REVIEW

Disease-Associated Variants in *GRIN1*, *GRIN2A* and *GRIN2B* genes: Insights into NMDA Receptor Structure, Function, and Pathophysiology

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Summary

N-methyl-D-aspartate receptors (NMDARs) are a subtype of ionotropic glutamate receptors critical for synaptic transmission and plasticity, and for the development of neural circuits. Rare or *de-novo* variants in *GRIN* genes encoding NMDAR subunits have been associated with neurodevelopmental disorders characterized by intellectual disability, developmental delay, autism, schizophrenia, or epilepsy. In recent years, some disease-associated variants in *GRIN* genes have been characterized using recombinant receptors expressed in non-neuronal cells, and a few variants have also been studied in neuronal preparations or animal models. Here we review the current literature on the functional evaluation of human disease-associated variants in *GRIN1*, *GRIN2A* and *GRIN2B* genes at all levels of analysis. Focusing on the impact of different patient variants at the level of receptor function, we discuss effects on receptor agonist and co-agonist affinity, channel open probability, and receptor cell surface expression. We consider how such receptor-level functional information may be used to classify variants as gain-of-function or loss-of-function, and discuss the limitations of this classification at the synaptic, cellular, or system level. Together this work by many laboratories worldwide yields valuable insights into NMDAR structure and function, and represents significant progress in the effort to understand and treat *GRIN* disorders.

Keywords

NMDA receptor • *GRIN* genes • Genetic variants • Electrophysiology • Synapse • Animal models

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Introduction

N-methyl-D-aspartate receptors (NMDARs), encoded by *GRIN* genes, are ionotropic glutamate receptors present at virtually all excitatory synapses in the central nervous system. Classical NMDARs have a characteristic biophysical signature, with a requirement for the binding of two agonists (glutamate and glycine/D-serine), strong block by Mg^{2+} at resting membrane potentials, high Ca^{2+} permeability, and relatively slow activation and deactivation kinetics [1]. These properties enable NMDARs to serve as coincidence detectors of presynaptic glutamate release and postsynaptic depolarization that removes the Mg^{2+} block. The resulting NMDAR-mediated Ca^{2+} influx is a key signal regulating activity-dependent changes in synaptic strength [2] that underlie the development of neural circuits and their

ability to process and store information [1]. Given this physiological role, dysfunction of NMDAR signaling is considered to be a significant factor in the etiology of diverse neurological disorders. In recent years, rare or *de-novo* variants of *GRIN* genes have been identified in patients with neurodevelopmental syndromes characterized by intellectual disability, developmental delay, with features of autism, schizophrenia, or epilepsy [3], sometimes collectively referred to as *GRIN* disorders.

There are seven NMDAR genes, *GRIN1*, *GRIN2A-D* and *GRIN3A-B*, encoding GluN1, GluN2A-D and GluN3A-B subunits, respectively. NMDARs are assembled as heterotetramers of two obligatory GluN1 subunits and two GluN2 and/or GluN3 subunits [1]. Aside from the GluN1 subunit, GluN2A and GluN2B are the most abundant subunits in principal neurons in the forebrain [4], with the GluN2B expression beginning early during embryonic development, followed by the expression of GluN2A postnatally; the expression of both GluN2A and GluN2B persists until and throughout adulthood [1]. NMDAR complexes can be diheteromeric (i.e., containing two GluN1 and two identical GluN2 subunits) or triheteromeric (containing two GluN1 and two different GluN2 subunits). Receptors containing different combinations of GluN2 subunits have different functional and pharmacological properties [5] and likely play different physiological roles [6]. It has been suggested that the majority of NMDARs in the adult forebrain are GluN1/GluN2A/GluN2B triheteromers [7,8] (but see [9]), whose structure and function has been explored in recent years, thanks to new methods for selectively expressing or isolating triheteromeric receptor complexes [5,10,11].

