

## Review

# Behavioral phenotypes of genetic mouse models of autism

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**More than a hundred *de novo* single gene mutations and copy-number variants have been implicated in autism, each occurring in a small subset of cases. Mutant mouse models with syntenic mutations offer research tools to gain an understanding of the role of each gene in modulating biological and behavioral phenotypes relevant to autism. Knockout, knockin and transgenic mice incorporating risk gene mutations detected in autism spectrum disorder and comorbid neurodevelopmental disorders are now widely available. At present, autism spectrum disorder is diagnosed solely by behavioral criteria. We developed a constellation of mouse behavioral assays designed to maximize face validity to the types of social deficits and repetitive behaviors that are central to an autism diagnosis. Mouse behavioral assays for associated symptoms of autism, which include cognitive inflexibility, anxiety, hyperactivity, and unusual reactivity to sensory stimuli, are frequently included in the phenotypic analyses. Over the past 10 years, we and many other laboratories around the world have employed these and additional behavioral tests to phenotype a large number of mutant mouse models of autism. In this review, we highlight mouse models with mutations in genes that have been identified as risk genes for autism, which work through synaptic mechanisms and through the mTOR signaling pathway. Robust, replicated autism-relevant behavioral outcomes in a genetic mouse model lend credence to a causal role for specific gene contributions and downstream biological mechanisms in the etiology of autism.**

Keywords: Anxiety-like, autism, cognition, genes, hyperactivity, mice, mutant models, neurodevelopmental, repetitive behavior, sensory reactivity, sociability, social behavior, vocalizations

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Autism spectrum disorder (ASD) is a neurodevelopmental syndrome with a prevalence of over 1% of the population (CDC 2014; Elsabbagh *et al.* 2012; Kim *et al.* 2011). Diagnosis by the current Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria is based on two categories of behavioral symptoms: (1) unusual reciprocal social interactions and impaired social communication; and (2) stereotyped and repetitive patterns of behaviors, with restricted interests and activities (American Psychiatric Association 2013; Lord & Bishop 2015). Associated symptoms, which are present in subsets of individuals with ASD, include intellectual disabilities, anxiety, seizures, hyperactivity, sleep disruption and unusual reactivity to sensory stimuli. Early behavioral interventions are the current standard of care and offer the best long-term outcomes at present (Lord & Jones 2013; Rogers *et al.* 2012). Intensive behavioral intervention is highly effective in teaching young children to improve their social skills and redirect their repetitive behaviors. However, even the best behavioral therapies do not work for all, are expensive, are labor- and time-intensive and are not available in many geographic regions. Understanding the causes of autism is the first step in the development of effective medical therapeutics to improve symptoms and reverse the disorder's trajectory.

Hypotheses about the genetic causes of ASD originally arose from observations of a male–female bias, 4:1 or greater, and high concordance between identical twins, 50–90%, as compared to less than 10% for non-identical twins and siblings (Fombonne 2009; Hallmayer *et al.* 2011; Miles 2011; Nordenbaek *et al.* 2014; Ritvo *et al.* 1985; Sandin *et al.* 2014; Smalley *et al.* 1988). Intensive searches for the genes causing ASD employed genome-wide association approaches in the early 2000s, progressed to analyses of copy-number variants (CNVs), and are now proceeding with exome and whole genome sequencing in thousands of individuals. Early findings quickly revealed that ASD is not a monogenic disorder. In contrast to disorders such as Huntington's disease and Fragile X syndrome, there is no one specific gene responsible for all cases of autism. Rather, a growing number of *de novo* single gene mutations and CNVs have been identified in people with autism (Alarcon *et al.* 2008; Bucan *et al.* 2009; Butler *et al.* 2005; Buxbaum *et al.* 2007; Cook & Scherer 2008; Crepel *et al.* 2014; Glessner *et al.* 2009; Iossifov *et al.* 2014; Krumm *et al.* 2015; Kumar *et al.* 2009; Lawson-Yuen *et al.* 2008; Leblond *et al.* 2014; Michaelson *et al.* 2012; Morrow 2010; Neale *et al.* 2012; O'Roak *et al.* 2011; Pinto *et al.* 2010; Szatmari *et al.* 2007; Vernes *et al.* 2008; Wang *et al.* 2009; Yuen *et al.* 2015). Mutations in

common gene variants and *de novo* coding mutations may be responsible for up to 50% of ASD cases (Gaugler *et al.* 2014; Iossifov *et al.* 2014; Miles 2011). Over 100 risk genes and CNVs for ASD have been published, each one appearing in only a relatively small number of individuals (Butler *et al.* 2005; Coe *et al.* 2014; De Rubeis *et al.* 2014; Gaugler *et al.* 2014; Iossifov *et al.* 2014; Li *et al.* 2014; Parikshak *et al.* 2013; Pinto *et al.* 2014; Willsey & State 2015). Epigenetic risk factors have been implicated in ASD, including chromatin remodeling and methylation mechanisms, such as *CHD8* (Bernier *et al.* 2014; Cotney *et al.* 2015; O’Roak *et al.* 2012; Wilkinson *et al.* 2015), *HDAC* (Foley *et al.* 2012; Moldrich *et al.* 2013) and *MECP2* (Shibayama *et al.* 2004; Theoharides *et al.* 2015). Further, environmental risk factors, such as parental age (Kong *et al.* 2012) and atypical maternal autoantibodies (Braunschweig *et al.* 2013; Brimberg *et al.* 2013; Diamond *et al.* 2013; Piras *et al.* 2014), are associated with a higher incidence of ASD.

One of the most intriguing aspects regarding the genetics of ASD is the enigma of how these many risk factors converge to result in the same general cluster of symptoms diagnosed as ASD. One possibility is that there are underlying convergent downstream mechanisms which contribute to ASD symptomatology. No definitive biomarkers have yet been identified across all diagnosed cases. Rather, subsets of biological factors may define subgroups of individuals with ASD. Stratification by subgroup, either by behavioral category or biomarker, may offer the best strategy for focused clinical trials. Intensive searches are underway to define abnormalities in neurophysiology, neuroanatomy, brain chemistry, immune markers and other key biological systems (Ecker *et al.* 2013; Jeste & Geschwind 2014; Levitt & Veenstra-VanderWeele 2015). High heterogeneity of symptoms across cases suggests that autism is actually multiple disorders, analogous to the plural concept of ‘cancers’, with different genetic etiologies and biological defects, to be treated with different classes of therapeutics. The concept of ‘autisms’ is implicit in the current use of the term ASD, implemented in the 2013 edition of the DSM-5.

