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Richard Delorme, Elodie Ey, Roberto Toro, Marion Leboyer, Christopher Gillberg, et al.. Progress toward treatments for synaptic defects in autism.. Nature Medicine, 2013, 19 (6), pp.685-94. 10.1038/nm.3193 . pasteur-01470299

HAL Id: pasteur-01470299

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Submitted on 26 Feb 2019

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Progress toward treatments for synaptic defects in autism

Richard Delorme¹⁻⁴, Elodie Ey¹⁻³, Roberto Toro¹⁻³, Marion Leboyer^{5,6}, Christopher Gillberg⁷⁻⁹ &

Q1 Thomas Bourgeron^{1-3,6} 

Q8 Autism spectrum disorder (ASD) encompasses a range of disorders that are characterized by social and communication deficits and repetitive behaviors. For the majority of affected individuals, the cause of ASD remains unknown, but in at least 20% of the cases, a genetic cause can be identified. There is currently no cure for ASD; however, results from mouse models indicate that some forms of the disorder could be alleviated even at the adult stage. Genes involved in ASD seem to converge on common pathways altering synaptic homeostasis. We propose, given the clinical heterogeneity of ASD, that specific ‘synaptic clinical trials’ should be designed and launched with the aim of establishing whether phenotype ‘reversals’ could also occur in humans.

ASD comprises a heterogeneous group of disorders with different etiologies, phenotypic outcomes and ages of onset. In a subset of patients with ASD, mutations of genes related to synaptic function have been identified, suggesting that abnormal neuronal homeostasis is a risk factor for ASD^{1,2}. Studies of the human brain transcriptome have shown that genes associated with synaptic functions may be expressed early during intrauterine development (3–6 months gestational age)³. However, the consequences of these alterations in gene expression are only detected as ASD much later, during the first 3 years of life. It is not known whether neuronal circuits expressing these genes can be preserved despite the presence of genetic mutations. An increasing number of studies in mouse models of ASD have shown that certain neuronal defects can be reversed in the mature mouse brain, either by restoring the gene function, decreasing mRNA translation or modulating the balance between excitation and inhibition (Table 1 and Supplementary Table 1). These results challenge the notion that all forms of ASD are irreversible neurodevelopmental disorders⁴. Given the clinical heterogeneity of ASD, we argue that specific synaptic clinical trials should be designed and launched with a view to establishing whether or not similar reversals could also occur in humans.

The clinical trajectory and heterogeneity of ASD

The very early (premorbid) signs of ASD are still largely unknown, which brings into question whether reversibility is possible and whether there might be a discrete window for reversing the pathological process. Among clinicians, there is agreement that autistic symptoms are relatively stable over time⁵. Nevertheless, over the past decades, several reports have described individuals with autism who were clearly affected in early childhood but emerged as adolescents and adults with relatively ordinary lives and no profound behavioral deficits. In a recent prospective study of 6,975 children with ASD followed from diagnosis through age 14, six distinct common trajectories of social, communication and repetitive behavior were identified⁶. For example, 21.4% of the subjects never showed repetitive behaviors, and 8.1% presented with repetitive behaviors at age 3 but improved as they grew older (Fig. 1). Most of the trajectories remained stable from 4 to 14 years, but with different levels of severity. Interestingly, about one in ten of children with ASD experienced rapid gains in communication and social abilities, moving from severely affected to high functioning (‘bloomers’).

The core symptoms of ASD are rarely seen in isolation and usually coexist with other psychiatric and medical conditions, including intellectual disability, language disorders, epilepsy, motor control problems, attention-deficit/hyperactivity disorders, tics, anxiety, sleep disorders, gastrointestinal problems and abnormal (too high or too low) response to sensory stimuli. Intensive behavioral interventions have been shown to improve the cognitive and adaptive behaviors of young children with ASD⁷. However, treatments for ASD need to take into account the extreme degree of etiological and clinical heterogeneity of these conditions and also the possibility that multiple treatments might be needed to target different types of symptoms. The concept of ESSENCE (early symptomatic syndromes eliciting neurodevelopmental clinical examinations) was recently introduced to better take into account the clinical heterogeneity and syndromic overlap of developmental symptoms that impair children under the age of 3–5 years⁸.

The relationship between brain development and function

Neuronal circuits are shaped by genes and the environment throughout life, but there are some critical periods when brain development is particularly sensitive to experience⁹. During early life, these key periods of high brain plasticity involve sensory systems, but also motor functions and cognition¹⁰. After these ‘windows in development’, the level of plasticity is reduced^{9,11}. This reduced plasticity is linked to the development of myelin or perineuronal networks that drastically curtail neurite outgrowth in the mature brain and also to functional modifications in the excitation–inhibition balance^{9,11}. For example,

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Q7 Received 3 December 2012; accepted 11 April 2013; published online XX XX 2013; doi:10.1038/nm.3193

Table 1 Improvement of the phenotypes in mouse models of autism spectrum disorders

