Disease Markers 33 (2012) 225–239
DOI 10.3233/DMA-2012-0917
IOS Press

Mutant mouse models of autism spectrum disorders

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Abstract. Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental diseases characterized by a triad of specific behavioral traits: abnormal social interactions, communication deficits and stereotyped or repetitive behaviors. Several recent studies showed that ASDs have a strong genetic basis, contributing to the discovery of a number of ASD-associated genes. Due to the genetic complexity of these disorders, mouse strains with targeted deletion of ASD genes have become an essential tool to investigate the molecular and neurodevelopmental mechanisms underlying ASD. Here we will review the most relevant genetic mouse models developed by targeted inactivation of ASD-associated genes, and discuss their importance for the development of novel pharmacological therapies of these disorders.

Keywords: Autism, brain development, knockout mouse, animal model

1. Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of brain diseases with a well recognized genetic and neurodevelopmental origin. Due to the complexity of these pathologies, the importance of animal models in ASD research has been widely recognized in recent years. Namely, the generation of mouse strains with targeted deletion of ASD-associated genes has become an essential tool to investigate the neurodevelopmental basis of ASD, as well as the molecular, cellular, anatomical, electrophysiological and behavioural consequences of gene dysfunction in ASD. Several genetic mouse models of ASD have been developed, whose complete phenotypic characterization is still under investigation in several laboratories [21, 116,130]. In this review, we will describe only the most relevant mouse models that were generated by targeted inactivation of ASD-associated genes. ASD-associated genes will be presented following the classification proposed in the SFARIgene database of the Simon Foundation Autism Research Initiative (https://gene.sfari.org): syndromic ASD genes, strong candidate genes and genes with suggestive or minimal evidence of ASD association. Congenic mouse strains showing ASD features, as well as experimentally-induced mouse models will not been discussed in this review. For a comprehensive list of the available mouse models for ASD, the reader is referred to the SFARIgene database (https://gene.sfari.org).

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2. Syndromic genes

Syndromic ASD genes are defined as those genes predisposing to autism in the context of a syndromic disorder (e.g. fragile X syndrome) (https://gene.sfari.org). These include CNTNAP2, FMR1, MECP2, NF1, PTEN, SHANK3, TSC1/2 and UBE3A (Table 1).

2.1. CNTNAP2

Contactin-associated protein-like 2 (CNTNAP2; also known as CASPR2) is a member of neuronal neurexin superfamily involved in neuron-glia interac-

ISSN 0278-0240/12/$27.50

Table 1
Mutant mouse models of autism spectrum disorders

Gene	SFARI classification	Mouse model	Phenotype	References
CNTNAP2	syndromic	*Cntnap2*$^{-/-}$ mice	ASD core behavioural symptoms, hyperactivity and seizures, loss of GABAergic neurons in the cortex, striatum and hippocampus	118
FMR1	syndromic	*Fmr1* KO mice	Attentional dysfunction	112
			Social anxiety and impaired social cognition	105,109,134
			Ultrasound vocalization deficits	123
			Seizures	50
			Reduced expression of $GABA_A$ receptor subunits	2,49
			Altered GABAergic transmission	27,43
			Loss of PV cortical interneurons	125
MECP2	syndromic	*Viaat-Mecp2* conditional mutant mice	ASD-like repetitive and stereotyped behaviours, EEG abnormalities and seizures, reduced GAD65/67 mRNA in the cerebral cortex, decreased cortical miniature inhibitory postsynaptic currents	29
NF1	syndromic	*Nf1*$^{+/-}$ mice	Deficits in early-LTP and spatial learning	38,39,131
		Mice lacking *Nf1* in interneurons	Over-activation of Ras signaling in inhibitory interneurons during learning, abnormal enhancement of GABA release	41
PTEN	syndromic	Mice lacking *Pten* in the cerebral cortex and hippocampus	Altered social behaviour and inappropriate responses to sensory stimuli	100
SHANK3	syndromic	*Shank3B* mutant mice	ASD-like features (repetitive grooming, reduced social behaviours)	117
		Shank3A mutant mice	Milder phenotype than *Shank3B* mice (normal social behaviours and reduced recognition of social novelties)	117
		Shank3(*e4-9*) mutant mice	Abnormal social and repetitive behaviours, impaired learning and memory	117
TSC1	syndromic	*Tsc1*$^{+/-}$ mice	Deficits in learning, memory and contextual fear conditioning, reduced levels of social exploration	56,68
TSC2	syndromic	*Tsc2*$^{+/-}$ mice	Deficits in learning, memory and contextual fear conditioning, normal social behaviour, altered ultrasonic vocalization	56,68.153
			Dowregulation of mGluR5 signaling contributes to behavioural deficits	6
UBE3A	syndromic	*Ube3A*$^{-/-}$ mice	Impaired motor function, inducible seizures, learning deficits, abnormal hippocampal EEG and impaired LTP	110
		Transgenic mice with triple dosage of *Ube3A*	Defective social interaction, impaired communication, and increased repetitive stereotypic behaviour	132
CNTN4	strong candidate	*Cntn4*$^{-/-}$ mice	No data available about ASD-like traits	59
NRXN1	strong candidate	*Nrxn1*$\langle^{-/-}$ mice	Impaired spatial memory, increased repetitive behaviours	15,35
			Differences in novelty responsiveness in male *Nrxn1*$\langle^{+/-}$ mice	92
EN2	suggestive or minimal evidence	*En2*$^{-/-}$ mice	Decreased play, reduced sociality, impaired spatial learning/memory	30
			Decreased interneuron connectivity, increased seizure susceptibility	145
FOXP2	suggestive or minimal evidence	*Foxp2*$^{-/-}$ mice	Motor impairment, absence of ultrasonic vocalizations in pups, cerebellar defects upon maternal separation	129
GABRB3	suggestive or minimal evidence	*Gabrb3*$^{-/-}$ mice	ASD-like traits (reduced sociability, reduced social novelty testing, attention deficits), learning and memory deficits, poor motor skills, seizure suscetibility, tremors, hyperactivity, cerebellar hypoplasia	42,47,75

