Goorden et al., 2007; Sato et al., 2012). Communication and repetitive behaviors have not been formally evaluated. As for comorbidities, male mutants demonstrated reduced anxiety-like behavior in the light/dark test, and both sexes show impaired learning and memory in the MWM and contextual fear conditioning, with the learning and memory deficits rescued by rapamycin. Locomotor activity in the open field and motor performance on the rotarod were normal (Goorden et al., 2007; Sato et al., 2012). Interestingly, cKO mice targeting exons 17–18 of *Tsc1* (JAX 005680) solely in cerebellar Purkinje cells demonstrate ASD-like behaviors including reduced sociability and social novelty in the 3-chamber test, increased pup USVs, increased self-grooming, and impaired reversal learning. They also showed comorbid behaviors including ataxic gait and impaired motor learning, but normal levels of locomotor activity. Treatment with rapamycin rescued social behavior and reversal learning in the MWM task (Tsai et al., 2012). These findings suggest an important role of *Tsc1* in cerebellum in ASD-like behaviors but how the cerebellar circuit contributes to ASD-like behaviors, particularly social behaviors, remains unknown.

***Tsc2* heterozygous mice and conditional knockout mice—**Several lines of *Tsc2* mutant mice have also been made (targeting exon 2 available as JAX 004686 or exons 2–5 available from Dr. Kobayashi), and again the homozygous KO mice are not viable. *Tsc2* heterozygous mice also show some ASD-related phenotypes including reduced sociability (females) and reduced preference for social novelty (males) in the 3-chamber test, and reduced interaction in pairs (Reith et al., 2013; Sato et al., 2012). Abnormal pup USVs were observed in heterozygous or wild-type pups born to *Tsc2* heterozygous dams, although adult USVs have not been tested (Young et al., 2010). Conventional heterozygous *Tsc2* mice did not show increased repetitive behavior in marble burying or reversal learning of the MWM (Reith et al., 2013). Results from comorbid behavioral tests included normal levels of anxiety in the open field, impaired motor performance on the rotarod, and impaired learning and memory in the MWM and contextual fear conditioning, with the impaired memory phenotype rescued by treatment with rapamycin (Ehninger et al., 2008; Sato et al., 2012). Similar to *Tsc1*, mice with a conditional allele of *Tsc2* were also made by floxing exons 2–4 of the *Tsc2* gene ($Tsc2^{flox}$) (mice available from Dr. Gambello, University of Texas Health Science Center). By crossing the $Tsc2^{flox}$ allele to a Cre line expressed in cerebellar Purkinje cells and to the conventional *Tsc2* heterozygotes, mice with *Tsc2* haploinsufficiency in all

tissues but complete deficiency in Purkinje cells were produced. This manipulation is capable of recapitulating more severe ASD-related behaviors than those found in the conventional heterozygote, including reduced sociability and social novelty in the 3-chamber test, increased marble burying and poor motor learning, although tests for reversal learning in the MWM did not reveal a deficit (Reith et al., 2013).

Assessment—TSC represents one of the more common causes of syndromic ASD. The conventional heterozygous *Tsc1/Tsc2* mice have high construct validity for recapitulating haploinsufficiency of *TSC1* or *TSC2* in human patients, and they demonstrate ASD-relevant social phenotypes and sensitivity to pharmacological intervention with rapamycin. However, these mice need further validation in the domains of communication and repetitive behavior, which make them weaker mouse models than some of the others presented in this overview. On the other hand, both *Tsc1/Tsc2* cKO mice demonstrate both abnormal social and repetitive behavior and sensitivity to treatment with rapamycin, although how cerebellar circuitry gives rise to these ASD-relevant behaviors remains less understood. However, the reproducibility of several key behaviors across labs and the success of rapamycin for reversing both cellular and behavioral phenotypes underscore this potential therapeutic option for TSC and other ASDs, should they also be shown to be related to dysregulation of the mTOR pathway.

Dravet Syndrome

Genetics—Dravet syndrome is a rare developmental epilepsy syndrome caused by autosomal dominant mutations in the *SCN1A* gene encoding the voltage-gated sodium channel NaV1.1, and is characterized by intractable epilepsy, ID, and autism. About 25% of Dravet patients meet the full diagnostic criteria of ASD, although a greater proportion had autistic behavior (Li et al., 2011).

***Scn1a* heterozygous mice—**While initial efforts developing and characterizing a mouse model of *Scn1a* haploinsufficiency focused on the epilepsy-related phenotypes, a recent paper from Han et al (2012) demonstrated that *Scn1a* heterozygous mice (targeting the last coding exon; from Dr. Caterall at the University of Washington) demonstrate ASD-like behavior including reduced sociability and preference for social novelty in the 3-chamber test, reduced sociability in pair interactions, avoidance of novel and social odors, increased circling in the open field which may represent a motor stereotypy, and enhanced self-grooming. These mice also exhibit anxiety-like behavior in the open field and elevated plus maze, hyperactivity in the open field, and impaired learning and memory in the MWM, Barnes maze, and contextual fear conditioning. Treatment with the benzodiazepine, clonazepam, at low doses which did not induce sedation or anxiolysis, rescued social and learning/memory phenotypes in these mice. Also, conditional deletion of *Scn1a* in forebrain inhibitory neurons recapitulated many of the ASD-related behaviors. Taken together, this evidence supports a mechanism whereby reduced GABA-ergic tone in *Scn1a* mutants results in disrupted excitatory/inhibitory balance and thus mediates the ASD behaviors.

Assessment—*Scn1a* mutants demonstrate both social and lower order repetitive behaviors, as well as comorbid epilepsy and impaired learning and memory consistent with

Dravet syndrome patients. Also intriguing is the sensitivity of these animals to both pharmacological and genetic manipulations targeting the GABA neurotransmitter. Additional molecular studies are warranted in this direction. Further efforts to characterize communication and higher order repetitive behaviors as well as confirm the reproducibility of these findings across backgrounds, laboratories, and testing conditions may be very important. Given the frequent comorbidity of autism in epilepsy syndromes and vice versa, along with the face validity for both ASD-related behaviors and spontaneous seizures in the *Scn1a* mice, this model provides an opportunity for investigating how hyperexcitability or loss of inhibitory/excitatory balance can result in these often overlapping neuropsychiatric conditions.