NMDAR subunits have a modular design: each subunit contains an extracellular amino-terminal domain (ATD), an agonist-binding domain (ABD), a transmembrane domain (TMD), and a cytoplasmic carboxy-terminal domain (CTD), interconnected by polypeptide linkers [1]. Recent structural studies of NMDAR complexes in different conformational states [12] or exploring receptor conformational dynamics [13,14,15] have greatly improved our understanding of NMDARs at the molecular level. NMDAR gating is a coordinated process involving agonist-induced structural rearrangements of the core gating machinery formed by the ABDs and the TMDs of all four subunits. The gating mechanism is regulated by voltage-dependent channel block by Mg^{2+}, and by endogenous allosteric modulation by H^+, Zn^{2+}, Ca^{2+}, and several classes of naturally present small molecules [16]. All domains participate in endogenous allosteric modulation. The role of the ATDs in mediating the effects of H^+ and Zn^{2+} is particularly well described [17]. Our own recent work has contributed to the characterization of NMDAR modulation by endogenous neurosteroids [18,19,20,21,22] and membrane cholesterol [23,24], that interact primarily with the TMDs. Ca^{2+}-dependent NMDAR modulation involves membrane-proximal regions of the CTDs [25,26,21]. While the intrinsically disordered CTDs resist structural characterization, they are known to contain residues that can undergo post-translational modification, and binding sites for intracellular signaling and scaffolding proteins. The CTDs thus play an important role in regulating NMDAR trafficking, signaling, and even gating [27]. Disease-associated *GRIN* gene variants are found in all receptor domains, and the variant pathogenic effects may be related to any aspect of receptor function, including the gating process, as well as changes in receptor allosteric modulation, post-translational modification, protein-protein interactions or trafficking.

Either excessive or insufficient NMDAR function is associated with neurological or neuropsychiatric disorders, suggesting a narrow physiological window of NMDAR activity [4]. Consistent with this, *GRIN* genes have low frequencies of variation in the healthy population [28,29,3,1]. In recent years, many *GRIN* gene variants have been identified through high-throughput sequencing in patients with neurological and neuropsychiatric symptoms [28,30,3]. To date, over 700 disease-associated *GRIN* gene variants absent in the healthy population (https://gnomad.broadinstitute.org) have been described, distributed among the different NMDAR subunits, but predominantly found in GluN2A (311 variants, 43 %), GluN2B (258 variants, 35 %) and GluN1 (91 variants, 13 %) [1]. Several different databases collect information about disease-associated *GRIN* gene variants (https://www.ncbi.nlm.nih.gov/clinvar; https://webapp2.pharm.emory.edu/cferv; https://www.grin-database.de/gen_table; https://alf06.uab.es/grindb/home). Most frequent are missense variants in exon regions that result in a single amino acid substitution in the protein sequence. Some patients carry nonsense or frameshift variants that introduce a premature stop codon. Variants in 5′ or 3′ untranslated regions, introns, or splice sites, or large-scale chromosomal rearrangements have also been reported [31]. In an overwhelming majority of cases the variants arise *de novo* and the variant is present in only one allele of the affected gene [3].

To understand how different variants may lead to

disease, many groups have embarked on functional characterization of individual patient-derived *GRIN* gene variants. Here we review the published reports of the functional evaluation of disease-associated variants in *GRIN1*, *GRIN2A*, and *GRIN2B* genes. Most of the reports so far have examined recombinant receptors composed of GluN subunits heterologously expressed in non-neuronal cell types, such as *Xenopus* oocytes or HEK293 cells. Typically, these studies use well-established patch-clamp electrophysiology and immunofluorescence microscopy methods, allowing some comparison across studies and research groups. We have focused on four parameters most commonly used to assess NMDAR function: receptor affinity for glutamate, receptor affinity for glycine, channel open probability (P_o), and receptor cell surface expression. Based on such parameters, pathogenic variants can be classified as loss-of-function (LoF) or gain-of-function (GoF), and therefore as candidates for therapeutic positive or negative pharmacological modulation, respectively. We discuss whether and how the variant position within the subunit amino acid sequence may predict its receptor-level functional consequence(s) [32] and its LoF or GoF classification.

Individual variant pathogenicity at the receptor level, however, may not accurately predict its functional effects at the synaptic, cellular, or system level. Relatively fewer reports include functional data from *in-vitro* neuronal preparations and only a handful of *in-vivo* studies in animal models exist to date. We briefly summarize this work and argue for the need to include these more physiologically relevant experimental models in future studies of the etiology of *GRIN* disorders.