Table 1, continued

Gene	SFARI classification	Mouse model	Phenotype	References
NLGN1	suggestive or minimal evidence	*Nlgn1*$^{-/-}$ mice	Repetitive behaviours, deficits in hippocampus-dependent learning and memory, decreased hippocampal LTP	35
			Decreased GABAergic activity	15
NLGN3	suggestive or minimal evidence	*Nlgn*^{3R451C} knock-in mice	Increased inhibitory synaptic transmission, deficits in social interaction	141
		Nlgn3$^{-/-}$ mice	Partial loss of PV-positive basket cells in the cerebral cortex	67
NLGN4	suggestive or minimal evidence	*Nlgn4*$^{-/-}$ mice	Abnormalities in reciprocal social interaction	82
OXT	suggestive or minimal evidence	*Oxt*$^{-/-}$ mice	Social memory deficits, reduced ultrasonic vocalizations, increased aggression, failure to recognize familiar conspecifics after repeated social encounters	148
OXTR	suggestive or minimal evidence	*Oxtr*$^{-/-}$ mice	Impaired social recognition, reduced ultrasonic vocalization, increased aggression	142
			Reduced cognitive flexibility and increased seizure susceptibility	124
RELN	suggestive or minimal evidence	*Reeler* mice	ASD-like behaviours	101
			Disorganized cerebral cortex and cerebellum	44,45
			Increased seizure susceptibility	115
			Partial loss and unproper positioning of cortical GABAergic neurons	71,98,110, 150
			Decreased GABA turnover	25

The table reports the most relevant mouse models developed by targeted inactivation of ASD-associated genes, classified according to the criteria proposed by the Simon Foundation Autism Research Initiative (SFARI; https://gene.sfari.org). For a comprehensive list of ASD mouse models see https://gene.sfari.org and references in [130]. Abbreviations are as in the text.

tions, neuronal migration in the developing cortex and clustering of voltage-gated K channels at Ranvier's nodes [120,136]. Originally, CNTNAP2 was linked to the cortical dysplasia-focal epilepsy syndrome, a rare disorder characterized by cortical dysplasia, focal epilepsy and autism [136]. Subsequently, genetic studies provided additional evidence that rare and common variants of this gene may contribute to ASD or ASD-related endophenotypes [3,5,7]. Interestingly, speech-language impairment frequently occurs as a phenotype of people with polymorphisms in CNTNAP2. Moreover, the expression of this gene can be regulated by FOXP2, whose mutations lead to language and speech disorders [146]; see below, Section 4.2). Deletion of *Cntnap2* in mice results in core behavioural symptoms of ASD (stereotypic behaviours, social interactions and vocal communication) [118]. *Cntnap2*$^{-/-}$ mice also show hyperactivity and seizures, both features often associated with ASD patients [133]. Administration of risperidone (the first FDA drug approved for ASD treatment) ameliorates hyperactivity and repetitive behaviours but not social deficits in *Cntnap2*$^{-/-}$ mice. Neuropathological analyses revealed abnormal neuronal distribution in deep cortical layers postnatal day (P) 7 and adult *Cntnap2*$^{-/-}$ mice, and presence of ectopic neurons in the corpus callosum of mutant mice at P14. Interestingly, *Cntnap2*$^{-/-}$ mice display a reduced number of GABAergic interneurons in the cortex, striatum and hippocampus. Moreover, *in vivo* two-photon calcium imaging showed that *Cntnap2*$^{-/-}$ mice exhibit reduced cortical neuronal synchrony with respect to wild-type (WT) mice, an interesting finding considering that these abnormalities are similar to those seen in many people with ASD [118].

