



Molecular mechanisms for targeted ASD treatments

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The possibility to generate construct valid animal models enabled the development and testing of therapeutic strategies targeting the core features of autism spectrum disorders (ASDs). At the same time, these studies highlighted the necessity of identifying sensitive developmental time windows for successful therapeutic interventions. Animal and human studies also uncovered the possibility to stratify the variety of ASDs in molecularly distinct subgroups, potentially facilitating effective treatment design. Here, we focus on the molecular pathways emerging as commonly affected by mutations in diverse ASD-risk genes, on their role during critical windows of brain development and the potential treatments targeting these biological processes.

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Introduction

ASDs are a group of heterogeneous conditions characterized by social interaction and communication deficits accompanied by repetitive and stereotyped behaviors [1]. These core symptoms often coexist with a multitude of other clinical problems including attention deficit and hyperactivity disorder (ADHD), developmental delay, motor abnormalities, intellectual disability (ID) and epilepsy. The heterogeneous clinical presentation is mirrored by high genetic variability, rendering ASD diagnosis difficult and complicating the development of effective treatments valid for large groups of patients. In addition, while it is clear that ASDs have a genetic component, the underlying molecular and cellular mechanisms are still at the center of research. Therefore, there is virtually no approved drug targeting the core symptoms of ASDs.

Despite these issues, in the past few years we have made important advancements. Studies delving into the

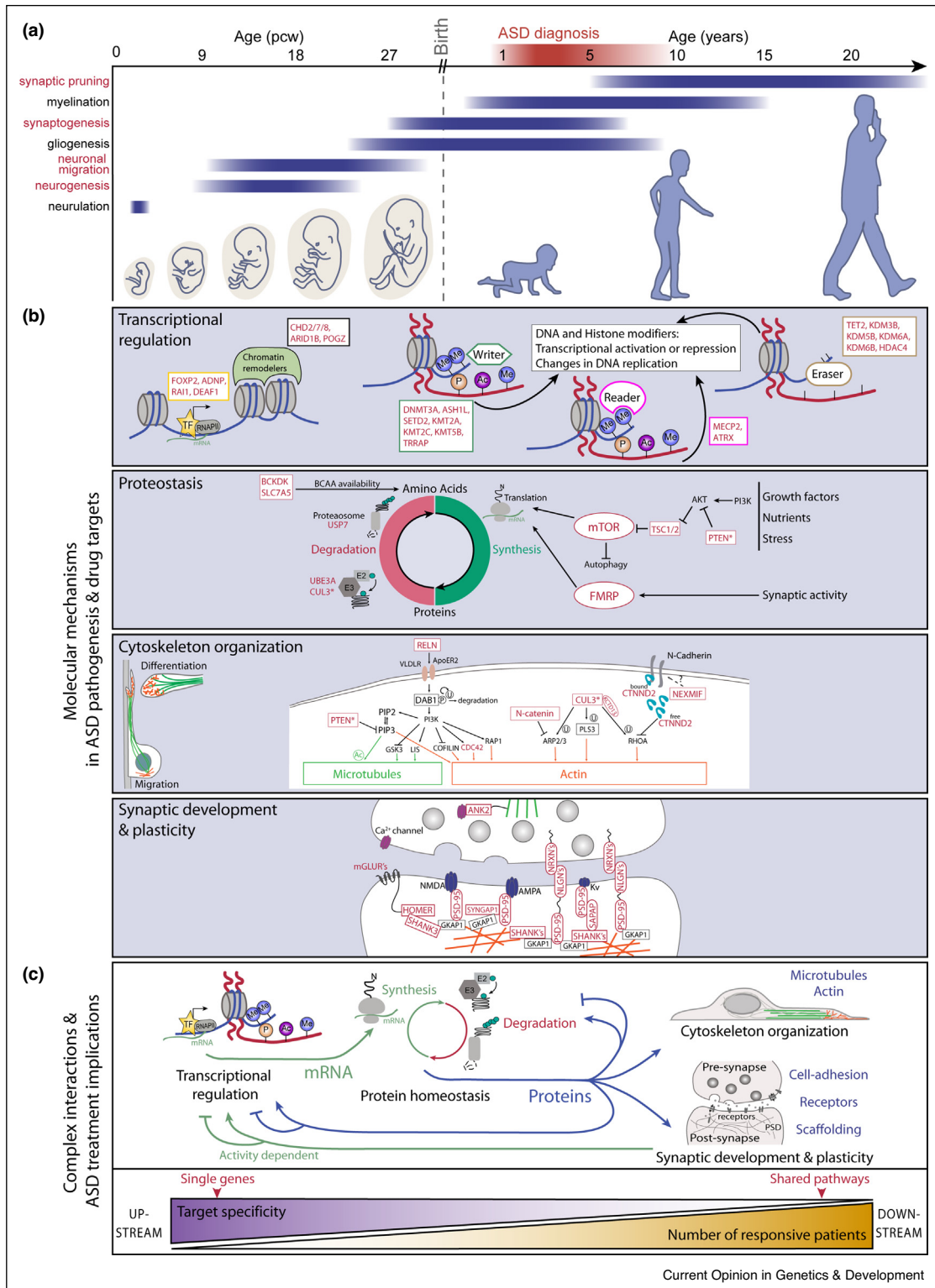
molecular causes of ASD changed our view of autism as a paradigmatic synaptopathy and uncovered novel potential targets for treatment. Furthermore, these analyses suggest that we should intensify research aimed to subdivide ASDs and to identify critical temporal windows. In this review, we guide the reader through the recent progresses in the understanding of molecular pathways implicated in ASDs, and the consequences of these discoveries for designing potential therapeutic interventions.