Models		Methods	Outcomes	
<i>Fmr1</i> ^{-/-}	Genetic	Overexpression of <i>FMR1</i> human gene ^{37*38*113*}	No effect: Motor learning; spatial learning and memory; anxiety; startle response (worse) Improvement: Macroorchidism; activity; social approaches; anxiety toward novel food; PPI	
		Reduction of mGluR5 expression ^{42*}	No effect: Macroorchidism Improvement: Extinction of inhibitory avoidance; plasticity and spine density in visual cortex; basal protein synthesis in hippocampus; audiogenic seizure; body growth	
	Pharmacology	Overexpression of Nlgn1 ^{41*}	No effect: Spatial learning; interest for novel objects Partial improvement: Positive puncta for excitatory synapses; number of inhibitory synapses Improvement: Social preference; social contact maintenance; activity; body weight	
		MPEP (mGluR5 antagonist) ^{43,114}	Partial improvement: Seizure Improvement: Anxiety in open field; activity; seizure	
		Minocycline (matrix metalloproteinase 9 inhibitor) ^{48*49*}	Improvement: Working memory in Y-maze; anxiety in plus maze; percentage of mushroom-shaped spines; dendritic spine length; percentage of short dendritic spines with larger heads; number of USV emitted by a male toward a female	
		CTEP (mGluR5 antagonist) ⁶⁸	No effect: Motor coordination Partial improvement: ERK activity; macroorchidism Improvement: Inhibitory avoidance; startle response to auditory stimuli; activity; spine density in visual cortex; hippocampal LTD (slices)	
		Arbaclofen (activation of GABA _B receptor) ⁷⁸	No effect: Distance in open field; marble burying; motor coordination Partial improvement: Seizure Improvement: Basal protein synthesis in hippocampus; AMPA receptor internalization; spine density	
		Lovastatin (ERK-mediated protein synthesis inhibitor) ⁷²	Partial improvement: Audiogenic seizures Improvement: Excessive protein synthesis, epileptiform activity in hippocampus (<i>in vitro</i>), hyperexcitability in visual cortex (<i>in vitro</i>)	
		Environment	Physical enrichment ³³	Improvement: Anxiety in open field; habituation to novel objects; basal dendritic branching; basal dendritic length; spine density; spine maturation; GluR1 levels in visual cortex
		<i>Nlgn3</i> ^{-/-}	Genetic	Reexpression of Nlgn3 in Purkinje cells ^{71*}
Pharmacology	D-Cycloserine (NMDAR partial agonist) ⁵²		Improvement: Self-grooming	
<i>Pten</i> ^{-/-}	Pharmacology	Rapamycin (mTOR inhibitor) ⁶⁴	No effect: Cell polarity Partial improvement: Macrocephaly Improvement: Social interactions; anxiety; soma hypertrophy	
<i>Shank2</i> ^{-/-}	Pharmacology	D-Cycloserine (NMDAR partial agonist) ⁵¹	No effect: Social recognition Partial improvement: Preference for social interactions Improvement: NDMA/AMPA ratio	
		CDPPB (mGluR5 positive allosteric modulators) ⁵¹	No effect: Social recognition; pup retrieval; repeated jumping; anxiety in plus maze; activity Partial improvement: Preference for social interactions Improvement: NMDA/AMPA ratio; LTP and LTD at hippocampal Schaffer-collateral-CA1-pyramidal synapses; NMDAR signaling in whole brain and synaptosomes	
<i>Cntnap2</i> ^{-/-}	Pharmacology	Risperidone (dopaminergic D2 receptor antagonist) ⁵⁴	No effect: Sensory hypersensitivity; preference for social interactions Partial improvement: Spontaneous alternations Improvement: Nesting behavior; self-grooming; hyperactivity	
<i>Scn1a</i> ^{+/-}	Pharmacology	Clonazepam (positive allosteric modulator of GABA _A) ⁷⁷	Improvement: Social interest; free social interactions; fear conditioning; inhibitory transmission	
<i>Eif4ebp2</i> ^{-/-}	Pharmacology	4EGI-1 (selective inhibitor which prevents eIF4E binding to eIF4G) ¹¹⁰	Improvement: Social preference; Nlgn protein amounts; excitation-inhibition balance	
<i>Tsc1</i> ^{-/-}	Pharmacology	Rapamycin (mTOR inhibitor) ⁶¹	Partial improvement: Survival; macrocephaly; hindlimb clasp	
<i>Tsc2</i> ^{-/-}	Pharmacology	Rapamycin (mTOR inhibitor) ⁶¹	Improvement: Spatial learning; context discrimination; L-LTP	
<i>Mecp2</i> ^{Y/-}	Genetic	Reactivation of <i>Mecp2</i> (<i>Cre-lox</i>) ^{4,115}	Partial improvement: Activity; gait; hindlimb clasp; tremor; respiratory function; (body weight) Improvement: LTP; activity; body weight; brain weight; neuronal size	
		Overexpression of BDNF ^{45*}	Partial improvement: Brain size Improvement: Activity	
	Pharmacology	Ampakine CX546 (AMPA agonist) ¹¹⁶	Partial improvement: BDNF levels Improvement: Respiratory function	
		Desipramine (norepinephrine reuptake inhibitor) ^{117,118}	No effect: Activity; body weight; head size Partial improvement: Respiratory function; lifespan Improvement: Tyrosine hydrolase expression in brainstem	
		Insulin-like growth factor 1 (IGF1R agonist) ^{34*}	Partial improvement: Lifespan; activity; respiratory function; heart rate; brain weight; PSD-95 concentration in motor cortex; spine density on motor cortex neurons; excitatory synaptic transmission in sensory motor cortex neurons; plasticity in cortical circuits	

(Continued)

Table 1 Continued

Models	Methods	Outcomes
	Insulin ⁸⁷	No effect: baseline breathing; breathing response to hypoxia Worsening: weight gain; heart rate decline; blood glucose; breathing response to hypoxia; lifespan
	Fingolimod (sphingosine-1 phosphatase receptor modulator) ⁵³	No effect: BDNF levels in cerebellum Partial improvement: Lifespan; hindlimb clasping; BDNF levels in cortex, hippocampus, striatum; wet weight of striatum Improvement: Locomotor activity; motor coordination
Environment	Physical enrichment ⁸¹	No effect: Motor coordination; BDNF levels in cerebellum
Del15q11-13 Genetic	Reduction of CaMKII inhibitory phosphorylation ^{117*}	Improvement: Motor coordination; spatial learning and memory; contextual fear conditioning; kinase activity; body weight; audiogenic seizure; LTP
Q29 <i>Ube3a^{m-p+}</i> Pharmacology	PD158780 and PD168393 (ERBBB inhibitor) ¹¹⁸	Improvement: LTP, long-term memory in fear conditioning
Dup17q11.2 Environment	Physical and social enrichment ⁸³	No effect: Social dominance; olfactory habituation/dishabituation; stereotypes Partial improvement: Social recognition; fear conditioning; anxiety Improvement: Motor coordination