2.2. *FMR1*

Fragile X syndrome (FXS) is the most frequent inherited cause of mental retardation and an identified cause of autism [52]. The Fragile Mental Retardation 1 locus (FMR1) resides in the X chromosome. FXS results from the expansion of triplet repeats in the untranslated region of the FMR1 gene, preventing synthesis of the FMR1 gene product FMRP. FMRP is a RNA-binding protein that modulates mRNA trafficking, dendritic maturation and synaptic plasticity [154]. The phenotype of *Fmr1* knockout (KO) mice has been extensively studied [13,108]. *Fmr1* KO mice show a complex behavioral profile, characterized by a number of ASD-like symptoms, including attention dysfunction [112], social anxiety and impaired social cognition [105,109,134], ultrasound vocalization

deficits [123] and seizures [150]. ASD-like features in *Fmr1* KO mice are dependent on the mouse genetic background [119,135]. These altered behaviors are accompanied by a series of anatomical and synaptic plasticity deficits, mainly affecting neurotransmission at the level of $GABA_A$ and group I metabotropic glutamate (mGluR1/5) receptors. Several studies showed a severe reduction in the expression of $GABA_A$ receptor subunit mRNAs and proteins in adult *Fmr1* KO mice [2,49], along with an abnormal GABAergic transmission [27, 43] and deficits of parvalbumin (PV) expressing cortical GABAergic interneurons [125]. A "metabotropic glutamate receptor (mGluR) theory" of FXS pathogenesis has also been proposed, based on a series of findings indicating that in the absence of FMRP, the FMRP-dependent consequences of mGluR5 activation are exaggerated [10]. In agreement with this hypothesis, *Fmr1* KO with a 50% reduction of mGlur5 (obtained by crossing *Fmr1* mutant mice with *Grm5* mutant mice) showed a complete rescue of anatomical, plasticity and behavioral deficits [50]. These findings led to propose mGlur5 as a pharmacological target for the cure of FXS and ASD (see also Section 5.1).

2.3. MECP2

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder typically emerging between 6–18 months of age characterized by the progressive loss of cognitive and motor functions, the emergence of ASD-like features and the occurrence of epileptic seizures [28]. RTT is caused by mutations in the gene encoding for the methyl-CpG binding protein 2 (MECP2), a transcriptional regulator involved in chromatin remodeling and splicing. Mice with truncated MeCP2 recapitulate many RTT features [23,32,70,126] and activation of the MeCP2 protein even at late stages of the disease can rescue the mutant phenotype [5, 64]. With respect to typical ASD features, the most interesting data were recently obtained in conditional mutants lacking *Mecp2* in inhibitory neurons expressing *Viaat* (Vesicular inhibitory aminoacid transporter, required to load GABA and glycine into synaptic vesicles) [29]. *Viaat-Mecp2* conditional mutants exhibit ASD-like repetitive and stereotyped behaviours, accompanied by electroencephalographic (EEG) abnormalities and seizures. *Viaat-Mecp2* conditional mutants show reduced levels of glutamic acid decarboxylase (GAD65/67) mRNA in the cerebral cortex, as well as decreased miniature inhibitory post-synaptic currents (mIPSC) in cortical slices, suggesting that *Mecp2* deficiency in GABAergic neurons might determine a reduction of GABA release as a consequence of reduced GAD synthesis in presynaptic terminals [29]. This study indicates that loss of *Mecp2* in inhibitory neurons might be a crucial determinant of severe forms of ASD.

2.4. NF1

Neurofibromatosis type 1 (NF1) is a neurocutaneous disorder caused by mutations in the NF1 gene [36, 55]. The NF1 gene encodes for a protein called neurofibromin, a GTPase activator involved in the regulation of the Ras/ERK signaling [8,102]. In patients, NF1 is frequently associated with intellectual and learning deficits [114,127], and a greater susceptibility to autism [103,104]. In mice, the complete loss of Nf1 is lethal, due to embryonic heart malformations [17, 79]. $Nf1^{+/-}$ mice are instead viable and show deficits in the early phase of long-term potentiation (LTP), as well as compromised spatial learning in the Morris water maze [38,39,41,131]. Interestingly, mice with *Nf1* deletion restricted to inhibitory neurons develop normally, do not show tumor predisposition but display learning deficits in the adult life [41]. Using these mice a cellular mechanism underlying Nf1-dependent learning deficits has been proposed, in which *Nf1* deficiency can lead to an over-activation of Ras signaling in inhibitory interneurons during learning, resulting in abnormal enhancement of GABA release from these neurons [41]. Spatial learning deficits and LTP can be rescued in $Nf1^{+/-}$ mice by using pharmacological/genetic approaches that inactivate Ras-ERK signaling or decrease GABA-mediated inhibition [39,41,97] (see also Section 5.4).

2.5. PTEN

The tumor suppressor Phosphatase and Tensin homolog on chromosome 10 (PTEN) is a negative regulator of the phosphatidylinositol 3-kinase (PI3K) signaling pathway, which mediates several processes in various tissues. PTEN is a phosphatase that dephosphorylates the second messenger phosphatidylinositol-(3,4,5)-trisphosphate (PIP3) in phosphatidylinositol-(4,5)-disphosphate (PIP2), reducing the activity of the protein kinases AKT/PKB and its downstream signaling cascade. AKT kinases can act on a large spectrum of substrates, including tuberous sclerosis complex 2 (TSC2/Tuberin; see Section 2.7), glycogen synthase kinase 3β (GSK3β), and the proapoptotic protein

BAD [100]. Mutations in the PTEN induce Cowden syndrome, characterized by prostate, skin and colon defects, spontaneous tumors and neurologic features such as autism and Lhermitte Duclos disease [147]. PTEN mutations have been reported in autistic individuals with macrocephaly [20,66,157]. In mice, conditional deletion of *Pten* in limited differentiated neuronal populations of the mouse cerebral cortex and hippocampus leads to abnormal dendritic and axonal growth, increased synapse number, neuronal hypertrophy and macrocephaly. *Pten* conditional mutant mice exhibit altered social behavior and inappropriate responses to sensory stimuli [100].