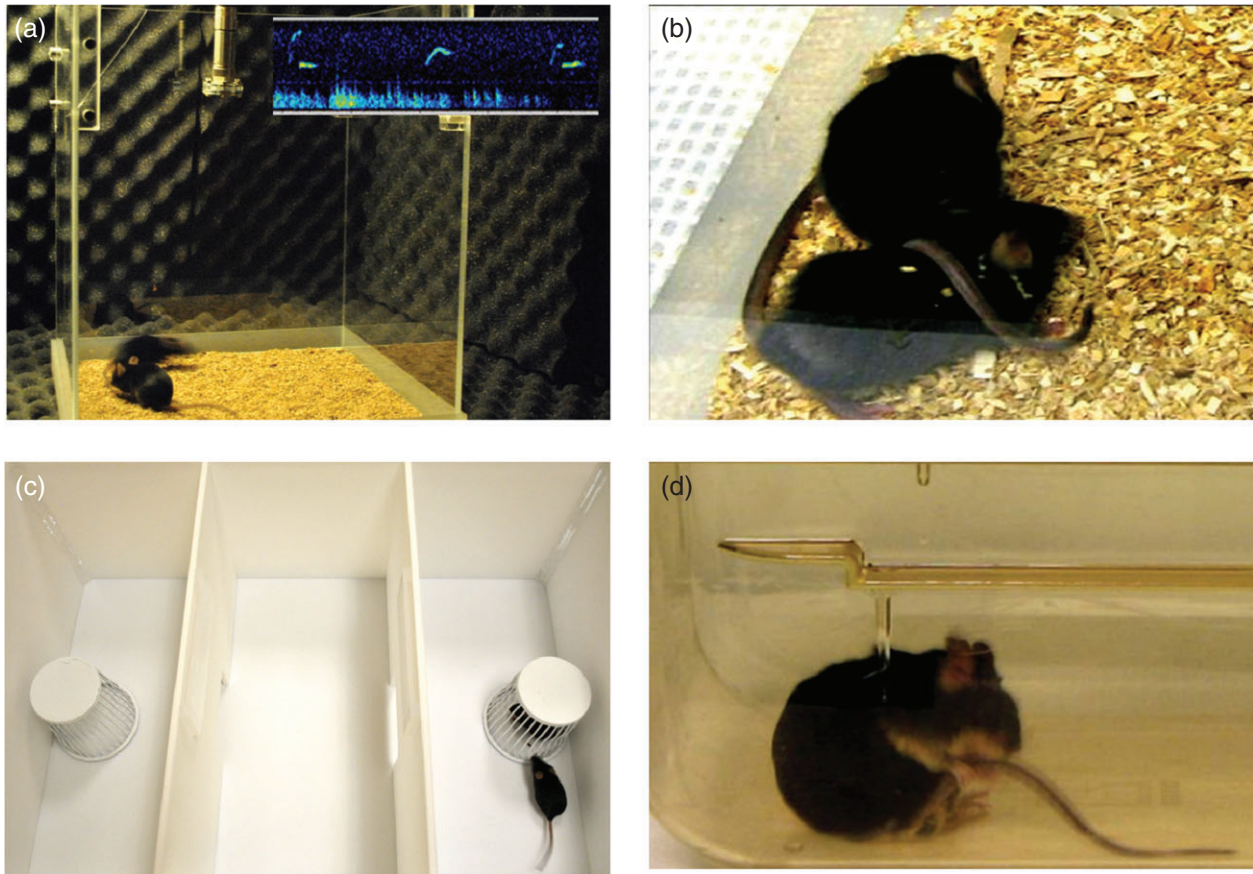
Readers of *Genes, Brain and Behavior* are well aware of methods to interrogate genetic hypotheses of human disorders by targeting the homologous mutation in another species and then explicating the consequent phenotypic outcomes. Knockout (KO) and humanized knockin mice, and more recently KO rats, have been generated for many of the single gene mutations and CNVs that were identified in ASD populations and for comorbid neurodevelopmental disorders such as Fragile X and tuberous sclerosis (TSC) (Baudouin *et al.* 2012; Ey *et al.* 2011; Silverman *et al.* 2010a; Zoghbi & Bear 2012). Some of these mutant mouse models are now being employed in preclinical testing of pharmacological targets to treat the core symptoms of ASD (Silverman & Crawley 2014; Spooren *et al.* 2012; Vorstman *et al.* 2014).

As genetic mouse models emerged, our behavioral neuroscience laboratory invested in methods development to design mouse behavioral assays with high relevance to the diagnostic symptoms of autism (Crawley 2004). Because the clinical phenotype of this uniquely human disorder is complex and heterogeneous, we initiated discussions with

autism clinical experts, to understand the critical symptoms that could be most meaningfully modeled in mice. Clinical researchers, including colleagues at the University of California Davis MIND Institute, Weill Cornell Medical College, University of North Carolina, University of Washington, University College London and the National Institute of Mental Health Intramural Research Program, kindly allowed us to observe diagnostic interviews and watch videotapes of children with ASD. Knowledge gained through these sessions, and from lectures and conversations with many other generous colleagues working with children, adolescents and adults with ASD, guided our thinking in the development of mouse behavioral assays that dovetail with the natural behavioral repertoire of mice. Considering the types of social approach abnormalities and inappropriate social behaviors that are common in ASD, we developed a mouse 3-chambered social approach assay (Moy *et al.* 2004; Nadler *et al.* 2004), refined methods for scoring reciprocal social interactions in juvenile and adult mice (McFarlane *et al.* 2008), adapted measures for the detection of responses to social olfactory cues (Yang & Crawley 2009), and developed call categories for ultrasonic vocalizations emitted in response to social cues during reciprocal social interactions (Scattoni *et al.* 2008, 2011). Further, we established observational scoring methods to quantify motor stereotypies and repetitive behaviors, such as self-grooming and digging, along with assembling a set of established behavioral assays relevant to anxiety, intellectual impairment, hyperactivity and sensory reactivity (McFarlane *et al.* 2008; Moy *et al.* 2008a,b; Rouillet & Crawley 2011; Silverman *et al.* 2010a,b, 2012, 2013, 2015; Wöhr *et al.* 2011a; Yang *et al.* 2011, 2012a, 2015), which are now widely used. A small subset of these assays is illustrated in Figure 1. This review presents examples and summaries of ASD-relevant phenotypes discovered by our lab and many other excellent behavioral genetics labs, revealing the phenotypic consequences of targeting mutations in ASD risk genes.

A remarkable number of risk genes for ASD code for synaptic proteins. Cell adhesion proteins, including contactin-associated proteins, neuroligins and neuroligins connect dendrites with axons to promote synapse formation. Postsynaptic scaffolding proteins, such as shanks and neuroligins, strengthen synapses and maintain synaptic transmission. Postsynaptic receptors, such as NMDA and metabotropic glutamate receptors, GABA receptors of varying subunit compositions, serotonin transporter and receptor subtypes, and oxytocin receptors, mediate excitatory and inhibitory synaptic signals. Sodium channels, potassium channels and downstream signaling pathways, such as the PTEN/PI3 kinase/Akt/mTOR pathway, mediate postsynaptic events and critical cellular functions. Mutations and common variants of the genes for these proteins have been identified in small numbers of individuals with autism and related disorders (Butler *et al.* 2005; Cheah *et al.* 2013; Frazier *et al.* 2014; Han *et al.* 2012; Krey *et al.* 2013; Rosander & Hallbook 2015; Tavassoli *et al.* 2014; Veenstra-VanderWeele *et al.* 2012; Weiss *et al.* 2003).

Mice with targeted mutations in many of these genes were generated by outstanding molecular genetics laboratories and generously donated to public repositories such as



**Figure 1: Examples of rodent behavioral assays with face validity to the diagnostic symptoms of autism.** (a) Two mice interacting in a Noldus Phenotyper 3000 reciprocal social interaction chamber equipped with an Avisoft ultrasonic microphone. The inset shows representative ultrasonic vocalizations recorded during adult male–female social interaction. (b) Close-up of two mice displaying ‘crawl over and under’ during the reciprocal social interaction test session. (c) 3-Chambered social approach apparatus offers automated scoring of time spent with a novel social partner vs. time spent with a novel object. (d) BTBR mouse engaged in repetitive self-grooming. Photos by Jane Hayes, Michael Pride, Jill Silverman and Mu Yang, MIND Institute, University of California Davis School of Medicine.

