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RESEARCH ARTICLE

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Rare heterozygous genetic variants of NRXN and NLGN gene families involved in synaptic function and their association with neurodevelopmental disorders

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Abstract

The interaction of neurexins (NRXNs) in the presynaptic membrane with postsynaptic cell adhesion molecules called neuroligins (NLGNs) is critical for this synaptic function. Impaired synaptic functions are emphasized in neurodevelopmental disorders to uncover etiological factors. We evaluated variants in *NRXN* and *NLGN* genes encoding molecules located directly at the synapse in patients with neuropsychiatric disorders using clinical exome sequencing and chromosomal microarray. We presented detailed clinical findings of cases carrying heterozygous *NRXN1* (c.190C > T, c.1679C > T and two copy number variations [CNVs]), *NRXN2* (c.808dup, c.1901G > T), *NRXN3* (c.3889C > T), and *NLGN1* (c.269C > G, c.473T > A) gene variants. In addition, three novel variants were identified in the *NRXN1* (c.1679C > T), *NRXN3* [c.3889C > T (p.Pro1297Ser)], and *NLGN1* [c.473T > A (p.Ile158Lys)] genes. We emphasize the clinical findings of CNVs of the *NRXN1* gene causing a more severe clinical presentation than single nucleotide variants of the *NRXN1* gene in this study. We detected an *NRXN2* gene variant (c.808dup) with low allelic frequency in two unrelated cases with the same diagnosis. We emphasize the importance of this variant for future studies. We suggest that *NRXN2, NRXN3*, and *NLGN1* genes, which are less frequently reported than *NRXN1* gene variants, may also be associated with neurodevelopmental disorders.

KEYWORDS

attention deficit hyperactivity disorder, autism spectrum disorder, intellectual disability, NLGN genes, NRXN genes

1 | INTRODUCTION

Synaptic dysfunction is implicated in the etiopathogenesis of neurodevelopmental disorders such as autism spectrum disorder (ASD), developmental delay (DD), intellectual disability (ID), and attention deficit hyperactivity disorder. Disorders resulting in altered synaptic function are referred to as synaptopathy (Molloy et al., 2023). Rare variants in genes expressed at the synapse are responsible for the genetic etiopathogenesis of synaptopathy.

Neurexins (NRXNs) are presynaptic cell surface receptors involved in synaptic signal transduction and regulating

synapse specification (Al Shehhi et al., 2019). Three *NRXN* genes, *NRXN1* (OMIM *600565), *NRXN2* (OMIM *600566), and *NRXN3* (OMIM *600567), are highly conserved among other species (Béna et al., 2013). This gene family uses the same intracellular domains but has different extracellular structures (Wu et al., 2023). Two main isoforms, alpha-NRXN and beta-NRXN, are encoded by independent promoters at different positions in the NRXN genes. These isoforms are mainly expressed in the brain (Béna et al., 2013). However, variable expression patterns exist due to the use of variable promoters and alternative splicing (Schaaf et al., 2012). *NRXN1* is the longest gene in the *NRXN* family with 24 exons, 23 coding exons, and a transcript length of 7578 bps according to Ensembl transcript ENST00000404971.5 (Xu et al., 2023).

NRXNs interact with neuroligin proteins (NLGNs) located in the postsynaptic membrane. NLGNs are encoded by four different *NLGN* genes: *neuroligin 1* (*NLGN1*, OMIM no *600568), *neuroligin 2* (*NLGN2*, OMIM no *606479), *neuroligin 3* (*NLGN3*, OMIM no *300336), and *neuroligin 4* (*NLGN4*, OMIM no *300427) (Uchigashima et al., 2020). NLGN1 is a postsynaptic adhesion molecule found in excitatory or glutamatergic synapses. In contrast, NLGN2 is localized at inhibitory or GABAergic synapses (Bemben et al., 2019; Chubykin et al., 2007). NLGN3 is involved in both synapse subtypes, especially in excitatory synapses (Molloy et al., 2023). NLGN4 is present at inhibitory glycinergic synapses (Marro et al., 2010). The interaction between NRXNs and NLGNs is critical for synaptic function, mainly synaptic transmission, synapse formation, and synaptic identity. These genes are not tolerant to loss-of-function variants (probability of loss-of-function intolerance (pLI) scores = .85–1 and have been reported as strong candidates for ASD in the SFARI database (https://gene.sfari.org).

Homozygous variants of *NRXN1* cause Pitt Hopkins-like syndrome 2 (PTHSL2, OMIM no 614325). Among the genes directly involved in synaptic function, the *NRXN1* gene has been the most implicated gene in previous reports of neurodevelopmental disorders and neuropsychiatric conditions (Blazekovic et al., 2022; Fromer et al., 2014; Satterstrom et al., 2020; Takata et al., 2018; Taşkıran et al., 2021; Truty et al., 2019; Wang et al., 2020).

There is limited information on the relationship between neuropsychiatric disorders and the *NRXN1* gene, and even less information on the association between neuropsychiatric disorders and other genes involved in synaptic function. In this study, we investigated the effect of *NRXN* and/or *NLGN* genes on the underlying genetic etiopathogenesis of neurodevelopmental disorders.

2 | MATERIALS AND METHODS

2.1 | Patients

The data of patients who underwent molecular genetic testing (clinical exome sequencing [CES] and chromosomal microarray analysis [CMA]) with a diagnosis of DD, ID, and/ or ASD between 01.10.2019 and 01.05.2023 were retrospectively evaluated. All molecular genetic testing was performed solo. All cases were included if they met the criteria for DD/ID and/or ASD in the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) (Blesson & Cohen, 2020). All subjects were recruited from the Balıkesir Atatürk City Hospital. We interviewed each participant and their parents and reviewed their medical records to collect their clinical information. This study was retrospective and cross-sectional.

CES data from cases were screened by creating custom gene sets for variants in each of the *NRXN1*, *NRXN2*, *NRXN3*, *NLGN1*, *NLGN2*, *NLGN3*, *NLGN4X*, and *NLGN4Y* genes involved in synaptic function. Patients with benign and likely benign variants in any of these genes are included in Table S1. Other variants and clinical findings were reported in Section 3. Family segregation analysis was performed by Sanger sequencing. Parental samples were available for Cases 2, 3, 4, 5, 7, 9, and 10.

2.2 | DNA extraction

Informed consent was obtained from all patients before the collection of blood samples. DNA extraction was performed from these 200 μL peripheral blood samples. We extracted DNA using the High Pure PCR Template Preparation Kit (Roche) protocol.

2.3 | Clinical exome sequencing (CES)

The obtained genomic DNA was enriched using two capture kits used for CES: the Twist CES kit and KAPA HyperCap DS CES Target Enrichment Probes (Roche). These were sequenced using the MGI-DNBSEQ-G400. In addition, WES was performed using the TruSeq Exome Enrichment Kit from Illumina following the manufacturer's protocol. It was sequenced using a HiSeq2500 Sequencer (Illumina). An average read depth of 20× and 95% coverage, including exon-intron junction boundaries (±10 bp), were evaluated. Furthermore, the amount of human genome covered by the Twist CES kit is 34.9 MB (in megabase pairs of DNA).

