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REVIEW ARTICLE

Neurexins in autism and schizophrenia—a review of patient mutations, mouse models and potential future directions

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Abstract

Mutations in the family of neurexins (*NRXN1*, *NRXN2* and *NRXN3*) have been repeatedly identified in patients with autism spectrum disorder (ASD) and schizophrenia (SCZ). However, it remains unclear how these DNA variants affect neurexin functions and thereby predispose to these neurodevelopmental disorders. Understanding both the wild-type and pathologic roles of these genes in the brain could help unveil biological mechanisms underlying mental disorders. In this regard, numerous studies have focused on generating relevant loss-of-function (LOF) mammalian models. Although this has increased our knowledge about their normal functions, the potential pathologic role(s) of these human variants remains elusive. Indeed, after reviewing the literature, it seems apparent that a traditional LOF-genetic approach based on complete LOF might not be sufficient to unveil the role of these human mutations. First, these genes present a very complex transcriptome and total-LOF of all isoforms may not be the cause of toxicity in patients, particularly given evidence that causative variants act through haploinsufficiency. Moreover, human DNA variants may not all lead to LOF but potentially to intricate transcriptome changes that could also include the generation of aberrant isoforms acting as a gain-of-function (GOF). Furthermore, their transcriptomic complexity most likely renders them prone to genetic compensation when one tries to manipulate them using traditional site-directed mutagenesis approaches, and this could act differently from model to model leading to heterogeneous and conflicting phenotypes. This review compiles the relevant literature on variants identified in human studies and on the mouse models currently deployed, and offers suggestions for future research.

Introduction

Disorders affecting brain development and behaviour (Neurodevelopmental Disorders) are highly heritable in nature [1, 2] and often present co-morbid phenotypes [3]. Two such neurodevelopmental disorders are autism spectrum disorder (ASD) and schizophrenia (SCZ), the first being diagnosed mostly in early childhood [4] and the latter detected usually in late adolescence/early adulthood, although it may also occur, rarely, in middle childhood [5, 6]. The last decades have witnessed considerable global efforts to identify genetic risk factors [7, 8] using state-of-the-art genotyping and sequencing techniques [9]. This has led to the identification of numerous, significantly associated single nucleotide polymorphisms (SNPs) [10] and fewer rare but highly penetrant variants (copy number variants (CNVs)) [11]. Interestingly, these studies have also highlighted genes that appear to predispose to both disorders. One prominent gene family that is identified in ASD and in SCZ, is the neurexin (*NRXN*) family of synaptic organisers. Because *NRXNs* are associated with ASD, SCZ and other neurodevelopmental diseases, such as intellectual disabilty [12, 13], it is important to study their function to confirm their potential role in mental disorders and obtain new insights into the biological mechanisms behind the development, progress and severity of mental disorders.

In this review, we have compiled the mutations identified to date in the family of *NRXN* genes in human patients with ASD and SCZ, listed all published mouse models together

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with details of their genotype and phenotype, and discussed the potential of future cellular and animal models to unveil the pathogenicity of *NRXNs* in mental disorders.

Neurexin family

NRXNs are conserved presynaptic transmembrane proteins predominantly functional at the cell surface of neurons and fundamental for synaptic transmission [14, 15]. In mammals, there are three *NRXN* genes (*NRXN1, NRXN2* and *NRXN3*) [16]. Each gene encodes for two major protein isoforms, a longer *α-NRXN* and a shorter *β-NRXN* with a common C terminus [17]. Both these isoforms are transcribed from distinct promoters. The promoter for the larger *α-NRXN* isoform located upstream of exon 1 generates a protein containing: (i) a signal peptide at the N-terminal; (ii) six laminin/neurexin/sex-hormone binding globulin (LNS) domains; (iii) three epidermal growth factor (EGF) like regions in the middle; (iv) a carbohydrate attachment region; (v) a transmembrane domain and (vi) a short cytoplasmic region (Fig. 1). The promoter for the shorter *β NRXNs* located downstream of exon 18 for *NRXN1* and exon 17 for *NRXN2* and *NRXN3* generates a protein that contains the same sequence as the *α* isoform beginning at the sixth LNS domain and with no EGF-like repeats [18]. Extensive alternative splicing, a hallmark of these genes at the six alternative splicing sites (SS1–6 in *NRXN1/3* and SS1–5 in *NRXN2*) in the *α* isoform, two of which are shared by the *β* isoform, gives rise to thousands of isoforms [15, 18, 19] (Fig. 1). These isoforms make this a very complicated and complex gene to study as depicted by the phenotypic diversity observed in both patients and mouse models presented below.

Neurexin mutations in autism spectrum disorder

The original discovery of two rare missense mutations in exon 1 of *NRXN1β* (p.S14L and p.T40S) after screening all three *β* isoforms of 131 Caucasian and 61 African-American patients with ASD and their absence in more than 500 controls provided a strong link between *NRXNs* and ASD [20]. In a landmark study, the Autism Genome Project Consortium examined 1181 families, each with at least two family members with ASD, identified a ~300 Kb hemizygous deletion in the coding region (2p16.3: 50,430–50,785) of the *α* isoform of *NRXN1* in two siblings [21]. Following this important result, Kim et al. [22] identified a disruption in intron 5 of the *NRXN1α* isoform in one subject with ASD and a translocation ~750 Kb upstream of the 5′ region of *NRXNα* in a second unrelated subject [22]. Guilmatre et al. [23] identified two deletions in unrelated ASD patients in a cohort of 260 ASD patients and 236 controls. Both these deletions were in the first two exons of *NRXN1α*. Zahir et al. reported a patient with developmental delay, autistic features and an unusual facial appearance who carried a ~320 Kb heterozygous mutation in the promoter region and first five exons of the *NRXN1α* gene. With the *β* isoform still intact, the author reported that a small reduction in the *NRXN1α* protein is enough to generate an ASD phenotype and suggested a possible gene-dose effect [24]. Consistent with this evidence, Duong et al. [25] postulated a bi-allelic loss, identifying a 451 Kb deletion of the first five exons affecting the promoter inherited from a mother with sub-diagnostic autistic traits along with type 1 diabetes, and a point mutation in *NRXN1* inherited from the father with SCZ. This patient with the inherited compound heterozygous mutation suffered from autism, mental

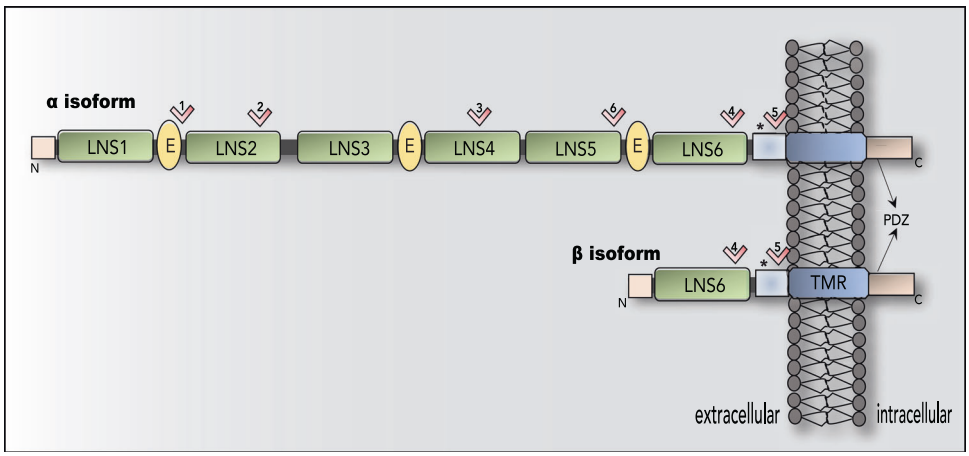

Fig. 1 Domain organisation of *α*-neurexins and *β*-neurexins. Schematics show six LNS (laminin/neurexin/sex hormones binding protein domain) and three EGF (epidermal growth factor) domains in the *α* isoform and one LNS domain in the *β* isoform. The red arrows mark the six alternative splice sites of *NRXN1* and *NRXN3* (only five in *NRXN2*) with two common in the *β* isoform, along with a highly glycosylated region(*), transmembrane region (TMR) and PDZ domain binding motif at the C terminal end.

retardation and epilepsy. The variable phenotype and family history in the proband exemplifies a gene-dose effect together with pleiotropy of this gene. These studies provide evidence that both pathogenic CNVs and single point mutations disrupting *NRXN1* have a deleterious effect.

Most of these studies have repeatedly identified *NRXN1* deletions with a lesser focus on *NRXN2* and *NRXN3* genes. However, a comprehensive study by Gauthier and colleagues [26] (cases: 379; controls 285) reported a novel truncating mutation in exon 12 of *NRXN2α* (no effect on *β*) in an autistic patient. Mutant *NRXN2α* is partially made but non-functional at the cell surface. Modelling this mutation in transfected COS cells revealed a failure of this protein (lacking the LNS 6, transmembrane and cytoplasmic domains) to anchor at the plasma membrane and induce synapse formation. Mohrmann and colleagues [27] observed a 0.57 Mb de novo deletion in the chromosomal region spanning the entire *NRXN2* gene to be associated with the patient's autistic traits and neurodevelopmental delay. In addition, Boyle et al. 2015, identified a de novo 1.6 Mb deletion at the *NRXN2* locus in a patient with autistic behaviour and severe intellectual disability [28]; both authors hypothesised that these *NRXN2* mutations could be responsible for those traits. A Canadian study [29] involving mutations in one or both isoforms of *NRXN3* (three of four cases had deletions in both isoforms) suggested the possible role of this *NRXN* in ASD. This study of four unrelated pedigrees provided inconclusive evidence for the mutation being linked to ASD as some of these mutations were also identified in unaffected siblings suggesting reduced penetrance. By contrast, a recent study by Yuan et al. [30] provided the first co-segregation evidence from a three generation Chinese family with an autistic boy carrying a 222 Kb deletion in exons 6–12 of the *NRXN3α*. Thus, there is inconsistent evidence for the potential pathogenic role of *NRXN2* and *NRXN3* genes in ASD.