The Jackson Laboratory. Behavioral phenotypes have been published for some of these mutant lines. In most cases, one original publication describes the behavioral, electrophysiological, neuroanatomical, and/or biochemical phenotypes of the new mouse model of autism. In some cases, the first findings have been replicated by the same laboratory in additional publications. In a few cases, behavioral phenotypes have been replicated by other laboratories. We summarize some of the strongest findings below. Table 1 provides descriptions of gene mutations associated with human ASD. Table 2 summarizes the behavioral phenotypes in the corresponding mouse models, focusing on a subset of ASD risk genes that are involved in synaptic function and the mTOR signaling pathway. Here, we will refer to mice without any functional alleles (homozygous null) as KO mice, mice with one functional allele as heterozygous (Het) mice, mice with targeted amino acid substitutions as knockin mice, and littermate controls with both functional alleles as wildtype (WT) mice.

### Mouse models of genetic risk factors for autism

The *CNTNAP2* gene, located on chromosome 7, encodes contactin-associated protein-like 2 (CASPR2), a member of the neurexin superfamily of proteins, functioning as a cell adhesion molecule and receptor (Rodenas-Cuadrado *et al.* 2014). This protein, which contains a putative PDZ binding domain, mediates interactions of neurons and glia during central nervous system development. It also is located in myelinated axons and directs potassium channel localization within differentiating neurons (Poliak *et al.* 1999, 2003). CASPR2 directly binds to the transcription factor FOXP2 (forkhead box protein P2), which has been implicated in speech and language development (Fischer & Hammerschmidt 2011; Vernes *et al.* 2008). Several mutations in the *CNTNAP2* locus, including rare, common and deletion variants, have been associated with ASD (Alarcon *et al.* 2008; Arking *et al.* 2008; Poot *et al.* 2010; Rossi *et al.* 2008; Strauss *et al.* 2006), although more recent studies indicate a limited contribution of *CNTNAP2*

**Table 1:** Examples of autism risk genes identified by human genetic studies

Gene	Identification method	Investigated population	Reference for genetic study
<b>CNTNAP2</b>			
Association with ASD	Linkage study	Old Order Amish families (4 children and 6 parents) with an autosomal recessive founder (null mutation)	Strauss <i>et al.</i> (2006)
	Linkage, association and gene expression studies	172 parent–child trios from the Autism Genetic Resource Exchange (AGRE) resource	Alarcon <i>et al.</i> (2008)
	Genome-wide linkage study, family based-association mapping	National Institute of Mental Health Autism Genetics Initiative Repository; Stage I: 72 multiplex families (148 affected, 292 controls); Stage II: 1295 parent–child trios (145 multiplex families with 303 affected children)	Arking <i>et al.</i> (2008)
	Cytogenetics	34 year old female	Rossi <i>et al.</i> (2008)
	Cytogenetics	11 year old male	Poot <i>et al.</i> (2010)
Limited association with ASD	Cytogenetics, linkage and resequencing	635 ASD cases and 942 controls	Bakkaloglu <i>et al.</i> (2008)
	Genetic association analyses; transmission disequilibrium test	186 multiplex (408 trios) and 323 simplex families with ASD from the AGRE resource	Sampath <i>et al.</i> (2013)
No association with ASD	Next generation sequencing	2704 ASD cases, 2747 controls	Murdoch <i>et al.</i> (2015)
<b>NLGN1</b>			
Association with ASD	Whole genome copy number variant study	859 ASD cases, 1409 controls; 1336 ASD cases, 1110 controls	Glessner <i>et al.</i> (2009)
Limited association with ASD	Mutation analysis, linkage analysis, association analysis	30 ASD cases; 19 families with 41 ASD cases; 100 families with 122 ASD cases	Ylisaukko-oja <i>et al.</i> (2005)
<b>NLGN3</b>			
Association with ASD	Amino acid sequencing, linkage study	36 ASD sibling pairs, 122 ASD trios, 350 unrelated controls	Jamain <i>et al.</i> (2003)
Limited association with ASD	Mutation analysis, linkage analysis, association analysis	30 ASD cases; 19 families with 41 ASD cases; 100 families with 122 ASD cases	Ylisaukko-oja <i>et al.</i> (2005)
<b>NLGN4</b>			
Association with ASD	Cytogenetic analysis, fluorescence in situ hybridization (FISH)	8 females, 3 with ASD	Thomas <i>et al.</i> (1999)
	Amino acid sequencing, linkage study	36 ASD sibling pairs, 122 ASD trios, 350 unrelated controls	Jamain <i>et al.</i> (2003)
	Linkage analysis and gene sequencing	10 members of a French family with ASD and intellectual disability, 200 controls	Laumonier <i>et al.</i> (2004)
	Direct sequencing and mutation analysis	148 unrelated ASD cases, 48 ADHD and bipolar cases, 288 unaffected controls	Yan <i>et al.</i> (2005)

Table 1: Continued

Gene	Identification method	Investigated population	Reference for genetic study
	Chromosome analysis	Family with 1 ASD proband, 96 controls	Lawson-Yuen <i>et al.</i> (2008)
	Single nucleotide polymorphism microarrays and karyotyping	427 unrelated ASD families	Marshall <i>et al.</i> (2008)
Limited association with ASD	Mutation analysis, linkage analysis, association analysis	30 ASD cases; 19 families with 41 ASD cases; 100 families with 122 ASD cases	Ylisaukko-oja <i>et al.</i> (2005)
No association with ASD	PCR genetic screen, denaturing high performance liquid chromatography	196 ASD cases from 183 multiplex and 13 simplex families	Vincent <i>et al.</i> (2004)
<b>NRXN1</b>			
	Gene scanning and sequencing	103 Caucasian ASD cases, 61 Afro-American ASD cases, 535 Caucasian controls	Feng <i>et al.</i> (2006)
	Linkage study, using comparative analysis of hybridization intensities	1496 multiplex ASD families (at least 2 affected individuals) (7917 family members)	Szatmari <i>et al.</i> (2007)
	Single nucleotide polymorphism microarrays and karyotyping	427 unrelated ASD families	Marshall <i>et al.</i> (2008)
	Cytogenic analysis	1 ASD case study	Zahir <i>et al.</i> (2008)
	Whole genome CNV study	859 ASD cases, 1409 controls; 1336 ASD cases, 1110 controls	Glessner <i>et al.</i> (2009)
	Comparative genomic hybridization microarrays	3540 cases with developmental disorders, ASD, intellectual disability	Ching <i>et al.</i> (2010)
<b>SHANK1</b>			
	Microarray	1158 Canadian and 456 European ASD cases, 15 122 controls	Sato <i>et al.</i> (2012)
	Meta-analysis of copy-number and coding-sequence variants	5657 ASD cases, 19 163 controls; 760–2147 ASD cases, 492–1090 controls depending on the SHANK gene	LeBlond <i>et al.</i> (2014)
<b>SHANK2</b>			
	Genome-wide microarray scan; DNA sequencing	396 ASD cases, 184 individuals with intellectual disability, 659 unaffected controls	Berkel <i>et al.</i> (2010)
	Dense genotyping arrays	996 European ASD cases, 1287 matched controls	Pinto <i>et al.</i> (2010)
	Meta-analysis of copy-number and coding-sequence variants	5657 ASD cases, 19, 163 controls; 760–2147 ASD cases, 492–1090 controls depending on the SHANK gene	LeBlond <i>et al.</i> (2014)
<b>SHANK3</b>			
	FISH analysis; direct sequencing	226 families with at least 1 ASD child, 270 controls	Durand <i>et al.</i> (2007)

