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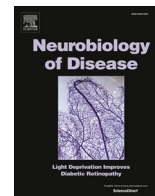
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Postsynaptic autism spectrum disorder genes and synaptic dysfunction

Paola Bonsi^{a,*}, Antonella De Jaco^{b,1}, Laurent Fasano^c, Paolo Gubellini^c

^a Laboratory of Neurophysiology and Plasticity, IRCCS Fondazione Santa Lucia, Via del Fosso di Fiorano 64, 00143 Rome, Italy

^b Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy

^c Aix Marseille Univ, CNRS, IBDM UMR7288, Parc Scientifique de Luminy, 163 avenue de Luminy Case 907, 13288 Marseille cedex 09, France

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ABSTRACT

This review provides an overview of the synaptic dysfunction of neuronal circuits and the ensuing behavioral alterations caused by mutations in autism spectrum disorder (ASD)-linked genes directly or indirectly affecting the postsynaptic neuronal compartment. There are plenty of ASD risk genes, that may be broadly grouped into those involved in gene expression regulation (epigenetic regulation and transcription) and genes regulating synaptic activity (neural communication and neurotransmission). Notably, the effects mediated by ASD-associated genes can vary extensively depending on the developmental time and/or subcellular site of expression. Therefore, in order to gain a better understanding of the mechanisms of disruptions in postsynaptic function, an effort to better model ASD in experimental animals is required to improve standardization and increase reproducibility within and among studies. Such an effort holds promise to provide deeper insight into the development of these disorders and to improve the translational value of preclinical studies.

1. Genetic and molecular aspects of autism spectrum disorder (ASD)

Autism spectrum disorder (ASD) comprises a heterogeneous group of rare diseases with different etiologies that share a common phenotype. ASD affects 1-1.5% of the worldwide individuals (Lord et al., 2020) and presents a complex genetic architecture with high heritability (Iakoucheva et al., 2019). A further level of complexity is added by an environmental component, that may add to the genetic susceptibility to affect common biological pathways and generate behavioral outcomes (Cheroni et al., 2020; Masini et al., 2020). As most psychiatric disorders, ASD is polygenic and strong genetic correlations between ASD and other psychiatric pathologies, including schizophrenia, have been reported,

confirming a genetic overlap and shared etiology (Gratten, Wray et al., 2014; Grove et al., 2019). In recent years, advances in large-scale genome-wide studies have been elucidating the complexity of genetic variations contributing to ASD etiology. These include copy number variations (CNVs) in around 15% of cases, and single nucleotide polymorphisms (SNPs), which are present in around 7% of patients. Total heritability has been estimated in the range of 50–60%, with common variants explaining the bulk of it (Turner et al., 2017). Nevertheless, the effect of common variants is relatively small, while rare variants, often CNVs, are highly penetrant with a large effect and can be either inherited or arise *de novo* in the germ line (Anney et al., 2012) (Gaugler et al., 2014). Although *de novo* mutations account for approximately 3-30% of autism risk (Satterstrom et al., 2020), currently identified

Abbreviations: AGC1, Solute Carrier Family 25 Member 12 (SLC25A12); AMPA, AMPA receptor; CAMP-GEFII, Rap Guanine Nucleotide Exchange Factor 4; Cav, voltage-gated calcium channel; CNTNAP, Contactin Associated Protein; CTTNBP2, Contactin Binding Protein 2; FMR1, FMRP Translational Regulator 1; FMRP, Fragile-X Mental Retardation Protein; FOXP1, Forkhead Box P1; FOXP2, Forkhead Box P2; FXR1, FMR1 Autosomal Homolog 1; GDP, Guanosine-5-diphosphate; GTP, Guanosine-5-triphosphate; HOXA1, Homeobox A1; Kv, voltage-gated potassium channel; MAGI, Membrane Associated Guanylate Kinase, WW and PDZ domain containing; MeCP2, Methyl CpG binding Protein 2; mGlu5, metabotropic glutamate receptor type 5; Nav, voltage-gated sodium channel; NF1, Neurofibromin 1; NLGN, Neuroligin; NMDA, NMDA receptor; NrCAM, Neuronal Cell Adhesion Molecule; NRXN, Neurexin; PSD-95, Postsynaptic Scaffolding Protein 95 kDa; PTEN, Phosphatase and Tensin homolog; RBFOX1, RNA Binding Fox-1 Homolog 1; SAPAP, Discs Large Homolog Associated Protein; SHANK, SH3 And Multiple Ankyrin Repeat Domains; SynGAP, Synaptic Ras GTPase Activating Protein; TBR1, T-Box Brain Transcription Factor 1; TSC, Tuberous Sclerosis Complex; TSHZ3, Teashirt Zinc Finger Homeobox 3.

* Corresponding author.

E-mail addresses: p.bonsi@hsantalucia.it (P. Bonsi), antonella.dejaco@uniroma1.it (A. De Jaco), laurent.fasano@univ-amu.fr (L. Fasano), paolo.gubellini@univ-amu.fr (P. Gubellini).

¹ These authors contributed equally.

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molecular pathways implicated in ASD are derived mainly from studies of these variants. Yet, more than half of all genes enriched for *de novo* mutations have yet to be discovered, and these variants may not account for the whole symptomatology. In addition, other variants are ultra-rare transmitted likely gene-disruptive (LGD), contributing to around 4.5% of autism risk in the human population, and converge on pathways shared by the *de novo* variants, but regulate a distinct set of ASD-linked genes (Wilfert et al., 2021). It is currently accepted that, for many risk alleles, both *de novo* and rare inherited (SNP and CNV) mutations contribute additively to the overall genetic risk (O’Roak et al., 2012). A large number of susceptibility genes for ASD has been now identified and most of them are available in large-genomic shared-datasets (Leblond et al., 2021). Nonetheless, an understanding of the neurobiology underlying ASD is still puzzling due to the modification of gene expression along the development and to the involvement of different brain regions. Lately, it is emerging that diverse genetic alterations associated with ASD can converge at multiple biological levels. Functional neurobiological studies have provided a map of the main dysregulated molecular pathways, that can be mainly divided in gene expression regulation (epigenetic regulation and transcription) and synaptic activity (neural communication and neurotransmission) (De Rubeis et al., 2014). Transcriptomic analyses indicate that the expression of ASD-related genes occurs early during development in combination with environmental insults, affecting brain development and the organization of neuronal pathways (Parikshak et al., 2013; Carroll et al., 2021). An enrichment in common variations is found in regulatory elements active in human corticogenesis (Grove et al., 2019), with ASD regulatory genes being more expressed prenatally in the cortex, while synaptic risk genes present the highest expression between late mid-fetal development and infancy in both excitatory and inhibitory neurons, likely impacting on neurotransmission and resulting in alterations of the excitatory-inhibitory balance (Satterstrom et al., 2020). This temporal window of expression of neuronal genes reflects the period for the interaction of presynaptic and postsynaptic neurons during the process of synaptogenesis, starting at the early fetal period to progress until birth and during prolonged postnatal periods (de la Torre-Ubieta et al., 2016; Courchesne et al., 2019).