Functional characterization of *GRIN* gene variants in non-neuronal systems

Our literature search found 74 studies [32a] of recombinant receptors heterologously expressed in non-neuronal systems (Tables 1, 2, 3) together reporting functional data for 45 disease-associated GluN1 variants, 72 GluN2A variants, and 70 GluN2B variants (49 %, 23 %, and 27 % of disease-associated GluN1, GluN2A, and GluN2B variants reported in [1], respectively). This represents a substantial body of work that is beginning to yield insights not only into the mechanisms of pathogenicity of different *GRIN* gene variants, but also into NMDAR structure and function more generally.

Importantly, patients are typically heterozygous for their *GRIN* gene variant, so any individual patient's NMDARs may be functionally heterogeneous: some may be composed entirely of unaffected subunits and some may include one or two subunits containing the potentially pathogenic variant combined with unaffected subunits of various types. It is therefore necessary to study not only diheteromeric receptors containing two variant subunits, but also triheteromeric receptors containing only one potentially pathogenic variant subunit. Typically, the function of triheteromeric receptors containing one variant subunit was found to be intermediate between the function of wild-type receptors and diheteromeric receptors containing two variant subunits [33,34,35,36,37,38]. For some variants, triheteromeric receptors show a functional deficit comparable to diheteromeric receptors (e.g., GluN2A-D731N [39]; GluN2A-N616K [40]). While studies of triheteromeric receptors are essential, here we focus on results obtained for diheteromeric receptors (Tables 1, 2, 3, Figs 1, 2, 3, 4), because these data represent the majority of the available information.

It is useful when studies directly compare effects of different variants, ideally with multiple functional parameters assessed in the same system [34,41,42,40,43,44,45]. These data often illustrate that individual variants can affect multiple functional parameters, sometimes in contradictory directions, complicating simple variant classification as LoF or GoF [43,44,45]. Some studies have proposed ways to integrate information about multiple functional parameters into one readout, for example by estimating how a variant would affect synaptic charge transfer or Ca^{2+} influx, to evaluate the overall impact of the variant on physiologically relevant NMDAR signaling [34,44,46].

Though imperfect, variant classification as LoF or GoF has prompted pharmacological investigations with the goal of identifying suitable NMDAR modulators that could correct the functional change associated with different disease-associated variants. Most generally, negative allosteric modulators show promise in treating certain GoF variants [47,48], while positive modulators may compensate for the effects of LoF variants [49,50]. Ultimately, pharmacological intervention should be more sophisticated and specifically target the functional parameters altered by the variant. Importantly, individual variants can show reduced or enhanced sensitivity to different modulators [51,42,52,53,50,48], further emphasizing the need to tailor therapy to the specific variant present. While we do not discuss rescue pharmacology of disease-associated *GRIN* gene variants any further in this review, the relevant references are

included. Several clinical case studies illustrate the benefit of pharmacological treatment for some carriers of pathogenic *GRIN* gene variants [54,28,55,56,57,58], and it is results like these that motivate the continued effort to characterize the functional impact of different *GRIN* gene variants in order to design appropriate therapy.

Glutamate and glycine affinity

The ABD of the GluN1 subunit binds the NMDAR co-agonist glycine or D-serine, while the ABDs of GluN2 subunits bind the agonist glutamate. NMDAR affinity for glutamate depends on the types of GluN2 subunits present in the receptor complex, with GluN2A associated with a lower glutamate affinity than GluN2B [1]. Receptor affinity for glutamate influences the rate of receptor deactivation following a brief synaptic-like glutamate transient [59], such that a lower-affinity receptor–glutamate interaction (typical for GluN2A-containing receptors) results in faster kinetics of receptor deactivation, while a higher-affinity interaction (characteristic for GluN2B-containing receptors) leads to slower deactivation. Receptor deactivation kinetics in turn influence the charge transfer and the Ca^{2+} influx through synaptically activated NMDARs, with consequences for downstream signalling [1]. In addition, the quality control of the nascent receptor complexes in the endoplasmic reticulum (ER) is based, in part, on glutamate and glycine affinity, and receptors with very low agonist and/or co-agonist affinity fail to reach the cell surface [60].

