Synaptopathology Involved in Autism Spectrum Disorder

Shiqi Guang1,2, Nan Pang1,2, Xiaolu Deng1,2, Lifen Yang1,2, Fang He1,2, Liwen Wu1,2, Chen Chen1,2, Fei Yin1,2* and Jing Peng1,2*

1 Department of Pediatrics, Xiangya Hospital, Central South University, Changsha, China, 2 Hunan Intellectual and Developmental Disabilities Research Center, Changsha, China

Autism spectrum disorder (ASD) encompasses a group of multifactorial neurodevelopmental disorders characterized by impaired social communication, social interaction and repetitive behaviors. ASD affects 1 in 59 children, and is about 4 times more common among boys than among girls. Strong genetic components, together with environmental factors in the early stage of development, contribute to the pathogenesis of ASD. Multiple studies have revealed that mutations in genes like NRXN, NLGN, SHANK, TSC1/2, FMR1, and MECP2 converge on common cellular pathways that intersect at synapses. These genes encode cell adhesion molecules, scaffolding proteins and proteins involved in synaptic transcription, protein synthesis and degradation, affecting various aspects of synapses including synapse formation and elimination, synaptic transmission and plasticity. This suggests that the pathogenesis of ASD may, at least in part, be attributed to synaptic dysfunction. In this article, we will review major genes and signaling pathways implicated in synaptic abnormalities underlying ASD, and discuss molecular, cellular and functional studies of ASD experimental models.

Keywords: autism, ASD, synapse, synaptogenesis, synapse elimination, synaptic transmission, synaptic plasticity

INTRODUCTION

The term “autism” as a unique disease concept was first used by Leo Kanner in 1943. He described eight boys and three girls, aged two to eight, who were unable to use language to communicate, displaying monotonous repetitions and preoccupation with objects (Kanner, 1995). Then in 1944, Hans Asperger identified a group of children who could not integrate into their social environment and showed certain stereotypic behaviors, but did not have the speech deficiency of typical autism (Hippler and Klicpera, 2003). In recent years, the term autism spectrum disorder (ASD) has gained general acceptance to describe a set of multifactorial neurodevelopmental disorders, grouping disorders including autistic disorder, Asperger Syndrome, pervasive developmental disorder-not otherwise specified (PDD-NOS) and child disintegrative disorder under the umbrella ASD. Although ASD has diverse behavioral manifestations with varying degrees of severity, it has a core dyad of impairments: (1) impaired social communication and social interaction, and (2) restricted and repetitive behavior, interests or activities. There are also a number of comorbidities associated with ASD, including intellectual disability (ID), epilepsy (Tuchman et al., 2010), anxiety, attention deficits hyperactivity disorder (ADHD), sleeping disorders, and gastrointestinal disorders (Doshi-Velez et al., 2014). ASD affects 1 in 59 children in the United States, and is about 4 times more common among boys than among girls according to the estimate from CDC's Autism and
Developmental Disabilities Monitoring (ADD M) Network (Baio et al., 2018). Large-scale surveys show the median worldwide prevalence of ASD is 1–2% (Kim et al., 2011; Blumberg et al., 2013). ASD represents a lifelong condition for patients and many of them require special educational and social support, so ASD imposes a huge financial and emotional burden on society (Lavelle et al., 2014; Leigh and Du, 2015). However, only limited treatment of ASD is available, in part because the causes of ASD have not yet been clearly elucidated.

Based on current studies, strong genetic components contribute to the pathogenesis of ASD, together with environmental factors in the early stage of development. Early twin studies showed that concordance of autism in monozygotic (MZ) twins (80–90%) was much higher than that in dizygotic (DZ) twins (0–10%) (Folstein and Rutter, 1977; Ritvo et al., 1985; Steffenburg et al., 1989; Bailey et al., 1995). However, these early studies have their limitations because of their small sample size, restricted follow-up time, and vicissitudes of diagnostic criteria. In recent years, multiple population-based cohort studies provide more reliable evidence for ASD genetic predisposition. The relative recurrence rate for MZ twins (153.0) is much higher than that for DZ twins (8.2) (Sandin et al., 2014) and estimates of the heritability of ASD range from 54% to 95% (Gaugler et al., 2014; Sandin et al., 2014; Colvert et al., 2015). In addition to genetic predisposition, known environmental risk factors of ASD include advancing parental (especially paternal) reproductive age (Gardener et al., 2009; Hultman et al., 2011; Sandin et al., 2012), complications during pregnancy (Brown et al., 2014; Estes and McAllister, 2016) and prenatal exposure to psychotropic drugs like valproic acid (VPA) (Rasalam et al., 2005; Meador et al., 2013; Boukrhis et al., 2016).

Recent advances in gene testing techniques have allowed identification of more than 1,000 candidate genes and copy number variants (CNVs) loci associated with ASD, according to the Simmons Foundation Autism Research Initiative (SFARI) gene database1. It is estimated that a genetic cause can be identified in up to 25% of ASD cases, chromosomal rearrangements (encompassing rare and de novo CNVs) and coding-sequence mutations making up ~10–20% and ~5–10% of ASD patients, respectively (Huguet et al., 2013; Zatts and Rennert, 2016). Numerous ASD candidate genes implicated in many aspects of basic cell function such as chromatin remodeling, metabolism, mRNA translation may impact neuronal processes ranging from neurogenesis to neuron migration, axon guidance, dendrite outgrowth, and synaptic formation and function (Gilbert and Man, 2017).

Synapses are highly specialized asymmetric cell-cell junctions that are fundamental units of brain communication. ASD is diagnosed at an early stage of life, usually before three years of age, a period when intense synaptogenesis is happening (Huttenlocher and Dabholkar, 1997). Multiple studies have found that mutations in genes like NRXN, NLGN, SHANK, TSC1/2, FMRI, and MECP2 (Table 1) converge on common cellular pathways that converge at the synapse (Wang T. et al., 2016; Stessman et al., 2017). ASD patients are found to carry a higher global burden of rare, large CNVs which can include known ASD associated synaptic genes, such as SHANK3 and CHD10 (Guo et al., 2017). These genes encode cell adhesion molecules, scaffolding proteins, and proteins involved in synaptic transcription, protein synthesis and degradation, which affect various aspects of synapses, including synapse formation and elimination, synaptic transmission and plasticity, suggesting that the pathogenesis of ASD, at least in part, may be attributed to synaptic dysfunction, which can lead to functional and cognitive impairments. Some environmental risk factors of ASD can also bring about synaptic defects. For instance, the patterns of long-term potentiation (LTP) of mice that are prenatally exposed to VPA pass from an enhanced LTP phenotype in early life to an impaired LTP phenotype in adulthood (Rinaldi et al., 2007). Synaptic impairments caused by gene mutations and environmental factors are implicated in many other neurodevelopmental diseases including epilepsy, ID, developmental delay, and attention deficit-hyperactivity disorder (ADHD). Some neuropsychiatric disorders, including schizophrenia (SCZ), bipolar disorder and obsessive-compulsive disorder (OCD) are also associated with synaptic dysfunction. Though rare non-syndromic gene mutations only account for a small fraction of ASD cases, they provide an excellent window to better understanding the synaptic abnormalities underlying ASD. Insight has also been gained from studies of the synaptopathology of monogenic diseases such as fragile X syndrome, tuberous sclerosis complex, and Angelman syndrome that share a high comorbidity with ASD. In this article, we will review abnormalities in synaptogenesis, synaptic elimination, synaptic transmission and plasticity that are caused by mutations of a number of ASD-associated genes, to gain a better understanding of the synaptic dysfunctions underlying ASD.

