Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

# Molecular mechanisms for targeted ASD treatments

Bernadette Basilico, Jasmin Morandell and Gaia Novarino

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.

**Address**
Institute of Science and Technology Austria, Klosterneuburg, Austria

Corresponding author: Novarino, Gaia (gaia.novarino@ist.ac.at)

**Current Opinion in Genetics and Development** 2020, **65**:126–137

This review comes from a themed issue on **Molecular and genetic bases of disease**

Edited by **Daniel H Geschwind** and **Joseph G Gleeson**

For a complete overview see the Issue and the Editorial

Available online 10th July 2020

**https://doi.org/10.1016/j.gde.2020.06.004**

## 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.

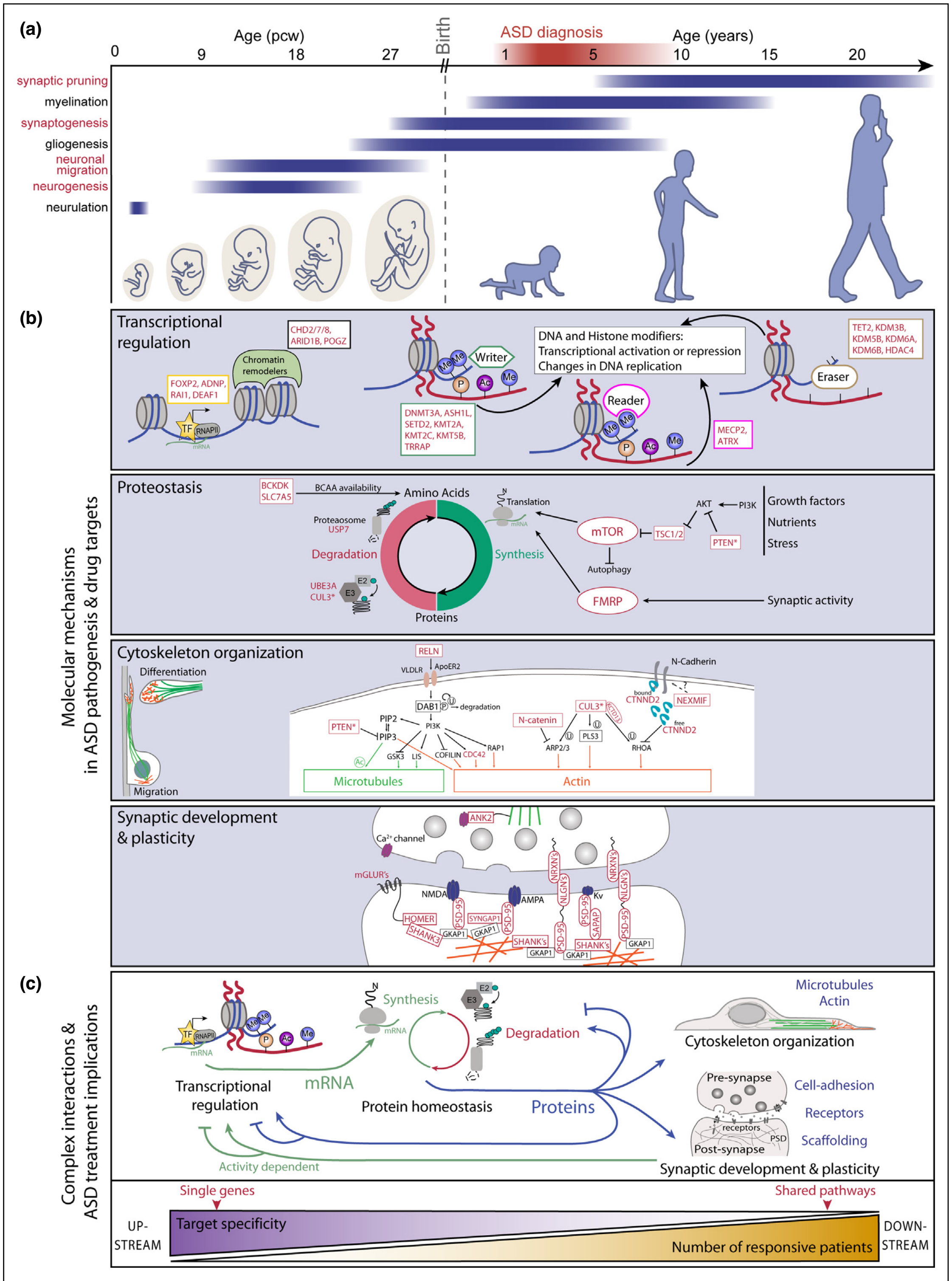
Despite these issues, in the past few years we have made important advancements. Studies delving into the molecular causes of ASD changed our view of autism as a paradigmatic synaptopathy and uncovered novel potential targets for treatment. Furthermore, these analyses suggest that we should intensify 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**



Current Opinion in Genetics & Development

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* knockdown 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 detyrosinated 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 *Nrxn*β 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.

## Acknowledgements

This work was supported by the ISTPlus postdoctoral fellowship (grant agreement No 754411) to B.B. and the Austrian Science Fund (FWF) to G. N. (W1232-B24 and SFB F7807-B).

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. APA: American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed.. 2013. 2013.

2. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL *et al.*: **De novo mutations revealed by whole-exome sequencing are strongly associated with autism**. *Nature* 2012, **485**:237-241.

3. Dong S, Walker MF, Carriero NJ, DiCola M, Willsey AJ, Ye AY, Waqar Z, Gonzalez LE, Overton JD, Frahm S *et al.*: **De novo insertions and deletions of predominantly paternal origin are associated with autism spectrum disorder**. *Cell Rep* 2014, **9**:16-23.

4. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Ercument Cicek A, Kou Y, Liu L, Fromer M, Walker S *et al.*: **Synaptic, transcriptional and chromatin genes disrupted in autism**. *Nature* 2014, **515**:209-215.

5. Iossifov I, O'Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, Stessman HA, Witherspoon KT, Vives L, Patterson KE *et al.*: **The contribution of de novo coding mutations to autism spectrum disorder**. *Nature* 2014, **515**:216-221.

6. Stessman HAF, Xiong B, Coe BP, Wang T, Hoekzema K, Fenckova M, Kvarnung M, Gerdts J, Trinh S, Cosemans N *et al.*: **Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases**. *Nat Genet* 2017, **49**:515-526.