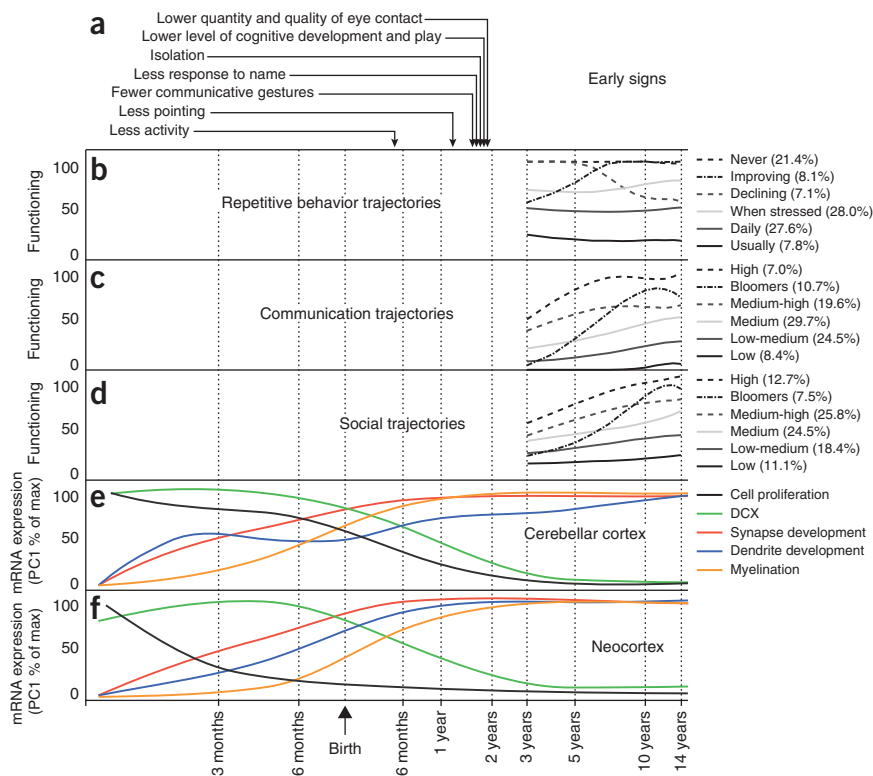
*Treatment administered during development. PPI, prepulse inhibition; LTD, long-term depression; LTP, long-term potentiation; PAG, periaqueducal gray; USV, ultrasonic vocalizations; DHPG, dihydroxyphenylglycine.

local GABAergic circuits modulate visual cortical plasticity in early postnatal life⁹, and similar fine-tuning of excitation and inhibition is also crucial for correct auditory cortical development¹². Autism was proposed as a critical-period disorder¹³ after it was shown that mutations in several genes associated with ASD may affect the early inhibition–excitation balance, thus shifting the timing of critical periods or even completely obliterating such periods of high neuronal plasticity. Dysfunction in auditory, visual and somatosensory information processing might even be the primary factor driving aberrant development of socialization and communication skills in ASD¹³. Thus, if a gene is required at a specific developmental stage, its reactivation at a later stage outside the critical period might be insufficient to reverse the consequence of its early loss.

Genetic studies have identified hundreds of genes associated with ASD^{2,14,15}. These genes have roles in various physiological processes, such as chromatin remodeling, metabolism, translation and synaptogenesis. It is speculated,

however, that these genes may converge into pathways affecting distinct neuronal functions². Neuronal and/or synaptic homeostasis could be one of these functions, which would help explain why opposite morphological or functional defects in ASD sometimes lead to similar clinical outcomes^{16,17}. For example, a reduction in the number of dendrites was observed in Rett and tuberous sclerosis syndromes, whereas a high density of dendrites was reported in fragile X syndrome¹⁶. These three genetic syndromes have a very high rate of associated ASD symptoms (up to 60% of affected individuals for fragile X and Rett syndromes, and up to 50% of individuals with tuberous sclerosis syndrome). Similarly, some mutations increase or reduce excitatory currents, whereas some increase or decrease inhibitory currents. For example, *Shank3* (encoding SH3 and multiple

Figure 1 Time course of gene expression in the human brain and clinical features observed in patients with ASD. **(a)** Early signs of ASD are detected in the first 2 years of life. **(b–d)** Trajectories of 6,975 children with ASD, followed from diagnosis through to the age of 14 years. Six distinct common trajectories were defined for each core symptom of ASD (social interaction, communication and restrictive pattern of interests). The y axis represents the level of functioning (from 0: low functioning to 100: high functioning). The percentages in parentheses indicate the proportion of subjects that exhibited the corresponding trajectory. Data are taken from ref. 6. **(e,f)** Developmental trajectories of genes associated with cell proliferation, doublecortin-immunopositive cells (DCX), dendrites, synapses and myelination in the cerebellar cortex and the neocortex of the human brain. The time course of gene mRNA expression is summarized by the first principal component (PC1) of the indicated set of genes (expressed as the percentage of the maximum) across age. Data are taken from ref. 3.



ankyrin-repeat domains 3) homozygous-knockout mice show a reduction in the frequency and amplitude of their excitatory post-synaptic currents¹⁸, whereas *Nlgn3* (encoding neuroligin 3) knock-in R451C mice show an increase in the frequency of their inhibitory post-synaptic currents¹⁹.

Interestingly, several genes associated with ASD are modulated by neuronal activity²⁰, suggesting that they may mediate experience-dependent circuit modifications². For example, a subset of genes associated with ASD (such as *NLGN3*, *NRXN1* (encoding neurexin 1), *SHANK3*, *PTEN* (encoding phosphatase and tensin homolog), *TSC2* (encoding tuberous sclerosis 2) and *NFI* (encoding neurofibromin 1)) are targets of the fragile X mental retardation protein (FMRP, encoded by *FMR1*) responsible for fragile X syndrome and regulation of neuronal translation²¹.

It remains largely unknown in which phases of development mutations found in ASD produce a detectable phenotype. In rodents, most genes involved in synaptogenesis are strongly expressed after birth and reach a plateau in adulthood^{22–24}. In contrast, in humans, the genes involved in dendrite and synapse formation are switched on very early *in utero*, and the amounts of transcripts reach a plateau at 6–9 months of fetal life³. This may also be the case for most ASD susceptibility genes. But, even if these genes are expressed at a very early stage of brain development, the impact of their mutation on brain function seems to be delayed and only observable at a later stage (Fig. 1). This delay between the initial expression of the gene and the functional impact of the mutation might have several different causes.

First, genetic redundancy might occur at early stages of development. Mutations usually affect a single allele of a gene, and the mutated gene may belong to a family of genes (for example, the *SHANK* family). Thus, the second allele, or other members of the same gene family (for example, *SHANK1*, *SHANK2* and *SHANK3*) might preserve the gene's functions early on, but its late functions cannot be compensated. In such a case, the basic wiring of the brain might not be affected. Such compensation and functional redundancy of synaptic proteins during development has been observed, for example, in *Dlg1* (also known as SAP97) knockout mice²⁵.