2.4 | Sanger sequencing

Before sequencing, the PCR products were purified using the Thermo Scientific GeneJET PCR Purification Kit (Thermo Fisher Scientific). After the completion of the thermal cycle step, the sequence reactions were purified according to the protocol of the ZR-96 DNA Sequencing Clean-up Kit (Zymo Research Corp.). Capillary electrophoresis of the purified sequence products was performed using ABI 3130 XL (Applied Biosystems Inc.). Variants were then analyzed using SeqScape 2.5.0 (Applied Biosystems Inc.) software.

2.5 | Data analysis

GenomizeSeq (Version 6.13.1) software was used for analysis with the reference human genome (hg19/GRCh37). Human Phenotype Ontology was used for phenotypic filters, and Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/) was used for gene sets. The Human Genome Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), Franklin (https://franklin.genoox.com/clinical-db/home), VarSome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and novel variants in the databases were checked. The pathogenicity score of new variants was interpreted using the in silico variant prediction programs Mutation Taster, Combined Annotation Dependent Depletion. The data were classified according to the American College of Medical Genetics and Genomics (ACMG) criteria (Richards et al., 2015).

2.6 | Chromosomal microarray analysis (CMA)

DNA isolated from the patient's sample was analyzed using the HumanCytoSNP-12 v2.1 BeadChip Kit (300K) (Illumina)/GenetiSure Cyto CGH Microarray Kit, 8 × 60K (Agilent). The Ensembl version 74 and GRCh37 Genome build names were used. The data obtained from the analyses were searched in Genomic Variants Databases (DGV, http://dgv.tcag.ca/dgv/app/home), DECIPHER (https://www.deciphergenomics.org/), OMIM, and other relevant databases. The Log*R* value of the microarray data obtained from the study was determined to be .15 (<.2), and the median call rate value was .98 (.98–1).

2.7 | Ethical publication statement

This study was conducted at Balıkesir Atatürk City Hospital. Ethical approval was obtained from the institutional Ethics Committee, dated May 04, 2023 and numbered 2023/2/22. The study was evaluated as a research file, and it was decided that it was scientifically and ethically appropriate.

2.8 | Homology modeling of mutant NRXN1, NRXN2, and NLGN1 variant analysis

Protein sequence information was obtained from the Uniprot database. Then, pdb format was obtained with Protein Homology/analogY Recognition Engine (Phyre2, V 2.0) (Kelley et al., 2015). Using this format, three-dimensional modeling of proteins was performed by the PyMOL Molecular Graphics System Version 2.5.5 (Schrödinger LLC).

3 | RESULTS

In this retrospective study, we presented detailed clinical and genetic data for 10 patients (n = 7 male, 70%; n = 3 female, 30%) carrying *NRXN1*, *NRXN2, NRXN3*, or *NLGN1* gene variants. The current mean age of these 10 patients was 7.3 years (interquartile range: 1–30 years). We found seven different single nucleotide variants (SNVs), including two different *NRXN1* gene variants (Case 1: c.190C > T, Case 2: c.1679C > T), and two different *NRXN2* gene variants from three patients (Case 3 and Case 4: c.808dup (n = 2), Case 5: c.1901G > T (n = 1)), one *NRXN3* gene variant (Case 6: c.3889C > T), and two different *NLGN1* gene variants (Case 7: c.269C > G, Case 8: c.473T > A) (Table 1). In addition, two copy number variations (CNVs), including the *NRXN1* gene, were detected using CMA analysis in Case 9 and Case 10 (Table 2). According to *NRXN* gene variant results, 66.6% (6/9) were missense type, 22.2% (2/9) were deletion type, and 11.1% (1/9) were frameshift type variants. All SNV variants detected in this study were heterozygous. We categorized SNVs as variants of uncertain significance and CNVs as likely pathogenic based on the ACMG criteria. Three novel variants were identified in the *NRXN1:*c.1679C > T (p.Thr560Ile), *NRXN3*:c.3889C > T (p.Pro1297Ser), and *NLGN1:*c.473T > A (p.Ile158Lys) genes. Most of the SNVs in this study had not been previously reported, according to the gnomAD genome.

The clinical results of patients with these variants and their families are shown in Table 1. Eight of these ten patients with the *NRXN1*, *NRXN2*, *NRXN3*, and *NLGN1* genetic variants had ID. Five patients (n = 5, 50%) had comorbid epilepsy. Dysmorphic facial features, ASD, and obesity were other findings.

In the CMA analysis of patient 9, a copy number loss of 293-kb was detected in the *2p16.3* chromosome region, which is paternally inherited. This case had early infantile epileptic encephalopathy, dysmorphic facial findings, and hypotonia. A de novo 320-kb copy number loss (1 copy) was detected in a

TABLE 1 Summary of neurexin (*NRXN*) and neuroligin (*NLGN*) gene variants detected in our study.

Case no	Age (y)	Gender	Gene transcript	Variation (inheritance)	Exon	Amino acid variation	Variant type	gnomAD frequency (genomes)	ACMG pathogenicity criteria	ClinVar	Zygosity	Associated phenotype
1	7	M	*NRXN1* (NM_004801.5)	c.190C > T (na)	2	p.Arg64Cys	Missense	No variant	PM2, BP1	Reported	Het	Autism, DD/ID
2	8	M	*NRXN1* (NM_004801.5)	**c.1679C > T (maternal)**	9	**p.Thr560Ile**	Missense	No variant	PM2, BP1	**Novel**	Het	ID/DD, epilepsy
3	3	F	*NRXN2* (NM_015080.4)	c.808dup (maternal)	5	p.Ala270GlyfsTer27	Frameshift	0.00003208	PVS1	Not reported	Het	Dysmorphic facial features, ID
4	10	M	*NRXN2* (NM_015080.4)	c.808dup (paternal)	5	p.Ala270GlyfsTer27	Frameshift	0.00003208	PVS1	Not reported	Het	ID/DD, epilepsy, obesity
5	2	F	*NRXN2* (NM_015080.4)	c.1901G > T (maternal)	10	p.Gly634Val	Missense	No variant	PM2, PP2	Not reported	Het	ID/DD, epilepsy, WEST syndrome
6	30	M	*NRXN3* (NM_001330195.2)	**c.3889C > T (na)**	19	**p.Pro1297Ser**	Missense	No variant	PM2, PP2	**Novel**	Het	Epilepsy
7	3	M	*NLGN1* (NM_001365925.2)	c.269C > G (de novo)	2	p.Thr90Arg	Missense	No variant	PM2	Not reported	Het	Speech disorder
8	3	M	*NLGN1* (NM_001365925.2)	**c.473T > A**	**2**	**p.Ile158Lys**	Missense	No variant	PM2, PP3	**Novel**	Het	ID/DD

Abbreviations: ACMG, American College of Medical Genetics and Genomics; ADHD, attention deficit hyperactivity disorder; DD, developmental delay; F, female; gnomAD, Genome Aggregation Database; Het, heterozygous; ID, intellectual disability; M, male; y, years.