7. Yuen RKC, Merico D, Bookman M, Howe JL, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N, Bingham J, Wang Z *et al.*: **Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder**. *Nat Neurosci* 2017, **20**:602-611.

8. • Grove J, Ripke S, Als TD, Mattheisen M, Walters RK, Won H, Pallesen J, Agerbo E, Andreassen OA, Anney R *et al.*: **Identification of common genetic risk variants for autism spectrum disorder**. *Nat Genet* 2019, **51**:431-444.

This study demonstrates a strong association between ASDs and a set of common risk variants, working on a larger sample size compared to previous papers and identifying five risk loci and seven additional loci shared with other psychiatric disorders.

9. Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An J-Y, Peng M, Collins RL, Grove J, Klei L *et al.*: **Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism**. *SSRN Electron J* 2019, **25**:18.

10. Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM, Blencowe BJ, Geschwind DH: **Transcriptomic analysis of autistic brain reveals convergent molecular pathology**. *Nature* 2011, **474**:380-384.

11. •• Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, Horvath S, Geschwind DH: **Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism**. *Cell* 2013, **155**:1008-1021.

This study identifies shared neurobiological functions of ASD genes during human brain development, demonstrating the presence of convergent pathways and gene-enrichment in specific cells and circuits.

12. Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, Thiruvahindrapuram B, Xu X, Ziman R, Wang Z *et al.*: **Convergence of genes and cellular pathways dysregulated in autism spectrum disorders**. *Am J Hum Genet* 2014, **94**:677-694.

13. Krishnan A, Zhang R, Yao V, Theesfeld CL, Wong AK, Tadych A, Volfovsky N, Packer A, Lash A, Troyanskaya OG: **Genome-wide prediction and functional characterization of the genetic basis of autism spectrum disorder**. *Nat Neurosci* 2016, **19**:1454-1462.

14. Parikshak NN, Swarup V, Belgard TG, Irimia M, Ramaswami G, Gandal MJ, Hartl C, Leppa V, Ubieta LDLT, Huang J *et al.*:

**Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism**. *Nature* 2016, **540**:423-427.

15. Li X, Zhang Y, Wang L, Lin Y, Gao Z, Zhan X, Huang Y, Sun C, Wang D, Liang S *et al.*: **Integrated analysis of brain transcriptome reveals convergent molecular pathways in autism spectrum disorder**. *Front Psychiatry* 2019, **10**.

16. • Meredith RM: **Sensitive and critical periods during neurotypical and aberrant neurodevelopment: a framework for neurodevelopmental disorders**. *Neurosci Biobehav Rev* 2015, **50**:180-188.
This review article summarizes the evidence for critical periods of brain development and their link with neurodevelopmental disorders.

17. •• Gallegos DA, Chan U, Chen L-F, West AE: **Chromatin regulation of neuronal maturation and plasticity**. *Trends Neurosci* 2018, **41**:311-324.
This review discusses how chromatin regulation acts locally to modulate the expression of specific genes and to coordinate gene expression programs during transitions between cellular states; it also highlights the importance of epigenetic transcriptional mechanisms in postmitotic neurons.

18. Ladd-Acosta C, Hansen KD, Briem E, Fallin MD, Kaufmann WE, Feinberg AP: **Common DNA methylation alterations in multiple brain regions in autism**. *Mol Psychiatry* 2014, **19**:862-871.

19. Wong CCY, Meaburn EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC, Plomin R, Mill J: **Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits**. *Mol Psychiatry* 2014, **19**:495-503.

20. Ellis SE, Gupta S, Moes A, West AB, Arking DE: **Exaggerated CpH methylation in the autism-affected brain**. *Mol Autism* 2017, **8**:6.

21. • Corley MJ, Vargas-Maya N, Pang APS, Lum-Jones A, Li D, Khadka V, Sultana R, Blanchard DC, Maunakea AK: **Epigenetic delay in the neurodevelopmental trajectory of DNA methylation states in autism spectrum disorders**. *Front Genet* 2019, **10**:907.
In this study, the authors describe the epigenetic delay in the trajectory of normal DNA methylation states, occurring during neurodevelopment. This may consequently lead to deleterious transcriptomic events in ASD and supports the hypothesis of an early developmental origin of ASD.

22. Sun W, Poschmann J, Cruz-Herrera del Rosario R, Parikshak NN, Hajan HS, Kumar V, Ramasamy R, Belgard TG, Elanggovan B, Wong CCY *et al.*: **Histone acetylome-wide association study of autism spectrum disorder**. *Cell* 2016, **167**:1385-1397 e11.

23. Zaghi M, Broccoli V, Sessa A: **H3K36 methylation in neural development and associated diseases**. *Front Genet* 2020, **10**:1291.

24. Nan X, Campoy FJ, Bird A: **MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin**. *Cell* 1997, **88**:471-481.

25. Cheng T-L, Wang Z, Liao Q, Zhu Y, Zhou W-H, Xu W, Qiu Z: **MeCP2 suppresses nuclear microrna processing and dendritic growth by regulating the DGCR8/Drosha complex**. *Dev Cell* 2014, **28**:547-560.

26. Young JI, Hong EP, Castle JC, Crespo-Barreto J, Bowman AB, Rose MF, Kang D, Richman R, Johnson JM, Berget S *et al.*: **Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2**. *Proc Natl Acad Sci U S A* 2005, **102**:17551-17558.

27. Shahbazian MD: **Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation**. *Hum Mol Genet* 2002, **11**:115-124.

28. Bedogni F, Cobolli Gigli C, Pozzi D, Rossi RL, Scaramuzza L, Rossetti G, Pagani M, Kilstrup-Nielsen C, Matteoli M, Landsberger N: **Defects during Mecp2 null embryonic cortex development precede the onset of overt neurological symptoms**. *Cereb Cortex* 2016, **26**:2517-2529.

29. Gulmez Karaca K, Brito DVC, Oliveira AMM: **MeCP2: a critical regulator of chromatin in neurodevelopment and adult brain function**. *Int J Mol Sci* 2019, **20**:4577.

30. Guy J, Gan J, Selfridge J, Cobb S, Bird A: **Reversal of neurological defects in a mouse model of Rett syndrome**. *Science (80-)* 2007, **315**:1143-1147.