Second, the functional consequences of the mutations may only be detectable when the networks are fully mature (that is, in postnatal life, at an early stage of development). In this case, defects in cellular differentiation, cellular migration or long-distance axon guidance might be detected only when a specific ability, such as spoken language, develops. Among the proteins associated with ASD that could affect neuronal connectivity are the contactins, the semaphorins and the SHANK proteins. Contactins and semaphorins are directly involved in neuritegenesis and axon guidance, but they are also found at the synapse. SHANK3 is important for dendrite formation but is also abundant in the growth cone of axons during migration, suggesting that it may also be necessary for long-distance connectivity²⁶.

In this case, the mutations might affect the hard wiring of neuronal networks, and recovery from an ASD after the brain has fully matured might be more difficult to achieve. This was recently shown for a mouse model lacking SYNGAP1, a synaptic protein crucial for dendritic spine development. Inducing SYNGAP1 mutations after the critical period of spine development had a minimal impact on spines, and repairing the defect in mouse adulthood did not improve behavioral phenotypes²⁷.

Third, the apparent delay between gene expression and phenotypic outcome might also be a result of the challenge of detecting early phenotypic features, for example, during pregnancy. After birth, most children with ASD experience a decline in the acquisition of

developmental milestones from 6 to 36 months²⁸, with less pointing by 12 months, fewer communicative gestures and responses to name by 18 months and less eye contact by 24 months (Fig. 1). Differences in patterns of head growth²⁹ and aberrant white matter organization³⁰ have been occurred in some cases during the first year of life, and differences in event-related potential to face processing³¹ and in electroencephalographic activity³² have been detected at as early as 6–10 months of age. These features are not specific to ASD but might be early signs of abnormal synaptic homeostasis and of a variety of neurodevelopmental dysfunctions.

To obtain the proof of principle for reversion of a specific neuronal defect, mouse models carrying similar mutations to those identified in individuals with ASD have been generated that allow the mutated genes to be conditionally reactivated in the mature brain. Such mice can be used to test the potential for reversibility, but species-specific differences might limit direct predictions in humans, and thus results from clinical trials are required to provide reliable information about the possibility of alleviating the disorder.

From mice to humans: developing treatments for ASD

Numerous studies have investigated the possibility of treating the phenotype in mouse models of fragile X syndrome and Rett syndrome as well in mice containing mutations of synaptic proteins associated with ASD (Table 1 and Supplementary Table 1). Strategies to alleviate the abnormal phenotype include genetic manipulation and cellular therapy, pharmacological intervention and environmental stimulation. Most studies have first focused on the comorbidities observed in mouse models of ASD, such as presence of seizures, abnormal locomotor activity and anxiety^{4,33,34}. Recent studies have undertaken a more comprehensive behavioral characterization of the core symptoms of ASD by exploring, for example, social interactions or ultrasonic vocalizations as a possible paradigm for speech deficiency^{35,36}.

Genetic and cellular therapeutic strategies. Experiments using genetic interactions (crossing mouse strains that carry mutations in different genes) have been used to modulate the expression of the genes and pathways of interest in ASD. In the fragile X model lacking the *Fmr1* gene, expression of a functional human *FMR1* gene restored social behavior and sensory gating^{37–39}. Interestingly, overexpression of neuroligin 1, a synaptic cell adhesion molecule mutated in some cases of ASD⁴⁰, can also improve social behavior in fragile X model mice but has no positive effect on learning or memory⁴¹. Similar experiments have been performed to compensate for the downstream effects of mutations: excessive mRNA translation following mGluR5 signaling, overexpression of amyloid- β protein or overexpression of the striatal-enriched protein tyrosine phosphatase in the fragile X mouse model^{42–44} and reduced expression of brain-derived neurotrophic factor (BDNF) in the Rett syndrome mouse model⁴⁵. These studies have achieved partial improvements: reduction in the number of seizures, reduction of body weight, decreased gait abnormalities, improved locomotor activity and reduced social and nonsocial anxiety (Table 1). Crossing mouse lines, however, provides no information on the reversibility of the phenotype in adult mice, as the compensation occurs from an early stage of development.

Genetic manipulation and cellular therapy strategies have also been used to reverse neuroanatomical and behavioral abnormalities in mice modeling ASD. In a pioneering experiment, the group of Adrian Bird created a mouse model of Rett syndrome in which the endogenous *Mecp2* gene (encoding methyl CpG-binding protein 2) was silenced but could be conditionally activated⁴. They showed that