Table 1: Continued

Gene	Identification method	Investigated population	Reference for genetic study
	DNA sequencing and microarray-based comparative intensity analysis	400 Canadian ASD cases; HapMap collection was used for comparison	Moessner <i>et al.</i> (2007)
	Single nucleotide polymorphism microarrays and karyotyping	427 unrelated ASD families	Marshall <i>et al.</i> (2008)
	Gene sequencing	427 ASD cases, 190 controls	Gauthier <i>et al.</i> (2009)
	Denaturing high performance liquid chromatography, direct sequencing, multiplex ligation-dependent probe amplification, array comparative genomic hybridization	3 cohorts: 133 American ASD cases; 88 Italian ASD cases; 104 American ASD cases; 560 American controls and 422 Italian controls	Boccutto <i>et al.</i> (2013)
	Meta-analysis of copy-number and coding-sequence variants	5657 ASD cases, 19, 163 controls; 760–2147 ASD cases, 492–1090 controls depending on the <i>SHANK</i> gene	LeBlond <i>et al.</i> (2014)
<b><i>PTEN</i></b>			
	Direct DNA sequencing	18 ASD cases with macrocephaly	Butler <i>et al.</i> (2005)
	Direct DNA sequencing, multiplex ligation-dependent probe amplification	88 ASD cases with macrocephaly	Buxbaum <i>et al.</i> (2007)
	Direct DNA sequencing	2 ASD cases with macrocephaly	Herman <i>et al.</i> (2007)
	Direct DNA Sequencing	114 cases of ASD, developmental delay or macrocephaly	Varga <i>et al.</i> (2009)
	Direct DNA sequencing	93 cases of ASD or developmental delays with macrocephaly	McBride <i>et al.</i> (2010)

dysregulation to ASD (Bakkaloglu *et al.* 2008; Murdoch *et al.* 2015; Sampath *et al.* 2013). Mice lacking *Cntnap2* exhibited reduced juvenile ultrasonic vocalizations, reduced social interaction time and increased repetitive behaviors (Penagarikano *et al.* 2011). In addition to these deficits in ASD-related behaviors, *Cntnap2* KO mice also displayed abnormal neuronal cortical migration, asynchronous neuronal firing in the cortex, a reduced number of inhibitory interneurons, behavioral perseveration in a cognitive task, hyperactivity and seizures (Penagarikano *et al.* 2011).

Neuroligins are cell adhesion molecules located at the postsynaptic side of the synapse, interacting with their presynaptic partner proteins, the neuroligins (Bang & Owczarek 2013). Neuroligins contribute to synaptic neurotransmission through their influence on synaptic formation and are distributed at excitatory and inhibitory synapses in an isoform-dependent manner (Hu *et al.* 2015). For example, neuroligin-1 is primarily located at excitatory synapses (Budreck *et al.* 2013; Chih *et al.* 2005; Song *et al.* 1999), while neuroligin-2 is found at inhibitory synapses (Varoqueaux *et al.* 2004); neuroligin-3 can be found at both these locations (Budreck & Scheiffele 2007). At the synapse, neuroligins bind to PSD-95, a scaffolding protein important

for postsynaptic strengthening and synapse organization, particularly with ion channels and receptors, such as the glutamatergic NMDA receptor (Bolliger *et al.* 2001; Irie *et al.* 1997; Kim *et al.* 1995, 2008; Kornau *et al.* 1995; Niethammer *et al.* 1996; Shipman & Nicoll 2012). Several studies suggest that neuroligins are involved in synapse modulation and specification rather than synapse formation (Chubykin *et al.* 2007; Krueger *et al.* 2012; Varoqueaux *et al.* 2006). Neuroligin proteins encoded by X-linked genes, such as *NLGN3* and *NLGN4* which map to Xq13 and Xp22.3, respectively, have been associated with ASD in large genome-wide scans (Auranen *et al.* 2002; Glessner *et al.* 2009; Philippe *et al.* 1999), but strong associations have not been found in all studies (Vincent *et al.* 2004; Ylisaukko-oja *et al.* 2005). Using amino acid sequencing in linkage and proband case studies, deletions and frameshifts in *NLGN3* and *NLGN4* sequences have been identified in individuals with ASD (Jamain *et al.* 2003; Laumonnier *et al.* 2004; Lawson-Yuen *et al.* 2008; Marshall *et al.* 2008; Thomas *et al.* 1999; Yan *et al.* 2005).

Neuroligin-1 KO mice exhibited minimal deficits in social behavior, but displayed increased grooming and spatial learning impairments, along with impaired hippocampal

**Table 2:** Examples of genetic mouse models of autism

Mouse model	Mouse phenotype	Reference
<i>Cntnap2</i> knockout mouse	<ul style="list-style-type: none"> <li>• Reduced social interactions</li> <li>• Increased repetitive behaviors</li> <li>• Reduced juvenile ultrasonic vocalizations</li> <li>• Hyperactivity</li> <li>• Seizures</li> <li>• Abnormal cortical migration</li> <li>• Asynchronous neurotransmission</li> <li>• Reduced number of interneurons</li> </ul>	Penagarikano <i>et al.</i> (2011)
Neurologin-1 knockout mouse	<ul style="list-style-type: none"> <li>• Minimally impaired social approach</li> <li>• Repetitive behavior</li> <li>• Spatial learning deficits</li> <li>• Impaired hippocampal long term potentiation</li> <li>• Reduced NMDA/AMPA glutamate receptor ratio at cortico-striatal synapses</li> </ul>	Blundell <i>et al.</i> (2010)
Neurologin-2* knockout mouse	<ul style="list-style-type: none"> <li>• Increased anxiety-like behavior</li> <li>• Decreased pain sensitivity</li> <li>• Decreased motor coordination</li> <li>• Reduced exploratory activity</li> <li>• Developmental milestone delays</li> <li>• Reduced ultrasonic pup vocalizations</li> <li>• Decreased inhibitory synaptic puncta, with no change in overall synapse number</li> <li>• Reduced inhibitory synaptic neurotransmission</li> </ul>	Chubykin <i>et al.</i> (2007), Blundell <i>et al.</i> (2009) and Wohr <i>et al.</i> (2013)
Neurologin-3* knockout mouse	<ul style="list-style-type: none"> <li>• Reduced fear conditioning</li> <li>• Olfactory impairments</li> <li>• Hyperactivity</li> <li>• Decreased total brain volume</li> </ul>	Radyushkin <i>et al.</i> (2009)
Neurologin-3* knockin mouse (R451C substitution)	<ul style="list-style-type: none"> <li>• Enhanced water maze spatial learning acquisition</li> <li>• Increased protein levels of inhibitory synaptic markers</li> <li>• Increased inhibitory neurotransmission in the somatosensory barrel cortex</li> <li>• Increase in AMPA-mediated excitatory neurotransmission and enhanced long-term potentiation in the hippocampus</li> <li>• Increased dendritic branching in the hippocampus</li> </ul>	Tabuchi <i>et al.</i> (2007) and Etherton <i>et al.</i> (2011)
Neurologin-3* knockin mouse (R451C substitution)	<ul style="list-style-type: none"> <li>• Reduced ultrasonic vocalizations in pups</li> <li>• Reduced acoustic startle to high decibel tones</li> <li>• Minor developmental differences in growth and righting reflexes</li> </ul>	Chadman <i>et al.</i> (2008)
<i>Nlgn4</i> knockout mouse (gene trap insertion downstream of exon I)	<ul style="list-style-type: none"> <li>• Reduced social interaction time</li> <li>• Impaired social approach and social novelty recognition</li> <li>• Reduced ultrasonic vocalizations</li> <li>• Reduced volume of total brain, cerebellum and brain stem</li> </ul>	Jamain <i>et al.</i> (2008) and El-Kordi <i>et al.</i> (2013)
<i>Nlgn4</i> * knockout mouse (gene trap insertion downstream of exon I)	<ul style="list-style-type: none"> <li>• No genotype differences in ultrasonic vocalizations, anxiety-related behaviors or locomotor activity</li> </ul>	Ey <i>et al.</i> (2012)
Neurexin 1 $\alpha$ * knockout mouse	<ul style="list-style-type: none"> <li>• Increased grooming</li> <li>• Decreased prepulse inhibition</li> <li>• Reduced excitatory synaptic neurotransmission</li> </ul>	Etherton <i>et al.</i> (2009)
Neurexin 1 $\alpha$ * knockout mouse	<ul style="list-style-type: none"> <li>• Reduced exploratory activity in novel environments</li> <li>• Increased aggressive behaviors</li> <li>• Mild increases in anxiety-like behavior</li> </ul>	Grayton <i>et al.</i> (2013)