Bold indicates novel variants that have not been previously reported in the literature. bold* novel variants.

TABLE 2 Summary of copy number variations (CNVs) including *NRXN1* gene.

Case	Age (y)	Gender	OMIM genes	Chromosomal location	Genomic coordinates (NCBI 37/hg19)	Type	Copy number	Size (bp)	Inheritance	Clinical significance in DECIPHER database	Associated phenotype
9	**1**	**F**	*NRXN1*	*2p16.3*	Chr 2: 51077569–51370461	Deletion	1	293 kb	Paternal	Likely pathogenic	ID/DD, epilepsy, WEST syndrome
10	**6**	**M**	*NRXN1*	*2p16.3*	Chr 2: 50959683–51279394	Deletion	1	320 kb	De novo	Likely pathogenic	Autism, ID

Abbreviations: DD, developmental delay; F, female; ID, intellectual disability; M, male; OMIM, Online Mendelian Inheritance in Man; y, years.

6-year-old male (Case 10) suffering from ASD and ID. There was no pathogenic or likely pathogenic variant in the CES analysis of Case 10. In this case, no problems were described during the perinatal period (an uneventful 36-week birth). Although he has been attending special education since the age of 2, no progress in his development has been described. He still has no the words, eye contact, or other social skills. No self-care skills had been acquired. In the psychiatric examination of the patient, the diagnosis of severe ASD and ID was accompanied by behavioral problems, including hyperactivity and self-mutilation. Additionally, the family described clinical worsening with the melatonin they had previously used due to sleep problems.

3.1 | Molecular findings

Because of the *NRXN1*: c.190C > T variant (Case 1), the positively charged arginine in this position becomes a neutrally charged and smaller cysteine amino acid. The mutant residue is more hydrophobic than the wild-type residue. The wild-type residue forms a salt bridge with glutamic acid at positions 176 and 178. This charge difference from the variant disrupts the ionic interaction of the original residue. The variant is located within a domain and is annotated in UniProt as Laminin G-like 1. The mutated residue is on the surface of a domain with an unknown function. The residue was not found to be in contact with other domains for which the function is known within the used structure. However, contact with other molecules or domains is still possible and may be affected by this variant. Case 2 (c.1679C > T): Variant of threonine at position 560 to isoleucine resulted in a more hydrophobic residue. This change is also predicted to affect hydrogen bond formation with aspartic acid at position 555 of the wild-type residue. This mutant residue was not among the other variants observed at this position in homologous proteins, which may indicate that the variant probably damages the protein (Venselaar et al., 2010). These *NRXN1* gene variants are located within the domain of Laminin G-like. To show the conformational changes in these protein structures, PyMOL software was used (Figure 1).

The variant, c.808dup: p.Ala270GlyfsTer27 (Case 3 and Case 4), introduces glycine at this position. Glycines are very flexible and can disturb the required rigidity of the protein at this position. This frameshift variant leads to a premature stop codon that results in a shorter protein product. Case 5: The *NRXN2* gene (c.1901G > T: p.Gly634Val) variant is located within a domain and is annotated in UniProt as Laminin G-like 3. The wild-type residue is glycine, which is the most flexible of all residues. This flexibility may be necessary for the protein's function. Variant of this glycine can abolish this function. The torsion angles for this residue are unusual. Only glycine is flexible enough to make these

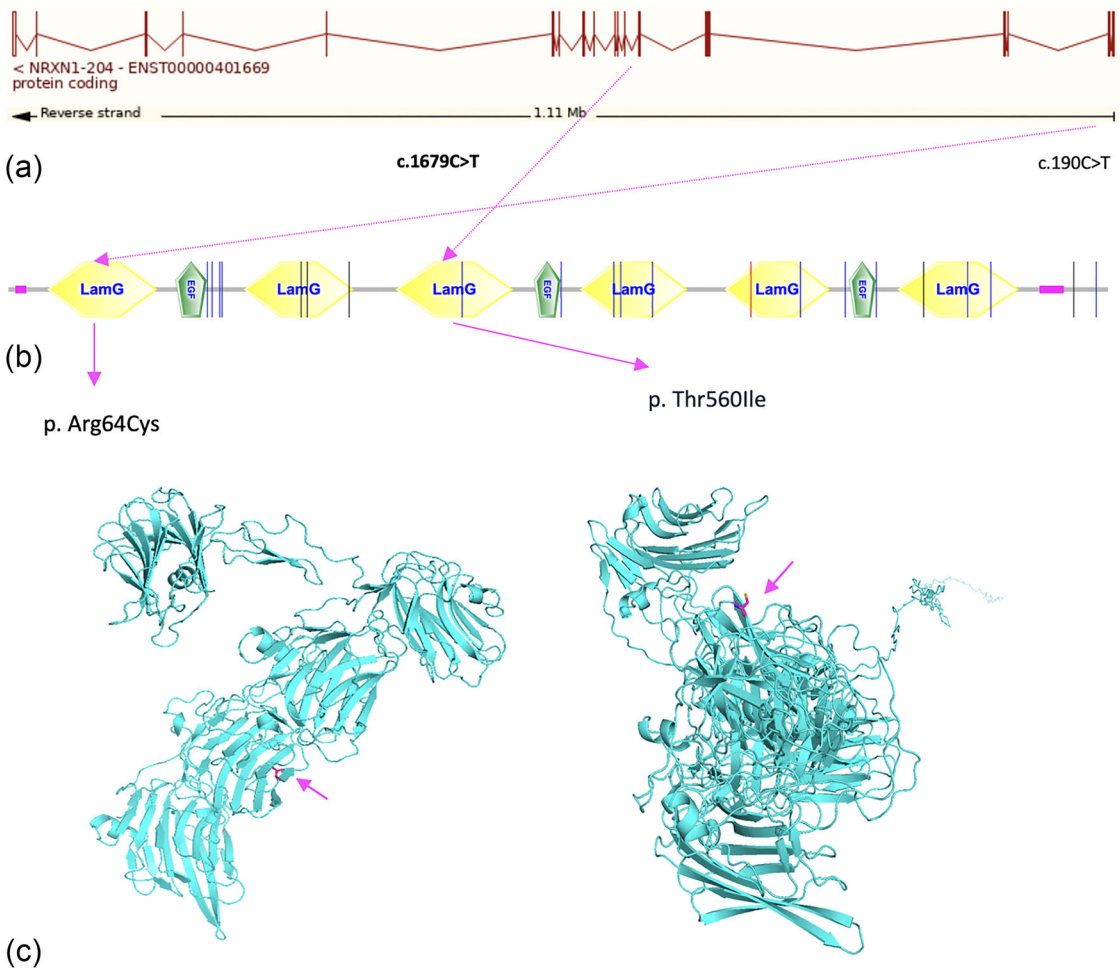

FIGURE 1 (a) Schematic representation of the *NRXN1* gene exons and introns according to the transcript of ENST00000401669. (b) Schematic representation of the protein domain structure with SMART diagram (predicted domains and motifs) showing the variant on the protein. (c) Overview of the mutated proteins (p.Arg64Cys and p.Thr560Ile, respectively) in cartoon presentations using the PyMOL program (ver2.5.5). The protein is colored cyan, and the side chain of the mutated residue is shown as small magenta sticks.

torsion angles variant into another residue, which will force the local backbone into an incorrect conformation and disturb the local structure (Venselaar et al., 2010) (Figure 2).