Convergent molecular pathways and potential treatments for ASDs

While numerous studies have bolstered the importance of genetics in ASDs, the analysis of large patient cohorts led to the identification of multiple types of genetic modifications associated with these conditions, ranging from chromosomal abnormalities and copy number variants to rare *de novo* mutations and rare combinations of common variants [2–7,8*,9]. Concomitantly, an increasing number of genes have been tightly associated with the pathogenesis of ASDs. These discoveries raise the question of how such a variety of different genetic backgrounds can lead to the core features of ASDs.

Excitingly, mounting evidence points to convergence of these genes onto shared biological pathways [4,10,11*,12–15] including neurogenesis and migration, synaptogenesis and synaptic plasticity, and activity-dependent transcription and translation (Figure 1). The hypothesis that ASD-risk genes converge along a limited number of molecular functions suggests that it may be valuable to stratify biologically defined ASDs into subtypes (Figure 1a,b). This endeavor may be complicated by the fact that a number of ASD-genes function as master regulators and/or are temporally and spatially ubiquitously expressed. However, when it comes to the identification of potential treatments, this approach grants the advantage to focus on a smaller number of molecular functions rather than a large number of genes and conditions. Analogously, functional genomic studies suggest that the multitude of ASD-risk genes might affect specific neurodevelopmental periods and certain brain regions. Although critical periods for developing sensorimotor networks have been well described, much less is known about the critical periods underlying complex behaviors and cognitive functions (reviewed in Ref. [16*]). Identifying these critical developmental periods may also be relevant to discover time windows when the interaction between genetic and environmental factors are most important to influence the maturation of neural circuits, thereby shaping mental development.

Figure 1



Molecular processes implicated in ASD pathogenesis. **(a)** Human brain development is an orchestrated process of time-limited developmental stages. While ASD-diagnosis typically occurs in childhood, the pathophysiological changes associated with ASDs may start already during embryonic development (ASD-associated neurodevelopmental processes in red). **(b)** A number of biologically diverse ASD-subtypes can be identified. Transcriptional regulation: the ASD-risk genes (red) belonging to this category are transcription factors (TF), chromatin modifiers and genes regulating DNA and histone modifications. Those can be

Regulation of gene transcription

Dynamic changes of gene expression patterns coordinate brain development and function, from neurogenesis to adult neural plasticity (reviewed in Ref. [17**]). Over the last few years, mutations in genes encoding transcription factors (e.g. *TBR1*, *FOXP2*, *ADNP*, *RAI1* and *DEAF1*) or chromatin modifiers (e.g. *CHD2*, *CHD7*, *CHD8*, *ARID1B*, *POGZ*, *SETD5*, *MECP2*, *KMT5B*, *KDM6B*) have been consistently identified in ASD patients [4]. Furthermore, analyses of global DNA methylation [18–20,21*] as well as histone modifications [22,23] revealed altered epigenetic signatures among patients with idiopathic ASDs.

