Human *GRIN2B* variants in neurodevelopmental disorders

Chun Hu[a], **Wenjuan Chen**[a], **Scott J. Myers**[a,b], **Hongjie Yuan**[a,b], and **Stephen F. Traynelis**[a,b,*]

[a]Department of Pharmacology, Emory University School of Medicine, Rollins Research Center, 1510 Clifton Road, Atlanta, GA 30322, USA

[b]Center for Functional Evaluation of Rare Variants (CFERV), Emory University School of Medicine, Rollins Research Center, 1510 Clifton Road, Atlanta, GA 30322, USA

Abstract

The development of whole exome/genome sequencing technologies has given rise to an unprecedented volume of data linking patient genomic variability to brain disorder phenotypes. A surprising number of variants have been found in the *N-methyl*-D-aspartate receptor (NMDAR) gene family, with the *GRIN2B* gene encoding the GluN2B subunit being implicated in many cases of neurodevelopmental disorders, which are psychiatric conditions originating in childhood and include language, motor, and learning disorders, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), developmental delay, epilepsy, and schizophrenia. The *GRIN2B* gene plays a crucial role in normal neuronal development and is important for learning and memory. Mutations in human *GRIN2B* were distributed throughout the entire gene in a number of patients with various neuropsychiatric and developmental disorders. Studies that provide functional analysis of variants are still lacking, however current analysis of de novo variants that segregate with disease cases such as intellectual disability, developmental delay, ASD or epileptic encephalopathies reveal altered NMDAR function. Here, we summarize the current reports of disease-associated variants in *GRIN2B* from patients with multiple neurodevelopmental disorders, and discuss implications, highlighting the importance of functional analysis and precision medicine therapies.

Keywords

NMDA receptor; GluN2B/NR2B; Neuropsychiatric disorders; Developmental delay; Intellectual disability

1. Introduction

N-methyl-D-aspartate receptors (NMDARs) are a family of ionotropic glutamate receptors that mediate a slow, Ca^{2+} permeable component of excitatory synaptic transmission in the

*Corresponding author. Department of Pharmacology, Emory University School of Medicine, Rollins Research Center, 1510 Clifton Road, Atlanta, GA 30322, USA. Fax: +1 404 727 0365. strayne@emory.edu (S.F. Traynelis).

Conflicts of interest

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central nervous system (CNS). NMDARs are a tetrameric assembly of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits. The GluN1 subunit is expressed throughout the CNS, whereas the four subtypes of GluN2 (A–D) subunits have differential temporal and spatial expression patterns (1,2). The GluN2B subunit encoded by *GRIN2B* is highly expressed prenatally, and its expression level starts to decline after birth in most brain regions (1). The early expression of this NMDAR subunit suggests it may play an important role in brain development, circuit formation, and perhaps cellular migration and differentiation, as well as synaptic plasticity (3). GluN2B expression dominates during rapid cortical synaptogenesis in late embryogenesis and early postnatal development (4). Neonate lethality of *GRIN2B* knock-out mice was observed (5), while overexpression of *GRIN2B* in the forebrain of mice enhanced hippocampal long-term potentiation and spatial memory performance (6).