### CELL ADHESION MOLECULES

**Neurexin/Neuroligin Complex**

Neurexins (Nrnx) are a family of presynaptic transmembrane proteins encoded by paralogous genes, NRXN1, NRXN2, NRXN3. Each of the three genes uses 2 independent promotors to generate 2 major protein isoforms, a long α-Nrnx and a short β-Nrnx. Loss-of-function variants in NRXN1 have been identified in patients with ASD in multiple cohort studies (Marshall et al., 2008; Morrow et al., 2008; Glessner et al., 2009). A wide spectrum of developmental disorders including ID without ASD (Camacho-Garcia et al., 2012; Yangngam et al., 2014), language delay, mild dysmorphic features, and hypotonia (Ching et al., 2010), as well as neuropsychiatric conditions like SCZ have also been reported in patients with NRXN1 variants. In contrast, variants in NRXN2 and NRXN3 are much rarer, but accumulating reports provide evidence supporting an association between these two genes and ASD, as well as other neurodevelopmental and neuropsychiatric conditions (Gauthier et al., 2011; Vaags et al., 2012; Yuan et al., 2018).

Neuroligins (Nlgn), encoded by five genes (NLGN1, NLGN2, NLGN3, NLGN4, and NLGN4Y) in the human genome, are a class of cell-adhesion molecules on the postsynaptic membranes,
### TABLE 1 | Critical ASD candidate genes and major molecular functions in synapse.

| Gene symbol | Chromosomal Locus | Protein | Major molecular functions in synapses | SFARI score |
|-------------|-------------------|---------|---------------------------------------|-------------|
| **Cell adhesion molecules** | | | | |
| NRXN1       | 2p16.3            | Neurexin 1 | Synaptogenesis, synaptic transmission, synaptic plasticity | 2 |
| NRXN2       | 11q13.1           | Neurexin 2 | | 4 |
| NRXN3       | 14q24.3-p31.1     | Neurexin 3 | | 3 |
| NLGN1       | Xp26.31           | Neuroligin 1 | | 3 |
| NLGN2       | 17p13.1           | Neuroligin 2 | | 4 |
| NLGN3       | Xq13.1            | Neuroligin 3 | | 2 |
| NLGN4       | Xp22.32-p22.31    | Neuroligin 4 | | 3 |
| NLGN4Y      | Yq11.221          | Neuroligin 4Y | | 4 |
| CNTNAP2     | 7q35-q36.1        | Contactin associated protein-like 2 | Synaptogenesis, synaptic transmission | 2/S |
| CDH8        | 16q21             | Cadherin 8 | Synaptogenesis, synaptic transmission | 4 |
| CDH11       | 16q21             | Cadherin 11 | | 4 |
| CNTN4       | 3p26.3-p26.2      | Contactin 4 | Synaptogenesis | 2 |
| CNTN6       | 3p26.3            | Contactin 6 | | 3 |
| PCDH10      | 4p28.3            | Protocadherin 10 | Synaptic elimination | 4 |
| **Scaffolding proteins** | | | | |
| DLG4        | 17p13.1           | Discs large MAGUK scaffold protein 4 | Synaptogenesis, synaptic elimination, synaptic plasticity | 5 |
| GPHN        | 14q23.3-24.1      | Gephyrin | Synaptogenesis | 3 |
| SHANK1      | 19q13.33          | Shank 1 | Synaptogenesis, synaptic transmission, synaptic plasticity | 3 |
| SHANK2      | 11q13.3-q13.4     | Shank 2 | | 3 |
| SHANK3      | 22q13.33          | Shank 3 | | 1/S |
| HOMER1      | 5q14.1            | Homer homolog 1 | Synaptogenesis, synaptic plasticity | 4 |
| RMR1        | Xp27.3            | Fragile mental retardation protein | Synaptogenesis, synaptic transmission, synaptic elimination | S |
| **Regulators of protein synthesis and degradation** | | | | |
| CYFIP1      | 15q11.2           | Cytoplasmic FMR1 interacting protein 1 | Synaptogenesis, synaptic transmission, synaptic plasticity | 3 |
| TSC1        | 9p34.13           | Tuberculous sclerosis 1 | Synaptogenesis, synaptic plasticity | S |
| TSC2        | 16p13.3           | Tuberculous sclerosis 2 | Synaptogenesis, synaptic transmission, synaptic plasticity | 3/S |
| EIF4E       | 4q23              | Eukaryotic translation initiation factor 4E | Synaptogenesis, synaptic transmission, synaptic plasticity | 4 |
| EIF4EBP2    | 10q22.1           | Eukaryotic translation initiation factor 4E binding protein 2 | Synaptogenesis, synaptic transmission, synaptic plasticity | 5 |
| PTEN        | 10q23.32          | Phosphatase and tensin homolog | Synaptogenesis, synaptic plasticity | 1/S |
| SYNGAP1     | 6p21.32           | Synaptic Ras GTPase activating protein 1 | Synaptogenesis, synaptic plasticity | 1/S |
| UBE3A       | 15q11.2           | Ubiquitin protein ligase E3A | Synaptogenesis, synaptic transmission, synaptic elimination | 3/S |
| **Regulators of remodeling and transcription** | | | | |
| MECP2       | Xq28              | Methyl CpG binding protein 2 | Synaptogenesis, synaptic plasticity | 2/S |
| MEF2C       | 5q14.3            | Myocyte enhancer factor 2C | Synaptic elimination | 4/S |

The SFARI score is used to evaluate the strength of association between candidate gene and ASD. (1) high confidence; (2) strong candidate; (3) suggestive evidence; (4) minimal evidence; (5) hypothesized but untested. S-syndromic genes leading to syndromes that share high comorbidity with ASD.