2.6. SHANK3

Shank family proteins (Shank1-3) are multidomain scaffold proteins forming the postsynaptic density complexes (PSD). Shank proteins have an important role to physically connect neurotransmitter receptors and other membrane proteins to actin cytoskeleton and signaling effectors in dentritic spines, contributing to synapse formation and spine maturation [90]. The functional importance of Shank scaffolding proteins is revealed by the fact that many mutations in *Shank* genes induce forms of mental retardation. Several studies found a correlation between ASD and single mutations in *Shank3* [53,63,111]. Two *Shank3* mutant mice strains have been generated, named *Shank3A* and *Shank3B* [117]. In *Shank3A* mutant mice, the *Shank3*α isoform was completely eliminated, whereas in *Shank3B* mutants both *Shank3* isoforms were deleted, also comprising a putative 3γ isoform. *Shank3B* mutants show ASD-like features, such as repetitive grooming and reduced social behaviors. Striatal hyperthrophy and reduced cortico-striatal glutamatergic transmission is also observed in these mutants. *Shank3A* mutant mice display a milder phenotype than *Shank3B* mice, characterized by a normal social behaviors and reduced recognition of social novelties [117]. *Shank3*(*e4-9*) mutant mice bear a deletion of exons 4 to 9. These mice display abnormal social and repetitive behaviors, as well as also impaired learning and memory. These behavioral deficits are accompanied by alterations in dendritic spines and impaired LTP [117]. Other studies shed light on the importance of Shank3 interactions with the scaffolding protein Homer; indeed, mutations affecting the Shank3-Homer binding site have been associated to ASD [53]. Heterozygous mice for a deletion of the Shank3-Homer binding site at the C terminus (*Shank3* + /ΔC mice) display a >90% reduction of Shank3 expression at synapses, reduced NMDAR-dependent synaptic responses in the frontal cortex and reduced social behaviors; *Shank3*+/ΔC mice show no changes in synapse number and morphology, and normal learning and memory abilities [9].

2.7. TSC1/2

Tuberous Sclerosis Complex (TSC) is a neurocutaneous syndrome characterized by benign tumors, early onset epilepsy, intellectual disability, and autism [74]. TSC results from loss-of-function mutations of TSC1 or TSC2 genes, which are crucially involved in the control of neuronal and glial cell proliferation during embryonic development. TSC1 encodes a protein (hamartin) containing two coiled-coil domains, while TSC2 encodes a GTPase activating protein (tuberin) that inhibits small G-proteins belonging to the Ras-related super-family. Hamartin and tuberin are both expressed in neurons and astrocytes of specific central nervous system (CNS) regions such as forebrain, cerebellum and brainstem, where they form a protein–protein complex that constitutively inhibits mTOR (mammalian target of rapamycin). Loss of TSC1/2 function leads to activation of the mTOR cascade and results in increased cell proliferation [74,86]. Mice with a heterozygous inactivating mutation in the Tsc genes ($Tsc1^{+/-}$ and $Tsc2^{+/-}$ mice) show deficits in learning, memory and contextual fear conditioning [56,68]. In addition to learning and memory deficits, $Tsc1^{+/-}$ mice have reduced levels of social exploration, while $Tsc2^{+/-}$ knockout mice show a normal social behavior [68,128] but altered ultrasonic vocalization [153]. In addition to behavioral changes, synaptic abnormalities are observed in the hippocampus of $Tsc2^{+/-}$ and in mice with a conditional homozygous deletion of *Tsc1* in astrocytes [56,68]. Furthermore, Tsc1 conditional knockout mice show abnormal dendritic spine morphology and density, enhanced cortical excitability and seizures [143,106]. In contrast with the main feature of human disease, $Tsc1^{+/-}$ or $Tsc2^{+/-}$ mice do not develop tubers [16,56,68,89], indicating that anatomical lesions are not involved in the pathogenesis of learning and social deficits. Recent studies also indicate that a dowregulation of mGluR5 signaling can contribute to synaptic and behavioural deficits in $Tsc2^{+/-}$ mice [6] (see also Sections 2.2 and 5.1). Taken together, these findings are interesting in light of the high prevalence of ASD in human TSC populations, pointing out the potential role of Tsc genes in the pathogenesis of syndromic ASD.

2.8. UBE3A

Angelman syndrome is a neurodevelopmental disorder characterized by mental retardation, absence of language development, EEG abnormalities and epilepsy. The genetic defects underlying Angelman syndrome are heterogeneous, including large maternal deletions of chromosome 15q11-q13, disomies of chromosome 15 and mutations in the E6-AP ubiquitin ligase gene UBE3A, located on chromosome 15 [84,88]. In mice, *Ube3a* is required for experience-dependent maturation of the neocortex [152] and a deficiency of the maternal allele of *Ube3a* results in impaired motor function, inducible seizures, learning deficits, abnormal hippocampal EEG and severely impaired LTP [110]. Increased gene dosage of *Ube3a* results in ASD-like traits and decreased glutamate synaptic transmission in mice. These results suggest that *Ube3a* gene dosage may contribute to the autism traits of individuals with maternal 15q11-13 duplication [132].