In light of the important role of *GRIN2B* in controlling NMDAR function, the time course of synaptic currents and plasticity, mutation-related disruption in the GluN2B subunit expression or function might give rise to a number of neurodevelopmental phenotypes that reflect alterations in circuit formation, neuronal connectivity, synaptic plasticity, and excitatory transmission. Since the identification of the first disease-causing mutations in NMDARs (7,8) over 60 variants in the *GRIN2B* have been reported in the literature identified in individuals from patient cohorts with a number of neurodevelopmental disorders, including intellectual disability (ID), developmental delay (DD), autism spectrum disorder (ASD), epileptic encephalopathy (EE), schizophrenia (SCZ), and to a lesser extent attention deficit hyperactivity disorder (ADHD), cerebral visual impairment (CVI), and Alzheimer's disease (AD) (Fig. 1 and Table 1). We expect the discovery of additional rare *GRIN2B* genetic variants and *de novo* mutations to grow as more clinics utilize exome sequencing as a tool in diagnostic programs to better understand the molecular basis for neurodevelopmental disorder(s) in pediatric patient populations. *GRIN2B* variants and de novo nonsynonymous mutations with neurological disease has recently been reviewed (55,56). Here we review all missense, nonsense, frameshift, or splice site *GRIN2B* mutations identified in individuals from patient cohorts with defined neurodevelopmental and psychiatric disorders such as ID and DD (7,9–15,17,18,21,29,54), EE (7,13,15,16,53,54), ASD (18–20,22–26,54), SCZ (19,20,26,27), AD(28), and CVI (11). The *GRIN2B* variants identified thus far occurred throughout the entire NMDAR subunit protein. That is, variants were found in the amino-terminal domain (ATD), agonist-binding domain (ABD), transmembrane domain (TM), and carboxyl-terminal domain (CTD) (Fig. 1). In spite of the increasing number of reports of new *GRIN2B* variants due to the recent advances of next-generation sequencing technologies, studies that provide thorough functional analyses of the effects of disease-causing mutations on NMDARs are still lacking. Such functional information is central to understanding the pathogenicity of these *de novo* mutations and rare variants, and thus is an important priority in future genetic studies. Here, we provide a domain-specific review of *GRIN2B* variants revealing that rare *de novo* mutations in the ABD and TM domains, but not the ATD and CTD domains, are absent in the exomes of a large control population sample (Table 1; 35). Furthermore, many of these ABD and TM rare variants result in significant alteration of NMDA receptor channel properties (7,13,16,24,34,54).

2. *GRIN2B* mutations with chromosome translocation

Chromosome translocation involves rearrangement of parts between non-homologous chromosomes. To date, two male subjects with *de novo* chromosome translocations in the *GRIN2B* gene have been reported: t(9;12)(p23;p13.1) and t(10;12)(q21;p13.1) (7). Fluorescence *in situ* hybridization (FISH) studies also found breakpoints in 12p13.1 in both translocations, disrupting the *GRIN2B* gene. Both subjects demonstrated moderate to severe mental disability, behavioral anomalies, and abnormal electroencephalogram (EEG). The 12-year-old subject also had other manifestations such as microcephaly and eye anomalies. Further, a *de novo* chromosome inversion (inv(12)(p13.1q21.31)) and three microdeletions with breakpoints in exon 1 or exon 2 of *GRIN2B* were identified in four patients with similar ID and DD symptoms (7,18,29,30). Thus, the *GRIN2B* gene seems to be critical for neuronal development, and translocation disruption in *GRIN2B* could lead to pronounced cognitive phenotypes. More importantly, functional studies on *GRIN2B* translocations are needed, since disruption in the *GRIN2B* gene might lead to receptor trafficking defects, NMDAR hypofunction, and alteration of endogenous modulator affinities. Interestingly, a translocation disrupting the *GRIN2A* gene (t(16;17)(p13.2;q11.2)), which encodes the GluN2A subunit, was also associated with severe mental disability and seizures in a 26-year-old male subject. This same translocation was identified in 3 similarly affected relatives (7). Further, a microdeletion encompassing the *GRIN2A* gene (16p13.2p13.13) was identified in three patients with variable mild to severe intellectual disability and seizures (8) and overlapping duplications of chromosome 12p points towards *GRIN2B* in a series of patients with a variable DD and ID phenotype (31). Thus, a comparative analysis of *GRIN* translocations and microdeletions could be informative.

3. *GRIN2B* mutations in Amino-Terminal domain (ATD)

The GluN2B ATD is a clamshell-like structure that harbors the binding site for the negative allosteric modulator Zn^{2+}. The GluN2B ATD also forms a heterodimer with the GluN1 ATD, the interface of which comprises a binding site for the negative allosteric modulator ifenprodil (32). Three *de novo* variants have been reported in the *GRIN2B* ATD region from patients with intellectual disability. Interestingly, all three variants are frameshift or splice site mutations leading to premature chain termination of *GRIN2B* transcripts and likely haploinsufficiency. Specifically, the first two *de novo* mutations involving frameshift in *GRIN2B* ATD were reported in Endele et al., 2010 (7), both patients had intellectual disability and behavioral anomalies. A 13-year-old female patient had a heterozygous nucleotide change of c.803_804delCA leading to an altered, p.Thr268SfsX15, GluN2B protein. A male patient also had a heterozygous mutation, c.411+1G>A, and presented with additional symptoms of microcephaly, EEG anomalies, and eye anomalies. The third variant in the *GRIN2B* ATD, p.Ser34GlnfsX25, reported in a patient with autism and intellectual disability, was an insertion of a single nucleotide in the gene leading to a frameshift mutation and early termination of the protein open reading frame (24). Five additional nonsynonymous ATD variants were identified in patient cohorts with schizophrenia symptomology with conservative amino acid changes (26,27). All five were transmitted from a parent with two variants, p.Val65Ile and p.Ala271Val, inherited from a parent that