Neurexin mutations in schizophrenia

The first study describing a role for *NRXN1* in SCZ came from Kirov and colleagues [31], where they screened the genome of 93 SCZ cases for CNVs and identified a 250 Kb deletion in the 5′ end of *NRXN1α* in an affected sibling pair and no deletion in 372 controls. A subsequent report identified a 115 Kb deletion at the 3′ end disrupting *NRXN1α* and *β* in an affected twin pair (2/233 cases; 0/268 controls) [32]. In a cohort of 54 SCZ patients, one individual was observed to carry a 389 Kb deletion in exon 1 of *NRXN1α* and, in a further cohort of 752 patients, four were observed to carry other deletions in exons and introns of *NRXN1α* [33]. Guilmatre et al. [23] screened 236 SCZ patients and 236 controls and found two deletions, one affecting exon 1–2 of *NRXN1α* and the second affecting both *NRXN1α* and *β*. On a much larger scale, Rujescu et al. [34] examined CNVs in 2977 European ancestry SCZ cases and 33,746 controls. They reported an excess of CNVs in *NRXN1* in SCZ (cases: 0.47% controls: 0.15%) and focused on exon-disrupting deletions or duplications concluding that the identified CNVs must impact disease predisposition. In total, they identified 12 deletions and 2 duplications present in the *NRXN1* gene or in its promoter region; this study did not identify any novel CNVs in *NRXN2* or *NRXN3*. In 2011, Gauthier et al. identified a de novo heterozygous frameshift mutation in exon 22 of *NRXN1*, affecting the *α* and *β* isoform in a female with disorganised SCZ. The 4- nucleotide insertion led to a premature stop codon, with a truncated protein lacking the transmembrane region and cytoplasmic tail [26]. Immunofluorescence assays and western blot analysis on neuronal cell cultures confirmed intracellular accumulation of a partial non-functional *NRXN1* protein resulting in a failure to form synapses similar to a *NRXN2α* study described earlier by the same authors. A landmark 2017 study by Marshall and colleagues [11] reported an analysis of the largest-to-date SCZ cohort (21,094 cases; 20,227 controls). A gene-based association test identified genome-wide significant association signals at 8 loci. *NRXN1* attracted the third highest level of statistical support, was the only single-gene locus identified and was observed to have one of the highest odds ratios (OR: 14.4; cases: 35; controls: 3) of all reported loci.

This odds ratio is consistent with the odds ratio (OR 9.74: cases 67; controls 15) of a genome-wide CNV analysis of *NRXN1* deletions [35]. This analysis represented samples from the United States [36–38], Japan [39], China [40] and many European countries [32, 41–52] including patients with childhood onset SCZ [53]. This reiterates the strong association of *NRXN1* deletions and increased risk in patients developing SCZ.

To date, there have been no studies associating *NRXN2* with SCZ. The first study to report an association of *NRXN3* with SCZ was in a Chinese Han population (1214 SCZ cases; 1517 controls). Of the seven genotyped SNPs, the three (rs7157669, rs724373 and rs7154021) observed to be associated with SCZ were located in intron 1 and 2 of the gene [54].

Neurexin KO/KI mouse models

The first *Nrxn* loss of function mouse models emerged in 2003, when Missler et al. [55] generated single, double and triple *Nrxn1α/2α/3α* KO mice by deleting the first exons of *α-Nrxns* to determine the importance of *αNrxn* LOF. Mice generated via this strategy were validated by immunoblotting for the full length *α-Nrxn* protein with an antibody that recognises the C-termini of all *Nrxn* proteins showing varying levels of the *α* protein in single and double KOs and

complete absence in the triple KOs. Variable survival rates and viability of different combinations of double and triple KO *α-Nrxns* compromised postnatal existence highlighting the fact that a minimum of two intact *α-Nrxns* is essential for survival. Mice with triple *α-Nrxn* KOs died due to difficulty in breathing. Intriguingly, these mice had normal brain morphology when born suggesting that *α-Nrxn* deficiency had no effect on neuronal development. A closer look at their synaptic function showed impairments in both excitatory and inhibitory neurotransmission in the brainstem, partly due to the loss of presynaptic Ca^{2+} influx [55, 56]. Behavioural analysis of *Nrxn1α* homozygous KO mice on a hybrid SV129/C57BL/6 background by Etherton and colleagues [57] found dramatic changes in motor learning abilities and an increase in repetitive grooming, both classic autistic behaviour domains [58], reduction in pre-pulse inhibition common amongst SCZ patients [59] and an impairment in nest building activities compared to their littermate controls. Surprisingly, a number of tasks involving social behaviours were normal, and because social interaction impairment is a core ASD symptom the authors declared a limited usage for this model. By contrast, Grayton et al. [60] investigated the behavioural effects of *Nrxn1α* KO on mice with a different background (pure C57BL/6J strain) and observed that these mice exhibited reduced locomotor activity with high levels of anxiety and aggression towards adult conspecifics with no loss of spatial or working memory. Several other studies reported a range of behavioural phenotypes using a combination of homozygous and heterozygous lines outlined in Table 1 [56, 61–66].

In mice, regardless of the continued expression of *β* isoforms, double and triple KO of *α-Nrxns* invariably lead to fatality suggesting that *β-Nrxns* do not partially compensate for the loss of *α-Nrxns* and either have unique functions dissimilar to *α-Nrxns* or are functionally redundant. To respond to these questions, Anderson et al. generated conditional (cKO) and constitutive KO mice of all three *β-Nrxn* genes and reported for the first time an association of *β-Nrxns* and endocannabinoid signalling [67]. Experiments described a new role of *β-Nrxn* in controlling tonic postsynaptic endocannabinoid signalling mediated by 2-arachidonoylglycerol, an endogenous agonist of CB_1 receptor, suggesting that *β-Nrxns* performed unique non-redundant functions. An approximate twofold decrease in excitatory synaptic transmission measured by electrophysiology experiments was surprising, given the low expression of *β-Nrxns* throughout the brain depicted by qRT-PCR analysis. The cKO mice were viable and fertile, however, inducing *β-Nrxn* KO led to infertility and significantly smaller mice. Loss of protein validation was performed by immunoblotting for tagged *β- Nrxn* specific sequences using anti-*Nrxn* antibody (see Table 1 for details).

Inclusion ($^+$) and exclusion ($^-$) of a highly conserved 90 bp exon at alternative splice site 4 (SS4) determines *NRXN* binding preference for its postsynaptic partners; for example, $SS4^+$ only binds to cerebellins [68, 69] and $SS4^-$ binds to dystroglycans [70], CIRL/latrophilins [71] and LRRTMs [72, 73]. The significance of this 4th canonical splice site was revealed after qPCR analysis of all *Nrxns* revealed a differential pattern of SS4 splicing in different regions of the mouse brain. For example, the striatum, cerebellar cortex, olfactory bulb, brainstem and spinal cord expressed $SS4^+$ forms of all three *Nrxns* whereas other regions such as the cortex and hippocampus expressed both $SS4^+$ and $SS4^-$ forms of *Nrxn1* and *Nrxn2* while the $SS4^-$ form of *Nrxn3* was predominant [74]. A genetic knock-in (KI) approach was then utilised to test the biological significance of *Nrxn* alternative splicing at SS4. Mice with a conditional and constitutively included $SS4^+$ exon (no longer subject to alternative splicing) in *Nrxn3* were generated. To validate this model mouse neuronal cultures from newborn hippocampi (WT and KI) were transfected with lentivirus (expressing active and inactive Cre). These produced sets of WT, $SS4^+$ and $SS4^-$ neurons validated by their respective mRNA levels. Hence, the authors proposed to have generated mutants in which neurons would produce $SS4^-$ variants upon Cre-directed recombination. In a later study [75], cKI mice for *Nrxn1* and *Nrxn2* similar to *Nrxn3* were made with a constitutively included SS4 exon that is no longer alternatively spliced but can be excised by Cre recombinase and converted to $SS4^-$ variants. Using these three lines simultaneously, the data summarised in Table 1 revealed that all three *Nrxn* $SS4^+$ variants had different effects on NMDAR and AMPAR responses.

Aoto et al. [76] used homologous recombination to flank exon 18 shared between *Nrxn3α* and *β* with loxP sites to create cKO mice observing their phenotype in two separate brain regions (hippocampus and olfactory bulb). Details of model validation can be found in Table 1. Constitutively active *Nrxn3α/β* homozygous KO mice died at birth and those that survived displayed a more severe phenotype than the other *Nrxn3* mice models [55, 74] for which the authors seek to investigate the consequences of *Nrxn3α/β* ablation on synaptic activity. By first examining cultured hippocampal neurons of *Nrxn3α/β* homozygous KO mice, results reported were an impairment in AMPAR mediated transmission but not in NMDAR mediated excitatory or GABAergic inhibitory responses. In the olfactory bulb, *Nrxn3α/β* deficiency revealed that *Nrxn3* is required for presynaptic GABA release [76].