To date, the most-studied transcriptional regulator associated with a condition presenting clinical features overlapping with ASDs is *MECP2*, whose mutations are the main cause of Rett syndrome (RTT). *MECP2* has multiple roles in the nucleus, ranging from transcriptional repression [24] to microRNA processing [25] and regulation of RNA splicing [26]. In the brain, *MeCP2* is expressed from early developmental stages to adulthood [27,28]. Constitutive *MeCP2* loss of function (loF) is associated with reduced morphological complexity in neurons, altered neuronal connectivity as well as synaptic transmission and plasticity. Conditional *MeCP2* ablation in adulthood causes similar synaptic defects, suggesting that *MeCP2* participates in maintaining neuronal functions in the adult brain (reviewed in Ref. [29]). *MeCP2* rodent models recapitulate clinical features observed in RTT patients, such as seizures, motor and cognitive defects, social deficits and anxiety-like behaviors. By employing these models, it has been demonstrated that the neurological and behavioral phenotypes associated with *MeCP2* mutations are reversible [30,31]. Thereafter, a number of groups have proposed potential therapeutic interventions, ranging from manipulation of neurotrophin levels [32–35] to gene therapies [36–38,39**,40*]. These approaches ameliorate phenotypic severity, extend mouse survival and recover the synaptic defects together with some of the RTT-associated behavioral abnormalities. While promising, these therapeutic strategies still have to overcome many challenges, such as identification of the specific cell type to target, toxicity of vehicles and side effects related to gene over dosage.

While the study of *MECP2* has a long history, many ASD-genes have been identified just recently. *CHD8* is one of the newly discovered but most frequently mutated genes in ASD patients. *CHD8* haploinsufficiency results in defects of neural proliferation, differentiation and function [41,42]; however, the underlying molecular mechanisms are still a matter of debate. Sparse *in utero* knock-down of *Chd8* in mice leads to downregulation of canonical Wnt- β -catenin signaling, premature neurogenesis and abnormal behaviors. Activation of Wnt signaling by expression of a stable form of β -catenin rescues the premature cell-cycle exit and behavioral abnormalities [43]. Constitutive *Chd8* haploinsufficiency, in contrast, leads to a slight activation of the Wnt- β -catenin pathway, whereas the overactivation of REST, a suppressor of neural gene transcription, has been proposed as the pathophysiological driver [41]. These discrepancies suggest distinctive cell-autonomous and non-cell-autonomous or dosage-dependent mechanisms of *Chd8* mutations and need to be taken into consideration when moving towards clinical applications.

Heterozygous mutations in *ARID1B*, encoding a component of the BAF chromatin-remodeling complex, have been associated with the Coffin-Siris syndrome, characterized by growth retardation, facial dysmorphism, ID and ASD [44–46]. *Arid1b* haploinsufficiency in mice leads to abnormal cognitive and social behaviors, as well as growth retardation phenotypes [47,48]. The observed ASD-relevant behaviors are associated with a reduced number of cortical parvalbumin-positive interneurons and a consequent alteration of the excitation/inhibition ratio. Treatment with clonazepam, a positive allosteric modulator of GABA_A receptors, rescues ASD behaviors, although information about the duration and age of treatment onset are missing [48]. Complementary manipulation of the GHRH-GH-IGF1 axis, a key modulator of growth and metabolism, by chronic GH supplementation is sufficient to rescue growth retardation and muscle weakness, but not behavioral phenotypes [47]. These findings emphasize the importance of combining multi-level therapeutic interventions when treating complex disorders, such as ASDs, to ameliorate different aspects of the disease.

(Figure 1 Legend Continued) further classified into ‘writers’ (catalyzing the post-translational modification), ‘readers’ (recognizing and interacting with specific variants) and ‘erasers’ (removing epigenetic marks). Proteostasis: protein homeostasis is regulated by the interplay of protein synthesis, controlled by different signaling cascades (e.g. mTOR and FMRP), and protein degradation, regulated by the ubiquitin proteasome system (UPS). Protein abundance is further limited by the availability of amino acids, such as the branched chain amino acids (BCAAs). ASD-genes encoding regulators of protein homeostasis are indicated in red, *Cul3** and *PTEN** appear twice. Cytoskeleton organization: cytoskeleton dynamics regulate fundamental brain developmental processes, such as neural migration and differentiation. Several ASD-genes (red) participate in molecular pathways that converge on the regulation of microtubules (green) and actin (orange) organization. Synaptic development and plasticity: a large number of cell-adhesion and post-synaptic density proteins have been implicated in the etiology of ASD. These proteins create an interconnected molecular network stabilizing both inhibitory and excitatory synapses. **(c)** Although ASDs can be grouped in molecularly distinct subtypes, the underlying pathways are interconnected. Treatments for ASDs may either target specifically affected molecular hubs (upstream) or intervene on general physiological processes (downstream).