Binding to the laminin/neurexin/sex hormone (LNS) binding domain of Nrxn through the extracellular cholinesterase-like domain. Nlg1 is expressed exclusively on excitatory synapses and binds to PSD-95, whereas Nlg2 is restricted to inhibitory synapses, preferentially binding to gephyrin (Song et al., 1999; Varoqueaux et al., 2004). Nlg3 and Nlg4 are present on both types of synapses (Budreck and Scheiffele, 2007).

Nrxn binds to its postsynaptic partner Nlg to form a Ca^{2+}-dependent neurexin/neuroligin complex, implicated in synaptogenesis and required for efficient neurotransmission. After the initial contact of an axon with a target cell, Nrxn and Nlg together recruit key synaptic components including neurotransmitter receptors and scaffolding proteins to promote synaptic assembly, maturation and differentiation (Krueger et al., 2012; Reissner et al., 2013; Sudhof, 2017). Overexpression of Nlg or Nrxn can increase the number of synapses, leading to an alteration in the excitatory/inhibitory (E/I) ratio (Chih et al., 2004; Chubykin et al., 2007; Dahlhaus...
et al., 2010). E/I balance is necessary for appropriate circuit development and synaptic plasticity, and its alteration impairs information processing and social function, contributing to the pathogenesis of ASD and many other neurodevelopmental diseases (Gatto and Broadie, 2010; Nelson and Valakh, 2015).

In Drosophila, Nlgn1 directly interacts with WAVE regulatory complex (WRC) and recruits WRC to postsynaptic sites to reorganize postsynaptic actin, thus maintaining normal synaptic formation and transmission in the Drosophila neuromuscular junction (N MJ) (Xing et al., 2018). Nlgn1 exerts its effect on differentiation and LTP of excitatory synapses via phosphorylation of intracellular tyrosine, which affects the recruitment of AMPARs (Letellier et al., 2018).

Nrxn- or Nlgn-deficient neurons display aberrant synaptic function, but not significant changes in the formation of synapses. Nrxn -1α deficient neurons show a selective reduction in the excitatory synaptic strength reflecting a decreased neurotransmitter release probability, leading to E/I imbalance (Etherton et al., 2009; Pak et al., 2015). Nrxn-1α acts as a positive regulator of Ca$_{2+}$ influx through α2δ-1 subunits that are responsible for trafficking and kinetic properties of Cav2.1 pore-forming subunits, ensuring proper vesicle release (Brochhaus et al., 2018). Moreover, NRXN1-mutant human neurons exhibit increased levels of CASK, a scaffolding protein binding to Nrxn1, and mutations in CASK are associated with ASD, brain malformation, mental retardation (Najm et al., 2008; Hackett et al., 2010). Mice lacking Nrxn-2α display deficits in social behaviors and increased anxiety-like behaviors, accompanied by impaired glutamatergic release in the neocortex (Dachtl er et al., 2014, 2015; Born et al., 2015). Mice with combined deletion of Nrxn-2α and Nrxn2β show defects of excitatory release similar to mice lacking Nrxn-2α, indicating that Nrxn-2β has no strong function in basic transmission (Born et al., 2015). In addition, there is a significant decrease of Munc18-1 in the hippocampus of mice lacking Nrxn-2α, suggesting deficiencies in presynaptic vesicular release (Dachtl er et al., 2014). Munc18-1 is involved in synaptic vesicle docking, priming and fusion (Rizzo and Xu, 2015), encoded by STXBP1, which is also associated with ASD (Wang T. et al., 2016; Uddin et al., 2017). The cytoplasmic tail of Nrxn captures Munc18 via a multiprotein complex with involvement of Mint1 to facilitate presynaptic vesicular release (Biederer and Sudhof, 2000).

Triple knockout mice lacking Nlgn1-3 die shortly after birth due to severely impaired synaptic transmission of both excitatory and inhibitory synapses, but the density of synaptic contacts is not altered (Varoquaux et al., 2006). Mice with Nlgn1 deletion show reduced NMDA/AMPR ratio in the striatum, accounting for a dramatic increase in repetitive, stereotyped grooming behavior, and the NMDAR partial co-agonist D-cycloserine can rescue this phenotype (Blundell et al., 2010). Mice with R451C substitution in Nlgn3 show an increased AMPAR- and NMDAR-mediated synaptic transmission and enhanced LTP in the hippocampus (possibly explaining the observed enhanced spatial learning abilities), but in the somatosensory cortex, inhibitory synaptic transmission is increased without apparent alteration in excitatory transmission (Tabuchi et al., 2007; Etherton et al., 2011a; Jaramillo et al., 2014). R451C substitution causes retention of Nlgn3 in the endoplasmic reticulum, resulting in decreasing delivery of Nlgn3 to the postsynaptic membranes (Comolletti et al., 2004). Strangely, mice with deletion of Nlgn3 do not exhibit any of these change (Tabuchi et al., 2007; Etherton et al., 2011b). It has been shown that knockout of Nlgn3 late in development produces a marked synaptic phenotype, but no detectable effects are seen after knockout during early development, apparently due to developmental compensation by cerebellin-1 (Clbn1), a secreted protein binding to Nrxn (Xing et al., 2018). This may explain why Nlgn3 KO mice have no phenotype in previous studies. R704C substitution in Nlgn3 results in a major and selective decrease in AMPAR-mediated synaptic transmission in pyramidal neurons of hippocampus, without altering NMDAR- and GABAR-mediated synaptic transmission, as well as presynaptic neurotransmitter release (Etherton et al., 2011b). Nlgn4 KO significantly decreases the peak amplitude and the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs), without changing mIPSCs, sEPSCs and mEPSCs in CA3 pyramidal cells, confirming that Nlgn4 knockout affects primarily inhibitory transmission (Hammer et al., 2015).