A triple *Nrxn1α/β, Nrxn2α/β* and *Nrxn3α/β (Nrxn123)* cKO mouse [77] was generated to describe broader functions of *NRXNs* by investigating synaptic activity in pre- and postsynaptic neurons in absence of *Nrxns*. Using a combination of qRT-PCR and western blot techniques, the

Table 1 Summary of current *Nrxn* KO/KI mouse models outlining details of their genotype such as mutation targets sites, validation and viability along with phenotyping on the basis of synaptic markers, electrophysiology and behaviour.

References	Target neurexin	Generation of Model/background	Viability of mice	Model validation	Synaptic/electrophysiological activity	Behaviour
1. Missler et al. [55]	*Nrxn1α/ Nrxn2α/Nrxn3α*	Generating homozygous KO mice by deletion of first exon of α isoforms on a mixed SV129/C57BL/6 background.	Single α neurexin deficient mice were viable. Double αKO mice only survived up to a week. Triple αKO mice displayed breathing abnormalities and died on the first day.	Loss of protein validation by immunoblotting using an antibody that binds to the C-termini of both isoforms of *Nrxn1,2* and *3*.	Impaired spontaneous and evoked neurotransmitter release. Loss of presynaptic Ca2+ influx. ↓ Number of GABAergic terminals at synapse.	Behaviour analysed in models below
2. Zhang et al. [56]					Selective alteration of N and P/Q type Ca2+ channels. No change in L-type Ca2+ channels.	
3. Sons et al. [61]					Impaired neurotransmitter release at mouse neuromuscular junction.	
4. Dudanova et al. [62]					Mild reduction of neuropil in olfactory bulb. No change in synapse formation or axon guidance in olfactory bulb projections.	
5. Etherton et al. [57]	*Nrxn1α*	Homozygous KO in hybrid SV129/ C57BL/6 background.			↓ Excitatory neurotransmission. No change in synapse number/density.	↑ Motor learning, grooming ↓ Pre-pulse inhibition (PPI) Normal social behaviours
6. Grayton et al. [60]	*Nrxn1α*	Homozygous KO in pure C57BL/6 background.				↓ Locomotor activity ↑Anxiety, aggression No effect on spatial/working memory
7. Dachtler et al. [63]	*Nrxn2α*	Commercially generated and validated homozygous KO mice created by deleting first exon of *Nrxn2α* in a C57BL/6 background. No effect on β.		Commercially generated.	↓ Munc-18 expression in frontal cortex and hippocampus.	Impaired sociability, social memory. ↑ Anxiety—thigmotaxis in open field, less time in open arm of elevated plus maze, more time in enclosure and less time exploring. Normal PPI and passive avoidance learning
8. Dachtler et al. [64]	*Nrxn1α and Nrxn2α*	Commercially generated and validated heterozygous KO mice.			No change in hippocampal Munc-18 expression.	No significant differences in *Nrxn1α* HETs. Normal PPI *Nrxn2α* HETs—deficits in sociability and social recognition. Female *Nrxn2α* KO mice spent longer time grooming and displayed ↓ sociability/social investigation than males. ↑ Anxiety in both sexes with no change in spatial memory
9. Born et al. [65]	*Nrxn2α*	*Nrxn2α* KO mice generated on a C57BL/6J background		Details found in one.	↓ Spontaneous transmitter release at excitatory synapses in mouse neocortex. No change in inhibitory synapses and synapse density.	
	Nrxn2α/β	*Nrxn2α/β* generated by deleting exon 23 common to 3′UTR of both isoforms	Nrxn2 KO grow to adulthood at 25% less body weight than healthy controls.	Northern blot analysis from mice brain showed no 2*β* mRNA but some 2*α*.	Altered NMDAR functions and glutamatergic release.	
10. Rabaneda et al. [66]	*Nrxn1β*	Generation of inducible mice using the Tet-off system. Transgenic animals were created on a C57BL/6J background under CamKIIa promoter activation of HA-tagged mutant Nrxn1β lacking part of the C-terminus.	Viable and healthy. Administration of doxycycline rescued/reversed mutant phenotypes.	Loss of protein validation using HA antibody.	↓ Frequency of mEPSCs and mIPSCs in cortical layer 5/6 pyramidal neurons.	↑ Self-grooming Impairment in sociability and social learning.
11. Anderson et al. [67]	*Nrxn1β* *Nrxn2β* *Nrxn3β*	Conditional (cKO) and constitutive KO by flanking first exon specific to *β* transcript with loxP sites. Epitope tags inserted into *β* specific sequences (EGFP for 1β and 3β and hemagglutinin for 2β).	*β* neurexin deficient mice were infertile and smaller in size.	Loss of protein validation in cKO by immunoblots of tagged *β*-neurexins using anti-EGFP and anti-neurexin (epitope detecting conserved C termini) antibodies.	↓ Approximately twofold in EPSC, neurotransmitter release, action potential induced Ca^{2+} influx at excitatory synapses. Regulating excitatory synaptic strength through tonic endocannabinoid signalling.	↓ Freezing behaviour specific for phenotyping contextual fear memory in hippocampal CA1 region.

Table 1 (continued)

References	Target neurexin	Generation of Model/background	Viability of mice	Model validation	Synaptic/electrophysiological activity	Behaviour
12. Dai et al. [75]	*Nrxn1* *Nrxn2*	Conditional knock-in (cKI) mice to selectively include/exclude exon flanked with loxP sites producing SS4^{+} and SS4^{-} variants of neurexins.	Viable and fertile.	Model validated by measuring mRNA levels from cultured hippocampal neurons (WT vs cKI) transfected with lentivirus (with inactive and active Cre).	SS4^{+}—↑ NMDAR EPSC, no effect on AMPAR SS4^{+}—no change in NMDAR and AMPAR EPSCs SS4^{+}—↓ AMPAR EPSCs, no change in NMDAR	↓ Contextual memory as measured in hippocampal CA1 region.
13. Aoto et al. [74]	*Nrxn3*					
14. Aoto et al. [76]	*Nrxn3αβ*	Conditional and constitutive KO by flanking exon 18 of *α* and *β* isoforms of *Nrxn3*.	*Nrxn3αβ* deficient mice died mostly at birth.	Model validated by western blots and qRT-PCR performed on mRNA isolated from WT, HET and HOM brains.	Essential for normal AMPARs levels in excitatory synapses in hippocampus and controlling inhibitory neurotransmitter release in olfactory bulb neurons.	Buried food finding assay to test inhibitory synaptic transmission in *Nrxn3αβ*; deficient olfactory bulb neurons reported that mice took more time to find buried food compared to the controls.
15. Chen et al. [77]	*Nrxn1,2,3*	Conditional KO of Nrxn1, 2 and 3 flanking the first common exon shared by *α* and *β* isoforms with loxP sites leading to global KO of neurexins.	Conditional KO of mice	Model validated by qRT-PCR of mRNA from cultured hippocampal neurons in the presence and absence of Cre.	↓ Synapse numbers of PV^{+} interneurons, ↓ Action potential induced Ca2+ influx in SST+ interneurons.	–

Each prefixed number 1-15 refers to a study of mutations mapped in Fig. 4.

model has been validated showing the absence of mRNA and protein levels from triple KO mice in the presence of Cre.

The KO phenotype analysed between two inhibitory interneurons (PV^{+} versus SST^{+}) that target the same post-synaptic neuron showed a decrease in synapse numbers of PV^{+} interneurons without any changes in synaptic transmission; whereas the SST^{+} interneurons maintained normal synapse numbers but induced a massive decrease in action potential induced Ca^{2+} influx. Sparse versus total pan-neuronal deletion in climbing fibres illustrated an important role of *NRXNs* in synapse formation. This result described a role of these genes in Ca^{2+} channel localisation at synaptic release sites similar to a previous study [55].

Discussion

Given the genetic association of variants in *NRXNs* and increased risk for neurodevelopmental disorders we are interested in trying to understand the neuronal function of these genes, and, most importantly, the causative role they could play in the onset, progression and/or severity of mental disorders. First, we have attempted to present a visual and systematic review of the known variants identified in human patients (Figs. 2 and 3) along with the different stable mutations already generated in mouse to date (Fig. 4). We also reviewed the genotype details of those lines as well as their phenotype (Table 1). We conclude that, although much information has been generated regarding the wild-type functions of *NRXNs*, it still remains unclear how the human variants predispose to their associated mental conditions. It is most likely that we will see further relevant information coming from the models presented in Table 1 in the future. At the same time, it may be valuable to interrogate the suitability of the current models to help understand the pathologic role of both the human variants and *NRXNs* in mental disorders.