31. McGraw CM, Samaco RC, Zoghbi HY: **Adult neural function requires MeCP2**. *Science (80-)* 2011, **333** 186–186.

32. Kline DD, Ogier M, Kunze DL, Katz DM: **Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in Mecp2-null mice**. *J Neurosci* 2010, **30**:5303-5310.

33. Johnson RA, Lam M, Punzo AM, Li H, Lin BR, Ye K, Mitchell GS, Chang Q: **7,8-dihydroxyflavone exhibits therapeutic efficacy in a mouse model of Rett syndrome**. *J Appl Physiol* 2012, **112**: 704-710.

34. Deogracias R, Yazdani M, Dekkers MPJ, Guy J, Ionescu MCS, Vogt KE, Barde Y-A: **Fingolimod, a sphingosine-1 phosphate receptor modulator, increases BDNF levels and improves symptoms of a mouse model of Rett syndrome**. *Proc Natl Acad Sci U S A* 2012, **109**:14230-14231.

35. Castro J, Garcia RI, Kwok S, Banerjee A, Petravicz J, Woodson J, Mellios N, Tropea D, Sur M: **Functional recovery with recombinant human IGF1 treatment in a mouse model of Rett syndrome**. *Proc Natl Acad Sci U S A* 2014, **111**:9941-9946.

36. Sztainberg Y, Chen HM, Swann JW, Hao S, Tang B, Wu Z, Tang J, Wan YW, Liu Z, Rigo F *et al.*: **Reversal of phenotypes in MECP2 duplication mice using genetic rescue or antisense oligonucleotides**. *Nature* 2015, **528**:123-126.

37. Gadalla KKE, Vudhironarit T, Hector RD, Sinnett S, Bahey NG, Bailey MES, Gray SJ, Cobb SR: **Development of a novel AAV gene therapy cassette with improved safety features and efficacy in a mouse model of Rett syndrome**. *Mol Ther - Methods Clin Dev* 2017, **5**:180-190.

38. Sinnett SE, Hector RD, Gadalla KKE, Heindel C, Chen D, Zaric V, Bailey MES, Cobb SR, Gray SJ: **Improved MECP2 gene therapy extends the survival of MeCP2-null mice without apparent toxicity after intracisternal delivery**. *Mol Ther - Methods Clin Dev* 2017, **5**:106-115.

39. •• Tillotson R, Selfridge J, Koerner MV, Gadalla KKE, Guy J, De Sousa D, Hector RD, Cobb SR, Bird A: **Radically truncated MeCP2 rescues Rett syndrome-like neurological defects**. *Nature* 2017, **550**:398-401.
In this study, the authors demonstrate that expression of truncated MeCP2 protein in *MeCP2*-deficient mice is associated with reduced RTT symptoms, thus, opening to potential therapeutic implications for gene therapy strategies.

40. • Le TTH, Tran NT, Dao TML, Nguyen DD, Do HD, Ha TL, Kühn R, Nguyen TL, Rajewsky K, Chu VT: **Efficient and precise CRISPR/Cas9-mediated MECP2 modifications in human-induced pluripotent stem cells**. *Front Genet* 2019, **10**:625.
In this study, the authors show the successful correction of *MECP2* mutation in human iPSCs, suggesting that CRISPR/Cas9-mediated correction could be a potential gene therapy approach for RTT.

41. Katayama Y, Nishiyama M, Shoji H, Ohkawa Y, Kawamura A, Sato T, Suyama M, Takumi T, Miyakawa T, Nakayama KI: **CHD8 haploinsufficiency results in autistic-like phenotypes in mice**. *Nature* 2016, **537**:675-679.

42. Gompers AL, Su-Feher L, Ellegood J, Copping NA, Riyadh MA, Stradleigh TW, Pride MC, Schaffler MD, Wade AA, Catta-Preta R *et al.*: **Germline Chd8 haploinsufficiency alters brain development in mouse**. *Nat Neurosci* 2017, **20**:1062-1073.

43. Durak O, Gao F, Kaeser-Woo YJ, Rueda R, Martorell AJ, Nott A, Liu CY, Watson LA, Tsai L-H: **Chd8 mediates cortical neurogenesis via transcriptional regulation of cell cycle and Wnt signaling**. *Nat Neurosci* 2016, **19**:1477-1488.

44. Santen GWE, Aten E, Sun Y, Almomani R, Gilissen C, Nielsen M, Kant SG, Snoeck IN, Peeters EAJ, Hilhorst-Hofstee Y *et al.*: **Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin-Siris syndrome**. *Nat Genet* 2012, **44**: 379-380.

45. Tsurusaki Y, Okamoto N, Ohashi H, Kosho T, Imai Y, Hibi-Ko Y, Kaname T, Naritomi K, Kawame H, Wakui K *et al.*: **Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome**. *Nat Genet* 2012, **44**:376-378.

46. Wieczorek D, Bögershausen N, Beleggia F, Steiner-Haldenstätt S, Pohl E, Li Y, Milz E, Martin M, Thiele H, Altmüller J *et al.*: **A comprehensive molecular study on Coffin–Siris and Nicolaides–Baraitser syndromes identifies a broad molecular and clinical spectrum converging on altered chromatin remodeling**. *Hum Mol Genet* 2013, **22**:5121-5135.

47. Celen C, Chuang J-C, Luo X, Nijem N, Walker AK, Chen F, Zhang S, Chung AS, Nguyen LH, Nassour I *et al.*: **Arid1b haploinsufficient mice reveal neuropsychiatric phenotypes and reversible causes of growth impairment**. *eLife* 2017, **6**.

48. Jung E-M, Moffat JJ, Liu J, Dravid SM, Gurumurthy CB, Kim W-Y: **Arid1b haploinsufficiency disrupts cortical interneuron development and mouse behavior**. *Nat Neurosci* 2017, **20**:1694-1707.

49. Sessa A, Fagnocchi L, Mastrototaro G, Massimino L, Zaghi M, Indrigo M, Cattaneo S, Martini D, Gabellini C, Pucci C *et al.*: **SETD5 regulates chromatin methylation state and preserves global transcriptional fidelity during brain development and neuronal wiring**. *Neuron* 2019, **104**:271-289 e13.

50. Mas-y-Mas S, Barbon M, Teyssier C, Déméné H, Carvalho JE, Bird LE, Lebedev A, Fattori J, Schubert M, Dumas C *et al.*: **The human mixed lineage Leukemia 5 (MLL5), a sequentially and structurally divergent set domain-containing protein with no intrinsic catalytic activity**. *PLoS One* 2016, **11**:e0165139.