Table 2: Continued

Mouse model	Mouse phenotype	Reference
<i>Shank1</i> * knockout mouse (targeted replacement of PDZ domain (exons XIV and XV))	<ul style="list-style-type: none"> <li>• Anxiety-like phenotype</li> <li>• Impaired contextual fear memory</li> <li>• Enhanced spatial learning</li> <li>• Impaired long term memory retention</li> <li>• Reduced dendritic spine size and smaller postsynaptic densities</li> <li>• Weaker basal synaptic neurotransmission</li> </ul>	Hung <i>et al.</i> (2008)
<i>Shank1</i> * knockout mouse (targeted replacement of PDZ domain (exons XIV and XV))	<ul style="list-style-type: none"> <li>• Mild anxiety-like phenotype</li> <li>• Reduced exploratory locomotion</li> </ul>	Silverman <i>et al.</i> (2011)
<i>Shank1</i> * knockout mouse (targeted replacement of PDZ domain (exons XIV and XV))	<ul style="list-style-type: none"> <li>• Reduced pup ultrasonic vocalizations</li> <li>• Reduced male scent marking and abnormal ultrasonic vocalizations in response to female pheromones</li> </ul>	Wohr <i>et al.</i> (2011b)
<i>Shank2</i> knockout mouse (lacking all <i>Shank2</i> isoforms)	<ul style="list-style-type: none"> <li>• Fewer social contacts during same-sex interactions</li> <li>• Reduced social novelty recognition</li> <li>• Repetitive grooming</li> <li>• Abnormal ultrasonic vocalizations</li> <li>• Hyperactivity</li> <li>• Increased anxiety-like behavior</li> <li>• Reduced dendritic spines</li> <li>• Impaired glutamatergic neurotransmission</li> </ul>	Schmeisser <i>et al.</i> (2012)
<i>Shank2</i> knockout mouse (exon VI and VII microdeletion and a frameshift for both <i>Shank</i> splice variants)	<ul style="list-style-type: none"> <li>• Reduced home-cage social interaction</li> <li>• Reduced social preference</li> <li>• Repetitive jumping</li> <li>• Reduced ultrasonic vocalizations</li> <li>• Impaired spatial learning</li> <li>• Decreased glutamatergic NMDA receptor function</li> </ul>	Won <i>et al.</i> (2012)
<i>Shank3A</i> * heterozygous mouse (ankyrin repeat domain deletion (exon IV-IX))	<ul style="list-style-type: none"> <li>• Reduced social sniffing in male–female reciprocal social interactions</li> <li>• Reduced ultrasonic vocalizations</li> <li>• Reduced long term potentiation</li> <li>• Transient dendritic spine expansion during long term potentiation</li> <li>• Reduced basal AMPA neurotransmission</li> </ul>	Bozdagi <i>et al.</i> (2010)
<i>Shank3A</i> * heterozygous and knockout mice (ankyrin repeat domain deletion (exon IV-IX))	<ul style="list-style-type: none"> <li>• Reduction of select parameters (e.g. sniffing, following, front approach and push-crawl) in juvenile reciprocal social interaction in male heterozygous and knockout mice</li> <li>• Increased repetitive grooming in male heterozygous and knockout mice</li> <li>• Impaired novel object recognition in knockout mice</li> <li>• Mild motor learning deficits in knockout mice</li> <li>• Lower pain sensitivity in knockout mice (C57BL/6 background)</li> <li>• Impaired synaptic transmission, induction and long-term potentiation in knockout mice</li> </ul>	Yang <i>et al.</i> (2012b) and Drapeau <i>et al.</i> (2014)
<i>Shank3A</i> knockout mouse (ankyrin repeat domain deletion (exon IV-IX))	<ul style="list-style-type: none"> <li>• Reduced social interaction time</li> <li>• Repetitive behaviors</li> <li>• Aberrant ultrasonic vocalizations</li> <li>• Reduced activity and motor learning in males</li> <li>• Impaired acquisition in Morris water maze</li> <li>• Reduced postsynaptic protein and glutamate receptor protein levels</li> <li>• Longer dendritic spines</li> <li>• Reduced long term potentiation</li> </ul>	Wang <i>et al.</i> (2011)



Table 2: Continued

Mouse model	Mouse phenotype	Reference
<i>Shank3A</i> * knockout mouse (ankyrin repeat domain deletion)	<ul style="list-style-type: none"> <li>• Reduced preference for social novelty</li> </ul>	Peca <i>et al.</i> (2011)
<i>Shank3B</i> knockout mouse (PDZ domain deletion)	<ul style="list-style-type: none"> <li>• Reduced social interaction and social novelty recognition</li> <li>• Self-injurious repetitive grooming</li> <li>• Increased anxiety-like behavior</li> <li>• Reduced postsynaptic proteins and glutamate receptor protein levels</li> <li>• Neuronal hypertrophy and reduction in dendritic spines</li> <li>• Increased caudate volume</li> <li>• Reduced neurotransmission in corticostriatal circuits</li> </ul>	Peca <i>et al.</i> (2011)
<i>Shank3</i> * knockout mouse (exon 21 deletion, including the Homer binding domain)	<ul style="list-style-type: none"> <li>• Increased repetitive grooming in older knockout mice</li> <li>• Impaired spatial learning</li> <li>• Avoidance of inanimate objects</li> <li>• Impaired motor coordination</li> <li>• Increased pain sensitivity</li> <li>• Decreased excitatory neurotransmission and reduced long-term potentiation</li> <li>• Increased mGluR5 receptor levels in synaptic fractions</li> </ul>	Kouser <i>et al.</i> (2013)
<i>Nse-Cre Pten</i> conditional knockout mouse ( <i>Pten</i> deletion restricted to subsets of postmitotic cortical and hippocampal neurons)	<ul style="list-style-type: none"> <li>• Decreased social interaction with juvenile mice</li> <li>• Reduced sociability and preference for social novelty</li> <li>• Spatial learning deficits</li> <li>• Reduced exploratory activity in the center of the open field</li> <li>• Increased startle responses and reduced sensorimotor gating</li> <li>• Spontaneous seizures</li> <li>• Macrocephaly, including hippocampal dentate gyrus enlargement</li> <li>• Hippocampal granule cell and cortical neuron hypertrophy</li> </ul>	Kwon <i>et al.</i> (2006), Ogawa <i>et al.</i> (2007) and Zhou <i>et al.</i> (2009)
<i>Pten</i> heterozygous mouse	<ul style="list-style-type: none"> <li>• Reduced sociability and preference for social novelty</li> <li>• Increased repetitive behavior</li> <li>• Decreased aggression and reduced social investigation in the resident-intruder test</li> <li>• Reduced sensorimotor gating</li> <li>• Increased depression-like behavior in male heterozygous mice</li> <li>• Macrocephaly and higher brain mass</li> </ul>	Page <i>et al.</i> (2009); Clipperton-Allen and Page (2014) and Clipperton-Allen and Page (2015)