Single-Gene Non-syndromic Models

Contactin-associated protein-like 2 (CNTNAP2)

Genetics—Homozygous or compound heterozygous mutations in contactin-associated protein-like 2 (*CNTNAP2*), a member of the neurexin super-family of neuronal cell adhesion molecules, were originally associated with a rare syndrome characterized by epilepsy, cortical dysplasia, and ID. Further genetic evidence implicates heterozygous variants in this gene with ASD or autism-related endophenotypes such as impaired language, although the contribution of these mutations to the prevalence of ASDs is likely quite small (Alarcon et al., 2008; Arking et al., 2008).

Cntnap2* mutant mice—Cntnap2* homozygous mice (targeting exon 1, JAX 017482) demonstrate reduced sociability in the 3-chamber test as well as during a juvenile play paradigm, reduced pup isolation USVs, enhanced self-grooming, and poor reversal learning in the MWM, in addition to seizures and hyperactivity (Penagarikano et al., 2011). This mouse model also demonstrates abnormal neuronal migration, reduced numbers of interneurons, and abnormal neuronal synchrony which may underlie the observed behavioral deficits. Treatment with risperidone, one of two FDA approved drugs for treatment of ASD-related behavior, reduced the hyperactivity and repetitive behavior phenotypes in *Cntnap2* homozygous mice, but did not improve their sociability defect. Heterozygous mice did not show any abnormal behaviors or neuropathological findings.

Assessment—While human genetic evidence supports heterozygous *CNTNAP2* mutations as associated with ASD, only mice homozygous for *Cntnap2* mutations recapitulate ASD-related behavioral deficits in all three domains, with heterozygotes appearing normal. In addition to efforts to reproduce behavioral findings and identify the pathophysiological mechanisms underlying *CNTNAP2* mutations, further work is warranted to identify why mice with haploinsufficiency of *Cntnap2* did not have significant ASD-related phenotypes, unlike in humans with heterozygous *CNTNAP2* mutations, and to determine whether this gene falls within a pathway shared with other causes of ASDs.

Neurexin1

Genetics—A number of studies have associated single gene mutations in and copy-number variations of the gene *NRXN1*, encoding neurexin1, with idiopathic autism, as well as other

neuropsychiatric disorders including ID and schizophrenia (Bena et al., 2013). Neurexins are a family of pre-synaptic cell-surface proteins which serve as synaptic cell-adhesion molecules through binding to post-synaptic neuroligins, which have also been implicated in ASDs and will be covered below.

Nrxn1α* mutant mice—Nrxn1*contains two promoters, which produce two major classes of isoforms, *Nrxn1α* and *Nrxn1β*. Targeting the first coding exon produced mice lacking *Nrxn1α* isoforms (JAX 006377). These mice demonstrated a relatively mild behavioral phenotype with normal social behavior including normal social interaction with both juvenile and adult partners, normal social preference in the 3-chamber test, and intact social recognition; however, the mice did demonstrate impaired nest building and enhanced self-grooming. Anxiety-like behaviors in the elevated plus maze, locomotor activity in the open field, and memory/learning in the MWM were reported to be normal. Interestingly, they demonstrated improved motor learning in a rotarod task. *Nrxn1α* mutant mice also demonstrated impaired PPI, a phenotype seen in schizophrenia patients and variably reported to be found in ASD patients as well (Etherton et al., 2009). It is critical to note that this study was conducted using 129/SvJ x C57BL/6J hybrid mice. A recent study of social behaviors of *Nrxn1α* KO mice on a backcrossed C57BL/6J background revealed enhanced sociability (both sexes) and preference for social novelty (females only) in the 3-chamber test, increased aggression in free interaction with adult and juvenile conspecifics (males only), as well as hypoactivity (females only) and anxiety-like behavior (males only) (Grayton et al., 2013). The reason for sex specific difference in various behaviors is interesting because a similar phenomenon, but not exactly the same direction, is well documented in human ASDs.

Assessment—To date, the ASD-relevant social and repetitive phenotypes of *Nrxn1α* have not been shown to be particularly robust or reproducible across laboratories or genetic backgrounds. These conflicting data emphasize the importance of these factors in altering possible both the expression of behavioral phenotypes as well as the sensitivity of behavioral testing paradigms. However, given the strong genetic evidence implicating *NRXN1* as an ASD causative gene, further experiments, such as examination of their ultrasonic vocalizations or other higher-order repetitive behaviors, are warranted in order to further validate this model before promoting its use for pre-clinical research into therapeutic interventions.

Neuroligin Family

Genetics—The neuroligin gene family (*NLGN1-4*) encodes post-synaptic cell-adhesion molecules, and like their pre-synaptic binding partners, the neurexins, are critical for synaptic function (Sudhof, 2008). A few studies have identified an enrichment of *NLGN1* variants in ASD patients relative to controls or rare *de novo* mutations in *NLGN1* identified only in single individuals with ASD (Glessner et al., 2009; O'Roak et al., 2012b; Ylisaukko-oja et al., 2005). Several studies have implicated mutations in *NLGN3* and *4* with ASDs, although incidence of these mutations is unknown but is thought to be quite low (Jamain et al., 2003; Laumonnier et al., 2004; Ylisaukko-oja et al., 2005; Yu et al., 2011).

Nlgn*1 mutant mice—Nlgn1* homozygous mice, targeting the first two coding exons (JAX 008136), demonstrated a mild ASD-related phenotype with reduced social approach to a caged conspecific, although their 3-chamber sociability and social novelty was normal, as was free interaction with a juvenile. They also demonstrated impaired nest-building and increased self-grooming, the latter being rescued by treatment with D-cycloserine, an NMDA receptor agonist. These mice also demonstrated altered sensitivity to pain (increased threshold) and heat (decreased threshold) stimuli although auditory stimuli were perceived normally, which may be related to the sensory processing abnormalities seen in ASD patients. As far as comorbidities go, these mice also demonstrated impaired spatial learning and memory in the MWM task, although their fear memory and learning was normal, with no increases in anxiety-like behavior in the open field, elevated plus maze, or light/dark test, normal activity levels in the open field, and normal motor performance on the rotarod (Blundell et al., 2010).

Nlgn2* mutant mice—Nlgn2* mutant mice, targeting the first coding exon (JAX 008139), have been tested for ASD-related behaviors and demonstrate abnormal communication with reduced number of pup USVs. However, tests of social behavior were normal as were tests of repetitive behavior. These mice did demonstrate several comorbid behaviors including delayed development (including growth parameters, eye opening, teeth eruption, and the acquisition of several reflexes), increased anxiety-like behavior, reduced activity and exploration, altered sensory sensitivity, and motor incoordination. However, it was noted that these phenotypes were not consistent across three different groups analyzing the mice emphasizing the importance of experimental conditions and genetic background once again (Blundell et al., 2009; Wohr et al., 2013).