was asymptomatic (26,27). Of the ATD variants identified, only the frameshift variants p.Thr268SfsX15 and p.Ser34GlnfsX25 were reported to be pathogenic in ClinVar.

4. *GRIN2B* mutations in agonist-binding domain (ABD)

The NMDAR ABD is highly conserved across different subunits and is shaped like a clamshell with two opposite halves of the bilobed domain largely (but not perfectly) corresponding to the S1 and S2 regions of the polypeptide chain (32,33). Glutamate binding to the GluN2B ABD induces a cleft closure within the bilobed ABD, triggering subsequent transition of NMDARs into an open state. Thus far, all six variants identified in the S1 region are missense *de novo* mutations in patients with ID, EE, and ASD (12,13,16,24,34,54), whereas 4 of 13 variants in the S2 domain produce a frameshift or introduce a stop codon. Eleven of twelve variants in S2 are *de novo* mutations in patients with mild to severe ID, DD, ASD or cerebral visual impairment with or without epileptic features (7,11–13,15,17,20,23,24,26,29,54). None of these S1 or S2 ABD mutations are found in the Exome Aggregation Consortium (ExAC) dataset (35, Table 1, Fig. 1).

The *de novo* mutation p.Glu413Gly occurred in a female patient with severe intellectual disability and behavioral anomalies (34). The Glu413 residue in the GluN2B ABD resides in close proximity to several key amino acid residues that make direct atomic contact with the agonist glutamate. Substituting Glu413 with glycine is predicted to disrupt the glutamate binding pocket. Consistent with this prediction, p.Glu413Gly decreased glutamate potency by ~50 fold without altering glycine potency (34,54). Five additional *de novo* mutations, p.Cys461Phe, p.Cys436Arg, p.Cys456Tyr, p.Asp524Asn, and p.Arg540His were reported in the S1 domain of patients with ID, ASD, and childhood onset epilepsy (12,13,16,54). Cys461 resides in the highly conserved glutamate binding pocket wherein mutations on conserved residues often produce changes in receptor function. As expected for variation in this critical region, p.Cys461Phe decreased glutamate potency (54). p.Arg540His is found to produce a mild increase in glutamate and glycine potency, with decreased Mg^{2+} block and elevated Ca^{2+} permeability (13,54), all of which could increase excitatory drive in the absence of homeostatic compensatory mechanisms and contribute to the patient's epileptic phenotype. The p.Cys456Tyr variant resulted in mixed effects on glutamate and glycine potency and the p.Cys436Arg variant appears to be poorly expressed (54).

Thirteen variants in the S2 ABD domain have been implicated in largely the same neurodevelopmental phenotypes as observed for S1. The *de novo* missense mutation, p.Arg682Cys, was found in a male patient with moderate intellectual disability and behavioral anomalies (7,29) and the study of agonist concentration-response curves revealed a slight increase in glycine potency (2-fold) and glutamate potency (1.7-fold) (7,54). The p.Arg696His variant identified in a female patient with severe ID and DD with features of autism also caused an increase in glutamate potency (~5 fold) without altering glycine potency (54). Three frameshift variants(c.2172-2A>G, c.2351-2A>G and c.2360-2A>G) and a mutation introducing a stop codon (p.Gln711X) were reported that ultimately produce truncated proteins (7,20,23,24). All of these individuals had intellectual disability, and these *GRIN2B* alterations are expected to have haploinsufficiency effects, indicating that GluN2B loss-of-function likely contributes to intellectual disability and autism. Although no