3. Strong candidate genes

Strong ASD candidate genes are defined as those genes for which a rigorous statistical comparison between cases and controls has been performed in a series of independent studies, yielding genome-wide statistical significance of ASD association (categories 1 and 2 in the SFARIgene database; https://gene.sfari.org). These include CNTN4 and NRXN1 (Table 1).

3.1. CNTN4

Several rare variants have been described in the CNTN4 gene in association with neurodevelopmental disorders including ASD [59,122]. Contactin4 (CNTN4), also known as BIG2 (brain-derived immunoglobulin superfamily molecule 2), is an axonal cell adhesion molecule that belonging to the contactin family. CNTN4 is mainly expressed in the brain (cerebellum, thalamus, amygdala, and cerebral cortex) where it is thought to play an essential role in the formation of axonal connections during development [59]. No data are available regarding the presence of ASD-like traits in mice lacking *Cntn4*. *Cntn4*$^{-/-}$ mice display aberrant projection of olfactory sensory neurons to multiple glomeruli, suggesting that *Cntn4* is an axon guidance molecule required for establishment of the olfactory neural circuitry [87]. Interestingly, several studies have shown olfactory deficits in a range of psychiatric and neurodevelopmental disorders including schizophrenia and ASD [12,18,37,138].

3.2. NRXN1

Neurexins (NRXNs) are presynaptic cell adhesion proteins that form trans-synaptic cell-adhesion complexes with their postsynaptic counterpart neuroligins (NLGNs) [137]. The interaction between neuroligins and neurexins is controlled by alternative splicing of both neuroligin and neurexin genes [33,40]. There are three NRXN genes in mammals, NRXN1–3, each expressed as a long α- and a short β-isoform by two alternative promoters. NRXNs are localized presynaptically, and their distribution to excitatory or inhibitory synapses is regulated by alternative splicing[40]. Rare single gene variations in the NRXN1 gene have been found associated with autism [57,139]. Mice with a targeted deletion of the promoter and first exon of *Nrxn1*α show a complete loss of Nrxn1α protein but unaltered levels of *Nrxn1*β and can therefore be considered as a *Nrxn1*α knockout. Nrxn1α deletion results in electrophysiological changes, impaired spatial memory and increased repetitive behaviors, traits that are all consistent with cognitive impairments [15,35]. Interestingly, *Nrxn1*$\alpha^{+/-}$ mice show differences in novelty responsiveness that are observed only in male mice, indicating sex-specific differences of the behavioral phenotype [92].

4. Genes showing suggestive or minimal evidence of ASD association

Genes with suggestive or minimal evidence of ASD association are defined as those candidate genes for which only small/unreplicated genetic studies have been performed (categories 3 and 4 in the SFARIgene database; https://gene.sfari.org). These include EN2, FOXP2, GABRB3, NGLNs, OXT/OXTR and RELN (Table 1).

4.1. EN2

EN2 codes for the homeobox-containing transcription factor Engrailed-2, a key regulator of posterior brain (midbrain/hindbrain) embryonic development [85]. The human EN2 gene maps to a region of chromosome 7 implicated in ASD susceptibility, and genome-wide association (GWA) studies indicated EN2 as a candidate gene for ASD. Namely, two SNPs in the human EN2 gene have been associated to ASD, one of which (rs1861973, A-C haplotype) is functional: when tested in a luciferase reporter assay

in rat, mouse and human cell lines, this SNP markedly upregulates EN2 promoter activity [11]. Preliminary evidence indicate that the EN2 A-C haplotype is also functionanal *in vivo*, being able to upregulate reporter gene expression in transgenic mice [34]. *En2*$^{-/-}$ mice display cerebellar hypoplasia and a reduced number of Purkinje cells [85]. Importantly, ASD-like behaviours (decreased play, reduced sociality, impaired spatial learning and memory) were described in these mutants [30]. Recently we showed that *En2* is also expressed in the adult hippocampus and cerebral cortex; *En2*$^{-/-}$ mice also show an increased susceptibility to experimentally-induced seizures that is accompanied by altered GABAeregic connectivity in the hippocampus (namely, reduced PV staining on cell bodies of CA3 pyramidal neurons and reduced somatostatin staining in the stratum lacunosum moleculare). We proposed that *En2*$^{-/-}$ mice might be used as model to study the role of GABAergic system dysfunction in the genesis of autism and epilepsy [145].