While this class of ASD-genes has been continuously expanding in the last few years, for some of them establishing the molecular function has been less straightforward than for others. A notable example is *SETD5*. Although *SETD5* belongs to a family of histone methyl-transferases [49], three independent studies have found no experimental evidence of such catalytic activity [50,51,52]. Instead, *Setd5* regulates gene transcription via its interaction with the Hdac3 and Paf1 complexes [52]. This regulatory function appears to be important during learning, to adjust synaptic gene expression and, potentially, network activity. Because the brain architecture is not affected by *Setd5* haploinsufficiency, these findings suggest that interventions targeting the activity-dependent regulatory function of *Setd5* may be sufficient to ameliorate the associated behavioral defects, an emerging concept potentially applicable to other genes belonging to this class.

In summary, the above-mentioned examples illustrate that chromatin interacting and modifying proteins play a key role in ASD pathogenesis during brain development and in adulthood. Since most of the genes belonging to this group are often ubiquitously expressed, many of them (such as *CHD8*, *TBR1*, *ADNP*) may orchestrate expression of several gene networks at multiple time points [53–56]. While their broad regulatory action may complicate the development of very specific treatments, the understanding gained from detailed functional analysis of downstream effects and interactions, as exemplified above by *MeCP2*, may lead to effective treatment strategies.

Protein homeostasis

The fine control of protein synthesis and degradation is central for the assembly of brain circuits and synaptic plasticity [57–59]. Accordingly, genes encoding regulators of protein synthesis (e.g. *TSC1/2*, *PTEN*, *NF1*, *FMR1*) and degradation (e.g. *UBE3A*, *CUL3*) represent another common motive in ASDs.

The mammalian target of rapamycin (mTOR) signaling cascade is one of the major pathways regulating local protein synthesis and it is involved in a number of processes in the brain (reviewed in Ref. [60]). Mutations resulting in abnormal activation of the mTOR pathway, such as the ones identified in *TSC1/2*, *PTEN* and *CNTNAP2*, are linked to ASDs. Therefore, over the last few years a number of modulators of the mTOR complex 1 have been tested as potential treatments for these conditions [61,62]. In mouse models, these compounds not only yielded promising results in ameliorating ASD-associated phenotypes, but were also valuable to identify time-dependent effects [63,64,65,66]. For instance, inhibition of the mTOR pathway in animal models of tuberous sclerosis highlighted that early treatment completely prevents both neuroanatomical and

behavioral phenotypes [63,65], whereas manipulation of the same pathway in adult animals is less successful [64]. These studies demonstrate that even when potential therapeutic targets are identified, correction of ASD core symptoms may be time-sensitive; therefore, temporal windows should be carefully considered when designing clinical trials.

Similarly to RTT, fragile X syndrome (FXS) represents another disorder with autistic features extensively studied in the past. FXS is caused by the expansion of a trinucleotide repeated sequence at the promoter of the gene encoding the fragile X mental retardation protein (FMRP). As consequence, an abnormal activation of group 1 metabotropic glutamate receptors leads to exaggerated protein synthesis at the synapse [67,68]. Several mGluR5 antagonists (e.g. MPEP, CTEP, fenobam) have been successfully tested in mice ([69,70–72], reviewed in Ref. [73]) and inhibiting protein synthesis rescues some FXS symptoms, as demonstrated by lovastatin administration in mice [74,75] and humans [76]. As more and more targets of FMRP are emerging, however, it becomes evident that FXS patients will require a life-long multi-target pharmacological approach rather than a single treatment.

Since protein synthesis depends on the availability of amino acids, it was intriguing to find that abnormal regulation of branched chain amino acid (BCAA) levels is also associated with the development of autism core symptoms. In particular, homozygous loF mutations in genes regulating BCAA transport and catabolism (*SLC7A5* and *BCKDK* respectively) are linked to ASDs. Studies of *Slc7a5* and *Bckdk* animal models revealed that the neurological defects caused by mutations in these genes can be ameliorated by elevating brain BCAA levels, either by ventricular injections or diet supplementation [77,78]. Encouragingly, dietary intervention successfully improved neurological symptoms also in patients carrying *BCKDK* mutations [79], supporting the idea that the detection of precise molecular causes of ASDs is the first step toward the identification of ad-hoc treatments.