Firstly, there is a need for further clarification on how human variants impact the complex *NRXN* transcriptome. From this review, we believe this could be a critical feature of *NRXN* toxicity in patients, a result of haploinsufficiency and selective transcriptomic abnormalities/changes which the current mammalian models may not properly recapitulate. Indeed, most of the work conducted in vivo has focused on generating complete-LOF animal models, by either aiming to delete the entire gene function and/or the major isoforms, i.e. *α* and *β* (Fig. 1 and Table 1). This is definitively a genuine approach to study the wild-type function of the *NRXN* gene family but may not be appropriate to study their pathologic role in mental disorders. There is a growing body of evidence that the transcriptomic/splicing dysregulation of these genes is at play in

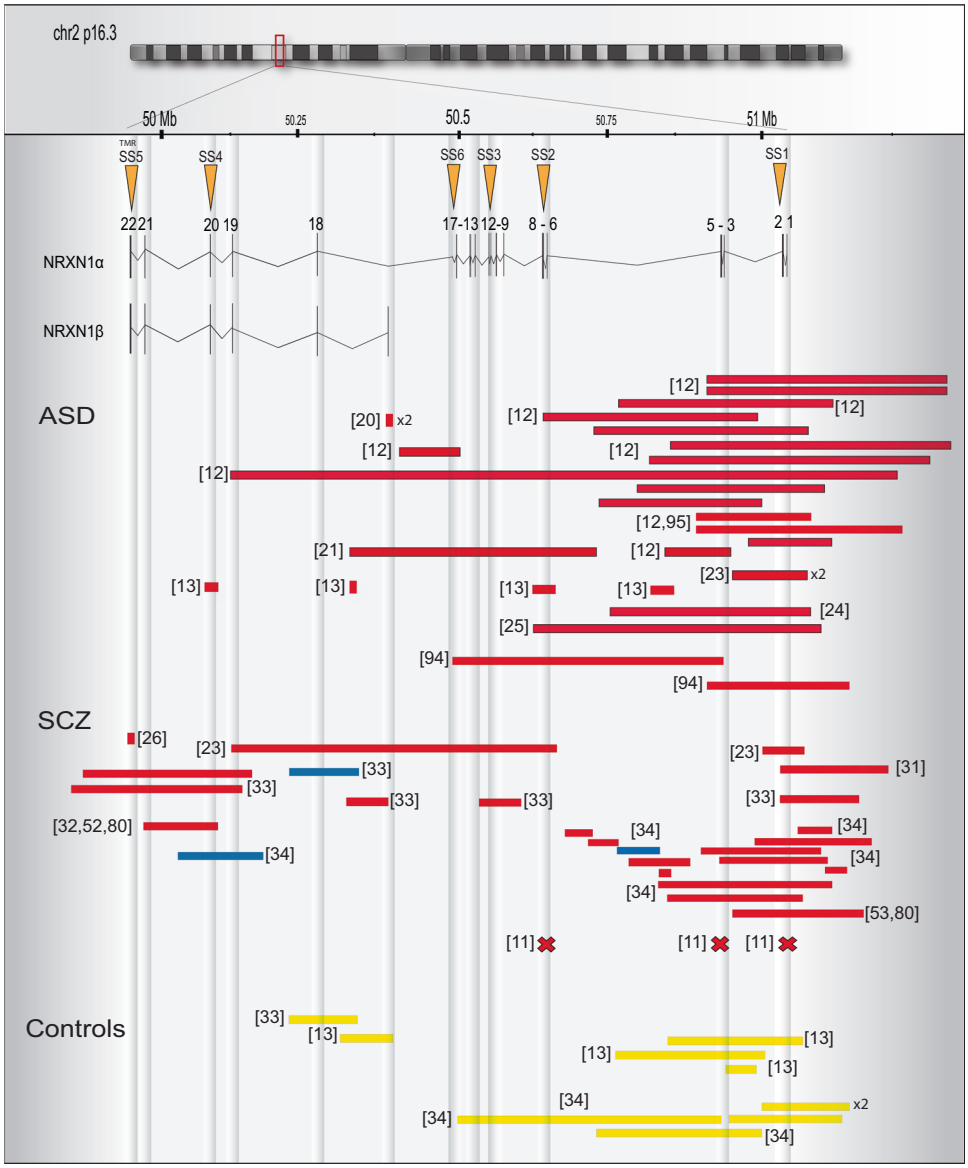

Fig. 2 Map of *NRXN1* mutations. Deletions (red solid lines), duplications (blue solid lines) and exonic deletions in controls (yellow solid lines) identified in human patients of ASD and SCZ. The crosses represent hotspots from [11] that have a breakpoint association value of >3. At the top is an idiogram showing chromosome 2p16.3 (UCSC genome browser, hg38 assembly) [109]. The reference transcripts of *NRXN1α* is NM:004801 and *β* NM:138735 with exons and the six splicing sites (orange arrows) numbered on the top of the gene. TMR transmembrane region, that is encoded by exon 22.

neurodevelopmental disorders [78–80]; not only in patients with rare variants as described here but also in non-carrier patients following environmental stressors, such as drug abuse [81–86]. The recent GWAS conducted on SCZ patients also revealed genes acting directly on *NRXN* transcriptomics, such as *miR-137* which is predicted to directly target *NRXN* isoforms [87, 88]. It is also worth noting that all the variants have been reported to act through haploinsufficiency. Taken together, these lines of evidence point toward complex and selective transcriptomic changes, presumably leading to partial-LOF and potentially coupled to gain-of-function (GOF, by the generation of new aberrant isoforms [80]), rather than complete-LOF as sought in animal models. GOF may be an underestimated contribution to the toxicity of genetic variants in patients. Indeed, although still poorly understood, genetic compensatory mechanisms are now known to be able to trigger transcriptomic alterations, such as exon skipping, use of cryptic splicing sites and/or overexpression of homologues to rescue potential mutations [89–92]. Considering the apparent splicing complexity and dynamics of *NRXNs*, these genes are most likely very prone to such mechanisms. For example, Flaherty et al. demonstrated that some heterozygote *NRXN1α* variants found in patients lead to (i) not only a selective (non-homogeneous) reduction of the wild-type isoforms but also to (ii) the generation of completely novel isoforms [80]. Interestingly, overexpression of these "de novo" isoforms decreased neuronal activity levels in wild-type iPSC-neurons, suggesting a significant toxicity of these mutant-transcripts. Similarly, it is also most likely that changes in one *NRXN* transcriptome may directly impact the expression and isoform diversity of the whole *NRXN* family

