Critical review

Human GRIN2B variants in neurodevelopmental disorders

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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.

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1. Introduction

N-methyl-D-aspartate receptors (NMDARs) are a family of ionotropic glutamate receptors that mediate a slow, Ca2+-permeable component of excitatory synaptic transmission in the 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
significantly alteration of NMDA receptor channel properties.

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 dysfunction, 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.
Table 1

| Variant | Genotype | Domain | Origin | Phenotype | Functional validation | Refs. | ExAc †, MAF | ClinVar significance, phenotype |
|---------|----------|--------|--------|-----------|-----------------------|-------|-------------|----------------------------------|
| p.Val181Le | c.52G>A   | ATD | Trans  | SCZ |  | 26 | 7, 0.0059% | Uncertain, NS |
| p.Ser134Gln|x.26C>T | ATD | DN | ASD, ID | 24 | 27, 0.0223% | Pathogenic, MR |
| p.Leu50Asn | c.149T>A | ATD | Trans  | SCZ |  | 26 |  |  |
| p.Val65Glu | c.193G>A | ATD | Trans  | SCZ |  | 27 | 1, 0.0008% |  |
| p.Asn1352del | c.4056_4058delCAA | ATD | DN | ID |  |  |  |  |
| p.Glu143Gly | c.4270C>G | ATD | DN | ID |  |  |  |  |
| p.Asn1024Val | c.3055G>A | ATD | DN | ID, DD | 21 |  |  |  |
| p.Asn1024Val | c.3055G>A | ATD | DN | ID, DD | 21 |  |  |  |
| p.Val1023Leu | c.3076G>T | ATD | DN | ID, DD | 21 |  |  |  |
| p.Glu1029Glu | c.3081C>T | ATD | DN | ID, DD | 21 |  |  |  |
| p.Asn1339Val | c.3993G>A | ATD | DN | ID |  |  |  |  |
| p.Asp505Glu | c.1516G>A | ATD | DN | ID, DD | 21 |  |  |  |
| p.Asn1024Val | c.3055G>A | ATD | DN | ID, DD | 21 |  |  |  |

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Table 1 (continued)

| Variant | Genotype | Domain | Origin | Phenotype | Functional validation | Refs | ExAC % | MAF | ClinVar significance, phenotype |
|---------|----------|--------|--------|-----------|-----------------------|------|--------|-----|------------------------------|
| 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) | (p13.1q21.13) | – | DN | ID, ASD, Epi | 18 | | | |
| chr microdeletion | 12p11.1 | exon 1-2 | DN, Trans | ID, DD, Epi | 30 | | | | |
| chr duplication | Multiple | 12p variants | | | | | | | |

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; \( \tau_w \): deactivation rates determined by fitting a two-component exponential function to determine a weighted \( \tau_w \).

f: 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.

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

The GluN2B ATD is a clasp-like structure that harbors the binding site for the negative allosteric modulator Zn\(^{2+}\). The GluN2A ATD also forms a heterodimer with the GluN1 ATD, the interface of which comprises a binding site for the negative allosteric modulator ifenprodil. 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, Phe268SerfsX15, 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. Five additional nonsynonymous ATD variants were identified in patient cohorts with schizophrenia symptomology with conservative amino acid changes. All five were transmitted from a parent with two variants, p.Val65Ile and p.Ala271Val, inherited from a parent that was asymptomatic. Of the ATD variants identified, only the frameshift variants p.Thr268SerfsX15 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 clasp with two opposite halves of the bilobed domain largely (but not perfectly) corresponding to the S1 and S2 regions of the polypeptide chain. 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, 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. 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. 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. Five additional de novo mutations, p.Cys461Phe, p.Cys436Arg, p.Cys456Tyr, p.Arg540His, and p.Arg540His were reported in the S1 domain of patients with ID, ASD, and childhood onset epilepsy. 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. p.Arg540His is found to produce a mild increase in glutamate and glycine potency, with decreased \( \mathrm{Mg}^{2+} \) block and elevated \( \mathrm{Ca}^{2+} \) permeability, of which could increase excitatory drive in the absence of homeostatic compensatory mechanisms and contribute to the patient’s epileptic phenotype. p.Cys465Tyr variant resulted in mixed effects on glutamate and glycine potency and the p.Cys436Arg variant appears to be poorly expressed. 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 and the study of agonist concentration—response curves revealed a slight increase in glycine potency (2-fold) and glutamate potency (1.7-fold). 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. 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 introducing a stop codon (p.Gln711X) were reported that ultimately 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 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 (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 pre-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 those subject to posttranslational modifications. The premature stop codon almost certainly creates a dysfunctional NMDAR protein product, particularly when those subject to posttranslational modifications. 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.

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 presence 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 co- horts 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 inhibitors (e.g. H+ , Mg2+ and Zn2+). 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.

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Conflicts of interest

S.F.T. is a cofounder of NeurOp Inc., and a paid consultant of NeurOp Inc, Janssen Pharmaceutica, and Pfizer Inc.

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