4.2. FOXP2

FOXP2 is a transcription factor containing a polyglutamine tract and a forkhead DNA-binding domain. Heterozygous mutations of the FOXP2 gene in humans cause severe speech and language disorders [60,93,94]. FOXP2 was recently associated to ASD by homozygous haplotype mapping [26]. While FOXP2 mutations were not directly associated to increased ASD risk, the language dysfunction that is central to ASD diagnosis may be influenced by FOXP2, therefore the FOXP2 gene has been considered as a potential autism susceptibility gene [61]. FOXP2 is expressed in multiple regions within the developing brain including the cortical plate, basal ganglia, thalamus, inferior olive, and cerebellum. *Foxp2*$^{-/-}$ mice show severe motor impairment, premature death, and an absence of ultrasonic vocalizations in pups upon maternal separation [129]. Histological analyses of these mice suggest that *Foxp2* mutation influences neuronal migration and/or maturation in the developing cerebellum [129]. *Foxp2*$^{+/-}$ mice show reduced synaptic plasticity of cortico-striatal circuits, associated with deficits in learning of rapid motor skills [69]. These motor deficits are consistent with the observed cerebellar phenotype, suggesting that the language dysfunction in these mice is due merely to motor function deficits [129]. However, recent studies have revealed novel cognitive deficits in *Foxp2*$^{-/-}$ mice that go beyond motor functions and extend to auditory-motor association learning [91]. The analysis of FOXP2 transcriptional targets identified a FOXP2-bound fragment in the first intron of another ASD candidate gene, CNTNAP2 (see Section 2.1). Further experiments confirmed that FOXP2 downregulates CNTNAP2 expression, strengthening the link between FOXP2 function and ASD [60,146].

4.3. GABRB3

The Angelman susceptibility region of chromosome 15q11 (see section 2.8) also contains the genes coding the $GABA_A$ receptor subunits $\alpha 5$, $\beta 3$ and $\gamma 3$ (GABRA5, GABRB3 and GABRG3, respectively); SNPs in these genes have been associated to ASD [73, 135]. *Gabrb3*$^{-/-}$ mice display high mortality rate, learning and memory deficits, poor motor skills, seizure suscetibility, tremors, hyperactivity and cerebellar hypoplasia [42,47,75]. *Gabrb3*$^{-/-}$ mice also show a series of ASD-like traits, including reduced sociability and reduced social novelty testing. These mice also display deficits across many attentional domains, including selective and sustained attention with the inability to shift and orient attention rapidly and accurately among spatial targets and between sensory modalities, as compared to WT mice [47]. These observations provide support for the face validity of the *Gabrb3*$^{-/-}$ mouse as a model of ASD.

4.4. NLGN1, 3 and 4

There are five known isoforms of the neurexin ligands neuroligins (NLGNs; see also section 3.2) in humans: NLGN1, NLGN2, NLGN3, NLGN4X and NLGN4Y [137]. Genetic association to ASD has been found for three of them: copy number variations in the NLGN1 gene and rare mutations in the NLGN3-4 genes [82]. In rodents, only four NLGNs exist (NLGN1–4), showing different synaptic localization: NLGN1 is localized exclusively to excitatory synapses, NLGN2 and 4 to inhibitory synapses NLGN3 to both inhibitory and excitatory synapses [19,76,95]. The effects of the targeted deletion of all NLGNs has been extensively studied in mice. With the exception of *Nlgn2*$^{-/-}$ mice, all *Nlgn* mutant mice show some ASD-like traits. *Nlgn1*$^{-/-}$ mice display repetitive behaviors, deficits in hippocampus-dependent learning and memory and decreased hippocampal LTP [35]. Recent studies also demonstrate that loss of *Nlgn1* leads to a small but significant reduction in NRXNs levels in the brain, decreased GABAergic/glycinergic activity and reduced glutamatergic activity [16]. Mice bear-

ing the R451C mutation found in the human NLGN3 gene (*Nlgn3*^{R451C} knock-in mice) show an increased inhibitory synaptic transmission without a change in excitatory transmission followed by a de?cit in social interaction [141]. Mice with a targeted deletion of *Nlgn3* (*Nlgn3*$^{-/-}$ mice) show a partial loss of PV-positive basket cells in the cerebral cortex [67]. Finally, *Nlgn4*$^{-/-}$ mice display abnormalities in reciprocal social interaction but they do not display repetitive behavior or impairments in other ASD-like symptoms such as sensory sensitivity, locomotion, exploratory activity, anxiety, or learning and memory [83].

4.5. OXT and OXTR

Oxytocin (OXT) is a nine-amino-acid peptide synthesized in the hypothalamus and released into the bloodstream through axon terminals in the neurohypophysis. Oxytocin stimulates uterine contraction during labor and milk ejection during nursing, and is involved in the central mediation of attachment behavior [77]. OXT effects are mediated by the OXT receptor (OXTR), a seven transmembrane rhodopsin-type G-protein coupled receptor. OXTRs are found in uterus and mammary tissue, but also in several regions of the brain including the hypothalamus, hippocampus, limbic and autonomic areas [65,78]. Reduced OXT plasma levels are observed in autistic children, and OXTR mRNA is decreased in postmortem samples of temporal cortex from ASD patients [78]. Conversely, intranasal infusion of OXT reduces stereotypic behavior and improves eye contact and social memory in autistic patients [4]. Genetic variations in OXTR have been associated with autism [24,54,80,149]. Mice with targeted deletion of the oxytocin (*Oxt*) or oxytocin receptor (*Oxtr*) gene have been generated, and their behaviour analyzed with respect to the presence of ASD-like traits. *Oxt*$^{-/-}$ mice have selective deficits in social memory. *Oxt*$^{-/-}$ pups emit fewer ultrasonic vocalizations upon maternal separation and *Oxt*$^{-/-}$ adults are more aggressive than WT mice. Furthermore, *Oxt*$^{-/-}$ mice fail to recognize familiar conspecifics after repeated social encounters, despite olfactory and non-social memory functions appear to be intact [148]. *Oxtr*$^{-/-}$ mice show severe impairments in social recognition, reduced ultrasonic vocalization of pups upon maternal separation and increased aggression [142]. In addition, *Oxtr*$^{-/-}$ mice have reduced cognitive flexibility and increased seizure susceptibility [124]. Interestingly, all these deficits can be rescued by pharmacological administration of oxytocin [124].