Case 6: The c.3889C > T variant in the *NRXN3* gene resulted in the conversion of a proline at position 1297 to a smaller amino acid serine. Prolines have a very rigid structure, sometimes forcing the backbone into a specific conformation. Possibly, variant changes a proline with such a function into another residue, thereby disturbing the local structure (Venselaar et al., 2010).

In Case 7, the *NLGN1* gene c.269C > G variant results in the substitution of threonine (neutrally charged) with arginine (positively charged) in the amino acid codon (Venselaar et al., 2010). The variant is located within a domain, annotated in UniProt as carboxylesterase type B (Krejci et al., 1991). Additionally, in Case 8, we identified a novel *NLGN1*:c.473T > A (p.Ile158Lys) gene variant (Figure 3). The wild-type residue charge was neutral, and the mutant residue charge was positive. Therefore, variant introduces a charge in a buried residue, which can lead to protein folding problems. In addition, the variant will cause a loss of hydrophobic interactions in the core of the protein.

4 | DISCUSSION

In this study, we presented detailed clinical findings of cases carrying heterozygous *NRXN1* (c.190C > T, c.1679C > T and two CNVs), *NRXN2* (c.808dup, c.1901G > T), *NRXN3* (c.3889C > T), and *NLGN1* (c.269C > G, c.473T > A) gene variants. Moreover, three novel variants were identified in the *NRXN1:*c.1679C > T (p.Thr560Ile), *NRXN3:*c.3889C > T (p.Pro1297Ser), and *NLGN1:*c.473T > A (p.Ile158Lys) genes.

We found four variants in the *NRXN1* gene (n = 2 SNV, n = 2 CNV). Of the total 491 variants in the *NRXN1* gene

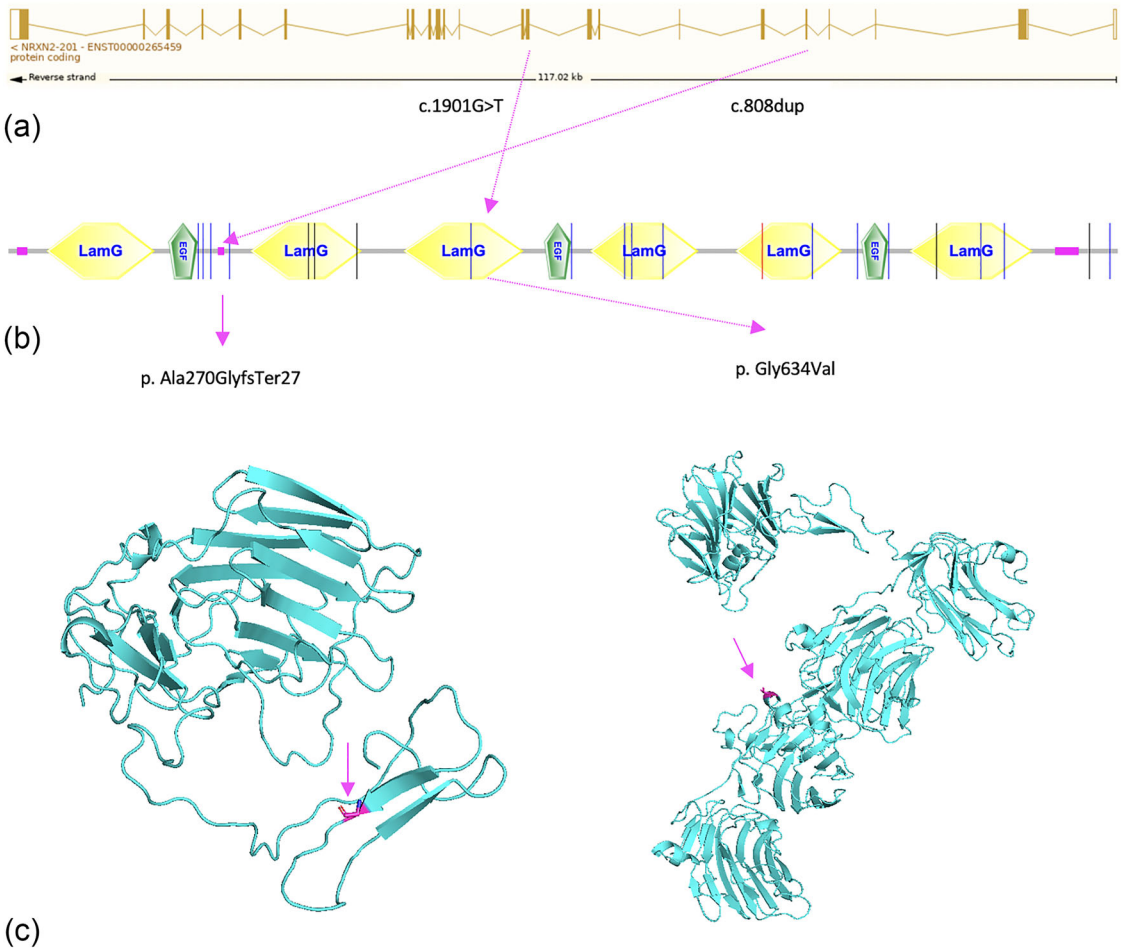

FIGURE 2 (a) Schematic representation of the *NRXN2* gene exons and introns according to the transcript of ENST00000265459. (b) Schematic representation of the protein domain structure with SMART diagram (predicted domains and motifs) showing the variant on the protein. (c) Overview of the mutated proteins (p.Ala270GlyfsTer27 and p.Gly634Val) in cartoon presentations using the PyMOL program (ver2.5.5). The protein is colored cyan, and the side chain of the mutated residue is shown as small magenta sticks.

reported to date in HGMD Professional 2023.2, 83 are missense/nonsense. These variants are mainly associated with ASD and ID/DD (Kim et al., 2008; Koshimizu et al., 2013; Satterstrom et al., 2020; Takata et al., 2018; Wang et al., 2020; Williams et al., 2019). We detected a missense heterozygous c.190C > T (p.Arg64Cys) variant in *NRXN1* in a 7-year-old boy with ASD (Case 1). This variant was not reported in Leiden Open Variation Database (LOVD), and updated HGMD, but was associated with Pitt Hopkins-like syndrome 2 in the ClinVar database. In in silico variant analysis programs such as Polyphen-2 and SIFT, this variant was predicted as disease-causing. This missense variant lies in exon 2 of *NRXN1* according to the NM_004801.5 transcript. We identified a novel missense variant in the *NRXN1* gene c.1679C > T: p.Thr560I in a different patient (Case 2) with ID/DD and epilepsy. This variant is maternally inherited, and a history of epilepsy has been reported in her mother. The variants have not been previously reported in the public population databases of the LOVD (https://www.lovd.nl) and Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/) and associated literature. Our findings are consistent with the previous findings indicating the potential effect of heterozygous *NRXN1* gene variants on neurodevelopmental disorders.