Spatio-temporal co-expression of ASD-genes has suggested that common gene regulatory networks in specific brain regions converge on shared functional modules related to synaptogenesis, apoptosis, and GABAergic signaling. Moreover, co-expression networks from the entire transcriptome show molecular interaction modules related to mitochondrial function, splicing, and protein turnover, suggesting that these pathways are related to each other (Mahfouz et al., 2015). Alterations in messenger transcript structure are also emerging from transcriptome data from ASD-brain samples, with co-expression modules enriched in isoform-level diversity due to use of alternative transcription start sites, polyadenylation, and splicing (Mahfouz et al., 2015). A down-regulation of alternative splicing in the expression of genes dependent by the neuronal activity was found in the cortex of ASD brain samples, along with higher expression of neuronal splicing factors in the same brain region (Parikshak et al., 2016). This could link some of the molecular features of ASD to the altered functional phenotypes. ASD-transcriptomic profiles also highlight the dysregulation of long coding RNA (lcrRNAs), specifically enriched in the brain (Parikshak et al., 2016). At the protein level, proteins encoded by ASD risk genes are highly interconnected to each other in protein-protein interaction (PPI) networks, in comparison to random proteins, and converge in processes related to cell cycle, mitochondria, chromatin remodeling, nervous system/synapse and transport (Leblond et al., 2021).

2. ASD-associated postsynaptic genes

ASD risk genes are often involved in the structural organization and functional activity of the synapse. One of the most studied pathways associated to ASD is the Neuroligin (*NLGN*)/Neurexin (*NRXN*), found at

the postsynaptic density (PSD), including more than 20 genes encoding for cell adhesion molecules, scaffolding proteins at glutamatergic synapse (*SHANK*), cytoskeletal organization molecules, neurotransmitter receptors that are found both at the glutamatergic and GABAergic synapses (Chen et al., 2014; Washbourne, 2015; Guang et al., 2018; Choquet and Hosy, 2020).

The molecules of this pathway are linked to each other, participating in the formation of a macromolecular postsynaptic complex crucial for the organization of the synapse, since it regulates and coordinates the localization of receptors and signaling molecules for proper synaptic development and function (Südhof, 2018). Trans-synaptic adhesion molecules and interacting proteins are central drivers of specificity of neuronal connectivity, with cell-adhesion molecules playing a central role in specifying the synaptic architecture (Südhof, 2008; Arendt, 2020; Sanes and Zipursky, 2020; Kim et al., 2021). NLGNs are the most extensively characterized synaptic cell-adhesion molecules in rodents and humans for their structure and function in modulating synapse maintaining and maturation, thereby modulating excitatory and inhibitory synaptic transmission (Varoqueaux et al., 2006). Different members of the NLGN family are cell-type specific and regulate differently the post-synaptic recruitment of neurotransmitter receptors at the excitatory and inhibitory synapses (Song et al., 1999; Varoqueaux et al., 2004; Budreck and Scheiffele, 2007). Moreover, members of the family are characterized by differential binding affinities for the NRXNs (Comoletti et al., 2003; Boucard et al., 2005; Chubykin et al., 2007). NLGN1, in addition to its trans-synaptic association with presynaptic NRXN, associates to post-synaptic scaffolding proteins via its cytoplasmic domain such as PSD-95, S-SCAM and SHANKs, and to AMPA and NMDA receptors within spines at the excitatory synapses (Barrow et al., 2009; Gerrow et al., 2006; Blundell et al., 2010; Budreck et al., 2013; Espinosa et al., 2015; Jiang et al., 2017; Haas et al., 2018; Luo et al., 2021). Intracellular partners of NLGN2, that is localized at the GABAergic synapses, are the post-synaptic scaffolding molecules gephyrin, collybistin and S-SCAM (Graf et al., 2004; Varoqueaux et al., 2004; Ali et al., 2020). In contrast to NLGN1 and NLGN2, restricted to glutamatergic and GABAergic synapses, respectively, NLGN3 is found in both synapse types and can interact with excitatory and inhibitory post-synaptic scaffolds (Budreck and Scheiffele, 2007; Nguyen et al., 2020; Uchigashima et al., 2021). NLGN4 was initially linked to inhibitory synapses in mice (Bolliger et al., 2008; Hoon et al., 2011) but, more recently, was suggested to regulate excitatory synapses in humans (Marro et al., 2019). Several single amino-acid substitutions in the X-linked genes *NLGN3* and *NLGN4* have been identified in patients with ASD (Jamain et al., 2003; Laumonier et al., 2004; Yan et al., 2005; Talebizadeh et al., 2006; Lawson-Yuen et al., 2008; Pampanos et al., 2009; Quartier et al., 2019).

At the level of the PSD, a major role is played by PSD-95, a post-synaptic scaffold protein involved in synaptic plasticity. Lately, by analyzing the motifs of interaction, it was proposed for PSD-95 to act as hub protein with high connectivity in the autism-cognition network (ACN) and its partners representing key targets in cognitive dysfunction in ASD (Farahani et al., 2020). *SHANK* is enriched at corticostriatal glutamatergic synapses and interacts with many PSD proteins such as SAPAP, which in turn binds to PSD-95 to form the PSD-95/SAPAP/*SHANK* postsynaptic complex. *SHANK* is also linked to group I metabotropic glutamate (mGlu) receptors, through their binding to HOMER (Kursula, 2019; Ledonne and Mercuri, 2020). Recurrent mutations in the *SHANK3* gene have been identified in individuals with ASD where alterations in *SHANK3* result in dysfunction of the corticostriatal synapse (Monteiro and Feng, 2017; Amal et al., 2020).

Dysfunction of neurotransmitter receptors has been shown to play a major role in ASD. Such dysfunction generally arises indirectly, for example due to mutations in the scaffolding proteins mentioned above or by other postsynaptic mechanisms, since receptor gene mutation is very rare (Chen et al., 2014). Until now, NMDA receptor mutations have been found in very few patients with ASD, in particular in *GRIN2B* and

GRIN2A subunits (Barnby et al., 2005; Tarabeux et al., 2011), as well as in *GRIK2* kainate receptor subunit (Jamain et al., 2002; Valbuena and Lerma, 2021). Also group I metabotropic glutamate (mGlu) receptors (mGlu1 and mGlu5) have been involved in ASD, but mutations occur rather at the level of genes coding for proteins of the mGlu receptor pathway than directly at these receptors (Kelleher et al., 2012).

Alterations in post-synaptic components or in their regulation can lead to perturbations of the homeostatic signaling involved in the activity-dependent events. The genetic alterations consist mainly of SNPs in coding and non-coding genomic regions that produce alterations in gene regulation, protein folding and trafficking. It is not clear how the genetics underlying synaptic dysfunctions converge to ASD-phenotypical outcomes, but ASD symptoms might result from deficits in relevant circuitry due to synaptic dysfunction arising from improper regulation of molecular pathways and protein-protein interactions.