4.6. RELN

The RELN gene codes for the extracellular matrix glycoprotein Reelin, which is involved in neuronal migration and lamination of the cerebral cortex during embryogenesis [62]. RELN maps to 7q22 human chromosome, a region linked to ASD [131]. Mice lacking Reelin (*reeler* mice) show a dramatic impairment of migration of cortical projection neurons, that results in a highly disorganized and dyslaminated cerebral cortex [44,45] and epileptiform activity [115]. *Reeler* mice also show deficits of the GABAergic system, namely decreased number of GAD67-positive cortical neurons [98] decreased GABA turnover [25] and improper layer positioning of GABAergic interneurons in the mature cerebral cortex [71,150]. Recently, ASD-like behaviors and loss of PV interneurons has also been reported in *reeler* mice [101].

5. Exploiting animal models for finding a cure

While knowledge grows about the pathophysiological mechanisms underlying the susceptibility to ASD, mouse models offer powerful translational systems for discovering therapeutic treatments. Early behavioral interventions are currently the only treatments that significantly improve core autistic symptoms (social interactions and communication deficits) [46,121]. Current medications only affect secondary symptoms such as hyperactivity or mood, but have not shown any effect on the core features of ASD. Preclinical studies have been reported for genetic and pharmacological rescue of "autistic-like" phenotypes in mouse models of ASD. Here we will summarize the most significant results in preclinical drug testing using ASD mouse models. As summarized in Table 2, successful drug candidates include mGluR5 modulators, growth factors, rapamycin, statins and oxytocin.

5.1. mGlu5 receptor modulators

A study of synaptic plasticity in the hippocampus and visual cortex of *Fmr1* KO mice suggested a novel connection between mGluR signaling and the FXS phenotype (the so-called "mGluR theory" of FXS pathogenesis; see also Section 2.2). In particular, the absence of FMRP was shown to cause an upregulation of mGluR5-mediated signaling. These findings indicated that many aspects of the phenotype of *Fmr1* KO mice, including behavioural abnormalities, cog-

Table 2
Pharmacological treatments tested in mouse models of autism spectrum disorders

Drug	Mouse models	Effect	References
mGlur5 antagonists	*Fmr1* KO mice	Reverted multiple phenotypes (including ASD-like behaviours)	48,140,144, 151
mGlur5 agonists	*Tsc2*$^{+/-}$ mice	Rescue of decreased hippocampal LTD and ASD-like behaviours	6
BDNF	*Fmr1* KO mice	Reducing BDNF expression ameliorates locomotor hyperactivity and deficits in learning and auditory responses	31,113,135
Rapamycin	Neural-specific *Pten* conditional KO mice	Reverted anatomical defects, reduced seizure frequency/ duration, improved social interaction	156
	Mice lacking *Tsc1* in neurons	Improved survival rate and rescued neuronal morphology	106
	Mice lacking *Tsc1* in glial cells	Prevented EEG abnormalities and seizures	155
	Tsc2$^{+/-}$ mice	Reverted synaptic plasticity and learning/memory deficits in adult mice (short-term treatment)	56
Statins	*Nf1*$^{+/-}$ mutant mice	Reverted learning/memory deficits	39,97
Oxytocin	*Oxt*$^{-/-}$ mice	Rescued social recognition deficits	58
	Oxtr$^{-/-}$ mice	Rescued ASD-like traits and cognitive deficits	124
Risperidone	*Cntnap2*$^{-/-}$ mice	Reverted ASD-core symptoms	118

The table reports novel pharmacological treatments (except risperidone, which is the only FDA-approved drug for ASD) tested in ASD mutant mouse models. Abbreviations are as in the text.

nitive deficits and dendritic spine changes, could be attributable to excessive mGluR5 signaling and could therefore be rescued through genetic downregulation of mGluR5 expression [50]. Indeed, several studies showed that a reduced mGluR5 signaling reversed some of the symptoms in Fmr1 KO mice [50,51]. Pharmacological treatment with mGluR5 negative modulators (MPEP, fenobam, AFQ056, and STX107) was effective in reversing multiple phenotypes in Fmr1 KO mice mouse models [48,140,144,151]. These results provided a rationale for developing mGluR5 antagonists as therapeutics. Fenobam was the first negative mGluR5 modulator tested in patients with FXS [14,93] and a phase II double-blind placebo-controlled trial of AFQ056 has been recently conducted in Europe with promising results [81]. More recently, a study performed in *Tsc2*$^{+/-}$ and *Fmr1* KO mice highlighted a more complex role of mGluR5 signaling in the pathogenesis of syndromic ASD. *Tsc2* and *Fmr1* mutations result in opposite hippocampal synaptic dysfunction in mice: increased long-term depression (LTD) in *Frm1* KO mice *vs.* decreased LTD in *Tsc2*$^{+/-}$ mice. Interestingly, treatments that modulate mGluR5 signaling in opposite directions are able to correct synaptic defects in these mutants [6], thus raising the possibility to use mGluR5 agonists (positive allosteric modulators) to revert ASD symptoms in TSC.