Deletion of *NRXN1* is associated with chromosome *2p16.3* deletion syndrome (OMIM no, 614332). This CNV partially overlaps with the *NRXN1* gene and is associated with autosomal dominant inherited "ID and autism (ClinGen)" (Riggs et al., 2022). In this study, the *2p16.3* deletion was detected in two different individuals. To date, mono/biallelic CNVs in *NRXN* genes have been mainly associated with incomplete penetrance and pleiotropy, DD, and neuropsychiatric disorders such as ASD (Dabell et al., 2013; Gerik-Celebi et al., 2023; Molloy et al., 2023). Homozygous deletion of the *2p16.3* region, including the *NRXN1* gene, has been better identified.

We emphasize the clinical findings of the two cases presented in Section 3 to indicate that monoallelic deletion of

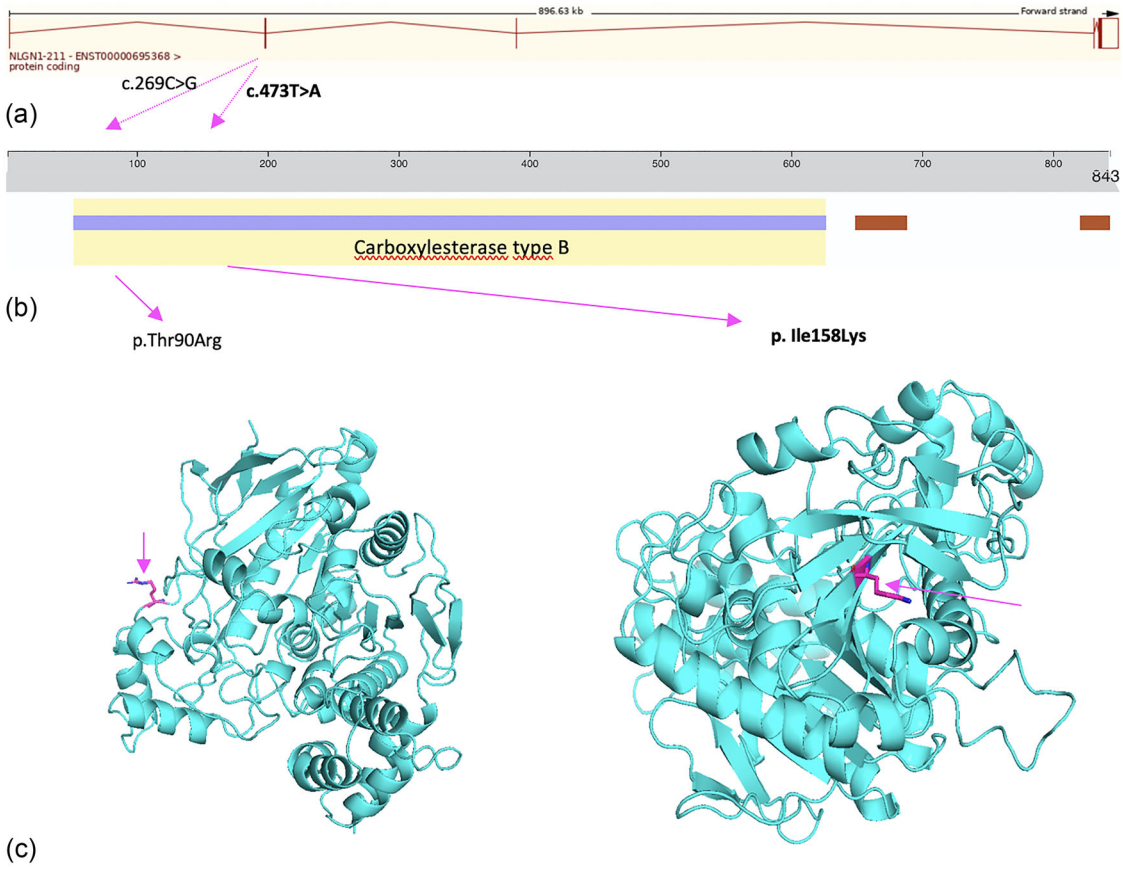

FIGURE 3 (a) Overview of the *NLGN1*: ENST00000695368. (b) Schematic representation of the protein domain structure with Uniprot. (c) Overview of the mutated proteins (p.Thr90Arg and p.Ile158Lys) in cartoon presentations using the PyMOL program (ver2.5.5). The protein is colored cyan, and the side chain of the mutated residue is shown as small magenta sticks.

the *NRXN1* gene also causes a severe clinical presentation. The first of these cases showed epileptic encephalopathy and severe DD, which started at the first age of her life and required intensive unit care. The second case had severe ASD, ID, and behavioral problems. The 6-year-old case was not responding to the special education program that he had started at the age of 2. He still has no word usage, eye contact, or other social skills. No self-care skills had been acquired. In previous reports, aggression, hyperactivity, and sleep problems were marked for deletions of the *NRXN1* gene in human studies, not in animal studies (Chawner et al., 2019; Molloy et al., 2023).

There are fewer studies on other synaptic function-related genes than *NRXN1*. However, *the NRXN2* gene has been reported as a high-confidence candidate for ASD with 11 ASD reports out of 16 reports according to the SFARI database (https://gene.sfari.org/database/human-gene/NRXN2). The association between ASD and *NRXN2* gene was first demonstrated by Gauthier et al. in 2011. In their study of individuals with ASD ($n = 142$), schizophrenia ($n = 143$), or non-syndromic ID ($n = 94$), they identified a *NRXN2* gene: NM_138732.2: c.2733delT variant in a patient with ASD (Gauthier et al., 2011). Furthermore, subsequent studies have also supported the association of the *NRXN2* with ASD (Cukier et al., 2014; Haile et al., 2023; Hu et al., 2023; Li et al., 2017; Lim et al., 2017; Wu et al., 2018). A total of 118 different *NRXN2* variants have been reported, including benign and likely benign variants (Haile et al., 2023). Supporting these previous findings, we detected a heterozygous *NRXN2* gene variant (c.808dup) in two unrelated cases diagnosed with ID/DD. *NRXN2*: c.808dup had a significantly low allelic frequency ($f = .00003208$) in the population dataset of gnomAD and was not present in the homozygous state. Because we detected this *NRXN2* gene variant (c.808dup) with low allelic frequency in two unrelated cases with the same diagnosis, we emphasize the importance of this variant for future studies.

There are limited studies examining the effects of the *NRXN3* gene on neuropsychiatric disorders, and these studies found an association with schizophrenia, attention-deficit/hyperactivity disorder, and ID (Hu et al., 2013; Prasad A et al., 2012; Vaags et al., 2012). These studies support that the *NRXN3* gene with a pLI score of 1 (observed/expected = 0.03 for LoF variants, z-score of 4.17

for missense variants) is also involved in a shared pathway in the etiopathogenesis of neurodevelopmental disorders. In our study, we detected a novel *NRXN3* gene variant in a case followed up with a diagnosis of epilepsy.