**SCAFFOLDING PROTEINS**

### 2.1 PSD-95

PSD-95, encoded by the DLG4 gene, is the central element of postsynaptic architecture of excitatory synapses. Rare nonsense mutations and CNVs in DLG4 may increase susceptibility for ASD and SCZ (Roberts et al., 2014; Wang J. et al., 2016; Xing et al., 2016). Recently, truncation variants in DLG4 have been reported to be responsible for ID with marfanoid features (Moutton et al., 2018). Through its three PDZ domains, an SH domain and a guanylate kinase homology (GK) domain, PSD-95 potentially binds to many key constituent PSD proteins, including AMPARs via Stargazin/TARP, NMDARs, adhesion molecules like Nlgn1, and other scaffolding proteins, such as GKAP. The binding diversity suggests that it is of critical importance for synaptic structural organization (Feng and Zhang, 2009). Overexpression of PSD-95 shifts the distribution of Nlgn2 from inhibitory synapses to excitatory synapses, resulting in a decrease in the total number of inhibitory synapses, demonstrating that levels of PSD-95 can manipulate the E/I balance (Levinson et al., 2005). RNAi knockdown of PSD-95 results in shortening and thinning of PSD and loss of putative AMPAR-type structures, confirming that PSD-95 is essential for sustaining molecular organization of PSD (Chen et al., 2011). Time-lapse imaging of axon-dendrite contacts shows that overexpression of PSD-95 reduces the turnover rates of pre- and postsynaptic structures, thus promoting the stabilization of putative synaptic contacts (Taft and Turrigiano, 2014).

PSD-95 exerts a strong influence on synaptic strength through its role in controlling neurotransmitter receptors. Complete genetic deletion of PSD-95 leads to the reduction in the number of AMPARs in synapses, forming silent synapses that contain a high abundance of NMDARs but no AMPARs. This bears...
important consequences for enhanced LTP, because during LTP expression, synaptic strength increases due to the incorporation of AMPARs into postsynaptic membranes (Béïque et al., 2006; Huang et al., 2015). In addition, PSD-95 downregulation increases the clustering of NR2B-containing NMDARs, which are characterized by slow kinetics and long decay time, resulting in a larger flow of Ca$^{2+}$ (Béïque et al., 2006; Bustos et al., 2014). NR2B subunit plays an essential role in the synaptic maturation and working memory functions, but its overabundance may lead to increased susceptibility to neuroexcitotoxicity (Monaco et al., 2015).

**Gephyrin**

Gephyrin, encoded by *GPHN* gene, is a key scaffolding protein at the inhibitory synapses, comprising three functional domains, G-, C- and E-domain. Rare de novo or inherited microdeletions and point mutations of *GPHN* have been reported in the patients with ASD, seizures and epileptic encephalopathy (Lionel et al., 2013; Dejanovic et al., 2014a, 2015; Hu et al., 2015). Cohort studies with large sample size add to the growing evidence that CNVs involving *GPHN* contribute to ASD (Prasad et al., 2012; Egger et al., 2014). Gephyrin directly interacts with β-subunit of glycine receptors (GlyR) and α-subunits of GABA$_A$ receptor (GABA$_A$R) via its E-domain. Nlgn2 preferentially binds to gephyrin through a conserved tyrosine residue to activate the gephyrin-interacting protein cobbistin, a neuron-specific GDP/GTP exchange factor. Gephyrin thus acts as a molecular hub to promote the clustering of inhibitory neurotransmitter receptors and regulate the formation and extension of the inhibitory postsynaptic specialization.

Unlike their counterparts at glutamatergic synapses, gephyrin and its binding partners lack canonical protein interaction motifs, suggesting that they rely on different regulation mechanisms such as posttranslational modification. Gephyrin monomers oligomerize to form a hexagonal lattice of gephyrin clusters underneath the cell membrane that slow the lateral mobility of GlyRs and GABA$_A$Rs, thus allowing their clustering at postsynaptic sites (Groeneweg et al., 2018). Phosphorylation of gephyrin on S270 by GSK-3β, on S268 by ERK and on S305 by CaMKII modulate the density and size of gephyrin clusters, thus affecting GABAergic transmission and homeostatic synaptic plasticity (Tyagarajan et al., 2011, 2013; Flores et al., 2015). It has been shown that small ubiquitin-like modifier (SUMO)-ylation acts upstream of phosphorylation on S268 of gephyrin (Ghosh et al., 2016). Palmitoylation of gephyrin, found to be GABA$_A$R activity-dependent, is crucial for the association of gephyrin with postsynaptic membranes and for gephyrin clustering, and also strengthens GABAergic synaptic transmission (Dejanovic et al., 2014b). Inhibition of nitric oxide synthase (nNOS) results in a loss of S-nitrosylation of gephyrin and the formation of larger gephyrin clusters, ultimately increasing the number of GABA$_A$Rs on the postsynaptic membranes (Dejanovic and Schwarz, 2014). PKC-dependent phosphorylation and calcineurin-dependent dephosphorylation of growth-associated protein 43 (GAP-43), an activity-dependent phosphoprotein mediating neurite growth, are also related to gephyrin folding and clustering (Wang et al., 2015). Expression of chemically induced LTP of inhibition (iLTP) requires synaptic recruitment of gephyrin from extrasynaptic areas to immobilize and confine GABA$_A$Rs at synapses, which in turn is promoted by CaMKII-dependent phosphorylation of GABA$_A$R-β3-Ser383 (Petrini et al., 2014).

**Shank**

Shank (also known as ProSAP) proteins consist of three major isoforms, Shank1, Shank2, and Shank3, which have similar sets of domains: N-terminal ankyrin repeats, a Src homology 3 (SH3) domain, a PSD-95/Discs large/ZO-1 (PDZ) domain, a long proline-rich region, and a sterile alpha motif (SAM) domain. As master scaffolding proteins at the PSD of excitatory synapses, Shank proteins interact with more than 30 synaptic proteins through these multiple domains, essential for synaptic formation, glutamate receptor trafficking and neuronal signaling (Monteiro and Feng, 2017). In *Drosophila*, both deletion and overexpression of Shank result in defects in synaptic bouton number and maturation via regulating the internalization of the Wnt receptor Frizzled-2 (Fz2) (Harris et al., 2016).

Shank3 is encoded by *SHANK3*, located on chromosome 22q13.3. *SHANK3* was first identified in the 22q13.3 deletion syndrome, also known as Phelan-McDermid Syndrome (PMS), which is characterized by global developmental delay (Phelan et al., 2001; Phelan and McDermid, 2012). More than 50% of the patients with PMS show autism and autistic-like behaviors. *SHANK3* variants including de novo deletions, insertion, splicing mutations and point mutations have been identified in ASD patients (Durand et al., 2007; Gauthier et al., 2009; Boccuto et al., 2013). Prevalence of *SHANK3* variants in patients with ASD and ID is estimated to 1–2%, whereas *SHANK1* and *SHANK2* mutations are much rarer (Boccuto et al., 2013; Leblond et al., 2014). Shank3-deficient neurons show reduced levels of PSD proteins including GKAP, Homer1b/c, AMPAR subunit GluA1 and NMDAR subunit NR2A (Peca et al., 2011; Wang et al., 2011). Disrupted interactions among Shank3, GKAP and Homer1b/c may account for the impaired activity-dependent redistribution of the GluA1 subunit, leading to reduced post-tetanic potentiation and hippocampal LTP (Wang et al., 2011). Neurons induced from pluripotent stem cells (iPSCs) in patients with PMS show significantly impaired NMDAR- and AMPAR-mediated synaptic transmission (Shcheglovitov et al., 2013). Shank3 deficient mice display self-injurious repetitive grooming and deficits in social interaction with significant reductions in frequency and peak amplitude of mEPSCs, which suggests a decreased number of functional synapses and reduced postsynaptic response from the available synapses, respectively (Peca et al., 2011). In contrast, overexpression of Shank3 leads to a dramatic increase in the amplitude of evoked AMPAR- and NMDAR-mediated EPSCs, as well as higher frequency of AMPAR-mediated mEPSCs (Arons et al., 2012).