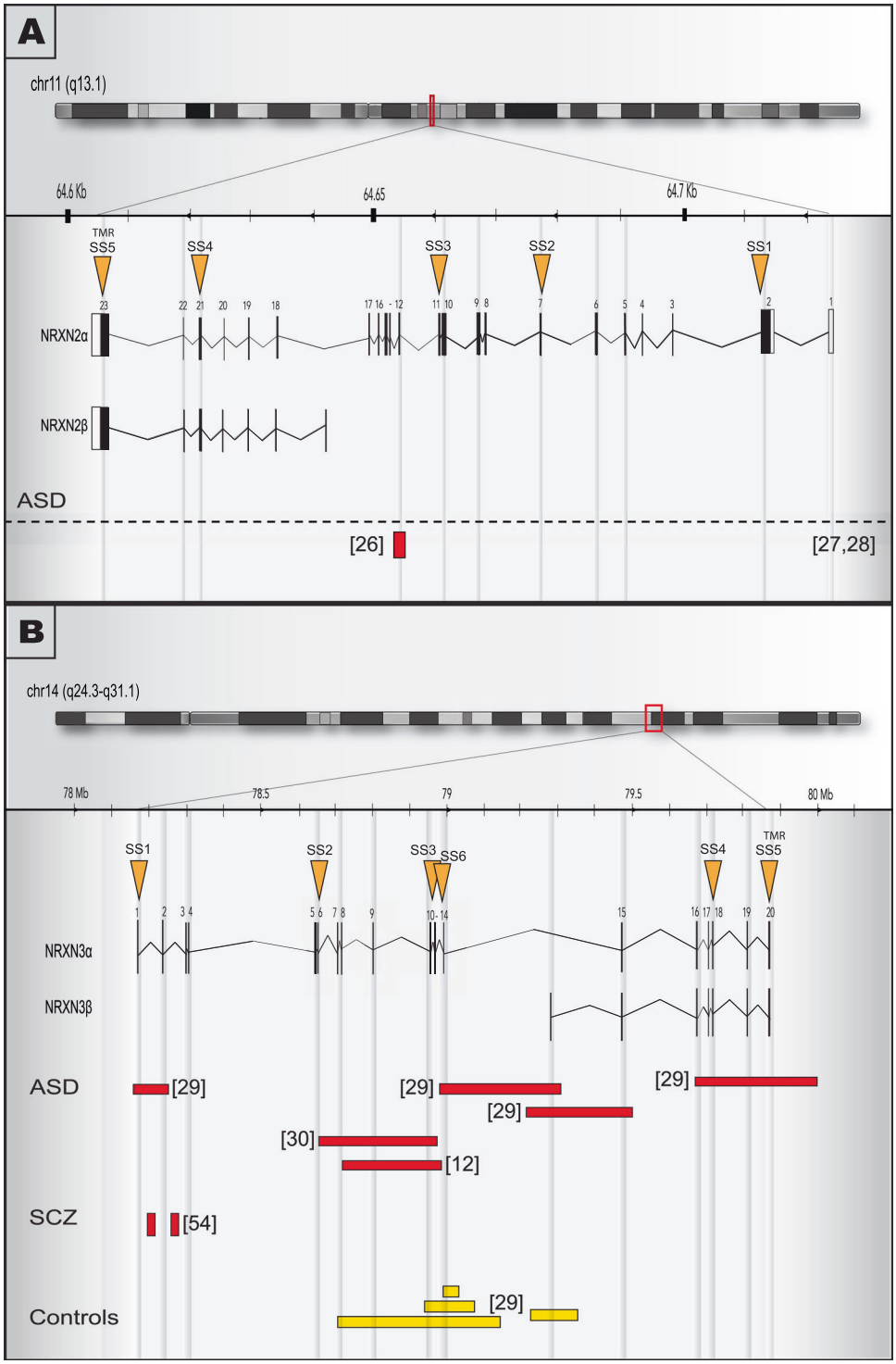

Fig. 3 Map of *NRXN2* and *NRXN3* mutations. a *NRXN2* mutations identified in ASD patients. At the top is an idiogram from the UCSC genome browser [109] showing chromosome 11q13.1 from hg38 assembly. The reference transcripts of *NRXN2α* is NM:015080.4 and *β* is NM:138734.2 with exons and the five splicing sites (orange arrows) numbered on the top of the gene. A single red box represents a mutation in exon 12 and dashed lines represent chromosomal spanning deletions. TMR transmembrane region, that is encoded by exon 23. **b** *NRXN3* mutations identified in ASD patients (red) and controls (yellow); SCZ patient mutations (red squares). At the top is an idiogram from the UCSC genome browser [109] showing chromosome 14q24.3-q31.1 from hg38 assembly. The transcript IDs of *NRXN3α* from Ensembl release 101 [110] is ENST634499.1 and *β* is ENST428277.6 with exons and the six splicing sites (orange arrows) numbered on the top of the gene. TMR transmembrane region, that is encoded by exon 20. Non-coding sequences (empty boxes), coding sequences (filled boxes).

through similar genetic compensatory mechanisms; that is, heterozygote variants of *NRXN1* may impact *NRXN2* and *NRXN3* transcriptome patterns as well [90, 93]. For example, some *NRXN1*-variants may produce non-sense mRNAs that would be subjected to decay with the potential to directly upregulate/disturb genes exhibiting sequence similarity, such as *NRXN2* and *NRXN3* [90].

Potential future directions

To study the variants' pathogenicity using animal models, it may be beneficial to attempt to replicate the associated human transcriptomic changes rather than aiming to study the total-LOF condition and/or focusing on replicating the human DNA mutations. Indeed, although the genomic

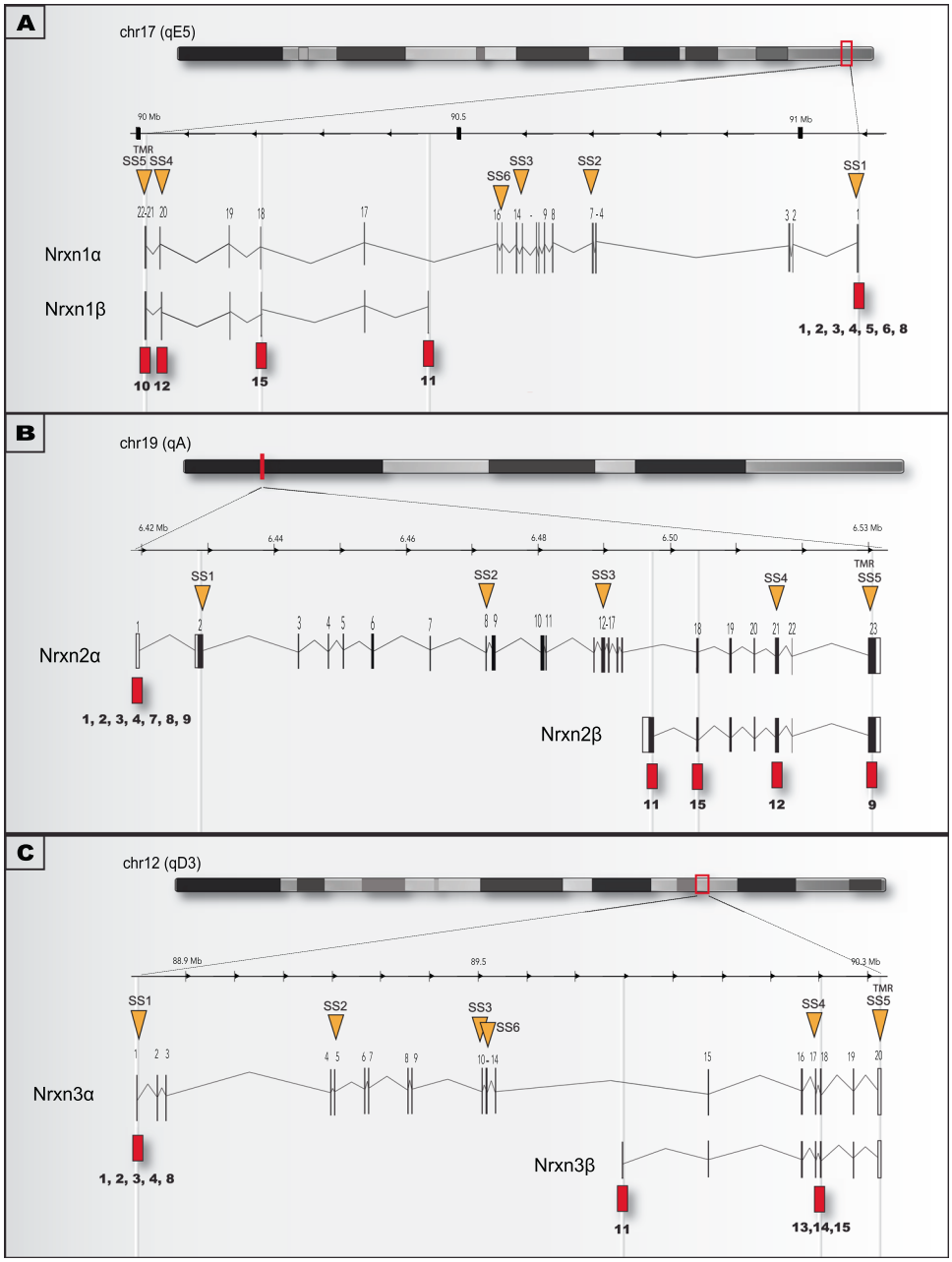

Fig. 4 *NRXN* mutations generated in mouse models. The numbers 1–15 represent studies detailed in Table 1. **a** *Nrxn1* (exons 1–22 and splicing sites marked by orange arrows illustrated on top) *Nrxn1α*; ENSMUST160844.9, *Nrxn1β*; ENSMUST159778.7 from Ensembl release 101 [110]. TMR transmembrane region, that is encoded by exon 22. **b** *Nrxn2* (exons 1–23 and splicing sites marked by orange arrows illustrated on top) *Nrxn2α*; ENSMUST77182.12, *Nrxn2β*; ENSMUST113458.7 from Ensembl release 101 [110]. TMR transmembrane region, that is encoded by exon 23. **c** *Nrxn3* (exons 1–20 and splicing sites marked by orange arrows illustrated on top) *Nrxn3α*; ENSMUST163134.7 and *Nrxn3β*; ENSMUST110130.3 from Ensembl release 101 [110]. Idiograms are taken from the UCSC genome browser [109] showing chromosomes 17, 19 and 12 from GRCm38/mm10 assembly. TMR transmembrane region, that is encoded by exon 20. Non-coding sequences (empty boxes), coding sequences (filled boxes).

organisation of *NRXNs* (including their major splicing sites, Fig. 1) are very well conserved across vertebrates, there is no guarantee that trying to replicate the human variants in those organisms would trigger similar transcriptomic changes and/or genetic compensatory mechanisms, such as the generation of specific de novo isoforms. Currently, too little is known about the complex *NRXN* neurodevelopmental transcriptional dynamics and about genetic compensatory mechanisms in general. Learning more about the normal and pathologic expression of this gene family might then be key to help conceptualise novel research approaches in the future. In this regard, further investigation into the transcriptomic effects of the known causative variants versus healthy carriers may represent a valuable approach. As recently demonstrated, one could generate iPSCs from patients to conduct in-depth transcriptomic studies on differentiated cells [80]. These studies should not be limited to the particular gene associated with the variant but to all *NRXNs*, thereby enabling the monitoring/unveiling of potential toxic genetic compensatory mechanisms. It would be interesting to study all known variants and compare their individual transcriptome fingerprints. Post-mortem brain tissue could also be utilised to complement these studies. It may also be informative to compare these data to (i) iPSCs from healthy carriers and (ii) "wild-type" laboratory human cell lines in which we would genetically replicate the

variants, using genome editing technology such as CRISPR/Cas9. This may unveil a potentially important role of the genetic background influencing the virulence/pathogenicity of each variant. Table 2 exemplifies the experimental range of cellular models [80, 94–97], especially human iPSCs. Generally, human cells may be more appropriate to study the splicing dynamics and the genetic effect of variants as well as the basic neural function of *NRXNs*. For these questions, cells lines are cheaper, easier to manipulate and allow one to work with a human genomic background. By contrast, animal models still play a crucial role in the exploration of mechanisms underlying mental disorders, most of which are considered neurodevelopmental in origin. It thus seems essential to study the pathogenic role of *NRXNs* during the formation of the brain and how these early alterations could trigger later aberrant nervous functions.