51. Osipovich AB, Gangula R, Vianna PG, Magnuson MA: **Setd5 is essential for mammalian development and the co-transcriptional regulation of histone acetylation**. *Development* 2016, **143**:4595-4607.

52. • Deliu E, Arecco N, Morandell J, Dotter CP, Contreras X, Girardot C, Käsper E-L, Kozlova A, Kishi K, Chiaradia I *et al.*: **Haploinsufficiency of the intellectual disability gene SETD5 disturbs developmental gene expression and cognition**. *Nat Neurosci* 2018, **21**:1717-1727.

This study demonstrates that *Setd5* haploinsufficiency causes ASD-relevant behaviors and impairs gene expression patterns associated with learning and memory.

53. Barnard RA, Pomaville MB, O'Roak BJ: **Mutations and modeling of the chromatin remodeler CHD8 define an emerging autism etiology**. *Front Neurosci* 2015, **9**:477.

54. Chuang H-C, Huang T-N, Hsueh Y-P: **T-Brain-1 - a potential master regulator in autism spectrum disorders**. *Autism Res* 2015, **8**:412-426.

55. Malishkevich A, Amram N, Hacohen-Kleiman G, Magen I, Giladi E, Gozes I: **Activity-dependent neuroprotective protein (ADNP) exhibits striking sexual dichotomy impacting on autistic and Alzheimer's pathologies**. *Transl Psychiatry* 2015, **5**:e501.

56. Notwell JH, Heavner WE, Darbandi SF, Katzman S, McKenna WL, Ortiz-Londono CF, Tastad D, Eckler MJ, Rubenstei JLR, McConnell SK *et al.*: **TBR1 regulates autism risk genes in the developing neocortex**. *Genome Res* 2016, **26**:1013-1022.

57. Sutton MA, Schuman EM: **Dendritic protein synthesis, synaptic plasticity, and memory**. *Cell* 2006, **127**:49-58.

58. Buffington SA, Huang W, Costa-Mattioli M: **Translational control in synaptic plasticity and cognitive dysfunction**. *Annu Rev Neurosci* 2014, **37**:17-38.

59. Louros SR, Osterweil EK: **Perturbed proteostasis in autism spectrum disorders**. *J Neurochem* 2016, **139**:1081-1092.

60. • Winden KD, Ebrahimi-Fakhari D, Sahin M: **Abnormal mTOR activation in autism**. *Annu Rev Neurosci* 2018, **41**:1-23.

This exhaustive review article summarizes evidence for mTOR pathway implication in ASDs.

61. Ehninger D, Silva AJ: **Rapamycin for treating Tuberous sclerosis and Autism spectrum disorders**. *Trends Mol Med* 2011, **17**:78-87.

62. Guertin DA, Sabatini DM: **The pharmacology of mTOR inhibition**. *Sci Signal* 2009, **2**.

63. Tsai PT, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, Steinberg J, Crawley JN, Regehr WG, Sahin M: **Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice**. *Nature* 2012, **488**:647-651.

64. •• Tsai PT, Rudolph S, Guo C, Ellegood J, Gibson JM, Schaeffer SM, Mogavero J, Lerch JP, Regehr W, Sahin M: **Sensitive periods for cerebellar-mediated autistic-like behaviors**. *Cell Rep* 2018, **25**:357-367 e4.

In a mouse model of tuberous sclerosis, the authors identify critical developmental periods to target ASD core symptoms. Using rapamycin, they highlight the existence of different time windows underlying different autistic behaviors.

65. Cox RL, Calderon de Anda F, Mangoubi T, Yoshii A: **Multiple critical periods for rapamycin treatment to correct structural defects in Tsc-1-suppressed brain**. *Front Mol Neurosci* 2018, **11**.

66. Xing X, Zhang J, Wu K, Cao B, Li X, Jiang F, Hu Z, Xia K, Li J-D: **Suppression of Akt-mTOR pathway rescued the social behavior in Cntnap2-deficient mice**. *Sci Rep* 2019, **9**:3041.

67. Bear MF, Huber KM, Warren ST: **The mGluR theory of Fragile X mental retardation**. *Trends Neurosci* 2004, **27**:370-377.

68. Dölen G, Osterweil E, Rao BSS, Smith GB, Auerbach BD, Chattarji S, Bear MF: **Correction of fragile X syndrome in mice**. *Neuron* 2007, **56**:955-962.

69. •• Michalon A, Sidorov M, Ballard TM, Ozmen L, Spooren W, Wettstein JG, Jaeschke G, Bear MF, Lindemann L: **Chronic pharmacological mGlu5 inhibition corrects Fragile X in adult mice**. *Neuron* 2012, **74**:49-56.

This study was the first demonstrating FXS phenotypic reversibility after symptom onset, treating juvenile *Fmr1* mice with the mGluR5 inhibitor CTEP.

70. Berry-Kravis EM, Lindemann L, Jønch AE, Apostol G, Bear MF, Carpenter RL, Crawley JN, Curie A, Des Portes V, Hossain F *et al.*: **Drug development for neurodevelopmental disorders: lessons learned from fragile X syndrome**. *Nat Rev Drug Discov* 2018, **17**:280-299.

71. Hagerman R, Jacquemont S, Berry-Kravis E, Des Portes V, Stanfield A, Koumaras B, Rosenkranz G, Murgia A, Wolf C, Apostol G *et al.*: **Mavoglurant in Fragile X syndrome: results of two open-label, extension trials in adults and adolescents**. *Sci Rep* 2018, **8**:16970.

72. Youssef EA, Berry-Kravis E, Czech C, Hagerman RJ, Hessl D, Wong CY, Rabbia M, Deptula D, John A, Kinch R *et al.*: **Effect of the mGluR5-NAM basimglurant on behavior in adolescents and adults with Fragile X syndrome in a randomized, double-blind, placebo-controlled trial: FragXis Phase 2 results**. *Neuropsychopharmacology* 2018, **43**:503-512.

73. Davenport MH, Schaefer TL, Friedmann KJ, Fitzpatrick SE, Erickson CA: **Pharmacotherapy for Fragile X syndrome: progress to date**. *Drugs* 2016, **76**:431-445.