Shank2 is encoded by *SHANK2*, located on chromosome 11q13.3-q13.4. *SHANK2* variations including deletions, missense mutations, truncations, and mutations in the *SHANK2* promoter regions have been identified, linking *SHANK2* variants to ASD.
and ID (Berkel et al., 2010). Single nucleotide polymorphisms (SNPs) in SHANK2 introns are also implicated in ASD risk (Bai et al., 2018). Discrepant effects on synaptic transmission and long-term plasticity are reported in two independently generated Shank2 KO mice, Shank2△e7−/− and Shank2△e6−7/−. Shank2△e7−/− mice (Schmeisser et al., 2012) display extreme hyperactivity and profound autism-like behavior, such as repetitive grooming and abnormalities in vocal and social behaviors, accompanied by reduced basal synaptic transmission, decreased frequency of mEPSCs and an increased NMDA/AMPA ratio consistent with increased levels of the NMDAR subunit GluN1 in the hippocampus and striatum. LTP of Schaffer collaterals is slightly enhanced in Shank2△e7−/− mice, but no evidence for alterations in LTD. Recently, Wegener et al. (2018) reproduced these results in Shank2△e6−7/− mice and further uncovered a developmental synapse phenotype, an excess of silent synapses, which explains the phenomena of decreased synaptic transmission and enhanced LTP. Shank2△e6−7/− mice (Won et al., 2012) exhibit hyperactivity and autism-like behavior too, though with a reduced NMDA/AMPA ratio and selectively decreased NMDAR-mediated synaptic transmission, as well as severely impaired LTP and LTD. Direct stimulation of NMDARs with D-cycloserine (a partial agonist of NMDARs), and a positive allosteric modulator of metabotropic glutamate receptor 5 (mGlur5) that enhances NMDAR function via mGlur5 activation can normalize NMDAR function and improves social interaction of Shank2△e6−7/− mice. Mice with conditional Shank2 deletion (exon 6-7) restricted to parvalbumin (PV)-positive neurons display moderate hyperactivity and enhanced self-grooming, suggesting that Shank2 deletion in PV-positive neurons contributes to the hyperactivity observed in global Shank2 KO mice (Lee et al., 2018). Interestingly, expression levels of Gabra2 and GABAAR-mediated inhibitory synaptic transmission are reduced in Shank2△e6−7/− mice, but not in Shank2△e7−/− mice, and enhancing inhibitory synaptic function with an allosteric modulator for the GABAAR Rs reverses spatial memory deficits in Shank2△e6−7/− mice (Lim et al., 2017).

Shank1 is encoded by SHANK1, located on chromosome 19q13.33. Deletions and point mutations of SHANK1 have been identified in ASD individuals, indicating the involvement of SHANK1 in ASD (Sato et al., 2012). Mice completely lacking all Shank1 protein isoforms (Shank1−/− mice) have increased anxiety-related behavior, impaired contextual fear memory and object recognition memory (Hung et al., 2008; Sungur et al., 2017), as well as social communication/interaction deficits (Wohr et al., 2011; Sungur et al., 2016). Adult, but not juvenile Shank1−/− heterozygous and Shank1+/− null mutant mice displayed slightly elevated levels of self-grooming behavior (Sungur et al., 2014). Silverman et al. (2011) found that motor functions were reduced on open field activity, rotarod, and wire hang in Shank1−/− mutants, extending the role of Shank1 to motor functions. Shank1−/− mice show smaller dendritic spines, thinner and smaller PSD, and altered PSD protein composition, as well as weaker basal synaptic transmission, affirming the importance of Shank1 for synaptic structure and function in vivo (Hung et al., 2008). Shank1 is highly expressed in parvalbumin-expressing (PV+) fast-spiking inhibitory interneurons in hippocampus, and loss of Shank1 causes increased E/I ratio by weakening inhibitory synaptic function, which is consistent with decreased expression of gephyrin (Mao et al., 2015).

**SYNAPTIC GENE TRANSCRIPTION, PROTEIN SYNTHESIS AND DEGRADATION**

It is well accepted that a stable proteome is important for synaptic plasticity, and dysregulation of synaptic protein synthesis, recycling, and degradation at the activated synapse is implicated in the pathogenesis of neurological diseases including ASD, ID and epilepsy (Cajigas et al., 2010; Klein et al., 2016; Louros and Osterweil, 2016). Of special interest is the phosphoinositide 3-kinase /AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway, which acts as a central regulator of a diverse array of cellular processes, including cell growth, proliferation and protein translation. Components of the mTOR pathway are present at synapses, where they play important roles in synaptogenesis, synaptic transmission and plasticity.

**TSC1/2**

Hamartin (TSC1) and tuberin (TSC2) are protein products of the TSC1 and TSC2 genes, respectively. These two proteins together with the recently identified protein TBC1D7 form a complex that inhibits the activity of mTOR complex 1 (mTORC1) through the inhibition of Ras homolog expressed in brain (Rheb), a GTPase located upstream of mTORC1. Spontaneous or inherited mutations in TSC1 and TSC2 genes lead to dysfunction of the TSC1/TSC2/TBC1D7 complex and disinhibition of mTORC1, thus resulting in tuberous sclerosis complex (TSC). TSC is a neurocutaneous disorder characterized by the formation of hamartomas in multiple organs, and neurological manifestations including epilepsy, ID and ASD. ASD is estimated to affect about 40% of patients with TSC (Richards et al., 2015), and epilepsy is one of the risk factors for the development of ASD in TSC patients (Vignoli et al., 2015; Mitchell et al., 2017).