3. RNA processing, local protein translation and folding of synaptic proteins

The polygenic nature of ASD is reinforced by mutations in the genes encoding chromatin remodelers, transcription factors (TFs) and RNA-binding proteins (RBPs) which can alter a variety of developmental programs rather than a single gene function (Ayhan and Konopka, 2019). Misregulation in gene regulatory processes during development can be driven by epigenetic factors resulting in altered synaptic functions and neuronal excitability (Vuong et al., 2016). A global misregulation of alternative splicing (AS) has been shown in ASD (Baralle and Giudice, 2017). The AS program of neural microexons enriched in genes with synaptic functions, is regulated by the neural specific factor *nSR100/SRRM4* and is dependent on neuronal activity. Reduced transcript levels of *nSR100/SRRM4* have been identified in individuals affected by ASD (Irimia et al., 2014; Quesnel-Vallières et al., 2016). Mutant mice for *nSR100/SRRM4* show altered synaptic transmission providing support for a role for *nSR100/SRRM4* as a regulatory “hub” that is impacted by the changes in neuronal excitability that underlie ASD (Quesnel-Vallières et al., 2016).

Despite the PTEN (phosphatase and tensin homolog) phosphatase is a tumor suppressor associated with cancer syndromes, there is a strong genetic association between germline PTEN mutation and ASD diagnosis (Yehia et al., 2020). The expression of *PTEN* is regulated by AS and, at the same time, nuclear PTEN can regulate AS in a phosphatase-independent manner (Shen et al., 2018). The disruption of *PTEN* affects the brain AS landscape with changes that are relevant to the pathophysiology of ASD (Thacker et al., 2020) as shown by the *Pten*^{m3m4} mouse model, that presents an autism-like behavior and molecular features of human ASD (Tilot et al., 2014, 2016; Frazier et al., 2015; Lee et al., 2019; Sarn et al., 2021). Changes in AS, in the *Pten*^{m3m4} mouse, lead to the decreased expression of neural-enriched splicing factors including *Celf* family genes, *Nova2*, *Rbfox1* and *nSr100/Srrm4* (Tilot et al., 2016; Thacker et al., 2020). Splicing changes are also found in targets of RNA binding proteins regulating synaptic transmission, such as *RBFOX1* and *FMRP* (Gandal et al., 2018). *RBFOX* proteins control AS, and dysregulated *RBFOX1* in *post-mortem* brains is a common feature of genetically distinct ASD cases (Voineagu et al., 2011). *RBFOX1* alterations interfere with the splicing of transcripts involved in ASD, such as *SHANK3*, *CACNA1C* and *TSC2* (Voineagu et al., 2011; Weyn-Vanhen-tenryck et al., 2014). Moreover, cytoplasmic *RBFOX1* targets are enriched in modules affecting synaptic function and ASD (Parikshak et al., 2013). In a co-expression analysis of the brain transcriptome, *RBFOX1*, *CNTNAP1*, *CHRM1*, *APBA2* were identified as genes highly connected in the ASD-associated co-expression module (Lee et al., 2016). *FMRP* regulates plasticity processes by interacting with the mRNA of synaptic and chromatin regulatory proteins and by controlling mTOR signaling pathway in embryonic and postnatal brains (D’Antoni et al., 2014; Brandalise et al., 2020; Casingal et al., 2020). *FMRP* is also involved in the activity-dependent synaptic remodeling processes in

different brain regions during critical periods (Doll and Broadie, 2015; Sawicka et al., 2019; Golovin et al., 2021). In the hippocampal CA1 neurons, *FMRP1* binds ASD-related transcripts encoding postsynaptic proteins such as *SHANK1*, *SHANK3*, *SYNGAP1* and *PSD-95* (Sawicka et al., 2019). In the *Fmr1* knockout (KO) mouse, while *FMRP* targets result mainly down-regulated, the up-regulation of proteins involved in proteasome and mitochondrial functions were reported, suggesting a link between potential mitochondrial deficits and impaired neuronal activity (Sawicka et al., 2019).

A large number of downregulated editing sites were found in the frontal cortex of patients with ASD (Tran et al., 2019). Main alterations occur during the transition from the fetal to the infantile phase of cortical development, a critical step for proper brain development. A convergence between RNA editing patterns in ASD and Fragile X syndrome has been reported, based on a correlation between the hypo-editing and the expression of *FMR1* and *FXR1* genes (Tran et al., 2019). The two proteins, *FMR1* and *FXR1*, also share common validated target sites, suggesting a synergistic regulation of the RNA editing process. A prominent role in human cognitive functions has been proposed for the family of *FOXP* transcriptional factors (Konopka and Roberts, 2016). *FOXP1* variants are linked to language impairments in association with intellectual disability (ID) and ASD (Lozano et al., 2015; Meerschaut et al., 2017). *FOXP2* is functionally linked to *FOXP1*, in association with speech and language disorders (Estruch et al., 2016). The protein *FOXP2* interacts with the transcription factor *TBR1* (Sakai et al., 2011; Deriziotis et al., 2014), which is encoded by *TBR1*, a category 1 high-confidence ASD-risk gene in SFARI (Simons Foundation Autism Research Initiative) (Abrahams et al., 2013; Notwell et al., 2016). In patients with ASD, most of the *de novo* pathogenic variants in *TBR1* disrupt the transcriptional repression activity and the interaction with *FOXP1*, *FOXP2* and *BCL11A* (Deriziotis et al., 2014; Fazel Darbandi et al., 2018; Nambot et al., 2020; Sapay-Triomphe et al., 2020), affecting the expression of genes associated with neurons, astrocytes, ribosomes and neuronal synapses (Yook et al., 2019). Among the transcriptional factors regulating ASD genes, the zinc-finger homeodomain transcription factor *TSHZ3* (teashirt zinc-finger homeobox family member 3) (Caubit et al., 2016) controls the cortical expression of more than 1000 genes, among them 173 genes found at the PSD (Chabbert et al., 2019). Analysis of RNA expression from the blood of ASD-pediatric individuals (1–4 years) revealed high enrichment in gene categories relevant to translation or translation initiation compared to the control patients (Pramparo et al., 2015). Synaptic translation is involved in several neuronal functions, such as neurite outgrowth, axon guidance, synapse formation, synaptic pruning, and synaptic plasticity, either long-term potentiation (LTP) or long-term depression (LTD) (Younts et al., 2016). In addition, rapid protein synthesis at the synapse plays a critical role in higher cognitive brain functions, through the regulation of signal transduction pathways, network connectivity, and axonal and synaptic morphology (Mofatteh, 2020). Local translation dysfunction may contribute to ASD through abnormal levels of synaptic proteins, including *NRXNs* and *NLGNs*, occurring in the synaptic area in neuronal activity dependent manner. Translation of the mRNAs for *NLGNs* is negatively regulated by *FMRP* (Chmielewska et al., 2019), and mRNAs encoding *SHANKs* include specific 3’ UTR, that, when mutated, interrupts local translation (Epstein et al., 2014) suggesting aberrant local translation in the pathophysiology of ASD (Joo and Benavides, 2021).

Several loss of function mutations have been described in individuals with ASD for *NRXN1*, *NLGN3*, and *NLGN4* genes (Tabuchi et al., 2007; Jamain et al., 2008). Regarding *NLGNs*, most of the reported mutations map to the extracellular protein domain (Fabrichny et al., 2012). Protein folding impairments caused by the substitutions affect trafficking of the mutant protein, diminishing its ability to modulate synaptic functional properties (Trobiani et al., 2020; Cast et al., 2021). *SHANK3* variants have been identified in patients with ASD, mainly non-synonymous mutations, while synonymous mutations or variations in regulatory regions were rarely reported (Bonaglia et al., 2011; Leblond et al., 2014;

Wang et al., 2020). Among the missense point mutations, L68P and R12C cause altered protein folding (Bucher et al., 2021).