74. Osterweil EK, Chuang S-C, Chubykin AA, Sidorov M, Bianchi R, Wong RKS, Bear MF: **Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of Fragile X syndrome**. *Neuron* 2013, **77**:243-250.

75. Asiminas A, Jackson AD, Louros SR, Till SM, Spano T, Dando O, Bear MF, Chattarji S, Hardingham GE, Osterweil EK *et al.*: **Sustained correction of associative learning deficits after brief, early treatment in a rat model of Fragile X syndrome**. *Sci Transl Med* 2019, **11**:eaao0498.

76. Çaku A, Pellerin D, Bouvier P, Riou E, Corbin F: **Effect of lovastatin on behavior in children and adults with fragile X syndrome: an open-label study**. *Am J Med Genet Part A* 2014, **164**:2834-2842.

77. Novarino G, El-Fishawy P, Kayserili H, Meguid NA, Scott EM, Schroth J, Silhavy JL, Kara M, Khalil RO, Ben-Omran T *et al.*: **Mutations in BCKD-kinase lead to a potentially treatable form of autism with epilepsy**. *Science (80-)* 2012, **338**:394-397.

78. Tărlungeanu DC, Deliu E, Dotter CP, Kara M, Janiesch PC, Scalise M, Galluccio M, Tesulov M, Morelli E, Sonmez FM *et al.*: **Impaired amino acid transport at the blood brain barrier is a cause of autism spectrum disorder**. *Cell* 2016, **167**:1481-1494 e18.

79. García-Cazorla A, Oyarzabal A, Fort J, Robles C, Castejón E, Ruiz-Sala P, Bodoy S, Merinero B, Lopez-Sala A, Dopazo J *et al.*: **Two novel mutations in the BCKDK (Branched-Chain Keto-Acid Dehydrogenase Kinase) gene are responsible for a neurobehavioral deficit in two pediatric unrelated patients**. *Hum Mutat* 2014, **35**:470-477.

80. Meng L, Ward AJ, Chun S, Bennett CF, Beaudet AL, Rigo F:
•• **Towards a therapy for Angelman syndrome by targeting a long non-coding RNA**. *Nature* 2015, **518**:409-412.
The authors developed an antisense oligonucleotide-based treatment to restore Ube3a protein level in neurons. This targeted therapy efficiently ameliorated cognitive deficits in a mouse model of AS.

81. Silva-Santos S, Van Woerden GM, Bruinsma CF, Mientjes E, Jolfaei MA, Distel B, Kushner SA, Elgersma Y: **Ube3a reinstatement identifies distinct developmental windows in a murine Angelman syndrome model**. *J Clin Invest* 2015, **125**:2069-2076.

82. Rotaru DC, van Woerden GM, Wallaard I, Elgersma Y: **Adult Ube3a gene reinstatement restores the electrophysiological deficits of prefrontal cortex layer 5 neurons in a mouse model of angelman syndrome**. *J Neurosci* 2018, **38**:8011-8030.

83. Dong Z, Chen W, Chen C, Wang H, Cui W, Tan Z, Robinson H, Gao N, Luo B, Zhang L *et al.*: **CUL3 deficiency causes social deficits and anxiety-like behaviors by impairing excitation-inhibition balance through the promotion of cap-dependent translation**. *Neuron* 2020, **105**:475-490 e6.

84. Amar M, Pramod AB, Herrera VM, Yu N-K, Qiu LR, Zhang P, Moran-Losada P, Trujillo CA, Ellegood J, Urresti J *et al.*: **Autism-linked Cullin3 germline haploinsufficiency impacts cytoskeletal dynamics and cortical neurogenesis through RhoA signaling**. *bioRxiv* 2020 http://dx.doi.org/10.1101/2020.02.07.939256.

85. Morandell J, Schwarz LA, Basilico B, Tasciyan S, Nicolas A, Sommer C, Kreuzinger C, Dotter CP, Knaus LS, Dobler Z *et al.*: **Cul3 regulates cytoskeleton protein homeostasis and cell migration during a critical window of brain development**. *bioRxiv* 2020 http://dx.doi.org/10.1101/2020.01.10.902064.

86. Reiner O, Karzbrun E, Kshirsagar A, Kaibuchi K: **Regulation of neuronal migration, an emerging topic in autism spectrum disorders**. *J Neurochem* 2016, **136**:440-456.

87. Hong SE, Shugart YY, Huang DT, Shahwan SAl, Grant PE, Hourihane JO, Martin NDT, Walsh CA: **Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations**. *Nat Genet* 2000, **26**:93-96.

88. Folsom TD, Fatemi SH: **The involvement of Reelin in neurodevelopmental disorders**. *Neuropharmacology* 2013, **68**:122-135.

89. Wasser CR, Herz J: **Reelin: neurodevelopmental architect and homeostatic regulator of excitatory synapses**. *J Biol Chem* 2017, **292**:1330-1338.

90. Weeber EJ, Beffert U, Jones C, Christian JM, Förster E, Sweatt JD, Herz J: **Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning**. *J Biol Chem* 2002, **277**:39944-39952.

91. Jakob B, Kochlamazashvili G, Jäpel M, Gauhar A, Bock HH, Maritzen T, Haucke V: **Intersectin 1 is a component of the Reelin pathway to regulate neuronal migration and synaptic plasticity in the hippocampus**. *Proc Natl Acad Sci U S A* 2017, **114**:5533-5538.

92. Niu S, Renfro A, Quattrocchi CC, Sheldon M, D'Arcangelo G: **Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway**. *Neuron* 2004, **41**:71-84.

93. Niu S, Yabut O, D'Arcangelo G: **The reelin signaling pathway promotes dendritic spine development in hippocampal neurons**. *J Neurosci* 2008, **28**:10339-10348.

94. Cobos I, Borello U, Rubenstein JLR: **Dlx transcription factors promote migration through repression of axon and dendrite growth**. *Neuron* 2007, **54**:873-888.

95. Mao R, Page DT, Merzlyak I, Kim C, Tecott LH, Janak PH, Rubenstein JLR, Sur M: **Reduced conditioned fear response in mice that lack Dlx1 and show subtype-specific loss of interneurons**. *J Neurodev Disord* 2009, **1**:224-236.

96. Gilbert J, Man H-Y: **The X-linked autism protein KIAA2022/KIDLIA Regulates neurite outgrowth via N-cadherin and δ-catenin signaling**. *eNeuro* 2016, **3** ENEURO.0238-16.2016.