In this regard, despite incomplete knowledge of the effects of underlying human genetic variation, animal models should still play a crucial role in understanding the functions of *NRXN* in both the normal and pathogenic brain. Together with robust gene editing tools (such as CRISPR/Cas9 [98]) and relatively inexpensive transcriptomic approaches to analyse/control their effects, there has never been a better time to manipulate these genes in model organisms. In parallel, considering the suspected haploinsufficient pathogenicity, one could deploy an RNAi (RNA interference gene silencing) approach, in an effort to target all *NRXN* isoforms simultaneously, avoiding the risk of triggering uncontrolled genetic compensatory mechanisms [90]. This approach would, moreover, offer the advantage to further control *NRXN* expression spatio-temporally, allowing independent interrogation of specific neurocircuitries. Interestingly, a versatile gene-silencing technology has been recently developed for the zebrafish animal model which has been used to study the role of the SCZ risk gene *miR-137* and *NAPRT1* [99–102]. Furthermore, the zebrafish is an attractive model to study the effect of *NRXNs* on brain development and function. This organism can be easily leveraged to observe, in vivo and in real time, the transition from neuronal progenitors to a fully wired brain including the formation and dynamics (such as pruning) of the synapses. In synergy, optogenetics approaches are relatively well established for this organism, allowing the functionality of specific connections and circuits to be tested [103–106] thereby providing clues to mechanisms involved in these complex diseases.

Although cellular and animal models are critical for the study of *NRXN* pathogenicity, one should not neglect detailed studies of patients with *NRXN1* mutations. These clinical studies continue to provide insights such as (i) pleiotropy: SCZ, intellectual disability, autism, and seizures have all been demonstrated to harbour *NRXN1* mutations

Table 2 Summary of hiPSC and ES based studies on *NRXN1* from ASD and SCZ patients outlining details of patient information, model generation, genotypes such as mutation sites and the emerging cellular phenotypes.

References	Target neurexin	Donor information and sample size	iPSCs and ESC model generation	Genotypes	Cellular phenotypes
Avazzadeh et al. [94]	*NRXN1*	Skin biopsies obtained from five healthy controls and three ASD patients with *NRXN1α* heterozygous deletions.	Seven control and six *NRXN1α* +/− hiPSCs differentiated into cortical excitatory neurons (day 100) using dual SMAD inhibition.	Patient 1—deletion in *NRXN1α* in exon 6–15. Patient 2—deletions in *NRXN1α* exon 1–5. Patient 3—mutation spanning chr2:50983186-51471321.	Altered ion transportation and transporter activity leading to ↑ of voltage-gated calcium channels.
Flaherty et al. [80]	*NRXN1*	Skin biopsies obtained from four cases with a diagnosis ranging from childhood onset schizophrenia, bipolar disorder with psychosis and adult onset schizoaffective disorder. Total of five controls (four unrelated controls + one related non-carrier control).	hiPSC - neural progenitor cells, hiPSC - neurons and *NGN2*-neurons	Patients 1 and 2: Mother and son carrying 5′ deletions (~115 Kb) in *NRXN1* [53]. Patients 3 and 4: monozygotic twins [31, 52] with 136 Kb deletions in 3′ end of *NRXN1* impacting both *αβ*.	↓ Neuronal activity, perturbation of neuronal maturation and identification of mutant isoforms.
Lam et al. [95]	*NRXN1*	One ASD patient carrying a bi-allelic mutation in *NRXN1α* and four healthy controls.	Neuroepithelial stem cells (NCS) differentiated to neurons (28, 49 and 70–75 days).	Compound heterozygote for *NRXN1α* inherited 1 allele with a paternal deletion of ~0.4 Kb and maternal deletion of 0.18 Kb both in exons 1–5 [11].	Calcium activity, impaired neuronal maturation and ↓ emergence of excitatory neurons. Enrichment in astroglia in differentiated cells (↑ glial fibrillary acidic protein—GFAP).
Pak et al. [96]	*NRXN1*	–	Converted mutant embryonic stem (ES) cells into cortical neurons.	Conditional deletion of exon 19 that genocopies a SCZ patient described in [25].	No changes in neuronal differentiation and synapse numbers Approximately twofold ↓ miniature excitatory postsynaptic currents (mEPSC) frequency. ↑ Levels of critical synaptic scaffolding protein (CASK)
Zeng et al. [97]	*NRXN1*	–	Two in vitro models—(1) hiPSCs from skin fibroblasts differentiated into neural stem cells, (2) human embryonic stem cells. *NRXN1* knockdown via shRNAmir based knockdown.	shRNAmir mediated knockdown targeting *α NRXN1*.	↓ Astrocyte marker GFAP correlating with *NRXN1* reduction.

See Fig. 2 for location of patient mutations.

[107] and (ii) the possible combined effect of mutations in more than one *NRXN* homologue as exemplified in a reported case of severe epilepsy with digenic mutations of *NRXN1* and *NRXN2* [108]. Observations such as these further enrich our knowledge of the potential mechanisms linking *NRXN* mutations to disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Bailey A, Lecouteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, et al. Autism as a strongly genetic disorder - evidence from a British Twin Study. Psychol Med. 1995;25:63–77.
2. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 2003;60:1187–92.
3. De Crescenzo F, Postorino V, Siracusano M, Riccioni A, Armando M, Curatolo P, et al. Autistic symptoms in schizophrenia spectrum disorders: a systematic review and meta-analysis. Front Psychiatry. 2019;10:78.
4. Lord C, Elsabbagh M, Baird G, Veenstra-Vanderweele J. Autism spectrum disorder. Lancet. 2018;392:508–20.
5. Asarnow RF, Forsyth JK. Genetics of childhood-onset schizophrenia. Child Adolesc Psychiatr Clin N Am. 2013;22:675–87.
6. Ahn K, An SS, Shugart YY, Rapoport JL. Common polygenic variation and risk for childhood-onset schizophrenia. Mol Psychiatry. 2016;21:94–96.
7. Vorstman JAS, Parr JR, Moreno-De-Luca D, Anney RJL, Nurnberger JI Jr., Hallmayer JF. Autism genetics: opportunities and challenges for clinical translation. Nat Rev Genet. 2017;18:362–76.
8. Foley C, Corvin A, Nakagome S. Genetics of schizophrenia: ready to translate? Curr Psychiatry Rep. 2017;19:61.
9. Schaaf CP, Wiszniewska J, Beaudet AL. Copy number and SNP arrays in clinical diagnostics. Annu Rev Genomics Hum Genet. 2011;12:25–51.
10. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014;511:421–7.
11. Marshall CR, Howrigan DP, Merico D, Thiruvahindrapuram B, Wu W, Greer DS, et al. Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. Nat Genet. 2017;49:27–35.
12. Bena F, Bruno DL, Eriksson M, van Ravenswaaij-Arts C, Stark Z, Dijkhuizen T, et al. Molecular and clinical characterization of 25 individuals with exonic deletions of NRXN1 and comprehensive review of the literature. Am J Med Genet B Neuropsychiatr Genet. 2013;162B:388–403.
13. Lowther C, Speevak M, Armour CM, Goh ES, Graham GE, Li C, et al. Molecular characterization of NRXN1 deletions from 19,263 clinical microarray cases identifies exons important for neurodevelopmental disease expression. Genet Med. 2017;19:53–61.
14. Sudhof TC. Synaptic neurexin complexes: a molecular code for the logic of neural circuits. Cell. 2017;171:745–69.
15. Ullrich B, Ushkaryov YA, Sudhof TC. Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. Neuron. 1995;14:497–507.
16. Ushkaryov YA, Petrenko AG, Geppert M, Sudhof TC. Neurexins: synaptic cell surface proteins related to the alpha-latrotoxin receptor and laminin. Science. 1992;257:50–56.
17. Tabuchi K, Sudhof TC. Structure and evolution of neurexin genes: insight into the mechanism of alternative splicing. Genomics. 2002;79:849–59.
18. Missler M, Sudhof TC. Neurexins: three genes and 1001 products. Trends Genet. 1998;14:20–26.
19. Treutlein B, Gokce O, Quake SR, Sudhof TC. Cartography of neurexin alternative splicing mapped by single-molecule long-read mRNA sequencing. Proc Natl Acad Sci USA. 2014;111:e1291–99.
20. Feng J, Schroer R, Yan J, Song W, Yang C, Bockholt A, et al. High frequency of neurexin 1beta signal peptide structural variants in patients with autism. Neurosci Lett. 2006;409:10–13.
21. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat Genet. 2007;39:319–28.
22. Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, Shen Y, et al. Disruption of neurexin 1 associated with autism spectrum disorder. Am J Hum Genet. 2008;82:199–207.
23. Guilmatre A, Dubourg C, Mosca AL, Legallic S, Goldenberg A, Drouin-Garraud V, et al. Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. Arch Gen Psychiatry. 2009;66:947–56.
24. Zahir FR, Baross A, Delaney AD, Eydoux P, Fernandes ND, Pugh T, et al. A patient with vertebral, cognitive and behavioural abnormalities and a de novo deletion of NRXN1alpha. J Med Genet. 2008;45:239–43.
25. Duong L, Klitten LL, Moller RS, Ingason A, Jakobsen KD, Skjodt C, et al. Mutations in NRXN1 in a family multiply affected with brain disorders: NRXN1 mutations and brain disorders. Am J Med Genet B Neuropsychiatr Genet. 2012;159B:354–8.
26. Gauthier J, Siddiqui TJ, Huashan P, Yokomaku D, Hamdan FF, Champagne N, et al. Truncating mutations in NRXN2 and NRXN1 in autism spectrum disorders and schizophrenia. Hum Genet. 2011;130:563–73.
27. Mohrmann I, Gillessen-Kaesbach G, Siebert R, Caliebe A, Hellenbroich Y. A de novo 0.57 Mb microdeletion in chromosome 11q13.1 in a patient with speech problems, autistic traits, dysmorphic features and multiple endocrine neoplasia type 1. Eur J Med Genet. 2011;54:e461–464.
28. Boyle MI, Jespersgaard C, Nazaryan L, Ravn K, Brondum-Nielsen K, Bisgaard AM, et al. Deletion of 11q12.3-11q13.1 in a patient with intellectual disability and childhood facial features resembling Cornelia de Lange syndrome. Gene. 2015;572:130–4.
29. Vaags AK, Lionel AC, Sato D, Goodenberger M, Stein QP, Curran S, et al. Rare deletions at the neurexin 3 locus in autism spectrum disorder. Am J Hum Genet. 2012;90:133–41.
30. Yuan H, Wang Q, Liu Y, Yang W, He Y, Gusella JF, et al. A rare exonic NRXN3 deletion segregating with neurodevelopmental and neuropsychiatric conditions in a three-generation