4. Novel directions in the investigation on synaptic dysfunction in ASD

ASD can be considered a disorder of connectivity, since ASD genes directly or indirectly affect a whole range of presynaptic and postsynaptic proteins, and synaptic dysfunction is common to both syndromic and idiopathic forms (Carroll et al., 2021). Several coding and non-coding variants of ASD genes may affect, with specific spatiotemporal features, the presynaptic and postsynaptic molecular assembly in the central (CNS) and peripheral (PNS) nervous systems. A recent review (Heavner and Smith, 2020) proposed a unified model that incorporates both developmental and synaptic signaling genes, on the basis of recent work showing that a critical developmental phase and lifelong activity-dependent synaptic homeostasis are largely controlled by the same sets of critical genes.

Therefore, in order to dissect the pathophysiology of ASD, it is important to determine both when and what components of the synaptic machinery are primarily affected, ultimately compromising the whole neuronal network.

4.1. Control of synaptic integration and dendritic excitability

Emerging evidence indicates that some ASD-associated genes lead to defects in the structure, integrative function and excitability of dendrites, causing synaptic alterations, and in turn influencing the whole network and the behavior (Nelson and Bender, 2021). On the other hand, changes in the expression, localization, and/or trafficking of synaptic proteins, ion channel function or expression, excitatory/inhibitory (E/I) balance, or dendritic morphology may affect dendritic excitability and integrative function, therefore interfering with neuronal

activity and synaptic plasticity (Nestor and Hoffman, 2012; Satterstrom et al., 2020).

High confidence ASD genes can control dendritic excitability and synaptic integration either directly, or indirectly. In particular, dendritic excitability can be directly affected by rare variants and common polymorphisms in ASD genes encoding voltage-dependent calcium, sodium, and potassium channels. Additionally, dendritic function can be impaired by high-confidence ASD genes encoding proteins which regulate synapse structure and function, e.g. NLGNs, SHANKs, AMPA and NMDA glutamate receptor subunits. Finally, dendritic activity may be affected by ASD genes encoding modifiers of chromatin, transcription, and translation, that can alter the expression of ion channels, receptors, and other synaptic proteins (Fig. 1).

As discussed in a recent review (Nelson and Bender, 2021), studies on the impact of many high-confidence ASD genes on dendritic structure, function, and integration in neocortical layer 5 and 6 pyramidal neurons, suggest that impaired dendritic excitability and integration may represent a central node of ASD gene convergence. Yet, the impact of ASD genes on dendritic function needs to be investigated in additional brain areas involved in ASD pathophysiology, including basal ganglia, hippocampus, and cerebellum, which show, with respect to the neocortex, different and specific characteristics of the dendritic function within the information processing.

4.2. Synaptic mitochondria

Increasing evidence obtained by multiple ASD models, patient post-mortem brains, clinical and genetic observations supports the view that mitochondrial dysfunction is involved in ASD pathophysiology (Rojas-Charry et al., 2021; Wen and Yao, 2021).

Indeed, neurons are characterized by a high metabolic demand, due to the maintenance of a hyperpolarized membrane potential and to the ATP-consuming synaptic activity. Accordingly, neuronal cells are

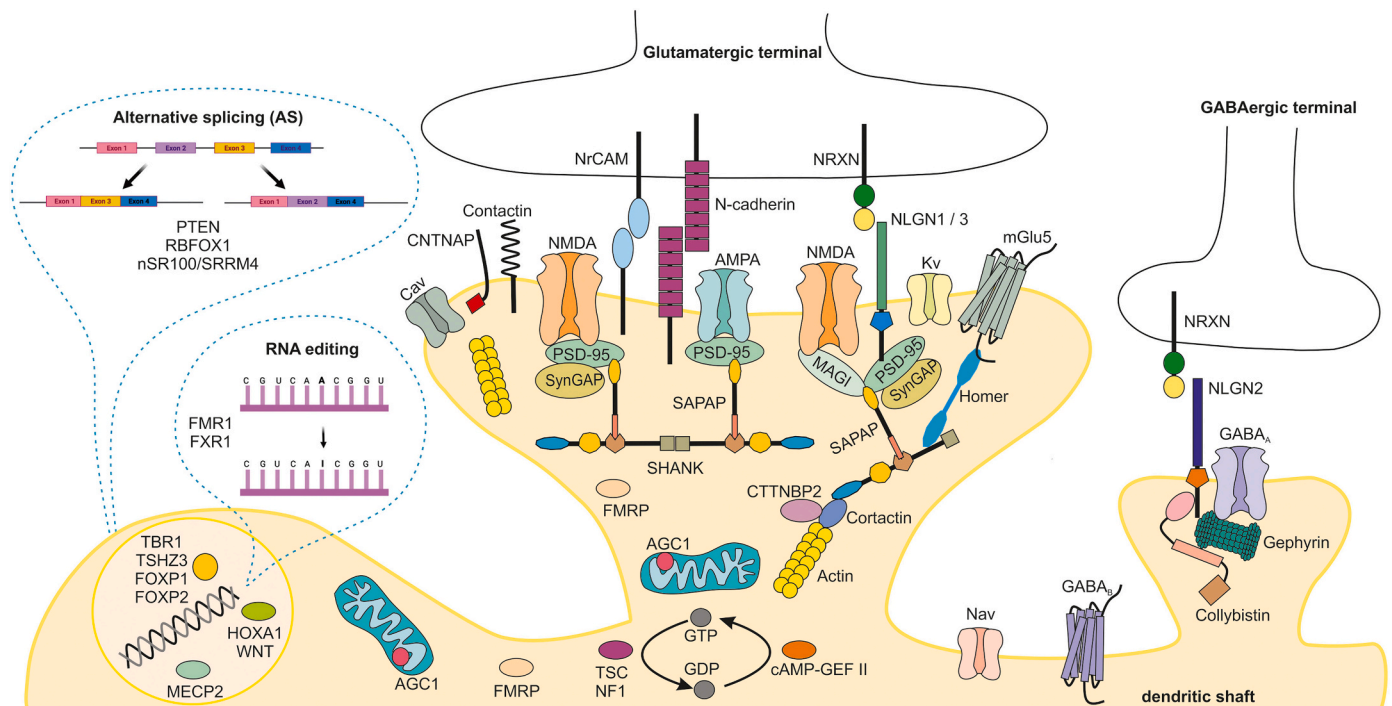


Fig. 1. Schematic illustration representing postsynaptic proteins associated to ASD. These proteins are involved in different synaptic functions, either directly (ion channels and glutamate receptors), or indirectly, including transmembrane heterophilic (NLGNs) and homophilic (NrcAM) cell-adhesion molecules, and scaffolding proteins (PSD-95, Shank, Homer), that link transmembrane and membrane-associated protein complexes with the underlying actin cytoskeleton. Additional cellular functions may influence synaptic activity in ASD, such as alternative splicing (PTEN, RFX1, nSR100/SRRM4), RNA editing (FMR1, FXR1), transcription (FOXP1, FOXP2, TBR1, TSHZ3), translation (FMR1), degradation (UBE3A), and mitochondrial activity (AGC1).