Both heterozygous and homozygous loss of Tsc1 in mouse cerebellar Purkinje cells (PCs) result in autistic-like behaviors, including abnormal social interaction, repetitive behavior, and vocalizations (Tsai P.T. et al., 2012). PCs in the model mouse show significantly reduced spontaneous EPSC (sEPSC) frequency and mEPSC frequency, indicating a decreased number of functional glutamatergic synapses; importantly, human neurons derived from TSC2-deficient iPSCs display the same defects (Costa et al., 2016; Sundberg et al., 2018). The expression levels of the PC-specific glutamate receptor δ2 (GRID2), the postsynaptic marker PSD-95, and presynaptic marker synaptophysin (SYP1) are all reduced, suggesting abnormal synaptic development (Sundberg et al., 2018). High-throughput RNA sequencing reveals a down-regulation of genes associated with neurogenesis...
and glutamate receptor signaling in cortical tubers of TSC patients (Mills et al., 2017).

Eker rats, which carry a spontaneous Tsc2 germline mutation, exhibit marked reduction in LTP and LTD elicited by different protocols in hippocampal synapses (von der Brelie et al., 2006). Mice with a heterozygous mutation of Tsc2 show a reduced threshold for late-phase of LTP in the CA1 region of hippocampus, which can lead to inappropriate storage of unrelated or unprocessed information, thus impairing hippocampal-dependent learning (Ehninger et al., 2008). Mutations in both TSCI and TSC2 abolish metabotropic glutamate receptor-dependent LTD (mGluR-LTD) in hippocampal CA1 synapses, without affecting NMDAR-mediating LTD (Auerbach et al., 2011; Bateup et al., 2011). Inhibition by negative allosteric modulator (NAM) of mGluR5 corrects hyperactivity, seizures and elevated synaptic protein synthesis in Tsc2 mutant mice, whereas positive allosteric modulation of mGluR5 results in the exacerbation of hyperactivity and epileptic phenotypes, suggesting a meaningful therapeutic potential for mGluR5 NAM in TSC (Kelly et al., 2018).

**PTEN**

PTEN (phosphatase and tensin homolog), encoded by the PTEN gene on chromosome 10q23, is a dual lipid/protein phosphatase, best known for its role in tumor suppression. PTEN dephosphorylates phosphatidylinositol 3,4,5-trisphosphate (PIP3) to generate the bisphosphate product phosphatidylinositol 4,5-bisphosphate (PIP2), further negatively regulating the PI3K/AKT/mTOR signaling pathway, ultimately inhibiting cellular survival and proliferation. PTEN is also highly expressed in the central nervous system, where it is intimately involved in neuronal growth and synaptic function. Loss-of-function mutations in PTEN contribute to macrocephaly, seizures, ASD and mental retardation (Butler et al., 2005; Buxbaum et al., 2001; Orrico et al., 2009; Varga et al., 2009), and incomplete loss of PTEN function is more commonly linked to ASD (Spinnelli et al., 2015). PTEN mutations are found in 7–27% of macrocephalic ASD individuals and 8% of macrocephalic ID patients (McBride et al., 2010; Hobert et al., 2014).

Conditional knockout (cKO) mice in which Pten is ablated in granule cells of dentate gyrus (DGC) were reported to show increased evoked synaptic transmission in both glutamatergic and GABAergic neurons, resulting from an increase in the number of synaptic vesicles available for release and upregulated synaptogenesis (Weston et al., 2012). However, others studying Pten-deleted neurons reported increased excitatory synaptic currents but unchanged IPSC amplitude, thus shifting the E/I balance, leading to hyperactivity of mutant neurons (Luikart et al., 2011; Williams et al., 2015). cKO of Pten in PCs results in autistic-like traits in adult Pten-mutant mice, and mutant PCs show structural abnormalities in dendrites and axons and decreased excitability, as well as altered excitatory postsynaptic currents (Cupolloilo et al., 2016). Although loss of PTEN during early development is associated with substantial structural abnormalities, it has been shown that the effects of PTEN on neuronal morphology and synaptic function are independent of each other. cKO mice with Pten deletion restricted to forebrain excitatory neurons displayed impaired LTP and LTD at excitatory hippocampal synapses without any changes in morphology, which coincides with impaired spatial memory (Sperow et al., 2012). Pten-deleted DGC synapses showed enhanced theta burst-induced LTP and impaired mGluR-LTD prior to the onset of gross morphological defects (Takeuchi et al., 2013). PTEN can modulate synaptic activity via a PDZ-dependent association between PTEN and PSD-95 triggered by NMDAR activation, the PTEN then becomes anchored to the postsynaptic membranes, driving depression of the AMPA receptor-mediated synaptic responses required for NMDAR-LTD (Jurado et al., 2010).

**FMRP**

Fragile mental retardation protein (FMRP), encoded by FMR1, functions as an mRNA-binding translational suppressor, and is also involved in RNA-, channel- and protein binding to modulate synaptic transmission and plasticity (Davis and Broadie, 2017). Fragile X syndrome (FXS), the most common form of inherited ID and the most common monogenic cause of ASD, is caused by the loss of FMRP, 60–74% of male and 16–45% of female FXS patients meet the diagnostic criteria of ASD (Klusek et al., 2014). Nearly 1,000 high-confidence FMRP-associated transcripts have been identified (Darnell et al., 2011; Tang et al., 2015), and there is substantial overlap between putative FMRP target mRNAs and ASD-related mRNAs (Muddashetty et al., 2011; Zhang et al., 2014). Therefore, the commonly observed ASD endophenotype of FXS might reflect a “multiple hit” effect on the expression of ASD-related genes caused by loss of FMRP.

FMRP plays a key role in the dynamic regulation of the synaptic proteome. FMRP associates with Nlgn1, Nlgn2 and Nlgn3 mRNAs; consequently, Fmr1 KO leads to elevated local translation of Nlgn mRNAs and increased targeting of Nlgn1 and Nlgn3 to postsynaptic membranes (Chmielewska et al., 2018). FMRP binds to a G-rich region of PSD-95 mRNA, and together with microRNA miR-125a, plays an important role in the reversible inhibition of PSD-95 mRNA translation in neurons (Muddashetty et al., 2011; Stefanovic et al., 2015). However, recently, it was revealed that FMRP enhances rather than represses the translation of large ASD-related proteins; this discrepancy may arise because many FMRP targets act negatively on translation, protein stability or cell growth (Greenblatt and Spradling, 2018).