Finally, several studies have linked ASDs to defects in protein degradation identifying mutations in genes encoding components of the ubiquitin proteasome system (UPS) (such as *UBE3A*, *CUL3*, *TRIP12* and *USP7*) in patients. For instance, loF of the *UBE3A* gene is the major cause of Angelman syndrome (AS). AS-causing mutations affect the maternally inherited allele, since the paternal allele is silenced by an *UBE3A* antisense transcript (*UBE3A-ATS*). In a mouse model of AS, the reduction of *Ube3a-ATS* levels via intra-hippocampal injection of antisense oligonucleotides results in partial restoration of Ube3a protein levels and improvement of some neurobehavioral abnormalities [80]. The efficacy of this treatment is time-sensitive, thus while embryonic

restoration of *Ube3a* expression fully prevents neurological phenotypes, reinstatement of *Ube3a* later in development only achieves a partial rescue [81,82]. Although gene therapy still faces major limitations in humans, these results point towards concrete opportunities for the treatment of AS.

The E3 ubiquitin ligase CUL3 is another member of the UPS associated with ASDs. A recent study on a neural lineage specific *Cul3* mutant mouse identified eIF4G1, an mRNA translation initiation factor, as a potential target of CUL3. Accordingly, pharmacological inhibition of eIF4G1 resulted in partial rescue of cellular and behavioral phenotypes [83]. While these results are intriguing, studies on constitutive *Cul3* mutant animals do not reveal changes in eIF4G1 levels [84,85] and highlight the importance of Cul3 during early developmental stages due to abnormal levels of cytoskeletal proteins, atypical actin-organization and defective neuronal migration [84,85]. Induction of *Cul3* haploinsufficiency in juvenile mice does not lead to the behavioral deficits observed in constitutive heterozygous mutants, emphasizing problems arising from the use of Cre-lines that might overcome critical developmental windows [85].

As outlined above, protein homeostasis in the central nervous system is achieved by a fine-tuned interplay of protein synthesis and protein degradation, in response to both intracellular and extracellular stimuli. Targeting this central player may therefore be a promising route to ASD treatment.

Cytoskeleton dynamics

Similarly to *CUL3*, several ASD-genes encode proteins implicated, either directly or indirectly, in the regulation of cytoskeletal organization (e.g. *DLX1/2*, *AUTS2*, *WDFY3*, *NDE1*, *RELN*, *NEXMIF*, *TBR1*, *PTEN*) [86]. In the developing brain, mutations in these genes are associated with defects in neuronal migration and differentiation, as well as axon guidance, causing alterations in brain cyto-architecture. For instance, reduced expression of the high-risk ASD-gene *RELN* has been linked also to lissencephaly and cerebellar hypoplasia [87,88]. Animal studies have shown that Reln is essential to regulate microtubule and actin cytoskeleton, orchestrating multiple steps in neuronal migration [89], and later on to modulate synaptic plasticity [90,91] and promote dendrite and spine development [92,93]. Migration is also affected by mutations in *NEXMIF* or *DLX1/2* genes, which, in mouse, lead to abnormal positioning of glutamatergic and GABAergic neurons, respectively, and neuronal morphology. These defects are associated with impaired actin organization, due to elevated levels of RhoA, in the case of *Nexmif*, or by increased expression of *Pak3*, in *Dlx1/2* mutants [94–98]. Similarly, activity of PTEN, another ASD-associated protein, is essential to control neuronal polarity through actin organization [99,100] and to

downregulate the level of deetyrosinated microtubules, preventing excessive axon outgrowth [101].

The observation of brain structural defects in animal models with mutations in this class of genes poses the problem of how to correct these abnormalities later in life. Interestingly, Manent *et al.* reported that postnatal re-expression of *Dcx* rescues subcortical band-heterotopia in *Dcx* knockdown rats and reduces seizure threshold [102]. These results are encouraging and hold the promise that activation of developmental programs later in life could be beneficial for ASDs as well.

Alternatively, early genetic testing could lead to embryonic treatments. For example, prenatal treatment with Tubastatin A, a specific inhibitor of HDAC6, rescued the migration phenotype and behavioral defects associated with CAMDI deficiency [103]. Along the same line, a recent study showed rescue of neuronal migration and neurite outgrowth deficits in cells lacking the ASD-gene *CTNNA2* by inhibition of ARP2/3, a regulator of actin cytoskeleton [104].