97. Pla R, Stanco A, Howard MA, Rubin AN, Vogt D, Mortimer N, Cobos I, Potter GB, Lindtner S, Price JD *et al.*: **Dlx1 and Dlx2 promote interneuron GABA synthesis, synaptogenesis, and dendritogenesis**. *Cereb Cortex* 2018, **28**:3797-3815.

98. Gilbert J, O'Connor M, Templet S, Moghaddam M, Di Via Ioschpe A, Sinclair A, Zhu L-Q, Xu W, Man H-Y: **NEXMIF/KIDLIA knock-out mouse demonstrates autism-like behaviors, memory deficits, and impairments in synapse formation and function**. *J Neurosci* 2020, **40**:237-254.

99. Adler CE, Fetter RD, Bargmann CI: **UNC-6/Netrin induces neuronal asymmetry and defines the site of axon formation**. *Nat Neurosci* 2006, **9**:511-518.

100. Ning K, Drepper C, Valori CF, Ahsan M, Wyles M, Higginbottom A, Herrmann T, Shaw P, Azzouz M, Sendtner M: **PTEN depletion rescues axonal growth defect and improves survival in SMN-deficient motor neurons**. *Hum Mol Genet* 2010, **19**:3159-3168.

101. Kath C, Goni-Oliver P, Müller R, Schultz C, Haucke V, Eickholt B, Schmoranzer J: **PTEN suppresses axon outgrowth by down-regulating the level of detyrosinated microtubules**. *PLoS One* 2018, **13**:e0193257.

102. Manent J-B, Wang Y, Chang Y, Paramasivam M, LoTurco JJ: **Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder**. *Nat Med* 2009, **15**:84-90.

103. Fukuda T, Nagashima S, Abe T, Kiyonari H, Inatome R, Yanagi S: **Rescue of CAMDI deletion-induced delayed radial migration and psychiatric behaviors by HDAC6 inhibitor**. *EMBO Rep* 2016, **17**:1785-1798.

104. Schaffer AE, Breuss MW, Caglayan AO, Al-Sanaa N, Al-Abdulwahed HY, Kaymakçalan H, Yılmaz C, Zaki MS, Rosti RO, Copeland B *et al.*: **Biallelic loss of human CTNNA2, encoding αN-catenin, leads to ARP2/3 complex overactivity and disordered cortical neuronal migration**. *Nat Genet* 2018, **50**:1093-1101.

105. Fife CM, McCarroll JA, Kavallaris M: **Movers and shakers: cell cytoskeleton in cancer metastasis**. *Br J Pharmacol* 2014, **171**:5507-5523.

106. Araki Y, Zeng M, Zhang M, Huganir RL: **Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP**. *Neuron* 2015, **85**:173-189.

107. Komiyama NH, Watabe AM, Carlisle HJ, Porter K, Charlesworth P, Monti J, Strathdee DJC, O'Carroll CM, Martin SJ, Morris RGM *et al.*: **SynGAP regulates ERK/MAPK signaling, synaptic plasticity, and learning in the complex with postsynaptic density 95 and NMDA receptor**. *J Neurosci* 2002, **22**:9721-9732.

108. Kim JH, Lee H-K, Takamiya K, Huganir RL: **The role of synaptic GTPase-activating protein in neuronal development and synaptic plasticity**. *J Neurosci* 2003, **23**:1119-1124.

109. Clement JP, Ozkan ED, Aceti M, Miller CA, Rumbaugh G: **SYNGAP1 links the maturation rate of excitatory synapses to the duration of critical-period synaptic plasticity**. *J Neurosci* 2013, **33**:10447-10452.

110. Ozkan ED, Creson TK, Kramár EA, Rojas C, Seese RR, Babyan AH, Shi Y, Lucero R, Xu X, Noebels JL *et al.*: **Reduced cognition in Syngap1 mutants is caused by isolated damage within developing forebrain excitatory neurons**. *Neuron* 2014, **82**:1317-1333.

111. Clement JP, Aceti M, Creson TK, Ozkan ED, Shi Y, Reish NJ, Almonte AG, Miller BH, Wiltgen BJ, Miller CA *et al.*: **Pathogenic SYNGAP1 mutations impair cognitive development by disrupting maturation of dendritic spine synapses**. *Cell* 2012, **151**:709-723.

112. Aceti M, Creson TK, Vaissiere T, Rojas C, Huang WC, Wang YX, Petralia RS, Page DT, Miller CA, Rumbaugh G: **Syngap1 haploinsufficiency damages a postnatal critical period of pyramidal cell structural maturation linked to cortical circuit assembly**. *Biol Psychiatry* 2015, **77**:805-815.

113. Guo X, Hamilton PJ, Reish NJ, Sweatt JD, Miller CA, Rumbaugh G: **Reduced expression of the NMDA receptor-interacting protein SynGAP causes behavioral abnormalities that model symptoms of schizophrenia**. *Neuropsychopharmacology* 2009, **34**:1659-1672.

114. Muhia M, Yee BK, Feldon J, Markopoulos F, Knuesel I: **Disruption of hippocampus-regulated behavioural and cognitive processes by heterozygous constitutive deletion of SynGAP**. *Eur J Neurosci* 2010, **31**:529-543.

115. Michaelson SD, Ozkan ED, Aceti M, Maity S, Llamosas N, Weldon M, Mizrachi E, Vaissiere T, Gaffield MA, Christie JM *et al.*: **SYNGAP1 heterozygosity disrupts sensory processing by reducing touch-related activity within somatosensory cortex circuits**. *Nat Neurosci* 2018, **21**:1-13.

116. Barnes SA, Wijetunge LS, Jackson AD, Katsanevaki D, Osterweil EK, Komiyama NH, Grant SGN, Bear MF, Nagerl UV, Kind PC *et al.*: **Convergence of hippocampal pathophysiology in Syngap+/- and Fmr1-/y mice**. *J Neurosci* 2015, **35**:15073-15081.

117. Hu W, Tian C, Li T, Yang M, Hou H, Shu Y: **Distinct contributions of Nav1.6 and Nav1.2 in action potential initiation and backpropagation**. *Nat Neurosci* 2009, **12**:996-1002.