In Fmr1 KO mice, mGluR-LTD in the CA1 region of hippocampus is exaggerated (Hou et al., 2006; Till et al., 2015), while mGluR5 is less associated with the long Homer isoforms and more associated with short Homer 1a (Giuifrida et al., 2005; Ronesi et al., 2012). The long Homer proteins, Homer 1b, 1c, 2 and 3, target mGluR5 to the PSD though their interaction with Shank proteins, whereas the short Homer 1a uncouples mGlu5R from postsynaptic membranes (Kammermeier and Worley, 2007). Therefore, mGluR5 is significantly more mobile at hippocampal synapses of Fmr1 KO mice, causing increased co-clustering of mGluR5 and NMDAR, which correlates with reduced amplitude of NMDAR currents and the absence of NMDAR-LTD (Aloisi et al., 2017). The
enhanced mGluR-LTD is likely caused by dysregulated NMDAR signaling. Blocking GluN2B rescued mGluR-LTD, suggesting that GluN2B-containing NMDARs are hyperactive in Fmr1 KO mice (Toft et al., 2016). Moreover, NMDAR-LTP is decreased in the hippocampus of Fmr1 KO rats and mice, and pharmacological or genetic blockage of GluN2A-containing NMDARs completely rescues LTP and mGluR-LTD (Tian et al., 2017; Lundbye et al., 2018). In addition to the mGluR hyperexcitability theory, GABAergic hypoinhibition that leads to E/I imbalance may cause disease symptoms. In mouse and Drosophila FXS models, the mRNA and protein levels of GABA_A and glutamic acid decarboxylase (GAD), the rate-limiting GABA synthesis enzyme, are downregulated (Adusei et al., 2010; Gatto et al., 2014). In the cerebellum of Fmr1 KO mice, excessive GABA release from basket cell synapses, which results from increased excitability and Ca^2+ transients in the presynaptic sites due to disruption of the interaction between FMRF and Kv1.2, suppresses the firing activity of Purkinje neurons (Yang et al., 2018).

**UBE3A**

Ubiquitin–protein ligase E3A (UBE3A) is encoded by UBE3A gene, located in an imprinted region on chromosome 15q11-q13. Loss of function of the maternally expressed UBE3A gene gives rise to Angelman Syndrome (AS), characterized by microcephaly, ID, motor abnormalities, profound speech impairment, epilepsy and a unique behavioral pattern, including a happy demeanor (Buiting et al., 2016). The duplication or triplication of maternally inherited 15q11-q13 is associated with ASD, accounting for 1–3% of ASD cases worldwide (Hogart et al., 2010; LaSalle et al., 2015). UBE3A transfers ubiquitin from an E2 ubiquitin-conjugating enzyme to the substrate proteins, thus tagging these substrate proteins for proteasomal degradation. Not surprisingly, function deficits of UBE3A can increase the synaptic abundance of its substrate proteins and dysregulate many synaptic processes including synapse development, elimination, and function.

Drosophila with dUbe3a mutations have excess synaptic boutons and endocytic defects at neuremuscular junction terminals due to enhanced level of bone morphogenetic protein (BMP) signaling, crucial to neurodevelopment throughout evolution (Li et al., 2016b). In Ube3am−/p+ neurons, Arc protein accumulates due to a lack of degradation, leading to the excessive internalization of AMPARs, eventually disrupting homeostatic synaptic scaling of AMPARs (Greer et al., 2010; Kuhnle et al., 2013; Pastuzyn and Shepherd, 2017). Ube3am−/p+ mice show strongly decreased inhibitory transmission and increased excitatory transmission, leading to marked E/I imbalance. This is accompanied by an abnormal accumulation of clathrin-coated vesicles at inhibitory axon terminals, suggesting a defect in vesicle cycling (Wallace et al., 2012; Judson et al., 2016; Rotaru et al., 2018). In contrast, increasing gene dosage of Ube3a suppresses glutamatergic, but not GABAergic, synaptic transmission as a result of reduced presynaptic release probability, synaptic glutamate concentration, and postsynaptic action potential coupling (Smith et al., 2011). Besides, excessive UBE3A dosage is found to impair retinoic acid-mediated neuronal homeostatic synaptic plasticity (Xu et al., 2018). UBE3Am−/p+ mice show severely impaired hippocampal LTP despite normal neuroanatomy and basal synaptic transmission, which may be the result of increased inhibitory phosphorylation of CaMKII and enhanced neuregulin-ErbB4 signaling, so both mutations that prevent inhibitory phosphorylation of αCaMKII and ErbB inhibitors can reverse these deficits in LTP (Jiang et al., 1998; van Woerden et al., 2007; Kaphzan et al., 2012). In UBE3A-deficient mice, the level of postsynaptic SK2, a member of the family of small-conductance calcium-activated potassium channels (SKs), is elevated, resulting in decreased NMDAR activation, thereby impairing hippocampal LTP (Sun et al., 2015). mGlu5 receptor-dependent LTD is enhanced in hippocampal slices of Ube3a m−/p+ mice. However, totally unlike the coupling changes observed in FXS mice, Ube3a m−/p+ mice show a reduced expression of Homer 1a and an increased coupling of mGluR5 and Homer 1c (Pignatelli et al., 2014).

**MeCP2**

Methyl-CpG binding protein 2 (MeCP2), encoded by MECP2 gene, is a nuclear protein that preferentially binds to methylated DNA over unmethylated DNA, leading to global transcriptional repression by recruiting corepressors, such as histone deacetylases and transcriptional silencing factors. Loss-of-function mutations in MECP2 are the major cause of Rett Syndrome (RTT), an X-linked progressive neurodevelopmental disorder that nearly exclusively affects female patients. RTT patients experience an apparently normal postnatal development up to 6–18 months of age, and then neurological symptoms appear, including ID, autistic features, epilepsy and deteriorated motor and sensory function. Gain-of-function mutations in MECP2 are associated with the neurodevelopmental disorder MECP2 duplication syndrome, marked by severe ID, stunted motor development and epilepsy, as well as autistic-like features and anxiety.

MeCP2 can regulate synapse development and synaptic function, which is crucial for normal E/I balance. Hippocampal glutamatergic neurons that lack MeCP2 display reduced synaptic responses, whereas neurons with doubling of MeCP2 exhibit an enhancement in synaptic responses, primarily due to the alteration in the number of synapses formed (Chao et al., 2007). Wild-type GABAergic neurons in cerebral cortex express ∼50% more MeCP2 than non-GABAergic neurons, indicating Mecp2 might be particularly important to GABAergic function (Chao et al., 2010). MeCP2-deficient GABAergic neurons show reduced inhibitory quantal size, consistent with a presynaptic reduction of glutamic acid decarboxylase-1 (GAD-1) and GAD-2 levels (Chao et al., 2010). The expression levels of GABA transporter 1 (GAT1) and vesicular GABA transporter (vGAT) are significantly lower in the frontal cortex of Mecp2 KO mice, implying a dysfunctional GABA recycling system (Barth et al., 2014). Loss of MeCP2 also accelerates the NMDAR subunit switch from Glu2B to 2A at excitatory synapses onto cortical PV cells, indicating an acceleration of NMDAR development, but delays this switch in pyramidal cells, suggesting a developmental delay (Mierau et al., 2016). The frequency and amplitude of mIPSCs
is decreased, while the amplitude of mEPSCs is significantly enhanced in hippocampal pyramidal neurons of Mecp2 KO male mice (Mecp2<sup>−/−</sup>) and Mecp2 A140V mutant mice (Ma et al., 2014; Calfa et al., 2015). Naive excitatory synapses in CA1 pyramidal neurons of Mecp2 KO mice show larger AMPAR-mediated synaptic currents that result from a higher expression of GluA1-containing receptors (Li et al., 2016a). In the somatosensory cortex of Mecp2-null mice, AMPAR- and NMDAR-excitation is not altered, while postsynaptic inhibition is enhanced, as demonstrated by an increase in the amplitude of GABA<sub>A</sub>R-mediated sIPSCs and mIPSCs (Lo et al., 2016).