In addition to the *NRXN* gene family, we investigated the *NLGN* gene family and detected a novel *NLGN1* [c.473T > A (p.Ile158Lys)] gene variant. Interestingly, an "Isoleucine" variant at this position, p.Thr90Arg, has been previously found, but the pathological significance of this variant in AUTS20 is unknown; it does not affect dendritic spine formation and has no effect on protein abundance; no effect on subcellular localization (Nakanishi et al., 2017). Reported variants associated with the *NLGN* gene family and neurodevelopmental disorders are less common. However, it maintains its importance in neurodevelopmental disorders because it is an important part of early neurodevelopment (Molloy et al., 2023). In particular, variants in the *NLGN3* and *NLGN4X* genes have been reported as leading impairments in synaptogenesis for neurodevelopmental disorders (Molloy et al., 2023; Nguyen et al., 2020). We also support the importance of the *NLGN1* gene variants in neurodevelopmental disorders.

As a conclusion, synaptopathies are a remarkable research topic, and homozygous *NRXN1* gene variants in particular are better defined in the literature. Homozygous variants of the *NRXN1* gene are described as Pitt-Hopkins-like syndrome-2, which is associated with a rare and severe neurodevelopmental disorder. In this study, we focused on the clinical presentations of heterozygous variants in the *NRXN1* gene and other synaptic function genes with limited reports on neurodevelopmental disorders. We emphasize the clinical findings of CNVs of the *NRXN1* gene causing a more severe clinical presentation than SNVs of the *NRXN1* gene. We support that *NRXN2, NRXN3*, and *NLGN1* genes, which are less reported than *NRXN1* gene variants, may also be associated with neurodevelopmental disorders. As we detected the *NRXN2* gene variant (c.808dup) with low allelic frequency in two unrelated cases with the same diagnosis, we emphasize the importance of this variant for future studies. Further studies focusing on synaptopathies, including *NRXN2, NRXN3*, and *NLGN1*, will increase our understanding of the neurobiological mechanisms related to neurodevelopmental disorders.

AUTHOR CONTRIBUTIONS

Gul Unsel-Bolat provided psychiatric evaluation. Hamide Betul Gerik-Celebi and Hilmi Bolat provided genetic evaluation. Gul Unsel-Bolat, Hamide Betul Gerik-Celebi, and Hilmi Bolat designed the study and then wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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REFERENCES

Al Shehhi, M., Forman, E. B., Fitzgerald, J. E., Mcinerney, V., Krawczyk, J., Shen, S., Betts, D. R., Ardle, L. M. C., Gorman, K. M., King, M. D., Green, A., Gallagher, L., & Lynch, S. A. (2019). NRXN1 deletion syndrome; phenotypic and penetrance data from 34 families. *European Journal of Medical Genetics*, *62*(3), 204–209. https://doi.org/10.1016/j.ejmg.2018.07.015

Bemben, M. A., Nguyen, T. A., Li, Y., Wang, T., Nicoll, R. A., & Roche, K. W. (2019). Isoform-specific cleavage of neuroligin-3 reduces synapse strength. *Molecular Psychiatry*, *24*(1), 145–160. https://doi.org/10.1038/s41380-018-0242-y

Béna, F., Bruno, D. L., Eriksson, M., van Ravenswaaij-Arts, C., Stark, Z., Dijkhuizen, T., Gerkes, E., Gimelli, S., Ganesamoorthy, D., Thuresson, A. C., Labalme, A., Till, M., Bilan, F., Pasquier, L., Kitzis, A., Dubourgm, C., Rossi, M., Bottani, A., Gagnebin, M., … Schoumans, J. (2013). Molecular and clinical characterization of 25 individuals with exonic deletions of NRXN1 and comprehensive review of the literature. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, *162B*(4), 388–403.

Blazekovic, A., Gotovac Jercic, K., Meglaj, S., Duranovic, V., Prpic, I., Lozic, B., Malenica, M., Markovic, S., Lujic, L., Petelin Gadze, Z., Juraski, R., Barišic, N., Baric, I., & Borovecki, F. (2022). Genetics of pediatric epilepsy: Next-generation sequencing in clinical practice. *Genes (Basel)*, *13*(8), 1466. https://doi.org/10.3390/genes13081466

Blesson, A., & Cohen, J. S. (2020). Genetic counseling in neurodevelopmental disorders. *Cold Spring Harbor Perspectives in Medicine*, *10*, a036533. https://doi.org/10.1101/cshperspect.a036533

Chawner, S. J. R. A., Owen, M. J., Holmans, P., Raymond, F. L., Skuse, D., Hall, J., & Van Den Bree, M. B. M. (2019). Genotype–phenotype associations in children with copy number variants associated with high neuropsychiatric risk in the UK (IMAGINE-ID): A case-control cohort study. *The Lancet Psychiatry*, *6*(6), 493–505. https://doi.org/10.1016/S2215-0366(19)30123-3

Chubykin, A. A., Atasoy, D., Etherton, M. R., Brose, N., Kavalali, E. T., Gibson, J. R., & Südhof, T. C. (2007). Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. *Neuron*, *54*(6), 919–931. https://doi.org/10.1016/j.neuron.2007.05.029

Cukier, H. N., Dueker, N. D., Slifer, S. H., Lee, J. M., Whitehead, P. L., Lalanne, E., Leyva, N., Konidari, I., Gentry, R. C., Hulme, W. F., Booven, D. V., Mayo, V., Hofmann, N. K., Schmidt, M. A., Martin, E. R., Haines, J. L., Cuccaro, M. L., Gilbert, J. R., & Pericak-Vance,

M. A. (2014). Exome sequencing of extended families with autism reveals genes shared across neurodevelopmental and neuropsychiatric disorders. *Molecular autism*, *5*(1), 1. https://doi.org/10.1186/2040-2392-5-1

Dabell, M. P., Rosenfeld, J. A., Bader, P., Escobar, L. F., El-Khechen, D., Vallee, S. E., Dinulos, M. B. P., Curry, C., Fisher, J., Tervo, R., Hannibal, M. C., Siefkas, K., Wyatt, P. R., Hughes, L., Smith, R., Ellingwood, S., Lacassie, Y., Stroud, T., Farrell, S. A., … Shaffer, L. G. (2013). Investigation of NRXN1 deletions: Clinical and molecular characterization. *American Journal of Medical Genetics. Part A*, *161*(4), 717–731. https://doi.org/10.1002/ajmg.a.35780

Fromer, M., Pocklington, A. J., Kavanagh, D. H., Williams, H. J., Dwyer, S., Gormley, P., Georgieva, L., Rees, E., Palta, P., Ruderfer, D. M., Carrera, N., Humphreys, I., Johnson, J. S., Roussos, P., Barker, D. D., Banks, E., Milanova, V., Grant, S. G., Hannon, E., … O'donovan, M. C. (2014). De novo mutations in schizophrenia implicate synaptic networks. *Nature*, *506*(7487), 179–184. https://doi.org/10.1038/nature12929