118. Yamagata T, Ogiwara I, Mazaki E, Yanagawa Y, Yamakawa K: **Nav1.2 is expressed in caudal ganglionic eminence-derived disinhibitory interneurons: Mutually exclusive distributions of Nav1.1 and Nav1.2**. *Biochem Biophys Res Commun* 2017, **491**:1070-1076.

119. Spratt PWE, Ben-Shalom R, Keeshen CM, Burke KJ, Clarkson RL, Sanders SJ, Bender KJ: **The autism-associated Gene Scn2a contributes to dendritic excitability and synaptic function in the prefrontal cortex**. *Neuron* 2019, **103**:673-685 e5.

120. Tatsukawa T, Raveau M, Ogiwara I, Hattori S, Miyamoto H, Mazaki E, Itohara S, Miyakawa T, Montal M, Yamakawa K: **Scn2a haploinsufficient mice display a spectrum of phenotypes affecting anxiety, sociability, memory flexibility and ampakine CX516 rescues their hyperactivity**. *Mol Autism* 2019, **10**:15.

121. Krueger DD, Tuffy LP, Papadopoulos T, Brose N: **The role of neurexins and neuroligins in the formation, maturation, and function of vertebrate synapses**. *Curr Opin Neurobiol* 2012, **22**:412-422.

122. Cao X, Tabuchi K: **Functions of synapse adhesion molecules neurexin/neuroligins and neurodevelopmental disorders**. *Neurosci Res* 2017, **116**:3-9.

123. Rabaneda LG, Robles-Lanuza E, Nieto-González JL, Scholl FG: **Neurexin dysfunction in adult neurons results in autistic-like behavior in mice**. *Cell Rep* 2014, **8**:338-346.

124. Baudouin SJ, Gaudias J, Gerharz S, Hatstatt L, Zhou K, Punnakkal P, Tanaka KF, Spooren W, Hen R, De Zeeuw CI *et al.*: **Shared synaptic pathophysiology in syndromic and nonsyndromic rodent models of autism**. *Science (80-)* 2012, **338**:128-132.

125. Rothwell PE, Fuccillo MV, Maxeiner S, Hayton SJ, Gokce O, Lim BK, Fowler SC, Malenka RC, Südhof TC: **Autism-associated neuroligin-3 mutations commonly impair striatal circuits to boost repetitive behaviors**. *Cell* 2014, **158**:198-212.

126. Kalbassi S, Bachmann SO, Cross E, Roberton VH, Baudouin SJ: **Male and female mice lacking neuroligin-3 modify the behavior of their wild-type littermates**. *eNeuro* 2017, **4** ENEURO.0145-17.2017.

127. Cao W, Lin S, Xia Q, Du Y, Yang Q, Zhang M, Lu Y, Xu J, Duan S, Xia J *et al.*: **Gamma oscillation dysfunction in mPFC leads to social deficits in neuroligin 3 R451C knockin mice**. *Neuron* 2018, **97**:1253-1260 e7.

128. Anderson GR, Aoto J, Tabuchi K, Földy C, Covy J, Yee AX, Wu D, Lee S-J, Chen L, Malenka RC *et al.*: **β-Neurexins control neural circuits by regulating synaptic endocannabinoid signaling**. *Cell* 2015, **162**:593-606.

129. Martella G, Meringolo M, Trobiani L, De Jaco A, Pisani A, Bonsi P: **The neurobiological bases of autism spectrum disorders: the R451C-neuroligin 3 mutation hampers the expression of long-term synaptic depression in the dorsal striatum**. *Eur J Neurosci* 2018, **47**:701-708.

130. Poliak S, Peles E: **The local differentiation of myelinated axons at nodes of Ranvier**. *Nat Rev Neurosci* 2003, **4**:968-980.

131. Poot M: **Connecting the *CNTNAP2* networks with neurodevelopmental disorders**. *Mol Syndromol* 2015, **6**:7-22.

132. Murdoch JD, Gupta AR, Sanders SJ, Walker MF, Keaney J, Fernandez TV, Murtha MT, Anyanwu S, Ober GT, Raubeson MJ *et al.*: **No evidence for association of autism with rare heterozygous point mutations in contactin-associated protein-like 2 (CNTNAP2), or in other contactin-associated proteins or contactins**. *PLoS Genet* 2015, **11**:e1004852.

133. Toma C, Pierce KD, Shaw AD, Heath A, Mitchell PB, Schofield PR, Fullerton JM: **Comprehensive cross-disorder analyses of CNTNAP2 suggest it is unlikely to be a primary risk gene for psychiatric disorders**. *PLoS Genet* 2018, **14**:e1007535.

134. Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Sonnenblick LI, Gruver R, Almajano J, Bragin A *et al.*: **Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits**. *Cell* 2011, **147**:235-246.

135. Anderson GR, Galfin T, Xu W, Aoto J, Malenka RC, Sudhof TC: **Candidate autism gene screen identifies critical role for cell-adhesion molecule CASPR2 in dendritic arborization and spine development**. *Proc Natl Acad Sci U S A* 2012, **109**:18120-18125.

136. Brunner D, Kabitzke P, He D, Cox K, Thiede L, Hanania T, Sabath E, Alexandrov V, Saxe M, Peles E *et al.*: **Comprehensive analysis of the 16p.2 deletion and null Cntnap2 mouse models of autism spectrum disorder**. *PLoS One* 2015, **10**:e0134572.

137. Gdalyahu A, Lazaro M, Penagarikano O, Golshani P, Trachtenberg JT, Gescwind DH: **The autism related protein contactin-associated protein-like 2 (CNTNAP2) stabilizes new spines: an in vivo mouse study**. *PLoS One* 2015, **10**:e0125633.

138. Varea O, Martin-de-Saavedra MD, Kopeikina KJ, Schürmann B, Fleming HJ, Fawcett-Patel JM, Bach A, Jang S, Peles E, Kim E *et al.*: **Synaptic abnormalities and cytoplasmic glutamate receptor aggregates in contactin associated protein-like 2 /Caspr2 knockout neurons**. *Proc Natl Acad Sci U S A* 2015, **112**:6176-6181.

139. Peñagarikano O, Lázaro MT, Lu X-H, Gordon A, Dong H, Lam HA, Peles E, Maidment NT, Murphy NP, Yang XW *et al.*: **Exogenous and evoked oxytocin restores social behavior in the Cntnap2 mouse model of autism**. *Sci Transl Med* 2015, **7** 271ra8-271ra8.