Nlgn3* mutant mice—Nlgn3* homozygous mice, targeting exons 2–3 (JAX 008394), demonstrate a mild ASD-related phenotype with normal social interaction in pairs, normal sociability but reduced preference for social novelty in the 3-chamber test, reduced adult USVs, and no perseveration on the holeboard test or inflexibility on the MWM. They did demonstrate locomotor hyperactivity in the open field, but they had normal levels of anxiety-like behavior in the elevated plus maze, normal motor performance on the rotarod, and normal learning and memory in the MWM or (Radyushkin et al., 2009).

***Nlgn3* R451C mice—**In addition to the *Nlgn3* knockout (KO) mouse, a knock-in (KI) was designed to recapitulate one of the mutations, R451C (JAX 008475), found in human ASD patients. Interestingly, the KI but not the KO mice demonstrated impaired sociability and enhanced spatial learning reproduced across two studies (Jaramillo et al., 2014; Tabuchi et al., 2007), whereas sociability was normal but USVs were reduced, and developmental delays and impaired motor learning were observed in another study of an independently generated KI line (Chadman et al., 2008). The explanation for these discrepancies is not immediately clear and may be due to genetic background or experimental conditions.

***Nlgn4* mutant mice—**Mice lacking *Nlgn4* (from Dr. Ehrenreich at the Max Plank Institute, Germany) also demonstrate ASD-related behaviors including reduced social interaction in pairs, a very mild and gender-dependent difference in sociability and

preference for social novelty in the 3-chamber test, and impaired nest-building. They also exhibited reduced adult USVs, increased self-grooming, compulsive marble burying and repetitive circling, although they did not show inflexibility in the reversal phase of the MWM. These mice demonstrated no increases in anxiety-like behavior, activity, learning and memory, or motor coordination (El-Kordi et al., 2013; Jamain et al., 2008). However, when Ey et al. (2012) examined later generations of this same line of mice in the same genetic background, they failed to recapitulate the social or communication deficits, again highlighting the possible impacts of genetic drift and variable experimental or environmental conditions on behavioral phenotypes.

Assessment—While the incidence of individual *NLGN* mutations is probably rare, the occurrence of pathogenic mutations in multiple gene family members and ASD-related behavior observed when disrupting each gene in mice highlights the importance of this gene family in normal synaptic function, and warrants future study of shared pathophysiological mechanisms between *NLGN* mutations as well as other ASD-causative genes which interact with members of this gene family including the *NRXNs* and *SHANKs*. However, the reduced robustness and reproducibility of the ASD-related behaviors across each of the *Nlgn* mutant lines may weaken their utility for testing pharmacological interventions at this time. Further investigation to understand the discrepancies among different studies may be helpful.

Shank Family

Genetics—The *SHANK* gene family includes 3 members encoding similar master scaffold proteins Src homology 3 and multiple ankyrin repeat domains (SHANK1-3), all of which, in particular *SHANK3*, have been strongly implicated from human genetic studies to contribute to ASD occurrence, accounting for 1–2% of cases. Most notably, deletions of human chromosome 22q13.3 including the *SHANK3* locus results in a neurodevelopmental disorder known as Phelan-McDermid syndrome which includes severe autistic behavior in the majority of patients. Idiopathic cases of ASDs have also been associated with point mutations in or microdeletions of all *SHANK* family members (Jiang and Ehlers, 2013).

Shank1* mutant mice—Shank1* mutant mice, targeting exons 14–15 (JAX 008108), were the first to be reported, and several groups have published behavioral characterizations revealing mild or absent ASD-related phenotypes including normal sociability in the 3-chamber test as well as a juvenile play paradigm, reduced pup and adult USVs and abnormal scent-marking which may represent an important murine mode of communication, and normal levels of grooming; although, paradoxically, they demonstrated enhanced reversal learning in a radial maze. Behavioral tests for comorbidities revealed anxiety-like behavior in the open field and light/dark test, developmental delays, hypoactivity in the open field, impaired motor performance on the rotarod, impaired contextual fear memory, but intriguingly, enhanced spatial learning and memory in the radial maze (Hung et al., 2008; Silverman et al., 2011; Wohr et al., 2011).

***Shank2* mutant mice—**Two different lines of *Shank2* mice were generated, with one line targeting exons 6–7 of *Shank2* (Dr. Kim, KAIST, Korea). These mice demonstrated ASD-related behaviors including impaired sociability but normal social novelty in the 3-chamber

test, reduced adult USVs, context-specific increases in self-grooming and repetitive jumping behavior, in addition to comorbidities including anxiety-like behavior in the elevated plus maze, profound hyperactivity in the open field test, and impaired learning and memory in the MWM. Treatment with the NMDAR agonist, D-cycloserine, and with the mGluR positive allosteric modulator, CPDDB, which can also enhance NMDAR function, improved the sociability phenotype as well as other biochemical and electrophysiological abnormalities (Won et al., 2012). The other line also had a similar mutation targeting exon 7 (Dr. Boeckers, Ulm University, Germany), and remarkably given the difficulty in replicating behavioral phenotypes in the same line of mice let alone in a different line of mice in different experimental conditions, these mice also demonstrated ASD-related behaviors. They exhibited reduced social contact in free interaction paradigms, increased pup USVs and more simplistic adult USVs, and increased self-grooming, as well as anxiety-like behavior in the light/dark test and profound hyperactivity in the open field test, but normal learning in memory in novel object recognition (Schmeisser et al., 2012).