particularly enriched in mitochondria. Nonetheless, the number of mitochondria in synaptic terminals and axons exceeds the predicted energy needs. This is not surprising, since besides energy production, also the calcium-buffering capacity of synaptic mitochondria is crucial during synaptic activity (Datta and Jaiswal, 2021). Neuronal activity causes calcium influx, activating mitochondrial uptake. An increase of mitochondrial calcium: i) sustains synaptic transmission by enhancing ATP production; ii) directly affects the firing probability of neurons; iii) buffers synaptic calcium, preventing dysfunction of neurotransmission and neurotoxicity; iv) intervene in the coordinate action of cytoskeletal and motor proteins, regulating mitochondrial motility. In fact, by means of balanced fusion and fission processes, and motility, mitochondria adapt their morphology and distribution in neurons to fulfill specific tasks. Of note, an involvement of mitochondrial fission in LTP has been recently documented in hippocampal pyramidal neurons, where NMDAR-dependent LTP induction promotes a rapid burst of dendritic mitochondrial fission and elevations of mitochondrial matrix calcium (Divakaruni et al., 2018). Accordingly, preventing fission impaired both mitochondrial calcium elevations and LTP. Mitochondria are found mainly in the dendritic shafts, but also in spines (Sheng and Hoogenraad, 2007). Notably, the mitochondrial content in dendrites is directly correlated to the number of spines and synapses (Li et al., 2004).

Some studies identified mitochondrial abnormalities in the BTBR phenotypic mouse model of ASD (Ahn et al., 2020), and in different brain areas of parvalbumin (PV)-KO mice (Janickova et al., 2020), which recapitulate ASD features, as well as in models of ASD-related neurodevelopmental disorders, such as DiGeorge, Rett, and Fragile X syndromes, Tuberous sclerosis complex, as well as Angelman and Down syndromes (Rojas-Charry et al., 2021).

SHANK3 interacts with several mitochondrial proteins in mouse synaptosomal preparations (Lee et al., 2017), and *Shank3* mutation induces aberrant protein S-nitrosylation signaling in mice, affecting a wide range of mitochondria-related proteins and processes that may contribute to ASD pathology (Kartawy et al., 2021). The *SHANK3* gene resides next to six mitochondrial genes in the 22q13 region, where deletions occur in Phelan McDermid syndrome (PMS) patients (Riccicardello et al., 2021). These genes include those essential for complex I (*NDUFA6*) and IV (*SCO2*) function of the electron transport chain, as well as mitochondrial DNA (*TYMP*), RNA (*TRMU*) and fatty acid (*CPT1B*) metabolism and tricarboxylic acid cycle function (*ACO2*). Accordingly, a study showed impaired activity of mitochondrial complexes I and IV in PMS patients (Frye et al., 2016). Dysregulation of mitochondrial genes has also been reported in the cortex (Chabbert et al., 2019) and the striatum (Caubit et al., 2021) of mice carrying conditional *Tshz3* deletions.

Mitochondrial abnormalities have been documented in genetic syndromes associated with ASD, such as *PTEN* mutations and tuberous sclerosis, Fragile X, Rett, 15q11-13 duplication, Angelman and Down syndromes (Frye et al., 2021).

The *SLC25A12* (solute carrier family 25 member 12) gene encodes the mitochondrial aspartate-glutamate carrier isoform 1 (AGC1; Fig. 1) and was initially identified as an autism-susceptibility gene through a linkage-directed association study (Ramos et al., 2008). Further studies confirmed an association between *SLC25A12* and restricted repetitive behaviors in ASD (Silverman et al., 2008; Kim et al., 2011). However, a meta-analysis reported inconsistent data regarding the association between ASD and *SLC25A12* SNPs (rs2292813 and rs2056202). In fact, sensitivity analyses including only studies with family-based design demonstrated significant association, while sensitivity analyses including only case-control design studies did not (Aoki and Cortese, 2016).

Another study investigated the expression and genetic association of genes related to diverse mitochondrial functions in the genomic DNA from postmortem brain tissues of patients with ASD. This study found that several nuclear genes (*SLC25A12*, *SLC25A14*, *SLC25A24*, *SLC25A27*, *DNAJC19*, *DNM1L*, *LRPPRC*, *TOMM20*, *MTX2*, *NEFL*)

showed brain region-specific expression alterations in patients with autism compared to controls (Anitha et al., 2012).

In addition to genomic DNA, mitochondrial DNA germline and somatic variants have been found in patients with ASD (Citrigno et al., 2020). Therefore, (Yardeni et al., 2021) tested the hypothesis that specific mtDNA mutations could induce ASD endophenotypes in mice. By introducing an mtDNA *ND6* gene missense mutation into a mouse germline, they found that the *ND6*^{P25L} mice exhibit impaired social interaction, compulsive behavior, and increased anxiety. These ASD-like behaviors correlated with impaired cortical and hippocampal mitochondrial respiration and increased reactive oxygen species production. Thus, these authors concluded that mitochondrial defects can be sufficient to produce ASD phenotypes.

In conclusion, many prenatal factors increasing ASD risk are associated with mitochondrial dysfunction (Frye et al., 2021). The role of mitochondria at the synapse is complex and goes beyond providing energy and stabilizing calcium concentration. Furthermore, mitochondrial function varies in different parts of the brain and neuronal types. Thus, further studies addressing these aspects will be necessary to unravel how synaptic mitochondria are affected by ASD genes and contribute to the pathophysiology of ASD.

4.3. Peripheral synaptic dysfunction in ASD

According to a “two-hit” hypothesis of ASD pathophysiology (Picci and Scherf, 2015), early genetic perturbation of brain connectivity may be considered as a “first-hit”, which sets up a neural circuitry that is prone to an environmental “second-hit” during late childhood. Indeed, human neurodevelopment can be affected by genetic and environmental factors during prenatal and postnatal critical periods for the establishment of the interaction of presynaptic axons and postsynaptic neurons.

ASD candidate genes involved in synapse formation and function (e.g., *CNTNAP2*, *FMR1*, *MECP2*, *NLGNs*, *NRXNs*, and *SHANKs*) are expressed also by primary sensory neurons. Accordingly, hypersensitivity to touch or abnormal pain sensitivity are common in people with ASD (Casio, 2010), and many genetic ASD mouse models show abnormal sensory behavior (Carroll et al., 2021). For instance, mutations in *Frm1*, *Mecp2*, and *Shank3* genes in mice impair touch discrimination and cause hypersensitivity to tactile stimuli (Orefice et al., 2016). These authors found that such sensory alterations were directly attributable to the altered synaptic function of peripheral neurons, which caused, in turn, additional ASD-like behaviors, suggesting that an aberrant sensory input may cause an altered synaptic development in patients with ASD. ASD gene disruption in primary sensory neurons during development or in adulthood causes similar alterations of sensory behavior, but removal of these genes during development additionally causes anxiety, reduced social behavior, and brain circuitry changes (Dawes et al., 2018; Orefice et al., 2019).