5.2. Brain-derived neurotrofic factor (*BDNF*)

Several studies suggest a correlation between BDNF signaling and the pathogenesis of FXS. Altered BDNF expression and signaling has been detected in the *Fmr1* KO mouse brain [99]. More importantly, locomotor hyperactivity and deficits in learning and auditory responses were ameliorated by reducing BDNF expression in *Fmr1* KO mice [31,113,135]. These results suggest that behavioural abnormalities syndromic ASD could be due to increased BDNF signalling and could theoretically be counteracted by reducing extracellular BDNF levels. Further investigation in models different than *Fmr1* KO mice is needed to strengthen this hypothesis.

5.3. Rapamycin

The mTOR inhibitor rapamycin has been proven to partially revert ASD-ike symptoms in mice with targeted deletion of *Tsc1/2* or the TSC positive regulator PTEN. In neural-specific *Pten* conditional KO mice, rapamycin was shown to revert anatomical defects, reduce seizure frequency and duration, and improve social interaction [156]. Postnatal rapamycin treatment was also effective in improving the survival rate and rescuing neuronal morphology in mice lacking *Tsc1* in neurons [107], and preventing seizures and EEG abnormalities in mice lacking *Tsc1* in glial cells [155]. Finally, a short-term (5 days) rapamycin treatment has been shown to revert synaptic plasticity and learning and memory deficits in adult *Tsc2*$^{+/-}$ mice [56]. These studies indicate that pharmacologically interfering with the PTEN-TSC-mTOR pathway via rapamycin admin-

istration partially ameliorates ASD-like freatures in mice. However, it remains to be clarified whether these achievements in mouse models may translate to therapy in human syndromic ASD patients. In particular, it must be considered that long-term rapamycin treatment is extremely problematic in humans due to its immunosuppressive effects. Novel drugs alternative to rapamycin should be therefore developed.

5.4. Statins

Studies performed in *Nf1* mutant mice indicate that memory and attention deficits result from Ras pathway activation following *Nf1* deficiency (see Section 2.4). For these reasons, much emphasis has been given to the potential pharmacological effects derived from inhibition of Ras-mediated signaling in NF1 patients with cognition deficits. In fact, a number of studies in recent years have shown that lovastatin (a drug belonging to the statin class which inhibits GTPases including Ras), can reverse the physiological and behavioral phenotypes of $Nf1^{+/-}$ mutant mice [39,97]. Statins are a class of relatively safe drugs used for the treatment of hypercholesterolemia. Another statin (simvastatin) has been shown to improve learning and memory deficits in a transgenic mouse model of Alzheimer's disease [96]. According to these results, preliminary findings in human patients suggest that lovastatin may be able to reverse some of the cognitive deficits in children with NF1 [1]. However, it is important to point out that further study and replication are required to confirm the potential effect of statins for the treatment of cognitive dysfunctions in humans.

5.5. Oxytocin

Oxytocin administration has been proven to rescue social recognition deficits in $Oxt^{-/-}$ mice [58] as well as cognitive deficits in $Oxtr^{-/-}$ mice [124].

6. Conclusions and future perspectives

In this review, we described the most relevant features of mice with targeted inactivation of ASD-associated genes (summarized in Table 1). Several considerations can be drawn from the comparative analyses of these models.

i) Different models show different type of ASD-like symptoms. This is due to genetic heterogeneity of the disease, and the difficulty of reproducing complex human disorders in mice. However, the use of each of these models is a powerful tool to unravel the contribution of each single gene to ASD.
ii) A careful, standardized phenotypic analysis of the different ASD mouse models is needed, with specific attention to standardized behavioral phenotyping of ASD "core symptoms" (social interactions, communication deficits and repetitive behaviors) [130]. A standardized approach would be of extreme importance for novel drug testing.
iii) The embryonic phenotype as well as the differences among young and adult animals should be carefully evaluated in all the available models, in order to carefully understand the neurodevelopmental basis of ASD. So far, these types of studies have been performed only in a limited number models.
iv) A careful investigation of the CNS transcriptome/proteome should be performed in the different models. These analyses should allow a better characterization of the molecular consequences of ASD gene dysfunction.
v) Cellular, anatomical and functional defects of the GABAergic interneurons are detected in many of the reported strains, indicating a possible role of these neurons in the pathogenesis of ASD.
vi) As a possible consequence of GABAergic dysfunction, seizures often occur in most of these models, reflecting the co-morbidity (and a possible common cause) of ASD and epilepsy.

Most importantly, a number of preclinical studies on ASD mouse models contributed to unravel novel drug candidates for the cure of ASD, including mGluR5 modulators, rapamycin, BDNF, statins and oxytocin (Table 2), thus confirming the relevance of this approach in translational ASD research.

Acknowledgements

P.S. is a postdoctoral fellow supported by Provincia Autonoma di Trento (Italy) under the Marie Curie-People cofunding action of the European Community. This work was funded by the Italian Ministry of University and Research (PRIN 2008 grant # 2008 94SYW2_002) and the University of Trento (CIBIO start-up) grants to Y.B.

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