Chinese family. Am J Med Genet B Neuropsychiatr Genet. 2018;177:589–95.
31. Kirov G, Gumus D, Chen W, Norton N, Georgieva L, Sari M, et al. Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. Hum Mol Genet. 2008;17:458–65.
32. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science. 2008;320:539–43.
33. Vrijenhoek T, Buizer-Voskamp JE, van der Stelt I, Strengman E, Genetic R. Outcome in Psychosis C et al. Recurrent CNVs disrupt three candidate genes in schizophrenia patients. Am J Hum Genet. 2008;83:504–10.
34. Rujescu D, Ingason A, Cichon S, Pietilainen OP, Barnes MR, Toulopoulou T, et al. Disruption of the neurexin 1 gene is associated with schizophrenia. Hum Mol Genet. 2009;18: 988–96.
35. Hu Z, Xiao X, Zhang Z, Li M. Genetic insights and neurobiological implications from NRXN1 in neuropsychiatric disorders. Mol Psychiatry. 2019;24:1400–14.
36. Todarello G, Feng N, Kolachana BS, Li C, Vakkalanka R, Bertolino A, et al. Incomplete penetrance of NRXN1 deletions in families with schizophrenia. Schizophr Res. 2014;155:1–7.
37. Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL, et al. A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS Genet. 2009;5:e1000373.
38. Levinson DF, Shi J, Wang K, Oh S, Riley B, Pulver AE, et al. Genome-wide association study of multiplex schizophrenia pedigrees. Am J Psychiatry. 2012;169:963–73.
39. Ikeda M, Aleksic B, Kirov G, Kinoshita Y, Yamanouchi Y, Kitajima T, et al. Copy number variation in schizophrenia in the Japanese population. Biol Psychiatry. 2010;67:283–6.
40. Li Z, Chen J, Xu Y, Yi Q, Ji W, Wang P, et al. Genome-wide analysis of the role of copy number variation in schizophrenia risk in Chinese. Biol Psychiatry. 2016;80:331–7.
41. Rees E, Walters JT, Georgieva L, Isles AR, Chambert KD, Richards AL, et al. Analysis of copy number variations at 15 schizophrenia-associated loci. Br J Psychiatry. 2014;204: 108–14.
42. Szatkiewicz JP, O'Dushlaine C, Chen G, Chambert K, Moran JL, Neale BM, et al. Copy number variation in schizophrenia in Sweden. Mol Psychiatry. 2014;19:762–73.
43. Morris DW, Pearson RD, Cormican P, Kenny EM, O'Dushlaine CT, Perreault LP, et al. An inherited duplication at the gene p21 Protein-Activated Kinase 7 (PAK7) is a risk factor for psychosis. Hum Mol Genet. 2014;23:3316–26.
44. Guha S, Rees E, Darvasi A, Ivanov D, Ikeda M, Bergen SE, et al. Implication of a rare deletion at distal 16p11.2 in schizophrenia. JAMA Psychiatry. 2013;70:253–60.
45. Buizer-Voskamp JE, Muntjewerff JW, Genetic R, Outcome in Psychosis Consortium M, Strengman E, Sabatti C, et al. Genome-wide analysis shows increased frequency of copy number variation deletions in Dutch schizophrenia patients. Biol Psychiatry. 2011;70:655–62.
46. International Schizophrenia C. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature. 2008; 455:237–41.
47. Magri C, Sacchetti E, Traversa M, Valsecchi P, Gardella R, Bonvicini C, et al. New copy number variations in schizophrenia. PLoS One. 2010;5:e13422.
48. Costain G, Lionel AC, Merico D, Forsythe P, Russell K, Lowther C, et al. Pathogenic rare copy number variants in community-based schizophrenia suggest a potential role for clinical microarrays. Hum Mol Genet. 2013;22:4485–501.
49. Rudd DS, Axelsen M, Epping EA, Andreasen NC, Wassink TH. A genome-wide CNV analysis of schizophrenia reveals a potential role for a multiple-hit model. Am J Med Genet B Neuropsychiatr Genet. 2014;165B:619–26.
50. Priebe L, Degenhardt F, Strohmaier J, Breuer R, Herms S, Witt SH, et al. Copy number variants in German patients with schizophrenia. PLoS One. 2013;8:e64035.
51. Van Den Bossche MJ, Johnstone M, Strazisar M, Pickard BS, Goossens D, Lenaerts AS, et al. Rare copy number variants in neuropsychiatric disorders: specific phenotype or not? Am J Med Genet B Neuropsychiatr Genet. 2012;159B:812–22.
52. Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, et al. Large recurrent microdeletions associated with schizophrenia. Nature. 2008;455:232–6.
53. Ahn K, Gotay N, Andersen TM, Anvari AA, Gochman P, Lee Y, et al. High rate of disease-related copy number variations in childhood onset schizophrenia. Mol Psychiatry. 2014;19:568–72.
54. Hu XF, Zhang JS, Jin C, Mi WF, Wang F, Ma WB, et al. Association study of NRXN3 polymorphisms with schizophrenia and risperidone-induced bodyweight gain in Chinese Han population. Prog Neuro-Psychoph. 2013;43:197–202.
55. Missler M, Zhang W, Rohlmann A, Kattenstroth G, Hammer RE, Gottmann K, et al. Alpha-neurexins couple Ca2+ channels to synaptic vesicle exocytosis. Nature. 2003;423:939–48.
56. Zhang W, Rohlmann A, Sargsyan V, Aramuni G, Hammer RE, Sudhof TC, et al. Extracellular domains of alpha-neurexins participate in regulating synaptic transmission by selectively affecting N- and P/Q-type Ca2+ channels. J Neurosci. 2005;25:4330–42.
57. Etherton MR, Blaiss CA, Powell CM, Sudhof TC. Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proc Natl Acad Sci USA. 2009;106:17998–8003.
58. Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. Nat Rev Neurosci. 2010;11:490–502.
59. Mena A, Ruiz-Salas JC, Puentes A, Dorado I, De Ruiz-Veguilla M, et al. Reduced prepulse inhibition as a biomarker of schizophrenia. Front Behav Neurosci. 2016;10:202.
60. Grayton HM, Missler M, Collier DA, Fernandes C. Altered social behaviours in neurexin 1alpha knockout mice resemble core symptoms in neurodevelopmental disorders. PLoS One. 2013;8:e67114.
61. Sons MS, Busche N, Strenzke N, Moser T, Ernsberger U, Mooren FC, et al. alpha-Neurexins are required for efficient transmitter release and synaptic homeostasis at the mouse neuromuscular junction. Neurosci. 2006;138:433–46.
62. Dudanova I, Tabuchi K, Rohlmann A, Sudhof TC, Missler M. Deletion of alpha-neurexins does not cause a major impairment of axonal pathfinding or synapse formation. J Comp Neurol. 2007;502:261–74.
63. Dachtler J, Glasper J, Cohen RN, Ivorra JL, Swiffen DJ, Jackson AJ, et al. Deletion of alpha-neurexin II results in autism-related behaviors in mice. Transl Psychiatry. 2014;4:e484.
64. Dachtler J, Ivorra JL, Rowland TE, Lever C, Rodgers RJ, Clapcote SJ. Heterozygous deletion of alpha-neurexin I or alpha-neurexin II results in behaviors relevant to autism and schizophrenia. Behav Neurosci. 2015;129:765–76.
65. Born G, Grayton HM, Langhorst H, Dudanova I, Rohlmann A, Woodward BW, et al. Genetic targeting of NRXN2 in mice unveils role in excitatory cortical synapse function and social behaviors. Front Synaptic Neurosci. 2015;7:3.
66. Rabaneda LG, Robles-Lanuza E, Nieto-Gonzalez JL, Scholl FG. Neurexin dysfunction in adult neurons results in autistic-like behavior in mice. Cell Rep. 2014;8:338–46.