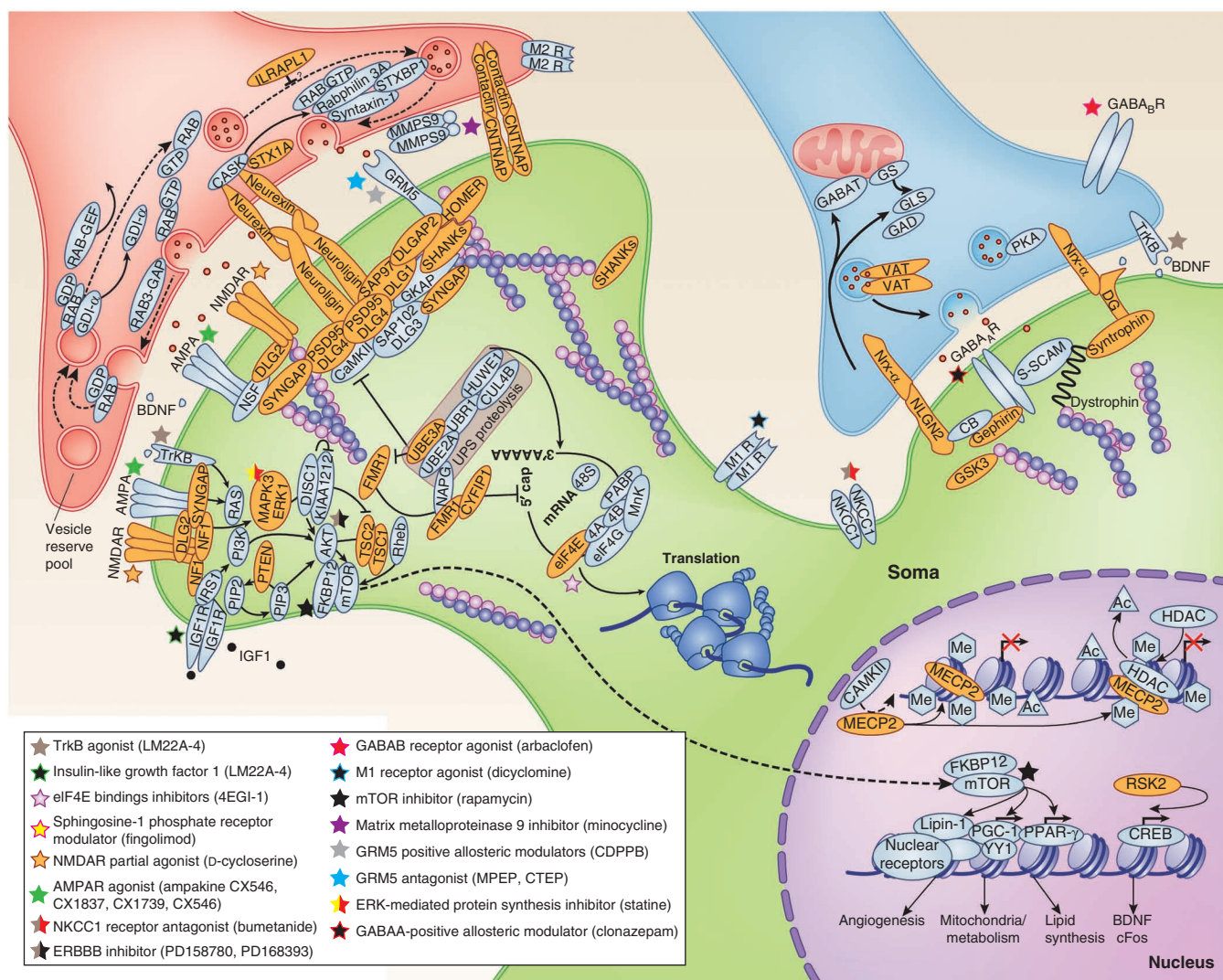


Figure 2 The synaptic proteins in ASD and the drugs that have been tested in mouse models of ASD or in clinical trials. Schematic representation of pre- and post-synaptic proteins located at glutamatergic and GABAergic synapses. Proteins reported to be associated with ASD are in orange. Factors involved in the pathways that the ASD-associated proteins participate in are shown in blue. Pharmacological compounds used to reverse core symptoms of autism in mouse models and patients are tagged with a star. The functional link between these proteins were shown experimentally (for example: FMRP targets distinct mRNA sequence elements to regulate expression of several proteins associated with ASD¹⁰⁹ and eIF4E overexpression leads to increased translation of neurologins¹¹⁰, which are postsynaptic proteins that are causally linked to ASDs¹¹¹). The pathways associated with ASD are reviewed in refs. 2,56,112.

activation of *Mecp2* in 70% of neurons in both immature and mature adult mice reversed many morphological defects in the motor cortex, including neuronal size and dendritic complexity. This led to a marked improvement in respiratory and sensorimotor functions, including breathing pattern, grip strength and balance-beam and rotarod performance⁴. In a follow-up study, reexpression of *Mecp2* only in catecholaminergic cells was sufficient to improve survival, general activity, motor coordination and seizure-like cortical activity in *Mecp2*^{+/-} mice⁴⁶. Recently, Derecki *et al.*⁴⁷ used transplantation of wild-type bone marrow to introduce microglial cells into the *Mecp2* knockout mouse model of Rett syndrome. This resulted in increased lifespan, normalization of breathing patterns, reduction of apnea, normalization of body weight and improved locomotor activity in these mice⁴⁷. Taken together, these findings support the view that MECP2 does not necessarily play a pivotal part in brain development but

may instead be required to maintain full neurological function once development has occurred⁴.

Pharmacological therapeutic strategies. Pharmacological interventions for ASD have been facilitated by the fact that most of the molecules tested in mice have already been used in humans for other purposes (for example, ampakine (CX546), dicyclomine, minocycline, desipramine, D-cycloserine, fingolimod). Lifespan, seizure, learning, memory and anxiety have been shown to be robustly ameliorated by pharmacological treatments^{48–53}. In a recent study on BTBR T+tf/J mice, a partial improvement of social interest was even possible with ampakine treatment³⁶. However, treatment of ASD core symptoms—decreased social interaction and increased stereotypy—may be more challenging. For example, in *Cntnap2* knockout mice, risperidone can alleviate increased repetitive behavior, but not social

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deficits⁵⁴, a dissociation similar to what is seen in humans treated with the same drug⁵⁵.

Empirical treatments that target two pathways in ASD (mRNA translation and excitation–inhibition balance) are now emerging and have shown some promise (Fig. 2). The possibility that targeting mRNA translation in ASD might be therapeutically beneficial was suggested by reports of aberrant mRNA translation of synaptic proteins in people with ASD⁵⁶. The evidence supporting this theory includes the identification of mutations affecting several proteins (NF1, TSC1, TSC2 and PTEN) that normally inhibit mRNA translation through the phosphoinositide 3-kinase–mammalian target of rapamycin (PI3K–mTOR) signaling pathway or in proteins directly involved in the inhibition of mRNA translation at the synapse (FMR1, CYFIP1 and ELF4E) in patients with ASD^{57–60}. In addition, an excess of mRNA translation (20%) was observed in the *Fmr1* knockout mouse model of fragile X syndrome. On the basis of these findings, two main strategies have been used to inhibit translation in mouse models of ASD.

The first strategy is repression of the PI3K–mTOR signaling pathway using molecules such as rapamycin. Rapamycin successfully reduced cognitive impairment and social behavior deficit in mouse models of tuberous sclerosis^{61–63} and improved behavior in adult mice with a deletion of PTEN⁶⁴. Unexpectedly, even gross anatomical abnormalities such as neuronal hypertrophy were reversed at the adult stage⁶⁴. In patients with tuberous sclerosis, rapamycin led to a marked reduction in the volume of astrocytomas and in seizure frequency, suggesting that this might be an alternative therapy to neurosurgical resection that could increase the quality of life for patients⁶⁵. However, the efficacy of rapamycin in ameliorating behavioral abnormalities, including those that are regarded as ASD core symptoms, remains to be further investigated, and the possibility of adverse events occurring in patients receiving such treatments also remains to be determined⁶⁶.