functional studies exist for the frameshift mutations in the ABD S2, it seems likely that protein that is made is either degraded, not trafficked to the cell surface, or is non-functional. It is notable and consistent with these protein truncation variants that many of the missense ABD variants, likewise, when expressed in *in vitro* systems, show substantial reductions in cell surface NMDAR expression (Table 1, 54). Multiple additional missense or nonsense de novo mutations in the ABD S2 (p.Gln662Pro, p.Arg682Cys, p.Thr685Pro, p.Pro687Arg, p.Gly689Ser, p.Arg696His, p.Met706Val, and p.Gln711X) have been identified in patients with ID, DD, ASD, cerebral visual impairment, and epilepsy with functional studies reported in just two cases, both which increase glutamate potency (11,12,15,17,21,26,54). Given these mutations reside in the ABD S2, and the variant mutation introduces non-conservative amino acids into the ABD, we anticipate many of these variants are likely to alter GluN2B agonist potency and GluN2B subunit function and thus may contribute to the patient phenotype. As for the S1 domain, ClinVar reports that several S2 variants are likely to be pathogenic, and none of these S2 mutations have been reported in ExAC (35), suggesting there is strong selective pressure against amino acid changes in both the S1 and S2 ABD domains.

5. *GRIN2B* mutations in transmembrane domains (TM)

The NMDAR transmembrane domain (TM) is highly conserved across all glutamate receptors and is connected to the ABD through three short linkers (32). The TM domain is comprised of three transmembrane helices M1, M3 and M4 as well as a reentrant loop M2 that contributes to the formation of the cation-permeable pore. The short linker region proceeding the M1 helix (pre-M1) is connected to ABD S1, oriented parallel to the plane of the plasma membrane, and in van der Waals contact with M3 and M4 in the closed channel (36–38). The transmembrane region is the most highly conserved region of the NMDA receptor family, and includes key determinants of gating and ion permeation. Therefore, mutations or alterations in the sequence of TM might be deleterious and thus result in neurodevelopmental phenotypes.

The missense *de novo* mutation p.Pro553Leu in the pre-M1 region was reported in a patient with intellectual disability(9). Since the pre-M1 helix is positioned as a cuff of the external surface of the NMDAR channel pore, the pre-M1 helix has been suggested to be involved in channel gating. Although no functional studies have been conducted, alterations in Pro553 might potentially influence channel gating, activation, deactivation, or desensitization time course. Additionally, a nonsense variant (p.Trp559X) in the M1 region was identified in a patient with intellectual disability and autism (24), and a missense de novo mutation (p.Val558Ile) was reported in an individual with intellectual disability (12). The premature stop codon almost certainly creates a dysfunctional NMDAR protein product, particularly when the mutation occurs at a position close to the translation initiation site in the sequence. Several missense variants were identified in the M1–M2 linker (p.Ala590Thr), the M2 domain (p.Trp607Cys, p.Gly611Val, p.Asn615Ile, p.Val618Gly, p.Val620Met), the M2–M3 linker (p.Ser628Phe), or the M3 domain (p.Ala636Val, p.Ala636Pro, p.Ala639Val), with the majority of these variants identified as de novo in individuals with ID and features of epilepsy, ADHD, and ASD (7,13,17,29,54). Two M2 missense mutations (p.Asn615Ile and p.Val618Gly) enhance channel function by reducing Mg^{2+} block and increasing Ca^{2+}

permeability of the channel, two effects that may contribute to the ID and West syndrome phenotype (13). The majority of these variants are reported in ClinVar to be likely pathogenic and none, except p.Ala590Thr, are in ExAC. Four *de novo* missense mutations have been identified in the M4 domain and a single de novo mutation in the S2-M4 linker in patients with ID, DD, epilepsy or autism. However, functional studies for these variants have not been reported (10,17,19,26,53). Similar to the ABD variants, transmembrane domain variants are rare (<0.0008%), given these transmembrane domain mutations are not present in ExAC.

6. *GRIN2B* mutations in carboxyl-terminal domain (CTD)