To conclude, functional studies and treatment for ASDs associated with abnormal regulation of the cytoskeleton are still limited. Much more, however, is known about these dysfunctions in the framework of other diseases (e.g. cell migration in cancer [105]) and some of the treatments identified in that context may be employed to ameliorate ASD symptoms.

Synaptic development and plasticity

Genes encoding synaptic proteins (e.g. *NLGNs*, *NRXNs*, *DLG4/PSD-95*, *ANK2*, *SYNGAP1* and *SCN2A*) were among the first to be associated with ASDs. While it is now clear that this class of genes is not the only driver of molecular defects in autism, it remains a central focus of the scientific community working on ASDs.

De novo SYNGAP1 mutations are associated with ID, epilepsy and ASD. Syngap1 is localized at excitatory synapses, where it regulates AMPA receptors trafficking [106] and synaptic plasticity [107–110]. In the developing brain, *Syngap1* haploinsufficiency leads to the premature maturation of spine structures [111,112] causing persistent deficits of neuronal network connectivity, cognition and behavior [107,110,111,113–115]. Interestingly, *Syngap1* heterozygous mice show also elevated activation of Ras and ERK1/2 pathways, with a consequent increase in basal protein synthesis [107,110,116]. Pharmacological manipulation of mGluR5 and Ras/ERK signaling, strategies already employed to rescue FXS symptoms, restores the exaggerated levels of protein synthesis in *Syngap1* mice [116].

Similarly, mutations in *SCN2A* have been associated with ASD, ID and epilepsy. *SCN2A* encodes the

Na_v1.2 channel, primarily expressed in the axon initial segment of glutamatergic neurons and in a subset of interneurons [117,118]. During early development, the Na_v1.2 channel contributes to action-potential generation and propagation, thus influencing neuronal excitability [117,119]. A recent study demonstrated that *Scn2a* plays a double role in synaptic development. While *Scn2a* haploinsufficiency impairs axonal excitability during the first postnatal week, it affects dendritic excitability later in development, leading to an excess of immature spines and impaired synaptic plasticity [119]. The synaptic defects are also associated with behavioral abnormalities, such as reduced sociability and anxiety-like behavior, and marked hyperactivity [119,120]. Recently, one study reported the reversibility of hyperactive behavior through manipulation of glutamatergic transmission [120].

The *trans*-synaptic NLGN/NRXN complex promotes synaptic assembly, maturation and maintenance [121]. Mutations in *NRXNs* and *NLGNs* genes are associated with strong impairments of synaptic transmission, leading to changes in excitation/inhibition ratio and ASD core symptoms [122]. Transgenic mice overexpressing mutant *Nrxn1β* protein show repetitive behaviors and impaired sociability, as well as an abnormal excitation/inhibition ratio. Inactivation of transgene expression promotes behavioral rescue in juvenile and adult animals, suggesting that the effects of *Nrxn* mutations are reversible [123]. Among the *NLGN* family, *Nlgn3* has been extensively studied in the context of ASDs and phenotypic reversibility. Several studies demonstrated that genetic reinstatement of *Nlgn3* in juvenile and adult mice ameliorates sociability and synaptic plasticity defects [124–127]. Conversely, pharmacological interventions targeting the NLGN/NRXN pathway are still missing. To date, the only pharmacological example is the manipulation of the endocannabinoid system. In mice, conditional triple *Nrxnβ* KO impairs excitatory synaptic transmission. Application of the CB1R-antagonist AM251 and inhibition of 2-arachidonoylglycerol synthesis restored the synaptic phenotype [128]. Similarly, the abnormal striatal synaptic transmission observed in mice carrying the human R451C mutation in the *NLGN3* gene is partially rescued by manipulating the endocannabinoid system [129]. This evidence identifies a potential signaling pathway that may exert beneficial effects on some aspects of the pathology.

The *CNTNAP2* gene encodes a NRXN-related cell adhesion protein involved in neuron-glia interactions and postnatal myelination [130]. Mutations in this gene have been associated with different neurodevelopmental disorders, including ASD, ID, Tourette syndrome, obsessive-compulsive disorder, cortical dysplasia-focal epilepsy (CDFE) syndrome and ADHD [131]. Although large sequencing studies suggest caution interpreting the role of *CNTNAP2* heterozygous variants in ASD onset

[132,133], *Cntnap2* KO mice show neuronal defects and ASD-associated behaviors [134–138]. Chronic treatment with risperidone in these mice rescues the hyperactivity and repetitive behavior but not the social deficits [134]. Whereas, treatment with oxytocin (OXT) early in development results in long-lasting beneficial effects on social behavior [139].