LTP and LTD are both attenuated in the hippocampus of Mecp2 KO mice (Asaka et al., 2006). The similar impairments are also observed in mice with truncated mutation of Mecp2 (Mecp2<sup>2908</sup>) and heterozygous female Mecp2-null mice (Mecp2<sup>stop/−</sup>) (Moretti et al., 2006; Weng et al., 2011). However, the underlying causes of this impaired LTP and LTD have not yet been thoroughly elucidated. One plausible mechanism for impaired LTP in Mecp2-null mice is the lack of activity-dependent trafficking of GluA1, since long-term plasticity is critically determined by activity-dependent insertion and removal of AMPARs to and from postsynaptic membranes (Li et al., 2016a). Moreover, an excess of free regulatory protein kinase A (PKA) subunits is seen in Mecp2<sup>−/−</sup>, binding to cAMP and buffering cAMP levels to restrict its availability for PKA activation, perhaps explaining the observed defective LTP in Mecp2<sup>−/−</sup> CA1 neurons (Balakrishnan et al., 2016). Mice with MeCP2 overexpression restricted in neurons (Ttau-Mecp2) also display impaired hippocampal LTP (Na et al., 2012). With regard to short-term plasticity, loss-of-Mecp2 function leads to reduced paired-pulse ratios (Asaka et al., 2006; Moretti et al., 2006; Weng et al., 2011), whereas gain-of-Mecp2 function augments paired-pulse responses (Collins et al., 2004; Na et al., 2012).

**SYNAPTIC ELIMINATION**

Besides abnormalities in synaptogenesis, synaptic neurotransmission, and synaptic plasticity caused by mutations in ASD-linked genes, several ASD risk genes including MEFC2, FMRI, DLG4, and PCDH10 are also involved in the process of synaptic elimination, so it is appropriate to discuss them as a whole (Ram Venkataraman et al., 2017), bearing in mind that completely different genetic causes can lead to a common cellular pathway leading to synaptic defects.

Maintenance of cognition and normal behaviors is heavily dependent on precise formation of mature neuronal circuits. During postnatal brain development, synaptic formation predominates at first, resulting in synapses in excess of functional need. Then in the subsequent postnatal period, activity-dependent elimination of redundant synapses decreases the number of synapses. In general, synaptic activity and the resulting neuronal depolarization and Ca<sup>2+</sup> influx activates the myocyte enhancer factor-2 (MEF2) family of transcription factors, inducing a rapid and robust synapse elimination (Wilkerson et al., 2014). MEF2C haploinsufficiency has been identified in patients who exhibit signs and syndromes that include ASD, ID and variable epilepsy (Novara et al., 2010; Paciorkowski et al., 2013). MEF2C acts as a convergent point in multiple regulatory pathways involved in the pathogenesis of ASD and ID (Parikshak et al., 2013; Gilissen et al., 2014).

MEF2-induced synapse elimination requires multiple autism-associated components. Deletion of MEF2C in mice causes a marked increase in the number of excitatory synapses, whereas neuronal expression of super-activating form of MEF2C leads to the reduced number of excitatory postsynaptic sites (Barbosa et al., 2008; Pfeiffer et al., 2010; Cole et al., 2012). However, MEF2 fails to eliminate functional or structural excitatory synapses in the hippocampus of Fmr1 KO mice, demonstrating FMRP functions downstream of MEF2-induced synapse elimination, consistent with the excess dendritic spines seen in Fmr1 KO mice and patients with FXS (Pfeiffer et al., 2010) FMRP and MEF2 cooperatively regulate the expression of protocadherin-10 (Pcdh10), a member of the cadherin superfamily of calcium-dependent cell adhesion molecules encoded by the PCDH10 gene. MEF2 activation leads to PSD-95 ubiquitination by the ubiquitin E3 ligase Murine Double Minute-2 (Mdm2), which is then delivered by Pcdh10 to the proteasome for degradation (Tsai N. et al., 2012). Exonic CNVs in Pten gene are associated with ASD (Morrow et al., 2008; Bucan et al., 2009). Mice lacking one copy of Pcdh10 (Pcdh10<sup>+/−</sup>) display reduced social approach behavior, as well as abnormal spine morphology, reduced levels of NMDAR subunits, and impaired gamma synchronization (Schoch et al., 2017). In neurons of Fmr1 KO mice, elevating levels of eukaryotic translation elongation factor 1-alpha (EF1α), an FMRP target mRNA, prevents phosphatase 2A (PP2A) interaction with and dephosphorylation of Mdm2 upon MEF2 activation, rendering Mdm2 hyperphosphorylated and trapped in the nucleus (Tsai N. et al., 2017).

**CONCLUSION**

Although the etiology of ASD is highly heterogeneous, numerous studies have shed light on common cellular pathways that converge at synapses. The functions of proteins encoded by ASD risk genes are so complex that in most cases, a single protein is implicated in multiple synaptic aspects, including synapse formation and elimination, synaptic transmission and plasticity. The data reviewed above suggests that the pathogenesis of ASD, at least in part, can be attributed to synaptic dysfunction. The ASD phenotype can be seen as the ultimate result of multiple hits of genetic and environmental factors. Further elucidation of the underlying synaptopathology, and advances in gene therapies and targeted drugs to modulate these processes, could lead to promising new therapies for the treatment of ASDs.

**AUTHOR CONTRIBUTIONS**

SG played a primary role in literature review and writing, revising of the manuscript. FH and CC participated in literature
searching. NP and LW helped with consultation of genetic information. XD and LY contributed to the qualitative coding and the writing of the manuscript. FY and JP developed the initial idea for this study, led the literature search, and critically reviewed manuscript drafts for intellectual content.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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