The NMDAR intracellular CTD is variable in sequence and contains phosphorylation sites and short docking motifs for binding proteins that play important roles in receptor trafficking and function (32). Currently, eleven missense variants (7,25–28), a frameshift (14), and a single amino acid deletion (26) in GluN2B CTD have been reported in patients with ID, ASD, and SCZ (See Table 1). The missense mutations, p.Gly1026Ser, p.Ala1267Ser, p.Thr1273Lys, or p.Met1339Val, in each of these patients was inherited from a parent that did not present with the same phenotype as their affected offspring. Three CTD variants, p.Thr1228Met, p.Thr1273Lys, and p.Met1339Val were not associated with risk for ASD by burden analysis when compared to controls, despite the finding that common synonymous variants (SNPs) in the same region showed significant risk for autism, suggesting that ASD may be modified by genetic regulation or environmental context for these *GRIN2B* variants (25). Ten of the reported CTD variants (Table 1) have also been reported in ExAC with varying minor allele frequencies, suggesting that selective evolutionary pressure across CTD domain variations is lower in the CTD than for TM and ABDs. Functional data for these CTD variants is lacking, but mutations in the CTD region may cause trafficking defects due to the residence of several ER retention signals in this region (39), which has been postulated to contribute to the phenotype of the p.Lys1293Arg *de novo* variant identified in an AD patient (28). In addition, regulation of NMDAR function through post-translational modification of the CTD is well known (32), and could be altered by missense mutations at sites of or adjacent to those subject to posttranslational modification. Numerous scaffolding proteins and intracellular proteins interact with the GluN2B CTD, including PDZ family proteins such as LIN7, PSD95, SAP97, and SAP102, cytoskeletal protein α-actinin-2, scaffold protein RACK1, and adaptor protein AP2 (40–48). Disrupting these interactions is another means by which missense mutations or truncation mutations could alter NMDAR localization or function.

7. Conclusion

The advent of whole exome/genome sequencing techniques over the last 20 years has provided researchers with unprecedented data relating genomic variations to the various neurodevelopmental disorder phenotypes, including autism, intellectual disability, epilepsy, and ADHD. These data have shown that the *GRIN2B* gene has less variation in the healthy population than normally expected, and is associated with epilepsy (49,50). Many of the mutations in *GRIN2B* that are associated with neurodevelopmental disorders, such as intellectual disabilities, are loss-of-function mutations resulting from frameshift, nonsense,

or missense mutations in key conserved residues. This early data raises the possibility that mutations that reduce expression or function of GluN2B may alter development of the central nervous system and contribute to intellectual delay or disability, epilepsy and autism. *De novo* missense mutations in critical TM and ABD regions, or frameshift or nonsense mutations leading to truncated proteins, were absent from SCZ patient cohorts. Rather, these cohorts reveal missense mutations in the ATD or CTD domains where ExAC allele frequencies trend higher and association with SCZ may be variable or low (27), consistent with a lack of association between clozapine response and *GRIN2B* variants in a SCZ patient population (51). Notably, the agonist binding domain of GluN2B exhibits significant intolerance to mutation based on absence of these variants in ExAC control exomes (Table 1, Fig. 1B). Furthermore, the ABD of GluN2B is less tolerant to variation than the analogous regions of GluN2A (54). Identification of a means of tailoring precision medicine for this target patient populations with *GRIN2B* variants will be challenging, since only 14% of the >60 published mutations in *GRIN2B* implicated in neurodevelopmental phenotypes have complementary functional analysis reported. Moreover, many of the functional studies are incomplete. In addition, there is only minimal information (5) about how *GRIN2B* deletion, truncation or mutation might alter the developing nervous system. It seems likely that compensatory mechanisms will alter expression of other NMDAR subunits in the absence of functional GluN2B. Furthermore, there are no FDA-approved compounds known to enhance NMDAR function; only polyamines, aminoglycosides, and neurosteroids, which show low-potency GluN2B-selective potentiation (32,52). Thus, in order to exploit precision medicine for this condition, the screening of FDA-approved drug libraries for compounds that can rectify mutation-associated functional changes is needed. Such screens would likely assess parameters such as co-agonist potency, channel open probability, receptor trafficking and surface expression, receptor desensitization, and the sensitivity of the receptor to known endogenous channel modulators (e.g. H^+, Mg^{2+} and Zn^{2+}). Identifying candidate compounds that might rectify some aspects of GluN2B hypofunction will provide a starting point for investigation of these agents in model systems, which will ultimately help to advance the future development of therapeutic strategies.