The second strategy is repression of mRNA translation by inhibiting the action of the group 1 metabotropic glutamate receptors mGluR1 and mGluR5. A 50% reduction in mGluR5 expression decreased excess translation and improved behavior in the fragile X mouse model⁴². The mGluR5 antagonist MPEP (2-methyl-6-(phenylethynyl)pyridine) can rescue altered dendritic spine morphology as well as behavioral and cognitive deficits in different models of fragile X syndrome (reviewed in ref. 67). Chronic treatments with CTEP, a high affinity, long-acting and orally bioavailable mGluR inhibitor, restored cognitive functions, ameliorated auditory hypersensitivity, aberrant dendritic spine density and overactive extracellular signal–regulated kinase (ERK) and mTOR signaling, and partially corrected the macroorchidism observed in male mouse models of fragile X syndrome⁶⁸.

In humans, a recent randomized, double-blind, crossover study of 30 males with fragile X syndrome—all of them with severe autistic symptoms—was performed to evaluate the effects of the mGluR5 antagonist AFQ056 (ref. 69). Despite a lack of improvement of the group of patients ($n = 30$) as a whole, secondary analysis revealed that a subset of patients with a fully methylated *FMR1* gene ($n = 7$) showed a benefit as measured with the aberrant behavior checklist (mean decrease -27.8 in patients with full methylation versus $+3.2$ in patients with partial methylation), highlighting the importance of identifying relevant biomarkers to assess therapeutic benefits. Three different mGluR5 antagonists, AFQ056 (Novartis), RO4917523 (Hoffmann-La Roche) and STX107 (Seaside Therapeutics) are currently being investigated in advanced clinical trials (phases 2–3) or will start to be investigated shortly. Studies in mice suggest that mGluR antagonists could also be efficacious for treating other synaptic defects. MPEP can reduce self-grooming behavior in BTBR mice, an inbred genetic

mouse model of ASD⁷⁰. *Nlgn3* knockout mice show ectopic synapse formation and perturbed mGluR-dependent synaptic plasticity, a hallmark of fragile X syndrome. These phenotypes could be rescued by reexpression of *Nlgn3* in juvenile mice, again highlighting the possibility of reverting neuronal circuit alterations in autism after the completion of development⁷¹. Lovastatin, an inhibitor of ERK-mediated protein synthesis currently used in treatment of hypercholesterolemia, has recently been shown to reduce audiogenic seizures in a mouse model of fragile X⁷². Finally, minocycline, a broad-spectrum tetracycline antibiotic, also represses mRNA translation⁴⁸. In fragile X mice, treatment with minocycline for 1 month after birth normalized the dendritic spine defects and improved behavior and cognition. In addition, the number of ultrasonic vocalizations of males in the presence of estrus females was restored to that of wild-type mice⁷³. Two open trials of minocycline in patients with fragile X syndrome demonstrated significant improvements, advocating for placebo-controlled trials of this drug^{74,75}.

A second pathway in ASD that could be modulated by drugs concerns the synaptic excitation–inhibition balance. Several genes associated with ASD seem to be involved in the formation of excitatory and inhibitory synapses, such as those encoding neuroligins and neuroligins. In addition, mutations in genes associated with epilepsy, such as *SCN1A*, which encodes a voltage-gated sodium channel, have been found in patients with ASD⁷⁶. In mice haploinsufficient for *Scn1a*, treatment with low-dose clonazepam, a positive allosteric modulator of GABA_A receptors, completely reversed the abnormal social behaviors and deficits in fear memory⁷⁷. Activation of the GABA_B receptor by arbaclofen decreased mRNA translation in the cortex of fragile X mice and corrected the increased spine density⁷⁸. In humans, a recent randomized, double-blind, placebo-controlled crossover study using arbaclofen in 63 subjects with a full mutation in the *FMR1* gene showed a slight improvement of social abilities measured by the aberrant behavior checklist social avoidance subscale but no significant benefit in the primary outcome measures quantified by the aberrant behavior checklist irritability subscale⁷⁹. Another example is provided by the *Nlgn1* knockout mouse, which shows a reduced NMDA/AMPA ratio at corticostriatal synapses⁵². In these mice, administration of the NMDA receptor (NMDAR) partial co-agonist D-cycloserine rescued the repetitive grooming phenotype. In *Shank2* knockout mice, direct stimulation of NMDARs with D-cycloserine normalized NMDAR function and improved social interaction⁵¹. Recently, an alternative and interesting new strategy to modulate the excitation–inhibition balance emerged that used the diuretic chloride-transporter antagonist bumetanide, which reduces intracellular chloride concentration, reinforcing GABAergic inhibition. A recent clinical trial in patients with ASD showed improvements calling for larger-scale randomized trials to test this drug⁸⁰.

Environmental and social enrichment strategies. Finally, environmental enrichment has been used to reverse abnormal phenotypes in mouse models of ASD. Changing objects and adding a running wheel in the cage decreased anxiety and restored dendritic length and branching, spine density and maturation in a mouse model of fragile X³³. The same strategy has been extensively used in mouse models of Rett syndrome and has restored locomotor activity, improved learning deficits and anxiety and increased BDNF expression⁸¹. Another strategy that might hold promise is social enrichment. Housing BTBR mice with highly social C57BL/6 mice has been reported to enhance the social interactions in the BTBR strain⁸². A combination of both social and physical enrichment rescued motor