These observations suggest that abnormal sensory experience during critical developmental periods may represent a pathophysiological mechanism in ASD.

Beside the aberrant sensory input at the level of the PNS mentioned above, impaired processing of sensory input in the cortex can also be involved in the abnormal sensitivity observed in patients with ASD.

5. Synaptic transmission dysfunction and behavioral abnormalities in experimental ASD models

It is now generally agreed that ASD can be considered a synaptopathy. Accordingly, a great number of studies of ASD mouse models have evidenced dysfunctions at the level of synaptic structure and transmission in several brain regions including the striatum, the cortex, the hippocampus and the amygdala, and report several behavioral abnormalities such as learning and motor deficits, social interaction impairments, anxiety, and stereotypies (Lepeta et al., 2016; Reig-Viader et al., 2018; Wang et al., 2018; Li and Pozzo-Miller, 2020; Yan and Rein,

2021). It is worth remembering here that ASD diagnosis is based exclusively on behavioral criteria, which comprise two domains defined as: i) deficit in social communication and interaction, and ii) stereotyped, repetitive patterns of behavior or activities, and restricted field of interests (American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, DSM-5, Fifth Edition* American Psychiatric Association: Washington, DC, 2013). For this reason, we will focus on behavioral abnormalities in ASD models mimicking the two ASD domains, even if the studies presented in this section reported other behavioral phenotypes. Also, it would be impossible here to discuss all the published papers due to their large number, and it is often difficult or impossible to compare them due to the heterogeneity of the results and methods, the different brain regions examined, as well as the multiple experimental ASD models utilized. We will therefore provide some examples of the most representative ASD models bearing mutations in these synaptic genes that have been characterized from a synaptic and an ASD-related behavioral point of view. The characterization of some of these models holds the promise to discover the biological mechanisms underlying ASD behaviors and to evaluate the efficacy of new potential treatments (Delorme et al., 2013; Kazdoba et al., 2016).

Striking examples of the abovementioned heterogeneity are provided for instance by *Nlgn* mutant mice among which *Nlgn3* R451C KI, but not *Nlgn3* KO, exhibit increased spontaneous inhibitory synaptic transmission (Tabuchi et al., 2007) and impaired endocannabinoid-mediated signaling in layer 2/3 of the somatosensory cortex (Speed et al., 2015b), paralleled by social interaction deficits (Tabuchi et al., 2007). Such alterations in synaptic transmission are region-specific (Etherton et al., 2011; Rothwell et al., 2014; Trobiani et al., 2018). An impairment of corticostriatal LTD was reported in the dorsal striatum of *Nlgn3* R451C KI mice, but was not investigated in the *Nlgn3* KO model (Martella et al., 2018). The *NLGN4X* R704C mutation enhances both AMPA and NMDA receptor-mediated synaptic transmission in the CA1 area of the hippocampus (Bemben et al., 2015). *Nlgn1* KO mice exhibit a decreased NMDA/AMPA ratio in the dorsal striatum and decreased LTP in the CA1 hippocampal area, associated to increased stereotyped grooming behavior. In these mice, administration of D-Cycloserine (a NMDAR partial co-agonist) improved the repetitive grooming phenotype (Blundell et al., 2010). Conversely, a striking similarity between different ASD models was shown by Antoine et al. (2019), who examined 4 genetically distinct mouse mutants (*Fmr1*^{-y}, *Cntnap2*^{-/-}, *16p11.2*^{del/+}, *Tsc2*^{+/-}) that exhibited impaired feedforward GABAergic transmission coupled to decreased glutamatergic inputs in layer 2/3 of the whisker barrel cortex, driving a common increase in E/I ratio and excitatory conductance.

More characterized models are those carrying *Shank3* mutations, which mainly result in several defects at the level of excitatory synaptic transmission, generally paralleled by the appearance of one or both ASD behavioral domains. For example, a model with heterozygous disruption of full-length *Shank3* showed decreased AMPA receptor-mediated transmission and impaired LTP in the hippocampus paralleled with reduced male/female social interaction. In these mice, injections of IGF-1 reversed deficits in hippocampal AMPA signaling and LTP as well as motor performance (Bozdagi et al., 2010). Mice with *Shank3* exon 11 deletion (*Shank3*^{Δ11-/-}) show impaired social behavior and loss of the mGlu5 receptor-mediated enhancement of NMDA receptor signaling in striatal medium spiny neurons; accordingly, the mGlu5 receptor positive allosteric modulator CDPPB [3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide] could restore this behavioral deficit (Vicidomini et al., 2017). In *Shank3B*^{-/-} (*Shank3*^{ex13-16-/-}) mice with exon 13-16 deletion, Peça et al. (2011) reported decreased corticostriatal synaptic transmission in the dorsal striatum as well as excessive grooming and robust social interaction deficits. In these mice, Fourie et al. (2018) showed that increased dietary zinc prevents ASD-associated behaviors by decreasing synaptic transmission through AMPA and NMDA receptors as well as by preventing LTP at corticostriatal synapses. Heterozygous and homozygous deletions of exon 13 (*Shank3*^{E13}) result in increased repetitive

grooming and deficits in social interaction, with impaired hippocampal long-term plasticity and reduced striatal glutamatergic synaptic transmission (Jaramillo et al., 2017). In these *Shank3*^{E13} mice, it was shown that ASD-like phenotype and synaptic defects can be rescued by genetic restoration of *Shank3*, obtained by removing a loxP-flanked STOP cassette inserted before exon 13 either at pre- or postnatal stages (Mei et al., 2016; Jaramillo et al., 2017). Another mutation consisting in exon 21 deletion (*Shank3*^{ΔC/ΔC} or *Shank3*^{G/G}) produced several behavioral deficits, but only minimal social abnormalities and increased grooming in adult but not in young mice, with decreased LTP and NMDA receptor mediated transmission in the hippocampus (Kouser et al., 2013; Speed et al., 2015a). Recently, (Moutin et al., 2021) reported in *Shank3*^{ΔC/ΔC} mice a decreased NMDA/AMPA ratio associated with repetitive stereotyped behavior. These synaptic and behavioral changes could be reversed by injecting an AAV containing a Homer-GluN2B chimera that restores a molecular bridge between NMDA and mGlu5 receptors. Mice with both homozygous and heterozygous deletion of *Shank3* exons 4-9 (*Shank3*^{e4-9}) show a reduction of NMDA/AMPA ratio at striatal medium spiny neurons and impaired hippocampal LTP, associated to abnormal social interaction and increased repetitive grooming (Wang et al., 2011; Jaramillo et al., 2016). Yoo et al. (2018) generated two *Shank3*-mutant lines with exons 14-16 deletion, either global or GABA neuron-specific (*Shank3*^{Δ14-16} and *Viaat-Cre;Shank3*^{Δ14-16}, respectively); they show similar decreases in excitatory striatal synaptic input and abnormal social behaviors, whereas repetitive behaviors are present only in *Shank3*^{Δ14-16}, suggesting that *Shank3* loss in GABAergic neurons affects specifically striatal excitatory synaptic transmission and social behavior. On the other hand, Wang et al. (2016) characterized mice with exon 4-22 deletion (*Shank3*^{Δe4-22}) which showed both impaired social behavior and stereotypies; moreover, they reported altered frontostriatal connectivity and lower NAc neuron firing *in vivo*, as well as loss of *in vitro* LTD in the dorsolateral striatum. Peixoto et al. (2016, 2019) describe precocious maturation of corticostriatal glutamatergic inputs in *Shank3B*^{-/-} mice that show increased grooming after postnatal (P) day 21 but not before P15, opening the issue about the role of such ASD-linked genes in early postnatal brain development, during which functional and structural changes may occur that will result in ASD-like phenotype, and which could represent a therapeutic window for early rescue.