***Shank3* isoform-specific knockout mice—**Five groups have made six different lines of *Shank3* mutant mice by targeting different portion of exons. Due to the complex structure of *Shank3*'s transcripts, all of these lines result in an isoform-specific but not complete KO of *Shank3*. As the behavioral profiles differ from line to line, they will be described separately. First, exons 4–9 of *Shank3* (JAX 017890) were targeted by Bozdagi et al. (2010) and further characterized by Yang et al (2012), finding an overall mild ASD-related phenotype including normal sociability and social novelty in the 3-chamber test, mild impairment in juvenile or male-female pair interactions, normal pup USVs but reduced adult USVs, increased self-grooming and inflexibility in the MWM. Tests of comorbid behavior revealed no anxiety-like behavior in the elevated plus maze or light/dark test, slight hypoactivity in the open field, impaired motor performance on the rotarod, and impaired learning and memory in novel object recognition, although acquisition of the MWM and fear conditioning were normal. It was also reported that IGF-1 can rescue the impaired motor performance in these animals, but its effects on ASD-related behaviors remain to be discovered (Bozdagi et al., 2013). Wang et al. (2011) also targeted exons 4–9 (JAX 017442) using a slightly different design, and found many ASD-related behaviors including impaired sociability and social novelty in the three chamber, reduced social interaction in freely interacting pairs, abnormal adult USVs, and increased self-grooming, perseveration in the holeboard test, stereotypic investigation of novel objects, and inflexibility in the reversal phase of the MWM. They also showed comorbid behaviors such as impaired motor performance on the rotarod and impaired learning and memory in social transmission of food preference, the MWM, and novel object recognition, although the mice displayed normal levels of anxiety and locomotor activity. Peca et al. (2011) generated one line targeting exons 4–7 (Dr. Feng, Massachusetts Institute of Technology) and another targeting exon 13–16 (JAX 017688), and due to the more profound phenotypes in the latter mutant, they focused their studies on exon 13–16 mutants. They found impaired sociability and preference for social novelty, as well as reduced pair interaction, and profound self-grooming leading to skin lesions although normal flexibility in MWM reversal learning. As well, they observed increased anxiety in the elevated zero maze and light/dark test, but normal activity in the open field, normal motor performance on the rotarod, and normal

acquisition of the MWM. Communication behaviors were not assessed. Schmeisser et al. (2012) targeted exon 11 (Dr. Boeckers), but reported very minimal behavioral characterization of this line, although prominent self-grooming and skin lesions did occur. Lastly, the Worley lab targeted exon 21 of *Shank3* (JAX 018398), and a recent paper by Kouser et al. (2013) tested ASD-related behaviors and found normal sociability but impaired social novelty in the 3-chamber test, normal social interaction and recognition when paired with a juvenile, impaired nest building, normal adult USVs, increased self-grooming and impaired reversal learning of the MWM. In addition, they observed increased anxiety-like behavior in the light/dark test but not in elevated plus maze or open field, reduced activity when initially presented to the open field, impaired motor performance on the rotarod, and impaired learning and memory in the MWM. In addition to experimental conditions and background which have been demonstrated to contribute to conflicting behavioral data in many of the other mutant models previously described, the question of targeting mutation design could explain some of the phenotypic variance seen in these mice. *Shank3* has multiple intragenic promotors and alternatively spliced exons giving rise to great transcriptional complexity leading to many protein isoforms. As each mutant targeted only a subset of *Shank3*'s coding exons, it is possible that each mouse only lacks a subset of Shank3 protein isoforms which may have differential functions across brain regions, developmental time periods, or cell types.

Assessment—The strong genetic evidence implicating *SHANKs* in ASDs, plus the SHANK family proteins' interaction with multiple other putative ASD-causative genes, provide support for the value of *Shank* mutant mice to model human ASDs. While the number of *Shank* mutant mice makes summarizing these models concisely a challenge, it is remarkable that the myriad *Shank2* and *Shank3* mice with different exonic deletions, genetic backgrounds, and testing paradigms reveal robust and relatively consistent findings of aberrant social, communicative, repetitive, and comorbid behavioral domains. This underscores these models as important for future investigation of pathophysiological mechanisms underlying mutations in synaptic genes and corresponding behavioral manifestations. These models may provide tools for the identification of therapeutic targets which may extend to other cases of ASD involving synaptic protein dysfunction. These mice also exemplify the importance of the specific targeting mutation in genes with such transcriptional complexity like the *Shank* family, which may result in different isoform-specific knockouts with varying consequences on synaptic and behavioral phenotypes. This principle may well apply to many other neuronal proteins with similar or even increased levels of isoform complexity, such as the neurexins and neuroligins. However, in order to better understand the human condition, complete knockout, rather than isoform-specific knockout mice are warranted, as microdeletions of *SHANK* genes are the genetic defect responsible for greater than 95% of *SHANK*-causing ASDs. A comprehensive study to compare different lines of isoform-specific mutant mice with complete *Shank3* KO mice may provide insight for correlating genotype and phenotype in humans.

Models of Human Copy Number Variations (CNVs)

Copy Number Variations at human chromosome 15q11-q13

Genetics—Human chromosome 15q11-q13 is a genetic locus with multiple variants associated with distinct disease entities including maternal deletions causing Angelman Syndrome, paternal deletions causing Prader-Willi Syndrome, and increased copy numbers associated with sporadic autism. While Angelman and Prader-Willi syndromes have prominent neurobehavioral features including autistic behaviors, this overview will focus on the third category, as duplication or even triplication of 15q11-q13 of maternal origin is one of the most commonly (estimated prevalence of 1–4%) occurring cytogenetic abnormalities associated with ASDs (Depienne et al., 2009; Moreno-De-Luca et al., 2013). Patients carrying triplications of 15q11-q13 with maternal origin have nearly 100% penetrance of autistic phenotypes. In contrast, increased gene copy number of paternal origin is usually associated with much milder developmental delay (Depienne et al., 2009). There are more than 15 genes, including maternally, paternally, and biallelically expressed genes in the 15q11-q13 region. The brain-specific and maternally expressed *UBE3A* gene is the causative gene for Angelman syndrome. Because of the strong association of maternal origin of this duplication with ASD, it has been hypothesized that the Angelman *UBE3A* gene is a strong candidate gene in the region responsible for the molecular pathogenesis of ASD phenotypes.

Dp(7)15 duplication mice—As mouse chromosome 7 has a syntenic region with conservation of the genes, gene order, and imprinting pattern of the homologous human 15q11-q13 linkage group, mutant mice with a duplication of a 6 Mb region homologous to human 15q11-q13 chromosomal region (mice available from Dr. Takumi, Hiroshima University, Japan) by chromosomal engineering technique were made (Nakatani et al., 2009). Unexpectedly, mice carrying a paternal duplication, but not maternal duplication, demonstrated face validity in many of the ASD behavioral domains including reduced sociability in the 3-chamber test, aberrant number, quality, and developmental profile of pup and adult USVs, and reduced behavioral flexibility in reversal phases of the MWM and Barnes maze. In addition, they demonstrated increased anxiety-like behavior in both generalized fear following fear conditioning and reduced exploration of the open arms of an elevated plus maze (Nakatani et al., 2009).