Acknowledgments

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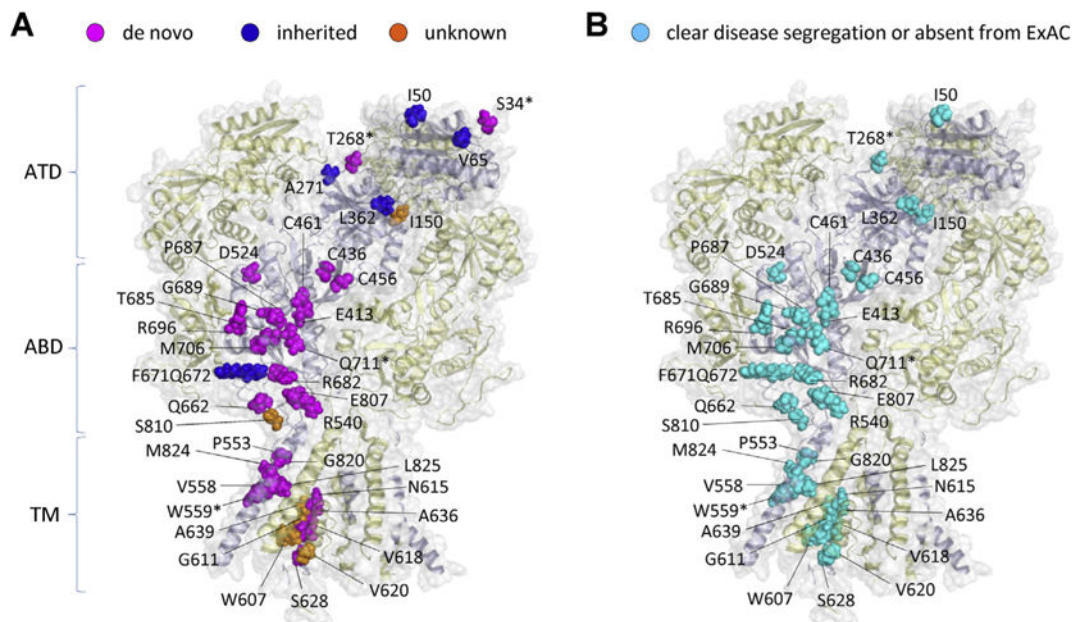
Fig. 1. Locations of GluN2B mutations

Ribbon structures of a tetramer GluN1/GluN2B receptor (37,38) illustrates receptor architecture (light yellow: GluN1; light blue: GluN2B). The amino terminal domain (ATD) is shown, the S1 and S2 regions describe two portions of the polypeptide chain that comprise the agonist binding domain (ABD), and three transmembrane helices (M1, M3, M4) and the M2 re-entrant pore loop comprise the transmembrane domain (TM). **A**, Residues harboring *de novo* mutations are highlighted in MAGENTA, transmitted mutations in BLUE, and variants of unknown origin in ORANGE. **B**, Residues harboring mutations in human patients with a clear disease segregation or absent from ExAC are highlighted in CYAN. In both panels an (*) indicates that the variant results in a truncated protein. The residue alanine at position 590 is not shown due to poor resolution of crystal structure in this region. The variants V18 in the signal peptide, and Q1014, G1026, R1099, T1228, A1267, T1273, K1293, M1331, M1339, N1352, S1415, L1424, S1452 in the carboxyl terminal domain (CTD) are not present in the crystal structure and therefore not shown.

Table 1

GRIN2B subunit variants identified in neurodevelopmental disorders.