The asterisk (\*) following the name of a mouse model indicates that its social behaviors were normal, using assays with high face validity to the types of social deficits which characterize autism.

long-term potentiation (Blundell *et al.* 2010). Neuroligin-2 heterozygous and KO mice showed normal social interactions in social approach, but displayed increased anxiety-like behavior, decreased pain sensitivity and poor motor coordination (Blundell *et al.* 2009; Wöhr *et al.* 2013). In addition, neuroligin-2 KO mice had decreased inhibitory neurotransmission, as well as decreased immunostaining of inhibitory synapse markers (Blundell *et al.* 2009; Chubykin *et al.* 2007). Neuroligin-3 knockin (R451C) mice, with an arginine to cysteine substitution at site 451, did not display robust autism-relevant behaviors, but rather had mild developmental differences, (e.g. slower righting reflexes), enhanced spatial learning acquisition and reduced acoustic startle (Chadman *et al.* 2008; Etherton *et al.* 2011; Tabuchi *et al.* 2007), suggesting that this ASD-related point mutation delayed

development, altered learning and reduced sensitivity to stimuli. Neuroligin-3 knockin mice also displayed increased inhibitory neurotransmission in the barrel cortex, increased excitatory neurotransmission and enhanced long-term potentiation in the hippocampus, increased dendritic branching in the hippocampus, as well as increased protein levels of inhibitory synaptic markers, while KO mice did not (Etherton *et al.* 2011; Tabuchi *et al.* 2007). Neuroligin-3 KO mice displayed normal sociability, but impairments in fear conditioning and olfaction, as well as hyperactivity and decreased total brain volume (Radyushkin *et al.* 2009). Characterization of *Nlgn4* KO mice revealed that loss of this neuroligin resulted in reduced sociability and ultrasonic vocalizations, as well as a reduction in total brain volume (El-Kordi *et al.* 2013; Jamain *et al.* 2008). However, phenotypic analysis of later

generations of the same line of *Nlgn4* KO did not find any genotype differences in sociability, ultrasonic vocalizations, anxiety-related behaviors or general locomotor activity (Ey et al. 2012).

Neurexins are a class of cell adhesion proteins found on the presynaptic terminal of synapses that bind to neuroligins (Bang & Owczarek 2013). Numerous association studies have identified mutations in the *NRXN1* gene, located on chromosome 2, in intellectual disabilities and other syndromes, including several cases of autism (Ching et al. 2010; Feng et al. 2006; Glessner et al. 2009; Gregor et al. 2011; Marshall et al. 2008; Szatmari et al. 2007; Zahir et al. 2008). Neurexin-1 $\alpha$  KO mice displayed increased grooming, reduced locomotor activity, reduced sensorimotor gating and increased aggression (Eherton et al. 2009; Grayton et al. 2013). Further studies are necessary to determine the exact contribution of specific neurexin and neuroligin mutations to ASD-relevant behaviors.

The *SHANK* family of genes located on chromosome 22q encodes scaffolding proteins that assist in the synaptic organization of excitatory glutamatergic neurons by binding to postsynaptic density proteins, signaling molecules, postsynaptic receptors and cytoskeletal proteins (Boeckers et al. 2002; Grabrucker et al. 2011; Lim et al. 1999; Naisbitt et al. 1999; Sheng & Kim 2000; Tu et al. 1999). *SHANK3* can bind to neuroligins, suggesting disrupted cell adhesion may contribute to ASD (Meyer et al. 2004). Genetic studies have identified *de novo* and inherited mutations in *SHANK1* (Sato et al. 2012), *SHANK2* (Berkel et al. 2010, 2012; Pinto et al. 2010) and *SHANK3* (Boccutto et al. 2013; Durand et al. 2007; Gauthier et al. 2009, 2010; Marshall et al. 2008; Moessner et al. 2007). A recent meta-analysis of *SHANK* mutations has suggested that ASD severity due to *SHANK* mutations may be related to which gene is mutated, such that *SHANK3* mutations have a higher frequency and penetrance in individuals with ASD, compared to *SHANK1* and *SHANK2* (Leblond et al. 2014). 22q13 deletion syndrome, also known as Phelan-McDermid syndrome, is caused by a deletion on the distal part of the long arm of chromosome 22 and is associated with ASD-like behaviors, including disrupted social behavior, repetitive behaviors, motor dysfunctions, seizures and moderate to severe intellectual disability (Kolevzon et al. 2014; Phelan & McDermid 2012). *SHANK3* is one of the most commonly mutated genes within the Phelan-McDermid critical region and is thought to underlie most of the neural consequences of this deletion (Phelan & McDermid 2012).

While *Shank1* KO mice do not display robust autism-relevant social deficits (Silverman et al. 2011), *Shank1* KO mice emitted fewer ultrasonic vocalizations as pups, exhibited reduced scent marking and abnormal vocalizations as adults and had motor impairments (Silverman et al. 2011; Wöhr et al. 2011b). *Shank1* KO mice also displayed dendritic spine abnormalities, including weaker basal synaptic neurotransmission (Hung et al. 2008). *Shank2* KO mice had abnormalities in several ASD-relevant behaviors, including reduced sociability, as measured by fewer social contacts during same-sex interactions, and reduced preference for social novelty, as well as higher levels of repetitive behaviors (e.g. grooming and jumping) and abnormal ultrasonic vocalizations (Schmeisser et al. 2012; Won et al. 2012). In addition,

*Shank2* KO mice had a reduced number of hippocampal dendritic spines and reduced glutamatergic neurotransmission in the hippocampus (Schmeisser et al. 2012; Won et al. 2012).

Mutant mice have been generated for each of the *Shank3* isoforms, with deletions in various domains of the *Shank3* gene, some of which displayed social deficits while others displayed normal sociability (Jiang & Ehlers 2013). Reduced sociability, reduced ultrasonic vocalizations and high levels of repetitive self-grooming were dependent on which isoform was deleted (Bozdagi et al. 2010; Drapeau et al. 2014; Kouser et al. 2013; Peca et al. 2011; Wang et al. 2011; Yang et al. 2012b). Reduced basal neurotransmission as well as abnormalities in neuronal morphology (e.g. neuronal hypertrophy, dendritic spine deficits) have been identified in most of these models (Bozdagi et al. 2010; Kouser et al. 2013; Peca et al. 2011; Wang et al. 2011; Yang et al. 2012b), underscoring the importance of the Shank proteins in maintaining normal synaptic function and neuronal structure. Therefore, several different ASD-relevant mutations in the *SHANK* gene family have been modeled in mice, and while the phenotypes differ between the specific models, it appears that complete or partial loss of Shank proteins may be detrimental to normal social behaviors and may induce high levels of repetitive behaviors in mice.