140. Mei Y, Monteiro P, Zhou Y, Kim J-A, Gao X, Fu Z, Feng G: **Adult restoration of Shank3 expression rescues selective autistic-like phenotypes**. *Nature* 2016, **530**:481-484.

141. Guo B, Chen J, Chen Q, Ren K, Feng D, Mao H, Yao H, Yang J, Liu H, Liu Y *et al.*: **Anterior cingulate cortex dysfunction underlies social deficits in Shank3 mutant mice**. *Nat Neurosci* 2019, **22**:1223-1234.

142. Orefice LL, Mosko JR, Morency DT, Wells MF, Tasnim A, Mozeika SM, Ye M, Chirila AM, Emanuel AJ, Rankin G *et al.*: **Targeting peripheral somatosensory neurons to improve tactile-related phenotypes in ASD models**. *Cell* 2019, **178**:867-886 e24.

143. Won H, Lee H-R, Gee HY, Mah W, Kim J-I, Lee J, Ha S, Chung C, Jung ES, Cho YS *et al.*: **Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function**. *Nature* 2012, **486**:261-265.

144. Bozdagi O, Tavassoli T, Buxbaum JD: **Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay**. *Mol Autism* 2013, **4**:9.

145. Lee E-J, Lee H, Huang T-N, Chung C, Shin W, Kim K, Koh J-Y, Hsueh Y-P, Kim E: **Trans-synaptic zinc mobilization improves social interaction in two mouse models of autism through NMDAR activation**. *Nat Commun* 2015, **6**:7168.

146. Sgritta M, Dooling SW, Buffington SA, Momin EN, Francis MB, Britton RA, Costa-Mattioli M: **Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder**. *Neuron* 2019, **101**:246-259 e6.

147. Rhine MA, Parrott JM, Schultz MN, Kazdoba TM, Crawley JN: **Hypothesis-driven investigations of diverse pharmacological targets in two mouse models of autism**. *Autism Res* 2019, **12**:401-421.

148. Won H, Lee H-R, Gee HY, Mah W, Kim J-I, Lee J, Ha S, Chung C, Jung ES, Cho YS *et al.*: **Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function**. *Nature* 2012, **486**:261-265.

149. Wang X, Bey AL, Katz BM, Badea A, Kim N, David LK, Duffney LJ, Kumar S, Mague SD, Hulbert SW *et al.*: **Altered mGluR5-homer scaffolds and corticostriatal connectivity in a Shank3 complete knockout model of autism**. *Nat Commun* 2016, **7**:11459.

150. Vicidomini C, Ponzoni L, Lim D, Schmeisser MJ, Reim D, Morello N, Orellana D, Tozzi A, Durante V, Scalmani P *et al.*: **Pharmacological enhancement of mGlu5 receptors rescues behavioral deficits in SHANK3 knock-out mice**. *Mol Psychiatry* 2017, **22**:689-702.

151. Kolevzon A, Bush L, Wang A, Halpern D, Frank Y, Grodberg D, Rapaport R, Tavassoli T, Chaplin W, Soorya L *et al.*: **A pilot controlled trial of insulin-like growth factor-1 in children with Phelan-McDermid syndrome**. *Mol Autism* 2014, **5**:54.

152. Zwanenburg RJ, Bocca G, Ruiter SAJ, Dillingh JH, Flapper BCT, van den Heuvel ER, van Ravenswaaij-Arts CMA: **Is there an effect of intranasal insulin on development and behaviour in Phelan-McDermid syndrome? A randomized, double-blind, placebo-controlled trial**. *Eur J Hum Genet* 2016, **24**:1696-1701.

153. • Qin L, Ma K, Wang ZJ, Hu Z, Matas E, Wei J, Yan Z: **Social deficits in Shank3-deficient mouse models of autism are rescued by histone deacetylase (HDAC) inhibition**. *Nat Neurosci* 2018, **21**:564-575.
In this study, a brief treatment with romidepsin alleviated social deficits in *Shank3*-deficient mice with a long-lasting effect ($\sim$3 weeks). Moreover, HDAC2 transcription was upregulated in these mice, and knockdown of HDAC2 in prefrontal cortex rescued their social deficits. These findings highlighted an epigenetic mechanism underlying social deficits linked to Shank3 deficiency.

154. Gigliucci V, Leonzino M, Busnelli M, Luchetti A, Palladino VS, D'Amato FR, Chini B: **Region specific up-regulation of oxytocin receptors in the opioid Oprm1-/- mouse model of autism**. *Front Pediatr* 2014, **2**:91.

155. Meziane H, Schaller F, Bauer S, Villard C, Matarazzo V, Riet F, Guillon G, Lafitte D, Desarmenien MG, Tauber M *et al.*: **An early postnatal oxytocin treatment prevents social and learning deficits in adult mice deficient for Magel2, a gene involved in Prader-Willi syndrome and autism**. *Biol Psychiatry* 2015, **78**:85-94.

156. Fujiwara T, Sanada M, Kofuji T, Akagawa K: **Unusual social behavior in HPC-1/syntaxin1A knockout mice is caused by disruption of the oxytocinergic neural system**. *J Neurochem* 2016, **138**:117-123.

157. Teng BL, Nikolova VD, Riddick NV, Agster KL, Crowley JJ, Baker LK, Koller BH, Pedersen CA, Jarstfer MB, Moy SS: **Reversal of social deficits by subchronic oxytocin in two autism mouse models**. *Neuropharmacology* 2016, **105**:61-71.

158. Harony-Nicolas H, Kay M, du Hoffmann J, Klein ME, Bozdagi-Gunal O, Riad M, Daskalakis NP, Sonar S, Castillo PE, Hof PR *et al.*: **Oxytocin improves behavioral and electrophysiological deficits in a novel Shank3-deficient rat**. *eLife* 2017, **6**.

159. Yamasue H, Domes G: **Oxytocin and autism spectrum disorders**. *Curr Top Behav Neurosci* 2018, **35**:449-465.

160. Gantois I, Khoutorsky A, Popic J, Aguilar-Valles A, Freemantle E, Cao R, Sharma V, Pooters T, Nagpal A, Skalecka A *et al.*: **Metformin ameliorates core deficits in a mouse model of fragile X syndrome**. *Nat Med* 2017, **23**:674-677.