Gauthier, J., Siddiqui, T. J., Huashan, P., Yokomaku, D., Hamdan, F. F., Champagne, N., Lapointe, M., Spiegelman, D., Noreau, A., Lafrenière, R. G., Fathalli, F., Joober, R., Krebs, M.-O., Delisi, L. E., Mottron, L., Fombonne, É., Michaud, J. L., Drapeau, P., Carbonetto, S., … Rouleau, G. A. (2011). Truncating mutations in NRXN2 and NRXN1 in autism spectrum disorders and schizophrenia. *Human Genetics*, *130*(4), 563–573. https://doi.org/10.1007/s00439-011-0975-z

Gerik-Celebi, H. B., Aydin, H., Bolat, H., & Unsel-Bolat, G. (2023). Clinical and genetic characteristics of patients with unexplained intellectual disability/developmental delay without epilepsy. *Molecular Syndromology*, *14*(3), 208–218. https://doi.org/10.1159/000529018

Haile, M. T., Khoja, S., De Carvalho, G., Hunt, R. F., & Chen, L. Y. (2023). Conditional deletion of Neurexin-2 alters neuronal network activity in hippocampal circuitries and leads to spontaneous seizures. *Translational Psychiatry*, *13*(1), 97. https://doi.org/10.1038/s41398-023-02394-6

Hu, C., Wang, Y. I., Li, C., Mei, L., Zhou, B., Li, D., Li, H., Xu, Q., & Xu, X. (2023). Targeted sequencing and clinical strategies in children with autism spectrum disorder: A cohort study. *Frontiers in Genetics*, *14*, 1083779. https://doi.org/10.3389/fgene.2023.1083779

Hu, X., Zhang, J., Jin, C., Mi, W., Wang, F., Ma, W., Ma, C., Yang, Y., Li, W., Zhang, H., Du, B. O., Li, K., Liu, C., Wang, L., Lu, T., Zhang, H., Lv, L., Zhang, D., & Yue, W. (2013). Association study of NRXN3 polymorphisms with schizophrenia and risperidone-induced bodyweight gain in Chinese Han population. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *43*, 197–202. https://doi.org/10.1016/j.pnpbp.2012.12.007

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. E. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, *10*, 845–858. https://doi.org/10.1038/nprot.2015.053

Kim, H.-G., Kishikawa, S., Higgins, A. W., Seong, I.-S., Donovan, D. J., Shen, Y., Lally, E., Weiss, L. A., Najm, J., Kutsche, K., Descartes, M., Holt, L., Braddock, S., Troxell, R., Kaplan, L., Volkmar, F., Klin, A., Tsatsanis, K., Harris, D. J., … Gusella, J. F. (2008). Disruption of neurexin 1 associated with autism spectrum disorder. *American Journal of Human Genetics*, *82*(1), 199–207. https://doi.org/10.1016/j.ajhg.2007.09.011

Koshimizu, E., Miyatake, S., Okamoto, N., Nakashima, M., Tsurusaki, Y., Miyake, N., Saitsu, H., & Matsumoto, N. (2013). Performance comparison of bench-top next generation sequencers using microdroplet PCR-based enrichment for targeted sequencing in patients with autism spectrum disorder. *PLoS ONE*, *8*(9), e74167. https://doi.org/10.1371/journal.pone.0074167

Krejci, E., Duval, N., Chatonnet, A., Vincens, P., & Massoulié, J. (1991). Cholinesterase-like domains in enzymes and structural proteins: Functional and evolutionary relationships and identification of a catalytically essential aspartic acid. *PNAS*, *88*(15), 6647–6651. https://doi.org/10.1073/pnas.88.15.6647

Li, J., Wang, L., Guo, H., Shi, L., Zhang, K., Tang, M., Hu, S., Dong, S., Liu, Y., Wang, T., Yu, P., He, X., Hu, Z., Zhao, J., Liu, C., Sun, Z. S., & Xia, K. (2017). Targeted sequencing and functional analysis reveal brain-size-related genes and their networks in autism spectrum disorders. *Molecular Psychiatry*, *22*(9), 1282–1290. https://doi.org/10.1038/mp.2017.140

Lim, E. T., Uddin, M., De Rubeis, S., Chan, Y., Kamumbu, A. S., Zhang, X., D'gama, A. M., Kim, S. N., Hill, R. S., Goldberg, A. P., Poultney, C., Minshew, N. J., Kushima, I., Aleksic, B., Ozaki, N., Parellada, M., Arango, C., Penzol, M. J., Carracedo, A., … Walsh, C. A. (2017). Rates, distribution and implications of postzygotic mosaic mutations in autism spectrum disorder. *Nature Neuroscience*, *20*(9), 1217–1224. https://doi.org/10.1038/nn.4598

Marro, S. G., Chanda, S., Yang, N., Janas, J. A., Valperga, G., Trotter, J., Zhou, B. O., Merrill, S., Yousif, I., Shelby, H., Vogel, H., Kalani, M. Y. S., Südhof, T. C., & Wernig, M. (2010). Neuroligin-4 regulates excitatory synaptic transmission in human neurons. *Neuron*, *103*(4), 617–626. e6. https://doi.org/10.1016/j.neuron.2019.05.043

Molloy, C. J., Cooke, J., Gatford, N. J. F., Rivera-Olvera, A., Avazzadeh, S., Homberg, J. R., Grandjean, J., Fernandes, C., Shen, S., Loth, E., Srivastava, D. P., & Gallagher, L. (2023). Bridging the translational gap: What can synaptopathies tell us about autism? *Frontiers in Molecular Neuroscience*, *16*, 1191323. https://doi.org/10.3389/fnmol.2023.1191323

Nakanishi, M., Nomura, J., Ji, X., Tamada, K., Arai, T., Takahashi, E., Bućan, M., & Takumi, T. (2017). Functional significance of rare neuroligin 1 variants found in autism. *PLoS Genetics*, *13*(8), e1006940. https://doi.org/10.1371/journal.pgen.1006940

Nguyen, T. A., Lehr, A. W., & Roche, K. W. (2020). Neuroligins and neurodevelopmental disorders: X-linked genetics. *Frontiers in Synaptic Neuroscience*, *12*, 33. https://doi.org/10.3389/fnsyn.2020.00033

Prasad, A., Merico, D., Thiruvahindrapuram, B., Wei, J., Lionel, A. C., Sato, D., Rickaby, J., Lu, C., Szatmari, P., Roberts, W., Fernandez, B. A., Marshall, C. R., Hatchwell, E., Eis, P. S., & Scherer, S. W. (2012). A discovery resource of rare copy number variations in individuals with autism spectrum disorder. *G3: Genes, Genomes, Genetics*, *2*(12), 1665–1685. https://doi.org/10.1534/g3.112.004689

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L., ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*, 405–424. https://doi.org/10.1038/gim.2015.30