## ASD risk genes in the mTOR signaling pathway

While many of the identified risk genes for ASD involve synaptic proteins, mutations in several components of the mTOR pathway are also implicated in ASD (Bourgeron 2009), suggesting that normal function of this intracellular signaling pathway is necessary for proper synaptic transmission and neuronal activity. The mTOR pathway is critical for protein synthesis, cellular proliferation and growth (Hershey et al. 2012; Laplante & Sabatini 2012). Activated tyrosine kinase receptors recruit and activate phosphoinositide 3-kinase (PI3K), which converts phosphatidylinositol (4,5)-biphosphate (PIP<sub>2</sub>) to phosphatidylinositol (3, 4, 5)-triphosphate (PIP<sub>3</sub>). PIP<sub>3</sub> recruits many proteins to the membrane through pleckstrin homology domains, including PDK1 and the serine/threonine kinase AKT, an important downstream effector of PIP<sub>3</sub>. Phosphatase and tensin homolog located on chromosome 10 (PTEN) is a lipid and protein phosphatase that negatively regulates Akt activity by working in opposition to PI3K, converting PIP<sub>3</sub> back to PIP<sub>2</sub> (Maehama & Dixon 1998; Stambolic et al. 1998). Akt is fully activated following phosphorylation by PDK1 and mTOR complex 2 (mTORC2), one of two complexes that involves the evolutionarily conserved serine/threonine kinase, mammalian target of rapamycin (mTOR) (Bayascas & Alessi 2005; Sarbassov et al. 2005). Phosphorylated Akt is involved in a wide range of cellular processes, including cell proliferation, survival and growth, via a myriad of downstream signaling proteins, including TSC1 and TSC2. Activated Akt inhibits a protein complex composed of TSC1 (hamartin) and TSC2 (tuberin) through phosphorylation (Huang & Manning 2009; Tee et al. 2002). This disinhibits the GTPase Rheb and activates mTOR complex 1 (mTORC1)

(Sato *et al.* 2008). Once activated, mTORC1 upregulates protein synthesis by phosphorylating several key proteins, including S6 kinase 1 (S6K1), which subsequently regulates Fragile X mental retardation protein (FMRP) through phosphorylation (Narayanan *et al.* 2008). Several key proteins, such as PTEN, TSC1, TSC2 and FMRP, are strongly implicated in subsets of ASD cases, and are discussed below. However, additional components of the mTOR pathway are also under investigation for their contribution to ASD-related deficits, such as the mTOR substrate eIF4E (Gkogkas *et al.* 2013).

*PTEN* mutations are most often associated with a variety of hamartoma syndromes, including Cowden syndrome (Eng 2003), which are characterized by benign focal malformations. *PTEN* was identified as an ASD candidate gene after several case and prospective studies revealed an association in individuals with ASD and macrocephaly (enlarged head size) (Butler *et al.* 2005; Buxbaum *et al.* 2007; Herman *et al.* 2007; McBride *et al.* 2010; Varga *et al.* 2009). Modeling *PTEN* mutations in mice has focused on two distinct strategies: conditional KO mice, in which distinct cell types lack the gene and protein product, and heterozygous null mice that possess constitutive haploinsufficiency. Conditional KO mice with a *Pten* deletion restricted to a subset of post-mitotic hippocampal and cortical neurons exhibited deficits in social interaction, sociability and preference for social novelty as well as impaired performance in the Morris water maze and macrocephaly (Kwon *et al.* 2006; Zhou *et al.* 2009). Similarly, heterozygous KO mice with a constitutive *Pten* mutation exhibited macrocephaly and deficits in sociability (Clipperton-Allen & Page 2014), but these sociability impairments were limited to female mice in one study (Page *et al.* 2009). Additionally, Allen, Page and colleagues also showed that *Pten* heterozygous mice exhibited elevated repetitive behaviors (i.e. digging, self-grooming). To date, the results obtained with *Pten* mutant mice suggest that *Pten* dysfunction leads to several ASD-relevant behaviors, although more extensive behavioral characterization of the various *Pten* conditional mouse models would further elucidate this phosphatase's contribution to ASD.

Tuberous sclerosis is a genetic condition resulting from mutations in the *TSC1* or *TSC2* gene, which negatively regulate mTOR activity (Curatolo & Maria 2013). Individuals that carry mutations in either *TSC* gene have a higher than expected occurrence of ASD-like features (50%) or an ASD diagnosis (29%) (Curatolo *et al.* 2010). *Tsc1* heterozygous mice exhibited deficits in social interactions, hippocampus-dependent contextual fear conditioning, and hidden platform Morris water maze tasks (Goorden *et al.* 2007). Social deficits were not identified in *Tsc2* heterozygous mice, except when the mice were also treated prenatally with Poly I:C, a maternal immune activation model (Ehninger *et al.* 2012). However, *Tsc2* mutations did produce learning and memory deficits in Morris water maze hidden platform performance and contextual fear conditioning (Ehninger *et al.* 2008). A dominant negative *Tsc2* mutation that binds to *Tsc1* and inhibits its activity also led to reduced social interactions and a reduced preference for social novelty, although these mice exhibited normal sociability in the 3-chambered social approach task (Chevere-Torres *et al.*

2012). A specific *Tsc1* disruption limited to cerebellar Purkinje cells produced deficits in sociability, repetitive behavior and cognitive flexibility (Tsai *et al.* 2012). Similarly, *Tsc2* disruption limited to cerebellar Purkinje cells replicated deficits in sociability and social preference, which were accompanied by increased repetitive behaviors (Reith *et al.* 2013). The study of these *Tsc1* and *Tsc2* mutant mice has provided knowledge as to how these single gene mutations can model some ASD-relevant phenotypes and contribute to the overall ASD phenotype.

Fragile X syndrome (FXS) is caused by a hypermethylated CGG repeat expansion in the *FMR1* gene that leads to a drastic reduction in its protein product, FMRP. FMRP is an RNA binding protein that has been implicated in regulating protein expression (Bagni & Greenough 2005; Chen & Joseph 2015; Crawford *et al.* 2001; Kazdoba *et al.* 2014). Individuals with FXS are characterized by intellectual disability and a variety of physical abnormalities, and frequently display social dysfunction, anxiety and repetitive behaviors (Berry-Kravis *et al.* 2002; Lightbody & Reiss 2009). The diagnostic criteria for ASD commonly occur in individuals with FXS, with recent estimates ranging from 18% to 47% (Clifford *et al.* 2007; Demark *et al.* 2003; Hatton *et al.* 2006; Kaufmann *et al.* 2004; Rogers *et al.* 2001). Conversely, the proportion of FXS in the ASD population has been estimated at 3–8% (Cohen *et al.* 1991; Fombonne *et al.* 1997). The first and most widely tested mouse model of FXS is the Dutch-Belgian Consortium *Fmr1* KO mouse, which has been maintained on multiple background strains. Recent work with the *Fmr1* KO mouse has evaluated ASD-relevant phenotypes, including social deficits and repetitive behaviors. *Fmr1* KO mice on a C57BL/6 (B6) background exhibited decreased sociability in some cases (Dahlhaus & El-Husseini 2010) or decreased sniffing during a social approach test when maintained on a B6/FVB hybrid background (McNaughton *et al.* 2008). In contrast, other studies revealed normal sociability and reduced preference for social novelty in *Fmr1* KO mice maintained on FVB and B6 backgrounds (Liu & Smith 2009; Pietropaolo *et al.* 2011). In tests of direct social interaction, *Fmr1* KO mice on a B6 background showed deficits in social interaction in some studies (Mineur *et al.* 2006), but as with much of the *Fmr1* mouse behavioral literature, there are caveats to these behavioral impairments (Kazdoba *et al.* 2014). Paylor and colleagues demonstrated increased social interaction in *Fmr1* KO mice on a B6 background (Spencer *et al.* 2005, 2008). Importantly, the Paylor team presented data interpreted as higher social anxiety in these mice. *Fmr1* KO mice also exhibited elevated repetitive behaviors, another core symptom of ASD. Specifically, *Fmr1* KO mice on B6 or hybrid backgrounds had elevated levels of self-grooming, but not when they were maintained on an FVB background (McNaughton *et al.* 2008; Pietropaolo *et al.* 2011). *Fmr1* KO mice on B6 and B6 hybrid backgrounds also had higher levels of marble burying (Gholizadeh *et al.* 2014; Spencer *et al.* 2011; Veeraragavan *et al.* 2012). Intellectual disabilities which characterize FXS have been evaluated in a variety of cognitive tasks. In some reports, *Fmr1* KO mice demonstrated cognitive deficits consistent with the intellectual impairments that characterize FXS. Deficits have been observed in contextual, cued and trace fear conditioning and/or context discrimination in