SHANK proteins participate in regulating dendritic spine structure. Studies on *Shank* mutant mice revealed the possibility to reverse some of the observed ASD-phenotypes in adulthood by restoration of gene expression [140–142] and pharmacological interventions [143–147]. For instance, enhancing NMDA receptor function or using mGluR5 allosteric modulators rescue both synaptic physiology and ASD-phenotypes observed in *Shank2* [145,148] and *Shank3* mutant mice [149,150]. These findings have led to concrete clinical interventions in humans. Patients carrying *SHANK3* mutations are currently treated with IGF1 and show significant improvement in both social impairment and restrictive behaviors [151,152]. Moreover, recent evidence linked *Shank3* deficiency to abnormally low levels of histone acetylation, resulting from HDAC2 upregulation, and consequent aberrant transcription of downstream target genes, including NMDAR subunits and key actin regulators. In juvenile mice, brief treatment with a low dose of romidepsin, a brain-permeable HDAC inhibitor already approved for cancer treatment, leads to robust and long-lasting rescue of social deficits [153*]. It is important to observe that synaptic plasticity is tightly linked to the other molecular processes discussed in this review such as the activity in the nucleus, protein synthesis and cytoskeleton organization. Therefore, the development of effective treatments for one ASD subgroup may also be useful for ameliorating defects in other ASDs (Figure 1c).

Conclusions

About 30 years after the discovery of the first autism-risk gene, we are looking back and reflecting on what we have learned from functional studies of molecularly defined forms of ASDs. Although far from complete, the fuzzy picture of these heterogeneous disorders is slowly becoming clearer and the path to treatment more defined. For simplicity, in this review we divided ASD-risk genes into functionally distinct groups. Since developing targeted treatments for each single form of ASD seems currently out of reach, this classification, together with systematic studies, may be important to stratify patients and to establish how and when to treat patients. This task, however, is complicated by the fact that several ASD-genes are pleiotropic, that these groups are functionally interconnected (Figure 1c) and that often mutations in one gene lead to the abnormal expression of other ASD-genes. Additionally, not all the experimental observations are reproducible across laboratories even for a single gene. Discrepancies may arise from the models employed (e.g.

genetic background, different cell or Cre-driver lines), tissues and time points analyzed and the selected experimental read-outs. Thus, the community should find ways to standardize approaches and to intensify communication between clinicians and basic scientists to identify read-outs that could be more directly translated into clinical trials.

Furthermore, given the multiplicity of phenotypes observed and the complexity of the molecular mechanisms involved, it is becoming obvious that even monogenic forms of ASD will require multi-level pharmacological approaches. Alternatively, the development of precise targeted interventions, such as the use of oligonucleotide and CRISPR-mediated gene correction techniques, may lead to some valuable tools to correct defects associated with single gene mutations. On the other hand, targeting downstream signaling pathways might allow ameliorating ASD-core symptoms in a larger fraction of patients (Figure 1c), independently of the upstream molecular mechanisms. One prominent example is the administration of the 'social' hormone OXT to ease social interaction problems in ASD individuals. Preclinical studies carried out in different ASD mouse models showed beneficial effects of OXT on ASD-related behaviors [139,154–158]. However, the results obtained in ASD patients are more controversial (reviewed in Ref. [159]). Furthermore, the efficacy and reliability of OXT treatment remain limited by issues concerning the administration route and doses, duration and interval of administrations, as well as the optimal timing of treatment and a lack in reliable treatment readouts [159].

To further complicate the picture, frequently, ASDs are caused by rare combinations of common mutations. Thus, how can we model such complexity? And how can we develop treatments for these patients? While single gene studies are important starting points, there will be the need to develop screening-based strategies that allow investigating the molecular pathways affected on a case-by-case manner. Based on this concept, it will become more common to repurpose known drugs to treat ASDs, an approach already showing some successful results [153*,158,160]. Altogether, despite the hurdles, advancements in knowledge and technology promise a rapid increase in the diversity and availability of ASD treatments and an improvement in the quality of life of affected individuals and their families.

Conflict of interest statement

Nothing declared.

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