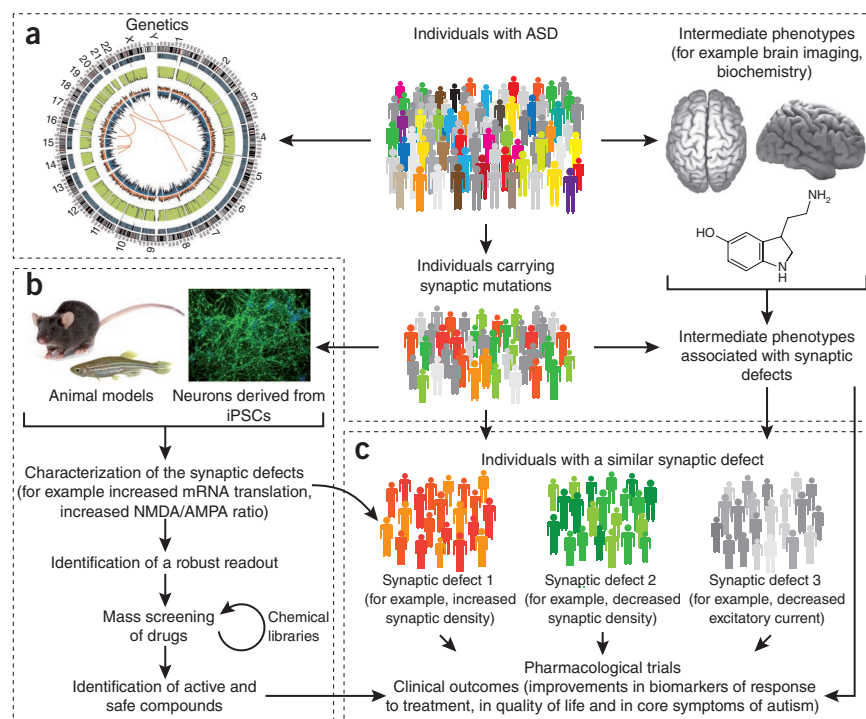
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Figure 3 Strategies for synaptic clinical trials in ASD. (a) To reduce genetic and clinical heterogeneity, individuals with ASD should be placed into groups on the basis of their affected genes and pathways by using whole-genome sequencing. In parallel, deep phenotyping of these subgroups of patients should be used to identify intermediate phenotypes and biomarkers more closely related to the synaptic defects.

(b) Animal models and iPSCs can be used to better characterize the synaptic defects present in patients. They are likely to be powerful tools for identification of robust assays that can provide the readout necessary for standardized high-throughput drug screening (safety and efficacy).

(c) Patients with similar synaptic defects should be enrolled in pharmacological trials to test the efficacy and the safety of compounds selected previously (in b). The categories of synaptic defects are taken from the results obtained in the current mouse models of ASD (increased synaptic density in the fragile X mouse model, decreased synaptic density in Rett syndrome, decreased excitatory current in the *Shank1* and *Shank3* knockout mouse). The outcome of such trials will be based on clinical instruments measuring the improvements in quality of life and core symptoms of autism, but also on biomarkers identified previously in this group of patients (in a).



Q26

defects, improved learning and memory, reduced aggressive behavior and relieved anxiety in a mouse model of Potocki-Lupski syndrome. This clinical syndrome is caused by a 3.7-Mb duplication in chromosome 17p11.2 and is characterized by neurobehavioral abnormalities, intellectual disability and ASD in 70–90% of affected individuals⁸³. Interestingly, in the mouse model study, social enrichment increased stereotypic repetitive behavior in both mutant and wild-type mice, suggesting that this phenotype is sensitive to overstimulation. To our knowledge, no studies have tested ‘behavioral strategies’, that is task repetition and regular training through repeated exposure with highly social strains as demonstrators or initiators, to improve the behavior of mutant mice in social interaction tasks. It would be interesting to test whether such behavioral interventions, which have demonstrated their efficacy in patients with ASD⁸⁴, could positively affect the behavior of mouse models of ASD.

Q19

Taken together, these results indicate that some synaptic and behavioral defects can be restored in adult mice even after the end of the critical periods of development. Nevertheless, some points remain to be further investigated. Many of these studies were performed on one genetic background, with small groups of mice per treatment, in some cases only in males (details in **Supplementary Table 1**). The stability of the improvement and the side effects of the treatments were not always documented. It was also shown that the behavioral consequence of a mutation might also be different across generations⁸⁵ or on different backgrounds⁸⁶. The investigation and publication of possible worsening of phenotypes in mouse models should also be encouraged to complement knowledge about treatments and dose effects⁸⁷. Finally, none of these studies investigated a combination of pharmacological intervention and a modified (enriched) environment to test for potential optimization of treatment. The key questions now are whether the positive results obtained in rodents can be translated to humans and how best to design trials to test therapeutic modalities that have shown promise in animal models of ASD.

Future directions for clinical trials

Q20

Only two drugs, risperidone and aripiprazole, have been approved by the US Food and Drug Administration for the treatment of ASD. Although these drugs serve to manage challenging and repetitive behaviors, they have little effect on the social communication deficit observed in ASD⁸⁸. Developing knowledge-based treatments for ASD will have to take into account the high genetic and clinical heterogeneity of these conditions⁸⁹. To date, the most frequently mutated genes affect less than 0.1–1% of the affected subjects, that is, approximately 8,000–80,000 individuals in all G8 countries. Standard randomized placebo-controlled clinical trials in hundreds of patients will therefore be difficult to achieve for ASD, and specific clinical trials designed for rare diseases need to be devised⁹⁰.

One of the main challenges for clinical trials in ASD is the considerable degree of heterogeneity, which could mask the effects of the tested treatment. Furthermore, the clinical scores most frequently used to measure treatment efficacy in clinical trials might be too focused on the core ASD symptoms and do not necessarily reflect improvements in quality of life⁹¹. For example, most of the pharmacological trials for ASD in ClinicalTrials.gov ($n = 153$) are using the clinical global instrument (33%), which measures clinical improvement, and the aberrant behavior checklist (31%), which measures the comorbid problem behaviors associated with ASD. Both instruments do not take into account the quality of life of patients, and an improvement measured by these instruments does not always correlate with better functioning in everyday life⁹². Therefore, one possibility would be to have quality-of-life scales as the primary outcome scales and autism checklists as the secondary ones. Or, when designing new studies, researchers could flag from the beginning that both types of scales should be preset as the (combined) primary outcome variable.

Q21

We propose that three major steps are needed to improve the efficacy of clinical trials for patients with ASD and synaptic defects (**Fig. 3**): (i) obtain more homogenous subgroups of individuals with ASD, as determined on the basis of the affected genes or pathways

rather than on the clinical phenotype; (ii) identify relevant clinical outcomes and biomarkers related to these subgroups (for example, neuronal, biochemical and brain imaging markers); and (iii) increase knowledge of the natural history of this subgroup of patients to identify, for example, periods of symptoms worsening.