***Ube3a* BAC transgenic mice—**Another group examined mice carrying duplications or triplications of the gene *Ube3a* by BAC (bacterial artificial chromosome) mediated transgenic mice (mice available from Dr. Anderson, Harvard University) to examine the sufficiency of increasing this gene's copy number to cause ASD-related behaviors (Smith et al., 2011). Indeed, mice carrying two additional transgenic copies of *Ube3a* demonstrated reduced sociability in the 3-chamber test, reduced adult USVs but normal pup USVs, and increased self-grooming without any abnormalities in activity or anxiety levels in the open field, motor performance on the rotarod, or cognition in a novel object recognition task. Mice with only one additional copy of *Ube3a* showed milder or normal behavior on ASD-related tests.

Assessment—As the 15q11-q13 locus demonstrates both relatively frequent prevalence and high penetrance for causing ASDs, these two lines of model mice represent excellent opportunities for exploring ASD mechanisms and pre-clinical tests of novel therapeutics. While the Dp(7)15 mice capture the genetic construct more precisely, by disrupting a similar number of genes as those found in most CNVs of human locus 15q11-q13, the opposite pattern of parental origin effect on this duplication between humans and rodents remains unexplained because other molecular features including parental origin expression of many genes in the region are highly conserved between humans and mice. The *Ube3a* triple copy mice offer the unique opportunity to examine whether dosage of the *UBE3A* gene alone is responsible for neurobehavioral phenotypes of this disorder, and similar work targeting other possible causative genes within the 15q11-q13 locus should be conducted. For both of these lines of mice, efforts to reproduce these behavioral findings across laboratories or genetic backgrounds, determine the pathophysiological mechanisms underlying the behavioral phenotypes, and test sensitivity to pharmacological, genetic, or environmental interventions are necessary.

Copy Number Variations at human chromosome 16p11.2

Genetics—Deletions and duplications at the 16p11.2 locus were first identified in ~1% of idiopathic ASD from genome-wide CNV analysis. Subsequently, these same CNVs have also been associated with a wide range of clinical presentation including ID, seizures, developmental delay, and obesity (Miller et al., 2011; Moreno-De-Luca et al., 2013). Similar to other CNVs associated with disorders, there are more than 20 genes mapped within the deleted or duplicated interval at 16p11.2 region. It is unclear which gene or genes are responsible for the major ASD-related clinical features.

Df(7)16 and Dp(7)16 mice—Taking advantage of the syntenic region on mouse chromosome 7, Horev et al. (2011) created mice carrying either a deletion (JAX 013128, **Df(7)16**) or the reciprocal duplication (JAX 013129, **Dp(7)16**) homologous to the human 16p11.2 region, and found some reciprocal behavioral phenotypes for these respective CNVs. Despite the strong genetic association of this locus in human ASD, both deletion and duplication mice showed no or relatively mild ASD-like behaviors, although behavioral phenotyping was limited to the 3-chamber test and observation in a novel, automated home cage environment. Df(7)16 mice demonstrated normal sociability and preference for social novelty in the 3-chamber test, but they did demonstrate prominent stereotypies in climbing behaviors in a modified home cage environment. While the deletion mice had impaired post-natal survival, those animals surviving weaning appeared grossly normal but demonstrated hyperactivity when introduced to a novel cage. Dp(7)16 mice demonstrated normal sociability and preference for social novelty in the 3-chamber test and no prominent stereotypies. The duplication mice did not have any viability problems, but they demonstrated hypoactivity in the modified home cage environment suggesting that the deletion and reciprocal duplication have opposite effects on some behavioral phenotypes.

Assessment—Given the prevalence of 16p11.2 CNVs and their frequent association with ASD in genetic studies, the 16p11.2 deletion and duplication mice clearly have strong molecular construct validity. However, these model mice are as of yet not validated across

the ASD-relevant behavioral domains, and their utility as pre-clinical models hinges on whether and how robustly they recapitulate social, communicative, and repetitive behaviors. In addition, identification of the dose-sensitive genes within this locus, and why both duplications and deletions can manifest in ASD diagnoses, remain critical areas of future study.

Potocki-Lupski Syndrome or human chromosome 17p11.2 duplication

Genetic Evidence—Like the genetic locus at human chromosome 15q11-13, reciprocal CNVs at human chromosome 17p11.2 have been associated with neurobehavioral syndromes, in particular, with deletions and duplications of 17p11.2 causing Smith-Magenis (SMS) and Potocki-Lupski syndromes (PLS), respectively, with the latter characterized by ASD in 80% of patients, developmental delay, ID, hypotonia, failure-to-thrive, and congenital cardiovascular abnormalities (Potocki et al., 2007). This region includes the dosage-sensitive gene, Retinoic Acid Inducible 1 (*RAI1*) which likely is responsible for many aspects of the phenotype, because point mutations in *RAI1* gene display major features of SMS. However, duplications of isolated *RAI1* have not been identified in PLS (Potocki et al., 2007).

Dp(11)17 Mice—Mice share a syntenic region to human chromosome 17 on murine chromosome 11 allowing for the construction of mouse models (available from Dr. Lupski, Baylor College of Medicine) by chromosomal engineering for this disorder with good molecular construct validity (Walz et al., 2003). Duplication of 17p11.2 (**Dp(11)17**) mice have demonstrated ASD relevant behaviors including reduced sociability and preference for social novelty in the 3-chamber test, enhanced social dominance in a tube test, enhanced aggression and reduced habituation in social cognition tests, altered developmental time course of pup USVs, and perseverative exploration of the holeboard (Lacaria et al., 2012; Molina et al., 2008; Ricard et al., 2010). Comorbid behaviors include anxiety-like behavior in the open field and elevated plus maze, hyperactivity in the open field, motor impairments on the rotarod, and impaired contextual fear memory (Lacaria et al., 2012; Molina et al., 2008; Walz et al., 2006). Rearing Dp(11)17 mice in an enriched environment rescued a subset of behaviors including aberrant social interaction, anxiety-like behavior, and impaired contextual fear memory, but exacerbated others such as perseverative holeboard exploration (Lacaria et al., 2012).