Concerning *Shank2*, KO mice show decreased glutamatergic synaptic transmission, increased NMDA/AMPA ratio and enhanced LTP in the CA1 region of the hippocampus (Schmeisser et al., 2012). The consequences of *Shank2* loss have also been characterized in the cerebellum, where it impairs the intrinsic properties of Purkinje cells and LTP at their synapse with parallel fibers (Peter et al., 2016), and reduces glutamatergic synaptic transmission onto these neurons (Ha et al., 2016). These *Shank2* KO mice present both social interaction deficits and stereotyped behaviors. Selective *Shank2* deletion in Purkinje cells leads to similar synaptic defects but only to some repetitive behavior, suggesting that *Shank2* is critical in these neurons for the correct establishment of their synaptic network (Ha et al., 2016). Another interesting input about the possible cellular substrates for the synaptic dysfunctions leading to ASD-like phenotypes in *Shank2* mutants is provided by the paper by Kim et al. (2018), which selectively deleted this gene in excitatory (*CamKII-Cre;Shank2*^{f/f}) or in GABAergic (*Viaat-Cre;Shank2*^{f/f}) neurons: the former deletion results in decreased glutamatergic synaptic transmission in CA1, as observed before (Schmeisser et al., 2012), while the latter in reduced GABAergic transmission in the striatum. Interestingly, *CamKII-Cre;Shank2*^{f/f} and *Viaat-Cre;Shank2*^{f/f} mice show similar but not identical ASD-like behavior, suggesting cell-type-specific *Shank2*-dependent regulation of neuronal synapses and behaviors (Kim et al., 2018). In mice with *Shank2* exons 6 and 7 deletion (*Shank2*^{-/-}), Won et al. (2012) showed ASD-like impaired social interaction and social communication, and repetitive jumping, associated to decreased NMDA/AMPA ratio and impaired LTP and LTD in the CA1 area of the hippocampus. Some of these synaptic and behavioral deficits could be restored by CDPPB or by

D-cycloserine, a partial agonist at the glycine-binding site of NMDA receptors (Won et al., 2012; Huang et al., 2014). Similarly, Lee et al. (2015) could improve social interaction, normalize NMDA/AMPA ratio and recover LTP in *Shank2*^{-/-} mice by clioquinol, a zinc chelator and ionophore, and this treatment was also effective in normalizing social behavior and NMDA receptor function in the lateral amygdala of *Tbr1*^{+/-} mice.

Shank1 has been given less attention than *Shank3* and *Shank2*. Two studies show reduced excitatory and inhibitory synaptic activity in the hippocampus and normal LTD and LTP in *Shank1*^{-/-} mice, but behavioral defects relevant to the two ASD domains were not reported (Hung et al., 2008; Mao et al., 2015). Another study using *Shank1*^{-/-} and *Shank1*^{+/-} mice could not pinpoint ASD-relevant behavior, mostly because control animals failed to show a normal social behavior (Silverman et al., 2011). Finally, a study characterizing *Shank1*^{-/-}/*Shank3*^{-/-} double KO showed reduced viability, drastic defects in brain structure and function, and ASD-like behavior (Mossa et al., 2021).

Less common ASD models include, for example, mice lacking either *Nrxn2α* or both *Nrxn2α* and β , which have impaired glutamate release and reduced NMDA/AMPA ratio in cortical layer 5, paralleled by similar ASD-like behavioral impairments, suggesting that the β -variant of NRXN2 has no strong function in basic transmission at these synapses (Born et al., 2015). Another example is provided by *Dlgap2*^{-/-} mice that present structural and functional synaptic defects in the orbitofrontal cortex and abnormal social behavior (Jiang-Xie et al., 2014). In *Cntnap2*^{-/-} mice, Penagarikano et al showed that Risperidone treatment decreases repetitive behavior (Peñagarikano et al., 2011). Moreover, Sacai et al. (2020) showed reduced excitatory and inhibitory synaptic transmission in prefrontal cortex layer 2/3 pyramidal neurons with a net increase of I/E ratio in *Cntnap2* knockdown mice. They also characterized *Ahi1* knockdown mice (*AHI1* is an ASD-linked gene whose mutation is also found in Joubert syndrome), which show increased NMDA/AMPA and I/E ratio, and decreased input/output relationship. Both *Cntnap2* and *Ahi1* knockdown mice show impaired social behavior that was restored by ampakine CX546, a positive allosteric modulator of AMPA receptors. As a last example, in *Fmr1*^{-/-} mice it was shown that NMDA receptor-dependent LTP in the CA1 area of the hippocampus is impaired, and it can be restored either by pharmacological inhibition of GluN2a subunit-containing NMDA receptors, or by *Grin2a* deletion obtained by crossing *Grin2a*^{-/-} with *Fmr1*^{-/-} mice (Lundbye et al., 2018).

There are several interesting papers characterizing the consequences of mutations in ASD-linked genes coding for non-synaptic proteins, but which lead to complex molecular and functional changes at synapses leading to behavioral deficits relevant to the two ASD domains. For example, mutation in the X-linked *MECP2* gene encoding the homonymous transcriptional regulator that cause Rett syndrome and several neurodevelopmental disorders including ASD has been modeled in *Viaat-Mecp2*^{-/-} mice, which lack *Mecp2* in GABAergic cells. These animals show reduced survival, decreased inhibitory transmission in layer 2/3 of the S1 cortex, and impaired hippocampal LTP (Chao et al., 2010). In *Fmr1*^{-/-} mice, the loss of this gene results in constitutive over-activation of mGlu5 receptor due to its aberrant interaction with Homer (Ronesi et al., 2012), which can be mimicked by a mutation on this receptor that disrupts such interaction (Guo et al., 2016). Another of such examples is provided by PREX1 (phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1), a protein that regulates cell maturation and motility: *PREX1* mutations have been found in Chinese Han patients with ASD, and *Prex1*^{-/-} mice show impaired NMDA receptor-dependent LTD in the hippocampus and the full ASD behavioral repertoire (Li et al., 2015). *Pten* KO mice show enhanced firing activity and excitatory synaptic transmission in the dentate gyrus (Williams et al., 2015). Another study with conditional *Pten* deletion restricted to subsets of postmitotic neurons in cortex and hippocampus (*Nse-Cre;Pten*^{loxP/loxP}) showed impaired social behavior in mutant mice, which was improved by rapamycin treatment (Zhou et al., 2009). A further example of conditional gene deletion is provided by a study on

Tshz3 KO (*Camk2a-Cre;Tshz3*^{fl/fl}) mice with postnatal loss of this gene in the cortex, which show impaired social interactions and repetitive behaviors with normal corticostriatal LTP but loss of LTD, the latter possibly due to increased NMDA receptor-mediated signaling (Chabbert et al., 2019). Strikingly, and again stressing the diversity of the synaptic changes observed in ASD mouse models, a decreased corticostriatal LTP is reported in heterozygous *Tshz3*^{+/-lacZ} mice that also show behavior defects similar to *Camk2a-Cre;Tshz3*^{fl/fl} mice (Caubit et al., 2016). Another interesting heterozygous mouse model is *Scn1a*^{+/-} mimicking Dravet's syndrome, a generalized epilepsy disorder due to the heterozygous mutation of the *SCN1A* gene (encoding for the voltage-gated sodium channel NaV1.1), which presents psychiatric comorbidities including ASD. *Scn1a*^{+/-} mice present impaired social behaviors and increased stereotypies, paralleled by an increased glutamate and decreased GABA release in the hippocampus; treatment with clonazepam, a positive allosteric modulator of GABA_A receptor, could completely rescue social behaviors (Han et al., 2012).