*Fmr1* KO mice on B6 and sighted FVB backgrounds (Auerbach et al. 2011; Ding et al. 2014; Paradee et al. 1999; Zhao et al. 2005). However, many other reports have described normal cognitive abilities in *Fmr1* KO mice maintained on B6, FVB/129 hybrid, and albino B6 backgrounds, including fear conditioning (Baker et al. 2010; Dobkin et al. 2000; Peier et al. 2000; Uutela et al. 2012; Van Dam et al. 2000). Similarly, spatial navigation and reversal deficits were observed during Morris water maze testing in *Fmr1* KO mice maintained on B6, albino B6 and FVB backgrounds in some labs (Baker et al. 2010; D'Hooge et al. 1997; Kooy et al. 1996; The Dutch-Belgian Fragile X Consortium et al. 1994) but these impairments were not seen in other studies (Paradee et al. 1999; Uutela et al. 2012; Yan et al. 2004). Thus, although the gene and its product are absent in *Fmr1* mice, behavioral phenotypes relevant to the human syndrome and to ASD appear to be variable, with phenotypes potentially dependent on a variety of methodological and environmental factors. While genetic background is one potential factor, there does not appear to be a clear segregation of behavioral outcomes on the B6 vs. FVB backgrounds, either in the social and repetitive behavioral domains most relevant to autism, or in the cognitive domains most relevant to FXS. In contrast to the inconsistent behavioral literature on *Fmr1* mice, investigations of neuroanatomical, electrophysiological, genetic and biochemical phenotypes in *Fmr1* KO mice have allowed researchers to gain considerable insight into biological mechanisms underlying Fragile X syndrome.

## Conclusions

The summary above and in Table 2 provides descriptions of behavioral and biological phenotypes in representative genetic mouse models of ASD. The small subset of genetic mouse models included herein focuses primarily on risk genes that mediate the formation and strengthening of synapses, and postsynaptic downstream signaling through the mTOR pathway. Our selection is presented for its possible usefulness in conceptualizing a cluster of genes with potentially interrelated actions through synaptic and postsynaptic intracellular mechanisms. The appeal of this convergence concept, proposed by many autism researchers (Delorme et al. 2013; Geschwind & State 2015; Silverman & Crawley 2014; Spooren et al. 2012), includes the possibility of developing pharmacological treatments for ASD that act through impaired synaptic mechanisms, perhaps using compounds repurposed from other uses involving synaptic dysfunction. Several intriguing preclinical studies with mouse models of ASD indicate improvements in social behaviors and/or reductions in repetitive behaviors and/or amelioration of cognitive deficits after pharmacological treatments. Promising results from pharmacological interventions in mouse models of ASD and FXS include mGluR5 antagonists (Michalon et al. 2012; Silverman et al. 2012; Tian et al. 2015), GABA agonists (Han et al. 2014; Henderson et al. 2012; Silverman et al. 2015), rapamycin (Burket et al. 2014; Ehninger & Silva 2011; Zhou et al. 2009), d-cycloserine (Burket et al. 2013; Yadav et al. 2012), BDNF and ampakines

(Lauterborn et al. 2007; Silverman et al. 2013), IGF-1 (Bozdagi et al. 2013) and oxytocin (Huang et al. 2014; Meziane et al. 2014; Modi & Young 2012; Penagarikano et al. 2015). Descriptions of additional mouse models of ASD are available in several other recent review articles (Ey et al. 2011; Kas et al. 2014; Silverman & Crawley 2014).

Caveats abound for the methods and interpretations of mouse behavioral phenotypes relevant to the core symptoms of autism. The first is genetic background. Just as humans with the same mutation may present with different symptoms, possibly due to protective or susceptibility genes in their genetic backgrounds, mice present with different phenotypes when a mutation is bred into different inbred strains, such as C57BL/6J, FVB/NJ and substrains of 129, each of which has its own idiosyncratic behavioral traits (Crawley et al. 1997). Varying behavioral phenotypes have been reported for mutant mouse models of autism and FXS, as described above, when outcomes of the mutation were placed on divergent backgrounds and directly compared (Moy et al. 2009; Pietropaolo et al. 2011; Spencer et al. 2011). The second is experimental design. Employing large Ns and using WT littermates as controls are essential to avoid over-interpretations of phenotypes which were actually caused by environmental influences that affect mouse behaviors. The third is statistical analysis of behavioral data. Two-way ANOVAs are often required, in which treatment is one factor and genotype is the other factor, rather than simple *t*-tests. Stringent post-hoc tests, such as Newman-Keuls, Dunnett's and Tukey's, avoid the false positives that may be obtained from more forgiving post-hoc tests such as Fisher's LSD. Statistical comparisons must match the original experimental design. For example, our 3-chambered social approach task is not sensitive enough to compare the absolute number of seconds spent with the novel mouse across genotypes, or across drug doses. Data on social approach are correctly analyzed with a simple paired *t*-test or equivalent, which compares time with the novel mouse vs. time with the novel object within genotype only, or within a drug dose only, to provide a yes-or-no outcome measure, i.e. sociability or absence of sociability. The fourth is corroboration within the behavioral domain. Conducting two or more assays that interrogate the same behavior (e.g. at least two social tasks, or two repetitive behavior assays, or two anxiety-related tests, or several learning and memory tasks that tap into different cognitive domains) allows for stronger interpretations and generalizability of a finding within a given domain. Including relevant controls, such as open field exploration for tasks that require locomotor activity, and pain or foot shock sensitivity for fear conditioning, will ensure that a motor or sensory artifact is not the cause of a significant effect in the behavioral domain of primary interest. Large numbers of mouse behavioral assays relevant to the diagnostic and associated symptoms of ASD are available to choose from (Crawley 2012; Silverman et al. 2010a), along with a wealth of relevant control tests (Crawley 2007).

One additional caveat, arguably the most important, is replicability. While all scientific discoveries require replication, behavioral findings require extra attention to reproducibility because of the strong influence of the various environmental factors on mouse behaviors. Stressors, such as construction

noise or rough handling, can greatly affect scores on sensitive behavioral assays for anxiety-related, social and cognitive phenotypes. These types of variable findings remain primarily anecdotal, because they are difficult to document and publish, although they are common knowledge among behavioral neuroscientists. Loss of behavioral phenotypes across breeding generations may occur due to attritional loss of the mutation or drift in background genes. Basic methodological issues, such as the age of the mice at testing, composition of the chow diet, properties of the testing equipment and testing room, social testing in single vs. mixed genotype dyads, cleaning of the equipment between subject mice, and simple random chance beyond  $P < 0.05$ , may create conditions that yield a positive finding that cannot be replicated. Following published procedures by expert behavioral neuroscientists can help alleviate some of these concerns. However, the best strategy to ensure high replicability is for the research team to conduct the entire experiment again in a separate cohort of mice prior to publishing. Further, to confirm the ultimate strength of a finding for the scientific community, several different laboratories should conduct essentially the same experiment with the same line of mice. When a social deficit in a genetic mouse model of ASD is replicated across cohorts and by many labs, the strength of a finding is ensured. Highly robust, well-replicated behavioral phenotypes relevant to the symptoms of ASD in a genetic mouse model can then effectively inform our understanding of the role of that gene in the symptomatology of ASD and serve as preclinical outcome measures for therapeutic discovery.

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