Assessment—The high penetrance of ASD behavior in Potocki-Lupski Syndrome patients together with the many ASD-related behavioral abnormalities in this mouse model support its use to investigate the pathophysiology of this CNV. As a series of studies of these mice have produced ASD-relevant findings, the reproducibility and robustness of this model's behavioral phenotype, as well as its amenability to an environmental intervention, makes it one of the stronger models of CNV-causing ASDs. Further work should be focused to explore which genes within the region represent dosage-sensitive targets for mediating neurobehavioral phenotypes, and whether pharmacological interventions can be identified to target these genes.

Non-genetic models: the example of BTBR T+*tff* J mice

In addition to the genetic engineering strategy for constructing mouse mutants recapitulating human mutations, many other mouse models for ASDs have been developed by examining behavioral variability between inbred mouse strains or by manipulating the environmental or toxicological exposures of non-genetic models. While this overview does not aim to cover the full spectrum of non-genetic models, attention is drawn to one of the best studied models of ASD-related behavior in an inbred strain of mice, the BTBR T+*tff* J mice (JAX 002282), as they compare to C57BL/6J mice, the most commonly used strain of mice for neurobehavioral studies. Extensive behavioral testing on this strain of mice has been conducted, and Meyza et al. (2013) reviews it in greater detail. As for selected ASD-related phenotypes, BTBR male mice regularly show a lack of sociability and preference for social novelty in the 3-chamber task (although females show more variable phenotypes), and both adult and juvenile BTBR mice also demonstrate reduced social interaction in pairs. USVs represent a more complicated picture, as isolated BTBR pups call more loudly and more frequently than pups of other inbred strains. However, adult male BTBR mice emit fewer USVs, with reduced complexity of the call structure, and they display reduced scent-marking behavior. BTBR mice also demonstrate increased self-grooming, perseverative marble burying, stereotyped object exploration, and impaired reversal learning on the MWM. When tested for comorbid behaviors, they demonstrate inconsistent performance in tests of anxiety-like behavior, hyperactivity in the open field, impaired motor performance on the rotarod, and normal learning and memory in the MWM acquisition phase. Many pharmacological and non-pharmacological interventions have been tested, and highlighted results include reduced self-grooming but unchanged sociability with MPEP (mGluR5 antagonist), GRN-529 (selective negative modulator of mGluR5) or risperidone (D2 antagonist) treatment; improved sociability and reduced self-grooming with D-cycloserine (NMDA receptor agonist) treatment; and improved sociability with rapamycin (mTOR pathway inhibitor) treatment (Burket et al., 2013; Burket et al., 2014; Silverman et al., 2010a). Interestingly, these drugs have had positive effects in other genetic mouse models of ASDs.

Assessment

While not originally bred with ASD-phenotypes in mind, many strains of inbred mice demonstrate variable behavioral phenotypes, including prominent ASD-related phenotypes in the BTBR strain. While these models do not recapitulate known genetic causes of ASDs in humans, and thus caution is warranted about how well findings from these mice will translate from pre-clinical studies to effective therapies before further genetic evidence emerges. Studies underway will possibly identify novel genetic variants in these mice which contribute to the development of ASD-related behaviors, with the hope of using this knowledge to better understand the causes of human autism. They also offer a unique opportunity to test pharmacological interventions discovered in genetically engineered ASD models to determine whether novel therapeutics can ameliorate ASD-related behaviors across models, and thus support shared pathophysiological mechanisms uniting these diverse etiologies.

Conclusions

This overview has highlighted the common genetic models of ASDs with the strongest human genetic and murine behavioral evidence to support their use for developing hypotheses about underlying pathogenic mechanisms and testing therapeutic interventions. The considerable molecular and clinical heterogeneity of ASDs in humans make it harder to generalize the knowledge learned from individual animal models. The challenging but urgent question is whether some shared molecular and circuit mechanisms can be defined in near future. Before that, selection of the most appropriate mouse model of ASDs depends on both the strength of the construct and face validity, but also the specific research question being asked and specific patient population to be targeted. In those models with the longest history of use or largest number of genetically engineered mice targeting specific gene families, there is more known about the robustness and reproducibility of behavioral findings, the underlying cellular and synaptic pathophysiology, as well as the sensitivity of these models to amelioration with pharmacological and environmental rescues. This may also emphasize the need for replication of preliminary findings and continued refinement of hypotheses about how a given mutation leads to the observed behaviors for newly developed models.

There also exist many opportunities for the creation and validation of other mouse models, as recent genetic studies have provided strong evidence for other ASD-causative genes such as *CHD8* or *MET*, although whether mice harboring these mutations recapitulate autistic phenotypes and prove to have usefulness and predictive validity remains to be seen. Likewise, there are existing mouse models with good face validity across the three domains of ASD-related behavior, such as *Reelin* or *Cadps2* mutant mice. However, pathological mutations of these genes have not been found in ASD patient populations in recent whole genome sequencing projects.

Modeling human ASDs in mice remains a significant challenge. The lack of reliable biomarkers or neuropathological hallmarks in human ASD is the major obstacle to dissecting the pathophysiology using these genetically engineered ASD mouse models. The current analytic paradigm for ASD mouse models is primarily focused on the analysis of behavioral impairments and defects in synaptic function. As illustrated by many models in this review, significant variability of behaviors occurs between different models and the sensitivity of behavioral assays poses many challenging questions for the translational value of these models in pre-clinical trials. Similarly, but not reviewed here, synaptic function studies in these models have also uncovered a wide range of synapse-specific or model-specific defects associated with a given genotype. These observations demand new analytic paradigms to better model human ASDs using genetically engineered mice.