In conclusion, synaptic dysfunctions in ASD models generally do not arise from direct receptor mutation, but from mutation in elements of the postsynaptic receptor scaffolding complex, which affect receptor function; moreover, synaptic defects emerge also when non-synaptic molecular pathways, which in some cases remain uncharacterized, are affected by a given mutation. Anyway, some general lines emerge from this literature, the most recurrent being changes in AMPA/GABA_A receptor-mediated synaptic transmission leading to the impairment of the E/I balance, and the change of NMDA vs. AMPA receptor contribution to glutamatergic signaling, which is likely to underlie the disruption of long-term synaptic plasticity. From a behavioral point of view, some models achieve to reproduce the two domains of the ASD repertoire, i.e. social interaction deficits and stereotyped behavior with restricted field of interest, some of them only one of the two. Other studies focus on behaviors such as spatial learning, habit formation and motor skills, although intellectual disability and motor abnormalities are not included among the ASD core features (they have a noticeable but incomplete prevalence in patients with ASD; Lai et al., 2014). In many cases, these behaviors are also impaired, suggesting widespread changes in brain circuitry and functioning in these ASD models.

6. Conclusions

In the last decade, progress in sequencing technologies and availability of large genomic datasets yielded the discovery of an enormous number of novel ASD-risk genes. The effort now is focused on the search for common pathological mechanisms shared by different genes. Multiple studies have indicated functional pathways where risk-genes are co-expressed and co-regulated. It is in fact emerging that ASD genes are connected within transcriptional and protein interaction networks in the developing brain (Hartl et al., 2021). Despite the genetic heterogeneity of ASD (over 1000 ASD-linked genes listed in SFARI) a high degree of convergence points towards molecular processes related to regulation of gene expression and synaptic function (Masini et al., 2020). Common patterns of mis-regulated gene expression and alternative splicing can dramatically affect neuronal processes from neurogenesis to mature synaptic function. Moreover, ASD-associated cellular processes controlling the formation and function of synapses are regulated by changes in neuronal activity, likely underlying the network disruption in brains from patients with autism. In this context, mutations in transcriptional factors and RNA-binding proteins have a prominent role since they function at the regulatory control level and can influence downstream processes producing developmental phenotypes (Quesnel-Vallières et al., 2016).

The studies presented in this review cover a large part of the literature on ASD-linked genes involved in postsynaptic functions, from a molecular to a functional point of view. Most of these genes have been targeted in rodent models to mimic human pathology and study the underlying molecular, cellular and synaptic processes. Among the data

collected from these ASD models, some key themes and issues emerge.

First, there is a great heterogeneity in the models. On one hand, this is inherent to the vast number of genes linked to ASD and the different mutations that a single gene can undergo. This is particularly true for *Shank3*, of which at least 7 models exist, with different DNA excisions targeting one or more exons aimed at disrupting the gene. On the other hand, such great variability makes it difficult to compare two different models targeting the same gene, and in general this can undermine the translational value of the results.

Second, these models have been heterogeneously characterized from a behavioral point of view, using different tests or variations of the same test, different conditions, etc. Often, the tests utilized were aimed at detecting behavioral abnormalities such as anxiety, spatial memory, etc. that do not define ASD. Moreover, a fair amount of studies have not reported a complete ASD-like phenotype (i.e., social interaction deficits and stereotyped, repetitive behaviors) but only one of the two categories: in some cases, both behavioral domains were tested, but only one was significantly affected, while in other cases only one domain was assessed. Therefore, a fair amount of the data provided by these studies is not relevant in the context of ASD.

Third, from a cellular point of view this heterogeneity is even greater, because the approaches utilized to study the consequences of ASD-linked gene disruption vary from molecular biology to histology and electrophysiology, with different methods and aims. Here we focused on synaptic dysfunction, and even in this case the variety among the studies is large. Most of them targeted the cerebral cortex (spanning from the prefrontal to the sensorimotor), but the corticostriatal pathway and the hippocampus are also well represented. The rationale of targeting a specific brain area (rather than another) is correlated to the phenotype, but not always. The parameters analyzed are also variable from one paper to another, but the most recurring are NMDA/AMPA ratio, E/I balance, and synaptic plasticity (LTP, LTD). In general, one or more of these parameters is altered in a given model, but results can surprisingly diverge even between very similar models. Such inconsistency can be due to experimental factors, including the genotypic background of animal models, and the sensitivity of synaptic function to subtle changes in its basal state.

Overall, such heterogeneity in models, methods and studied parameters results in the production of data that are seldom comparable and, in some cases, conflicting. This issue somehow limits our knowledge on gene mutation-dependent phenotypes and the underlying synaptic mechanisms, and calls for improving standardization of experimental analysis to increase reproducibility, allow better comparison among models and studies, as well as improving the translational value. Such improvement could come from new methods that allow real-time analysis of the behavior of mice. One of these systems has been recently used to characterize the behavior of *Shank2* and *Shank3* mutant mice, revealing differences in complex social interactions (de Chaumont et al., 2019).

In addition to the methodological limitations discussed above, recent studies suggest that more factors should be taken into consideration when studying ASD models. Indeed, the same sets of genes can control different functions depending on the timing and site of expression. In fact, the same genes may control either developmental processes or adult activity-dependent synaptic homeostasis. On the other hand, genes modifying the activity of ion channels or of mitochondria, besides affecting diverse cellular populations to a different extent, also exert a greater influence on the dendritic and synaptic compartments in neurons. Moreover, many ASD genes are expressed also in the PNS, adding further complexity to their influence on the CNS.

7. Future directions

In conclusion, specifically postsynaptic ASD genes, as well as ubiquitous ASD genes that particularly influence dendritic and synaptic activity, both profoundly affect the establishment and function of mature

neuronal networks. Therefore, future studies on animal models of monogenic ASD forms should take into consideration both the timing and the site where the gene mutation exerts its primary effect, ultimately compromising the whole neuronal network.

CRedit author statement

Paola Bonsi: Conceptualization, Writing – original draft, Writing – review & editing, Project administration; Antonella De Jaco: Conceptualization, Writing – original draft, Writing – review & editing; Laurent Fasano: Conceptualization, Writing – original draft, Writing – review & editing; Paolo Gubellini: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None.

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