Riggs, E. R., Bingaman, T. I., Barry, C.-A., Behlmann, A., Bluske, K., Bostwick, B., Bright, A., Chen, C.-A. N, Clause, A. R., Dharmadhikari, A. V., Ganapathi, M., Gonzaga-Jauregui, C., Grant, A. R., Hughes, M. Y., Kim, S. E. R., Krause, A., Liao, J., Lumaka, A., Mah, M., … Schaaf, C. P. (2022). Clinical validity assessment

of genes frequently tested on intellectual disability/autism sequencing panels. *Genetics in Medicine*, *24*(9), 1899–1908. https://doi.org/10.1016/j.gim.2022.05.001

Satterstrom, F. K., Kosmicki, J. A., Wang, J., Breen, M. S., De Rubeis, S., An, J.-Y., Peng, M., Collins, R., Grove, J., Klei, L., Stevens, C., Reichert, J., Mulhern, M. S., Artomov, M., Gerges, S., Sheppard, B., Xu, X., Bhaduri, A., Norman, U., … Walters, R. K. (2020). Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism. *Cell*, *180*(3), 568–584. e23. https://doi.org/10.1016/j.cell.2019.12.036

Schaaf, C. P., Boone, P. M., Sampath, S., Williams, C., Bader, P. I., Mueller, J. M., Shchelochkov, O. A., Brown, C. W., Crawford, H. P., Phalen, J. A., Tartaglia, N. R., Evans, P., Campbell, W. M., Chun-Hui Tsai, A., Parsley, L., Grayson, S. W., Scheuerle, A., Luzzi, C. D., Thomas, S. K., … Cheung, S. W. (2012). Phenotypic spectrum and genotype-phenotype correlations of NRXN1 exon deletions. *European Journal of Human Genetics*, *20*(12), 1240–1247. https://doi.org/10.1038/ejhg.2012.95

Takata, A., Miyake, N., Tsurusaki, Y., Fukai, R., Miyatake, S., Koshimizu, E., Kushima, I., Okada, T., Morikawa, M., Uno, Y., Ishizuka, K., Nakamura, K., Tsujii, M., Yoshikawa, T., Toyota, T., Okamoto, N., Hiraki, Y., Hashimoto, R., Yasuda, Y., … Matsumoto, N. (2018). Integrative analyses of de novo mutations provide deeper biological insights into autism spectrum disorder. *Cell Reports*, *22*(3), 734–747. https://doi.org/10.1016/j.celrep.2017.12.074

Taşkıran, E. Z., Karaosmanoğlu, B., Koşukcu, C., Ürel-Demir, G., Akgün-Doğan, Ö., Şimşek-Kiper, P. Ö., Alikaşifoğlu, M., Boduroğlu, K., & Utine, G. E. (2021). Diagnostic yield of whole-exome sequencing in non-syndromic intellectual disability. *JIDR*, *65*(6), 577–588. https://doi.org/10.1111/jir.12835

Truty, R., Patil, N., Sankar, R., Sullivan, J., Millichap, J., Carvill, G., Entezam, A., Esplin, E. D., Fuller, A., Hogue, M., Johnson, B., Khouzam, A., Kobayashi, Y., Lewis, R., Nykamp, K., Riethmaier, D., Westbrook, J., Zeman, M., Nussbaum, R. L., & Aradhya, S. (2019). Possible precision medicine implications from genetic testing using combined detection of sequence and intragenic copy number variants in a large cohort with childhood epilepsy. *Epilepsia Open*, *4*(3), 397–408. https://doi.org/10.1002/epi4.12348

Uchigashima, M., Leung, M., Watanabe, T., Cheung, A., Le, T., Pallat, S., Dinis, A. L. M., Watanabe, M., Kawasawa, Y. I., & Futai, K. (2020). Neuroligin3 splice isoforms shape inhibitory synaptic function in the mouse hippocampus. *Journal of Biological Chemistry*, *295*(25), 8589–8595. https://doi.org/10.1074/jbc.AC120.012571

Vaags, A. K., Lionel, A. C., Sato, D., Goodenberger, M., Stein, Q. P., Curran, S., Ogilvie, C., Ahn, J. W., Drmic, I., Senman, L., Chrysler, C., Thompson, A., Russell, C., Prasad, A., Walker, S., Pinto, D., Marshall, C. R., Stavropoulos, D. J., Zwaigenbaum, L., … Scherer, S. W. (2012). Rare deletions at the neurexin 3 locus in autism spectrum disorder. *American Journal of Human Genetics*, *90*(1), 133–141. https://doi.org/10.1016/j.ajhg.2011.11.025

Venselaar, H., Te Beek, T. A., Kuipers, R. K., Hekkelman, M. L., & Vriend, G. (2010). Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics*, [Electronic Resource], *11*, 548. https://doi.org/10.1186/1471-2105-11-548

Wang, T., Hoekzema, K., Vecchio, D., Wu, H., Sulovari, A., Coe, B. P., Gillentine, M. A., Wilfert, A. B., Perez-Jurado, L. A., Kvarnung, M., Sleyp, Y., Earl, R. K., Rosenfeld, J. A., Geisheker, M. R., Han, L., Du, B., Barnett, C., Thompson, E., Shaw, M., … Eichler, E. E. (2020). Large-scale targeted sequencing identifies risk genes for neurodevelopmental disorders. *Nature Communications*, *11*(1), 4932. https://doi.org/10.1038/s41467-020-18723-y

Williams, S. M., An, J. Y., Edson, J., Watts, M., Murigneux, V., Whitehouse, A. J. O., Jackson, C. J., Bellgrove, M. A., Cristino, A. S., & Claudianos, C. (2019). An integrative analysis of non-coding regulatory DNA variations associated with autism spectrum disorder. *Molecular Psychiatry*, *24*(11), 1707–1719. https://doi.org/10.1038/s41380-018-0049-x

Wu, D., Zhu, J., You, L., Wang, J., Zhang, S., Liu, Z., Xu, Q. U., Yuan, X., Yang, L., Wang, W., Tong, M., Hong, Q., & Chi, X. (2023). NRXN1 depletion in the medial prefrontal cortex induces anxiety-like behaviors and abnormal social phenotypes along with impaired neurite outgrowth in rat. *Journal of Neurodevelopmental Disorders*, *15*(1), 6. https://doi.org/10.1186/s11689-022-09471-9

Wu, J., Yu, P., Jin, X., Xu, X., Li, J., Li, Z., Wang, M., Wang, T., Wu, X., Jiang, Y., Cai, W., Mei, J., Min, Q., Xu, Q., Zhou, B., Guo, H., Wang, P., Zhou, W., Hu, Z., … Sun, Z. S. (2018). Genomic landscapes of Chinese sporadic autism spectrum disorders revealed by whole-genome sequencing. *Journal of Genetics and Genomics*, *45*(10), 527–538. https://doi.org/10.1016/j.jgg.2018.09.002

Xu, B., Ho, Y., Fasolino, M., Medina, J., O'brien, W. T., Lamonica, J. M., Nugent, E., Brodkin, E. S., Fuccillo, M. V., Bucan, M., & Zhou, Z. (2023). Allelic contribution of Nrxn1α to autism-relevant behavioral phenotypes in mice. *PLoS Genetics*, *19*(2), e1010659. https://doi.org/10.1371/journal.pgen.1010659

SUPPORTING INFORMATION

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