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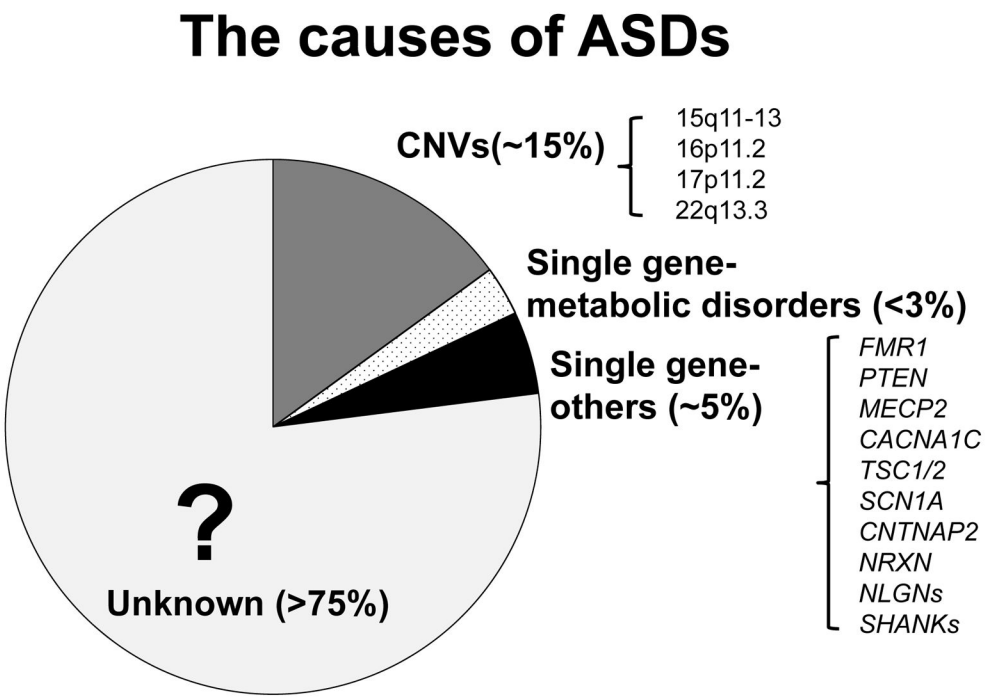

Figure 1. The causes of ASDs

The cause for the majority (>75%) of ASD cases is unknown. Human genetics research and clinical studies have implicated chromosomal copy number variations (CNVs) in ~15% and single gene mutations in ~8% of ASD cases. Recessive mutations in single gene resulting in metabolic disorders account for a small number (<3%) of ASD patients. Mutations in non-metabolic disease related genes, frequently *de novo* mutations in synaptic genes, account ~5% of ASD cases. Both CNVs and single gene mutations have been associated with syndromic and non-syndromic forms of ASDs.

Table 1

Summary of ASD-Related Behavioral Tests

Test	Description
Social Behavior	
3-Chamber Social Test	Test mouse explores partitioned arena with neutral center chamber and side chambers containing stimuli; sociability phase measures time spent in chamber containing caged mouse versus chamber containing empty cage; social novelty phase measures time spent in chamber containing novel mouse versus chamber containing familiar mouse
Partition Test	Test mouse explores one half of partitioned arena with novel mouse on the other side of the barrier; time spent near the partition is a measure of social approach
Habituation-Dishabituation	Test mouse is repeatedly introduced to stimulus mouse; decreasing time spent in social interaction represents social recognition of stimulus mouse; then novel mouse can be introduced as stimulus mouse and increased time spent in social interaction represents social recognition
Direct Interaction Tests	Test mouse freely interacts with stimulus mouse and number, duration, and types of social interactions measured; variations include neutral pairing, i.e. stimulus is ovariectomized female or juvenile male, or agonistic pairing, i.e. stimulus is adult males in novel arena social (social dyadic) or test mouse's home cage (resident intruder)
Juvenile Play	Pairs of juvenile mice allowed to freely interact; number, duration, and types of play behaviors can be measured
Nest Building	Test mouse is given pressed cotton material; amount of material shredded and arranged into nest is a measure of nest construction ability
Communication Behavior	
Pup Ultrasonic Vocalizations	Test mouse pup is briefly separated from dam and littermates; number, duration, and types of ultrasonic isolation calls recorded
Adult Ultrasonic Vocalizations	Test male mouse introduced to reproductive setting, i.e. estrus female or female urine, or agonistic setting, i.e. another male mouse (such as in resident-intruder paradigm); number, duration, and types of ultrasonic calls recorded
Scent-Marking	Test male mouse exposed to urine from estrus females; amount and proximity of male urine scent-marks to female urine stimuli is a measure of scent-marking communication behavior
Repetitive/Restricted Behavior	
Repetitive Grooming	Test mouse is observed for self-grooming in home cage or novel environments; duration and bouts of grooming are measures of repetitive behavior; skin lesions are measures of self-injurious behavior
Marble Burying	Test mouse introduced to cage containing marbles atop bedding; number of marbles buried is a measure of compulsive digging and bedding sifting behavior
Holeboard Exploration	Test mouse explores an arena with grid pattern of circular holes in floor allowing for head-dips into the holes; number and pattern of holes explored is a measure of perseverative exploration
Maze Reversal Learning	Test mouse must learn new location of hidden platform or escape hole in spatial mazes (see below); ability to learn new location is a measure of cognitive flexibility
Frequent Comorbidities	
Elevated Zero or Plus Mazes	Test mouse explores annular or cross-shaped arena with closed and open arms; time spent in open arms is a measure of anxiety-like behavior

Table 2

Summary of ASD Mouse Models

Model	Social Interaction	Communication	Repetitive	Comorbidities	Interventions	Source
Single-Gene Syndromic Models						
Fmr1 (−/Y)	± sociability − social novelty ↑social anxiety	↑pup calls	↑stereotypies ↑perseveration	±anxiety ↑activity ↓learning/memory	enrichment ↓ mGluR	JAX 004634 (FVB) JAX 003025 (B6)
Pten (cKO)	↓sociability ↓social novelty ↓social recognition	not tested	↑self-grooming	↑anxiety ↑activity ↓learning/memory ↑seizures	rapamycin	JAX 006440 (B6)
Mecp2$^{tm1.1Jae}$ (exon 3)	↑sociability ↑social novelty	↑pup calls	not tested	↓anxiety ↓activity ↓motor skills ↓learning/memory	enrichment Ampakine CX546 choline diet IGF-1	MMRRC 000415 (B6) UC Davis
Mecp2^{tm1Hzo} (308X)	−sociability −social novelty ↓pair interaction	↓pup calls	↑self-grooming	↑anxiety ↓activity ↓motor skills ↓learning/memory	none tested	JAX 005439 (B6)
Mecp2$^{tm1.1Joez}$ (T158A)	not tested	not tested	not tested	↓anxiety ↓activity ↓motor skills ↓learning/memory	none tested	JAX 017741 (B6)
Cacna1c (Ts2-Neo KI)	↓sociability −recognition	↓pup calls	↑self-grooming ↑inflexibility ↑perseveration	−anxiety ↓activity ↑learning/memory	none tested	JAX 019547 (B6)
Tsc1 (+/−)	↓pair interaction	not tested	not tested	↓anxiety (♂) −activity −motor skills ↓learning/memory	rapamycin	Dr. Cheadle of Cardiff University Dr. Kobayashi of Juntendo University
Tsc1 (cKO)	↓sociability ↓social novelty	↑pup calls	↑self-grooming ↑inflexibility	−activity ↓motor skills	rapamycin	JAX 005680 (B6)
Tsc2 (+/−)	↓sociability ↓social novelty (♂) ↓pair interaction (♀)	↑pup calls	−inflexibility −perseveration	−anxiety −activity ↓motor skills ↓learning/memory	rapamycin lovastatin	Dr. Kobayashi of Juntendo University JAX 004686 (B6)
Tsc2 (cKO)	↓sociability ↓social novelty	not tested	↑perseveration −inflexibility	±anxiety ↓motor skills	rapamycin	Dr. Gambello of University of Texas
Scn1a (+/−)	↓sociability ↓social novelty	not tested	↑self-grooming ↑circling	↑anxiety ↑activity ↓learning/memory	clonazepam	Dr. Catterall of University of Washington
Single-Gene Non-syndromic Models						