67. Anderson GR, Aoto J, Tabuchi K, Foldy C, Covy J, Yee AX, et al. beta-neurexins control neural circuits by regulating Synaptic endocannabinoid signaling. Cell. 2015;162:593–606.
68. Matsuda K, Yuzaki M. Cbln family proteins promote synapse formation by regulating distinct neurexin signaling pathways in various brain regions. Eur J Neurosci. 2011;33:1447–61.
69. Uemura T, Lee SJ, Yasumura M, Takeuchi T, Yoshida T, Ra M, et al. Trans-synaptic interaction of GluRdelta2 and Neurexin through Cbln1 mediates synapse formation in the cerebellum. Cell. 2010;141:1068–79.
70. Sugita S, Saito F, Tang J, Satz J, Campbell K, Sudhof TC. A stoichiometric complex of neurexins and dystroglycan in brain. J Cell Biol. 2001;154:435–45.
71. Boucard AA, Ko J, Sudhof TC. High affinity neurexin binding to cell adhesion G-protein-coupled receptor CIRL1/latrophilin-1 produces an intercellular adhesion complex. J Biol Chem. 2012;287:9399–413.
72. Ko J, Fuccillo MV, Malenka RC, Sudhof TC. LRRTM2 functions as a neurexin ligand in promoting excitatory synapse formation. Neuron. 2009;64:791–8.
73. Siddiqui TJ, Pancaroglu R, Kang Y, Rooyakkers A, Craig AM. LRRTMs and neuroligins bind neurexins with a differential code to cooperate in glutamate synapse development. J Neurosci. 2010;30:7495–506.
74. Aoto J, Martinelli DC, Malenka RC, Tabuchi K, Sudhof TC. Presynaptic neurexin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell. 2013;154:75–88.
75. Dai J, Aoto J, Sudhof TC. Alternative splicing of presynaptic neurexins differentially controls postsynaptic NMDA and AMPA receptor responses. Neuron. 2019;102:993–1008 e1005.
76. Aoto J, Foldy C, Ilcus SM, Tabuchi K, Sudhof TC. Distinct circuit-dependent functions of presynaptic neurexin-3 at GABAergic and glutamatergic synapses. Nat Neurosci. 2015;18:997–1007.
77. Chen LY, Jiang M, Zhang B, Gokce O, Sudhof TC. Conditional deletion of all neurexins defines diversity of essential synaptic organizer functions for neurexins. Neuron 2017;94:611–25 e614.
78. Gandal MJ, Zhang P, Hadjimichael E, Walker RL, Chen C, Liu S et al. Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. Science. 2018;362:693–7.
79. De Los Angeles A, Tunbridge EM. Unraveling mechanisms of patient-specific NRXN1 mutations in neuropsychiatric diseases using human induced pluripotent stem cells. Stem Cells Dev. 2020;29:1142–4.
80. Flaherty E, Zhu S, Barretto N, Cheng E, Deans PJM, Fernando MB, et al. Neuronal impact of patient-specific aberrant NRXN1alpha splicing. Nat Genet. 2019;51:1679–90.
81. Fuccillo MV, Foldy C, Gokce O, Rothwell PE, Sun GL, Malenka RC, et al. Single-Cell mRNA Profiling Reveals Cell-Type-Specific Expression of Neurexin Isoforms. Neuron. 2015;87:326–40.
82. Jahn K, Heese A, Kebir O, Groh A, Bleich S, Krebs MO, et al. Differential methylation pattern of schizophrenia candidate genes in tetrahydrocannabinol-consuming treatment-resistant schizophrenic patients compared to non-consumer patients and healthy controls. Neuropsychobiology. 2020;Jun 29:1–9.
83. Nussbaum J, Xu Q, Payne TJ, Ma JZ, Huang W, Gelernter J, et al. Significant association of the neurexin-1 gene (NRXN1) with nicotine dependence in European- and African-American smokers. Hum Mol Genet. 2008;17:1569–77.
84. Hishimoto A, Liu QR, Drgon T, Pletnikova O, Walther D, Zhu XG, et al. Neurexin 3 polymorphisms are associated with alcohol dependence and altered expression of specific isoforms. Hum Mol Genet. 2007;16:2880–91.
85. Panagopoulos VN, Trull TJ, Glowinski AL, Lynskey MT, Heath AC, Agrawal A, et al. Examining the association of NRXN3 SNPs with borderline personality disorder phenotypes in heroin dependent cases and socio-economically disadvantaged controls. Drug Alcohol Depend. 2013;128:187–93.
86. Stoltenberg SF, Lehmann MK, Christ CC, Hersrud SL, Davies GE. Associations among types of impulsivity, substance use problems and neurexin-3 polymorphisms. Drug Alcohol Depend. 2011;119:e31–38.
87. Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kahler AK, Akterin S, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet. 2013;45:1150–9.
88. Sarachana T, Hu VW. Genome-wide identification of transcriptional targets of RORA reveals direct regulation of multiple genes associated with autism spectrum disorder. Mol Autism. 2013;4:14.
89. El-Brolosy MA, Stainier DYR. Genetic compensation: a phenomenon in search of mechanisms. PLoS Genet. 2017;13:e1006780.
90. El-Brolosy MA, Kontarakis Z, Rossi A, Kuenne C, Gunther S, Fukuda N, et al. Genetic compensation triggered by mutant mRNA degradation. Nature. 2019;568:193–7.
91. Ma ZP, Chen J. Nonsense mutations and genetic compensation response. Yi Chuan. 2019;41:359–64.
92. Sztal TE, Stainier DYR. Transcriptional adaptation: a mechanism underlying genetic robustness. Development. 2020;147.
93. Trotter JH, Hao J, Maxeiner S, Tsetsenis T, Liu Z, Zhuang X, et al. Synaptic neurexin-1 assembles into dynamically regulated active zone nanoclusters. J Cell Biol. 2019;218:2677–98.
94. Avazzadeh S, McDonagh K, Reilly J, Wang Y, Boomkamp SD, McInerney V, et al. Increased Ca(2+) signaling in NRXN1alpha (+/−) neurons derived from ASD induced pluripotent stem cells. Mol Autism. 2019;10:52.
95. Lam M, Moslem M, Bryois J, Pronk RJ, Uhlin E, Ellstrom ID, et al. Single cell analysis of autism patient with bi-allelic NRXN1-alpha deletion reveals skewed fate choice in neural progenitors and impaired neuronal functionality. Exp Cell Res. 2019;383:111469.
96. Pak C, Danko T, Zhang Y, Aoto J, Anderson G, Maxeiner S, et al. Human neuropsychiatric disease modeling using conditional deletion reveals synaptic transmission defects caused by heterozygous mutations in NRXN1. Cell Stem Cell. 2015;17:316–28.
97. Zeng L, Zhang P, Shi L, Yamamoto V, Lu W, Wang K. Functional impacts of NRXN1 knockdown on neurodevelopment in stem cell models. PLoS One. 2013;8:e59685.
98. Komor AC, Badran AH, Liu DR. CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell. 2017;168:20–36.
99. Giacomotto J, Rinkwitz S, Becker TS. Effective heritable gene knockdown in zebrafish using synthetic microRNAs. Nat Commun. 2015;6:7378.
100. Giacomotto J, Carroll AP, Rinkwitz S, Mowry B, Cairns MJ, Becker TS. Developmental suppression of schizophrenia-associated miR-137 alters sensorimotor function in zebrafish. Transl Psychiatry. 2016;6:e818.
101. Periyasamy S, John S, Padmavati R, Rajendren P, Thirunavukkarasu P, Gratten J et al. Association of schizophrenia risk with disordered niacin metabolism in an indian genome-wide association study. JAMA Psychiatry. 2019;76:1026–34.
102. Laird AS, Mackovski N, Rinkwitz S, Becker TS, Giacomotto J. Tissue-specific models of spinal muscular atrophy confirm a critical role of SMN in motor neurons from embryonic to adult stages. Hum Mol Genet. 2016;25:1728–38.

103. Forster D, Kramer A, Baier H, Kubo F. Optogenetic precision toolkit to reveal form, function and connectivity of single neurons. Methods 2018;150:42–48.
104. Forster D, Dal Maschio M, Laurell E, Baier H. An optogenetic toolbox for unbiased discovery of functionally connected cells in neural circuits. Nat Commun. 2017;8:116.
105. Ji N, Freeman J, Smith SL. Technologies for imaging neural activity in large volumes. Nat Neurosci. 2016;19:1154–64.
106. Ahrens MB, Orger MB, Robson DN, Li JM, Keller PJ. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. Nat Methods. 2013;10:413.
107. Al Shehhi M, Forman EB, Fitzgerald JE, McInerney V, Krawczyk J, Shen S, et al. NRXN1 deletion syndrome; phenotypic and penetrance data from 34 families. Eur J Med Genet. 2019;62:204–9.
108. Rochtus AM, Trowbridge S, Goldstein RD, Sheidley BR, Prabhu SP, Haynes R, et al. Mutations in NRXN1 and NRXN2 in a patient with early-onset epileptic encephalopathy and respiratory depression. Cold Spring Harb Mol Case Stud. 2019;5:1–13.
109. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Res. 2002;12:996–1006.
110. Yates AD, Achuthan P, Akanni W, Allen J, Allen J, Alvarez-Jarreta J, et al. Ensembl 2020. Nucleic Acids Res. 2020;48:D682–8.