Variant[a]	Genotype	Domain[b]	Origin[c]	Phenotype[d]	Functional validation[e]	Refs.	ExAC[f] #, MAF	ClinVar significance, phenotype[g]
p.Val18Ile	c.52G>A	ATD	Trans[h]	SCZ		26	7, 0.0059%	Uncertain, NS
p.Ser34GlnfsX25	c.99dupC	ATD	DN	ASD, ID		24	27, 0.0223%	Pathogenic, MR
p.Ile50Asn	c.149T>A	ATD	Trans[h]	SCZ		26		
p.Val65Ile	c.193G>A	ATD	Trans	SCZ*		27	1, 0.0008%	
c.411 + 1 G>A (splice)	c.411 + 1 G>A	ATD	DN	ID		7		
p.Ile150Val	c.448A>G	ATD				54		
p.Ala271Val	c.812C>T	ATD	Trans	SCZ*		26,27	34, 0.0280%	Pathogenic, MR
p.Thr268SfsX15	c.803_804delCA	ATD	DN	ID, ADHD		7,29		Pathogenic, MR
p.Leu362Met	c.1084C>A	ATD	Trans[h]	SCZ		26		
p.Glu413Gly	c.1238A>G	ABD (S1)	DN	ID	↑ Glu EC$_{50}$, ↓ τ$_w$, ↓ expression	34,54		Pathogenic, MR
p.Cys436Arg	c.1306T>C	ABD (S1)	DN	ID	↓ expression	54		
p.Cys456Tyr	c.1367G>A	ABD (S1)	DN	ASD, ID	↓ Glu EC$_{50}$, ↑ Gly EC$_{50}$, ↓ expression	24,54		Pathogenic, MR
p.Cys461Phe	c.1382G>T	ABD (S1)	DN	ID, ASD, Epi	↑ Glu EC$_{50}$, ↓ Gly EC$_{50}$, ↓ τ$_w$, ↓ expression	16,54		
p.Asp524Asn	c.1570G>A	ABD (S1)	DN	ID		12		
p.Arg540His	c.1619G>A	ABD (S1)	DN	ID, Epi	↓ Glu EC$_{50}$, ↓ Gly EC$_{50}$, ↑ τ$_w$, ↓ expression, ↑ Mg^{2+} IC$_{50}$	13,54		Pathogenic, EE
p.Pro553Leu	c.1658C>T	linker	DN	ID		9		Pathogenic, MR
p.Val558Ile	c.1672G>A	M1	DN	ID		12		
p.Trp559X	c.1677G>A	M1	DN	ASD, ID		24		Pathogenic, MR
p.Ala590Thr	c.1768G>A	linker	Trans	ID		7	19, 0.0157%	Uncertain, NS
p.Trp607Cys	c.1821G>T	M2		DD, ID		21		
p.Gly611Val	c.1832G>T	M2				54		Likely pathogenic, NS
p.Asn615Ile	c.1844A>T	M2	DN	ID, West	↑ Mg^{2+} IC$_{50}$	13		Pathogenic, EE
p.Val618Gly	c.1853T>G	M2	DN	ID, West	↑ Mg^{2+} IC$_{50}$	13		Pathogenic, EE
p.Val620Met	c.1858G>A	M2				54		Likely pathogenic, EE
p.Ser628Phe	c.1883C>T	linker	DN	ID, DD		17		

Variant[a]	Genotype	Domain[b]	Origin[c]	Phenotype[d]	Functional validation[e]	Refs.	ExAC[f] #, MAF	ClinVar significance, phenotype[g]
p.Ala636Pro	c.1906G>C	M3	DN	ID, ADHD		29		
p.Ala636Val	c.1907C>T	M3				54		Likely pathogenic, NS
p.Ala639Val	c.1916C>T	M3				54		Pathogenic, IGD
p.Gln662Pro	c.1985A>C	ABD (S2)	DN	ID, Epi		15		
p.Phe671_Gln672del	c.2011-5_2011-4delTC	ABD (S2)	Trans	ID, Epi		13		
p.Arg682Cys	c.2044C>T	ABD (S2)	DN	ID	$\downarrow$ Glu EC$_{50}$, $\downarrow$ Gly EC$_{50}$, $\uparrow$ τ_w	7,29,54		Pathogenic, MR
p.Thr685Pro	c.2053A>C	ABD (S2)	DN			54		Pathogenic, EE
p.Pro687Leu	c.2060C>T	ABD (S2)				54		Likely pathogenic, NS
p.Pro687Arg	c.2060C>G	ABD (S2)	DN	ID, DD		17		
p.Gly689Ser	c.2065G>A	ABD (S2)	DN	CVI, ID		11		Likely pathogenic, MR
p.Arg696His	c.2087G>A	ABD (S2)	DN	ID, DD, ASD	$\downarrow$ Glu EC$_{50}$, $\uparrow$ τ_w, $\downarrow$ expression	54		
p.Met706Val	c.2116A>G	ABD (S2)	DN	ID		12		
p.Gln711X	c.2131C>T	ABD (S2)	DN	ASD		20		
c.2172-2A>G (splice)	c.2172-2A>G	ABD (S2)	DN	ASD, ID		23,24		Pathogenic, MR
c.2351-2A>G (splice)	c.2351-2A>G	ABD (S2)	DN	ASD, ID		23		
c.2360-2A>G (splice)	c.2360-2A>G	ABD (S2)	DN	ID		7		
p.Glu807Lys	c.2419G>A	linker	DN	ID, DD		17		
p.Ser810Arg	c.2430C>A	linker				54		Pathogenic, NS
p.Gly820Glu	c.2459G>A	M4	DN	ID		10		
p.Gly820Ala	c.2459G>C	M4	DN	ID, DD		17		Conflicting interpretations
p.Gly820Val	c.2459G>T	M4				54		Likely pathogenic, NS
p.Met824Arg	c.2471T>G	M4	DN	ID, DD, Epi		53		
p.Leu825Val	c.2473T>G	M4	DN	ASD		19,26		
p.Gln1014Arg	c.3041A>G	CTD	Trans[h]	SCZ		26		
p.Gly1026Ser	c.3076G>A	CTD	Trans	ASD, SCZ*		26,27	30, 0.0247%	Uncertain, NS
p.Arg1099AlafsX51	c.3295del	CTD	DN	ID		14		
p.Thr1228Met	c.3683C>T	CTD		ASD		25	3, 0.0033%	
p.Ala1267Ser	c.3799G>T	CTD	Trans	ID, SCZ*		7,27	40, 0.0330%	Uncertain, NS
p.Thr1273Lys	c.3818C>A	CTD	Trans	ASD*		25	52, 0.0429%	Uncertain, NS
p.Lys1293Arg	c.3878A>G	CTD	DN	AD		28	1, 0.0008%	
p.Met1331Ile	c.3993G>A	CTD		ID		7	6, 0.0049%	