Model	Social Interaction	Communication	Repetitive	Comorbidities	Interventions	Source
Cntnap2 (−/−)	↓sociability ↓juvenile play	↓pup calls	↑self-grooming ↑inflexibility	↑activity ↑seizures	risperidone	JAX 017482 (B6)
*Nrxn1*α (−/−)	± sociability ± social novelty −recognition	not tested	±self-grooming	± anxiety ± activity ↑motor skills −learning/memory	none tested	JAX 006377 (B6-129)
Nlgn1 (−/−)	↓sociability −social novelty	not tested	↑self-grooming	−anxiety −activity −motor skills ↓learning/memory	D-cycloserine	JAX 008236 (B6-129)
Nlgn2 (−/−)	−sociability	↓pup calls	−self-grooming	↑anxiety ↓development ↓activity ↓motor skills	none tested	JAX 008139 (B6)
Nlgn3 (−/−)	−sociability ↓social novelty	↓adult calls	−perseveration −inflexibility	−anxiety ↑activity −motor skills −learning/memory	none tested	JAX 008394 (B6-129)
Nlgn3 (R451C KI)	±sociability	↓pup calls	−perseveration	−anxiety ↓development −activity ↓motor skills ±learning/memory	none tested	JAX 008475 (B6)
Nlgn4 (−/−)	±sociability ±social novelty −juvenile play	−pup calls ±adult calls	±self-grooming ↑perseveration ↑stereotypies −inflexibility	−anxiety −development −activity −motor skills −learning/memory	none tested	Dr. Ehrenreich of the Max Planck Institute
Shank1 (−/−)	−sociability −juvenile play	↓pup calls ↓adult calls	−self grooming −inflexibility	↑anxiety ↓development ↓activity ↓motor skills ±learning/memory	none tested	JAX 008108 (B6)
Shank2 (ex6–7)	↓sociability −social novelty	↓pup calls	↑self-grooming ↑jumping	↑anxiety ↑activity ↓learning/memory	D-cycloserine CPDDB	Dr. Kim of KAIST, South Korea
Shank2 (ex7)	↓pair interaction	↓pup calls ↓adult calls	↑self-grooming	↑anxiety ↑activity −learning/memory	none tested	Dr. Boeckers of Ulm University
Shank3 (ex4–9^{B})	−sociability −social novelty ↓pair interaction	−pup calls ↓adult calls	↑self-grooming ↑inflexibility	−anxiety ↓activity ↓motor skills ±learning/memory	IGF-1	JAX 017890 (B6)

Model	Social Interaction	Communication	Repetitive	Comorbidities	Interventions	Source
Shank3 (ex4–9[J])	↓sociability ↓social novelty ↓pair interaction	↓adult calls (♀) ↑adult calls (♂)	↑self-grooming ↑inflexibility ↑stereotypy ↑perseveration	−anxiety −activity ↓motor skills ↓learning/memory	none tested	JAX 017442 (B6)
Shank3 (ex4–7)	−sociability ↓social novelty	not tested	−self-grooming	−anxiety	none tested	Dr. Feng of Massachusetts Institute of Technology
Shank3 (ex13–16)	↓sociability ↓social novelty ↓pair interaction	not tested	↑self-grooming −inflexibility	↑anxiety −activity −motor skills −learning/memory	none tested	JAX 017688 (B6)
Shank3 (ex11)	not tested	not tested	↑self-grooming	not tested	none tested	Dr. Boeckers of Ulm University
Shank3 (ex21)	−sociability ↓social novelty −pair interaction −social recognition	−adult calls	↑self-grooming ↑inflexibility	±anxiety ↓activity ↓motor skills ↓learning/memory	none tested	JAX 018398 (B6)
Copy Number Variation Models						
15q11-13dup (Dp(7)15)	↓sociability	↑pup calls ↓adult calls	↑inflexibility	↑anxiety ↓activity	none tested	Dr. Takumi of Hiroshima University
Ube3a BAC (triple copy)	↓sociability	−pup calls ↓adult calls	↑self-grooming	−anxiety −activity −motor skills	none tested	Dr. Anderson of Harvard University
16p11.2del (Df(7)16)	−sociability −social novelty	not tested	↑stereotypies	↑activity ↓survival	none tested	JAX 013128 (B6-129)
16p11.2dup (Dp(7)16)	−sociability −social novelty	not tested	−stereotypies	↓activity −survival	none tested	JAX 013129 (B6-129)
17p11.2dup (Dp(11)17)	↓sociability ↓social novelty	↓pup calls	↑perseveration	↑anxiety ↑activity ↓motor skills ↓learning/memory	enrichment	Dr. Lupski of Baylor College
Non-genetic Models						
BTBR	↓sociability (♂) ↓social novelty ↓pair interaction	↑pup calls ↓adult calls	↑self-grooming ↑inflexibility ↑stereotypy ↑perseveration	↑anxiety −activity ↓motor skills −learning/memory	D-cycloserine MPEP rapamycin risperidone	JAX 002282

Key:

↑ is increased and ↓ is decreased; − is no change; ± is conflicting reports; ♂ is male; ♀ is female; −/− is homozygous for null allele; +/− is heterozygous for null allele; cKO is conditional knockout; KI is knock-in; JAX is Jackson Laboratory Mouse Supplier; MMRRC is Mutant Mouse Regional Resource Center; for strains of mice: B6 is C57BL/6J; FVB is FVB/NJ; 129 is 129SV