The benefit of a clinical trial strategy focused on patients selected for their synaptic defects is to reduce the genetic, but also the phenotypic, heterogeneity. The strategy of selecting patients on the basis of the affected genes or pathways is currently used for ‘syndromic’ forms of ASD, such as tuberous sclerosis and fragile X syndrome. Using the same strategy, specific synaptic clinical trials should now be launched for ASD. As a first step, patients enrolled in these synaptic clinical trials should be selected using whole-genome sequencing to identify the causative or contributory synaptic mutations as well as putative modifier genes. In many cases, patients with inherited deletions of 16p or *de novo* SHANK2 mutations, for example, carry additional mutations, and the presence of multiple hits might be indeed the rule rather than the exception in ASD^{93,94}. A better knowledge of these modifier genes will inform clinicians and researchers on responses to treatment. For example, the identification of multiple genetic hits in a patient might explain why he or she will not fully respond to the treatment whereas other patients without these additional genetic mutations will.

Animal models and patient-specific induced pluripotent stem cells (iPSCs) should be used to inform researchers on the synaptic defects present in each patient and thereby enable them to enroll patients with different causative mutations but with apparently similar synaptic defects (for example, decreased or increased synaptic density, excitation–inhibition imbalance) in the clinical trials. Specific neural cell subtypes differentiated from patient-specific iPSCs are likely to be a powerful tool for evaluating the hierarchy of cellular vulnerability in these diseases and the potential efficacy of therapies being tested. As an example, a specific assay for dysfunction of synaptic proteins, such as SHANK, remains to be determined. Cellular models expressing deleterious SHANK3 mutations suggest that modifications of dendritic spine morphology via an actin-dependent mechanism are part of the mutant phenotype²⁶. Similarly, SHANK3 mutant mice show a hypertrophy of medium spiny neurons with an increase in the complexity of dendritic arbors and total dendritic length^{17,18}. An in-depth characterization of the synaptic defect associated with each type of genetic mutation is crucial for two main reasons: first, the identification of a robust readout is necessary for drug screening; second, patients with mutations in similar genes might actually show the opposite synaptic defects, as observed in *Shank2* (increased NMDA) and *Shank3* (decreased NMDA) knockout mice^{17,18,51}. If these results are confirmed in iPSCs from patients with these mutations, then patients with SHANK2 and SHANK3 mutations should be enrolled in two different synaptic clinical trials to test potential disease-modifying therapies.

Finally, a better knowledge on the natural history of each subgroup of patients is crucial for designing a synaptic clinical trial. For example, most patients with *de novo* or truncating mutations in SHANK3 show an early regression before 36 months and a worsening during adolescence, which correlates with a frequent emergence of epilepsy⁹⁵. SHANK3 mutations have also been identified in individuals developing adult-onset SHANK3 related-disorders, such as schizophrenia and bipolar disorders^{96,97}. To better understand this variability in clinical outcome and trajectories of the patients, large-scale initiatives are currently ongoing. The Simons Variation in Individuals Project (Simons VIP) aims to identify and study a large number of individuals with a deletion or duplication of chromosome 16p11.2 that increases the risk of developing neurodevelopmental disorders⁹⁸. The Rett Rare

Disease Clinical Research Center natural history study has enrolled more than 800 patients and led to a redefinition of the critical periods of deterioration of patients and the emergence of ritualized and stereotypic behaviors⁹⁹. These careful analyses of genetically defined ASD subtypes should allow detailed phenotypic comparisons within and among these groups to clarify genotype–phenotype correlations.

Recruiting the functional allele: a possible ASD therapy?

Q22

The proposed strategy of synaptic clinical trials for ASD should lead to personalized treatments by taking into account the genetic synaptic defect present in the patient. In this line, the observation that abnormal gene dosage (deletion or duplication) seems to play a key part in ASD could be an advantage for finding new treatments². In contrast to X-linked or autosomal recessive traits, for which the gene product is often absent, in most patients with ASD for which a genetic cause is known, only one allele is affected by copy number variations or two alleles carry weak mutations but are still functional¹⁰⁰. Given these observations, one promising route for treatment is to activate the expression of the remaining functional allele (in the case of a deletion) or to slightly repress the expression of the gene (in the case of a duplication). This strategy was recently used for Angelman’s syndrome, a disorder affecting an imprinted genomic region on chromosome 15q11–q13 that is caused by deletion or mutation of the maternal allele of *UBE3A*. Using an unbiased screen in primary cortical neurons from mice, Huang *et al.*¹⁰¹ identified several topoisomerase inhibitors that unsilence the paternal allele of *UBE3A*. Such molecules could therefore reactivate the functional, but dormant, allele of *UBE3A* in patients with Angelman’s syndrome. More generally, the amount of synaptic proteins is regulated by transcription¹⁰², splicing¹⁰³, mRNA transport¹⁰⁴, translation¹⁰⁵ and degradation¹⁰⁶. A better knowledge of the factors that regulate each of these regulatory steps might help to identify new targets for restoring the amount of specific synaptic proteins in patients. In addition, given that several genes associated with ASD are regulated by neuronal activity²⁰, it is most likely that a combination of pharmacological treatments (that restore the amount of synaptic proteins) and behavioral treatments (that stimulate specific neuronal circuits) might show the most benefit for patients.

Conclusions

There is increasing support for the notion that customized treatments could lead to recovery in some patients with ASD, which may spur revision of views on the stability of ASD phenotypes. In addition, further in-depth molecular pathophysiological characterization of ASDs that are not directly linked to synaptic dysfunction (for example defects in chromatin remodeling¹⁰⁷ or metabolism¹⁰⁸) should be carried out to investigate the possibility of reversing phenotypes. An integrated approach, which brings together cellular and animal models and knowledge-based clinical trials, will be key to furthering understanding of which affected genes and pathways in ASD can be efficiently recovered and will also provide a basis for developing new therapies.

Note: Supplementary information is available in the [online version of the paper](#).

ACKNOWLEDGMENTS

We thank D. DiGregorio and I. Cloez-Tayari for critical reading of the manuscript and G. Huguet for the illustrations. This work was funded by the Institut Pasteur, the Bettencourt-Schueller foundation, Centre National de la Recherche Scientifique, University Paris Diderot, ANR (ANR-08-MNPS-037-01-SynGen), Neuron-ERANET (EUHF-AUTISM), the Fondation de France, the Conny-Maeva Charitable Foundation, the Cognacq Jay Foundation, the Orange Foundation, the Fondamental Foundation and the Innovative Medicines Initiative Joint Undertaking under grant agreement no. 115300, the resources of which

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are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007 - 2013) and EFPIA companies in kind contribution.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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