Variant[a]	Genotype	Domain[b]	Origin[c]	Phenotype[d]	Functional validation[e]	Refs.	ExAC[f] #, MAF	ClinVar significance, phenotype[g]
p.Met1339Val	c.4015A>G	CTD	Trans	ASD*		25		
p.Asn1352del	c.4056_4058delCAA	CTD	Trans[h]	SCZ		26	1, 0.0008%	
p.Ser1415Leu	c.4244C>T	CTD	Trans[h]	ASD		26	1, 0.0008%	
p.Leu1424Phe	c.4270C>T	CTD	Trans[h]	SCZ		26	3, 0.0025%	
p.Ser1452Phe	c.4355C>T	CTD	Trans[h]	SCZ		26	2, 0.0016%	Uncertain, NS
chr translocation	t(9;12)(p23;p13)	–	DN	ID, ADHD		7,29		
chr translocation	t(10;12)(q21;p13)	–	DN	ID, ADHD		7,29		
chr inversion	inv(12) (p13.1q21.31)	–	DN	ID, ASD, Epi		18		
chr microdeletion	12p13.1	exon 1–2	DN	ID, DD, Epi		30		
chr duplication	Multiple 12p variants	–	DN, Trans	DD, ID		31		

[a] fs: frameshift, splice: donor or acceptor splice site, X: termination codon, del: deletion, chr: chromosome.

[b] ATD: amino terminal domain, ABD: agonist binding domain (S1) and (S2), M1, M2, M3, and M4: membrane-associated domains, CTD: carboxyl terminal domain.

[c] DN: *de novo* mutation, Trans: transmitted from parent.

[d] ID: Intellectual Disability, DD: Developmental Delay, AD: Alzheimer's Disease, ASD: Autism Spectrum Disorder, ADHD: Attention Deficit Hyperactivity Disorder, SCZ: Schizophrenia or Schizoaffective Disorder, Epi: epilepsy and/or seizures, infantile spasms, CVI: Cerebral Visual Impairment, WEST: West syndrome, * indicates the disorder phenotype was not present in the transmitting parent.

[e] Glu, glutamate; Gly, Glycine; upward, downward arrows indicate increase or decrease in measured value, respectively; τ_W, deactivation rates determined by fitting a two-component exponential function to determine a weighted tau (τ_W).

[f] ExAC dataset of 60,706 control exomes, release 0.3.1 March 2016; MAF = minor allele frequency.

[g] www.ncbi.nlm.nih.gov/clinvar; Access date 28 Aug 2016; MR = Mental retardation; EE = Epileptic encephalopathy; IGD = Inborn Genetic Disease; NS = Not Specified. ClinVar and ExAC only reported when entries were present; for ExAC, no entry suggests MAF <0.0008%.

[h] Transmission status by personal communication with the group of Dr. Guy A. Rouleau.