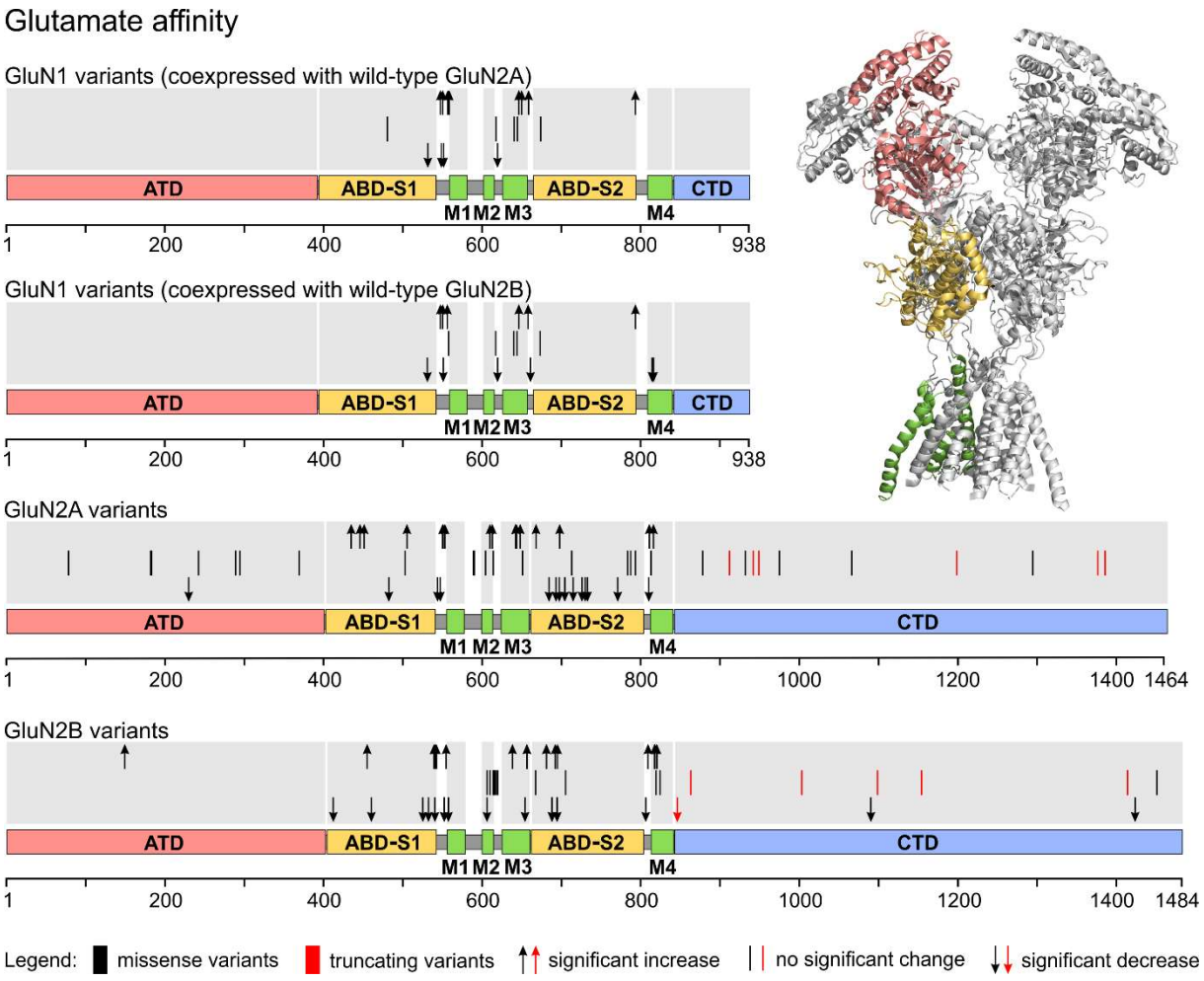

Fig. 1. Effects of disease-associated *GRIN* gene variants on glutamate affinity. Relationship between the amino acid positions of individual variants (scale shown below the schematic of the domain structure of each subunit) and the observed effect on glutamate affinity (increase, decrease, no change), as determined for diheteromeric recombinant receptors in non-neuronal cells. ATD, amino-terminal domain; ABD-S1 and ABD-S2, agonist-binding domain segments S1 and S2; M1–M4, transmembrane domain (TMD) helices; CTD, carboxy-terminal domain. Inset shows human GluN1/GluN2A diheteromeric complex (PDB: 7EU7) with GluN1 ATD, ABD, and TMD highlighted in colors corresponding to the schematic of the domain structure; the CTD is not included in this receptor structure.

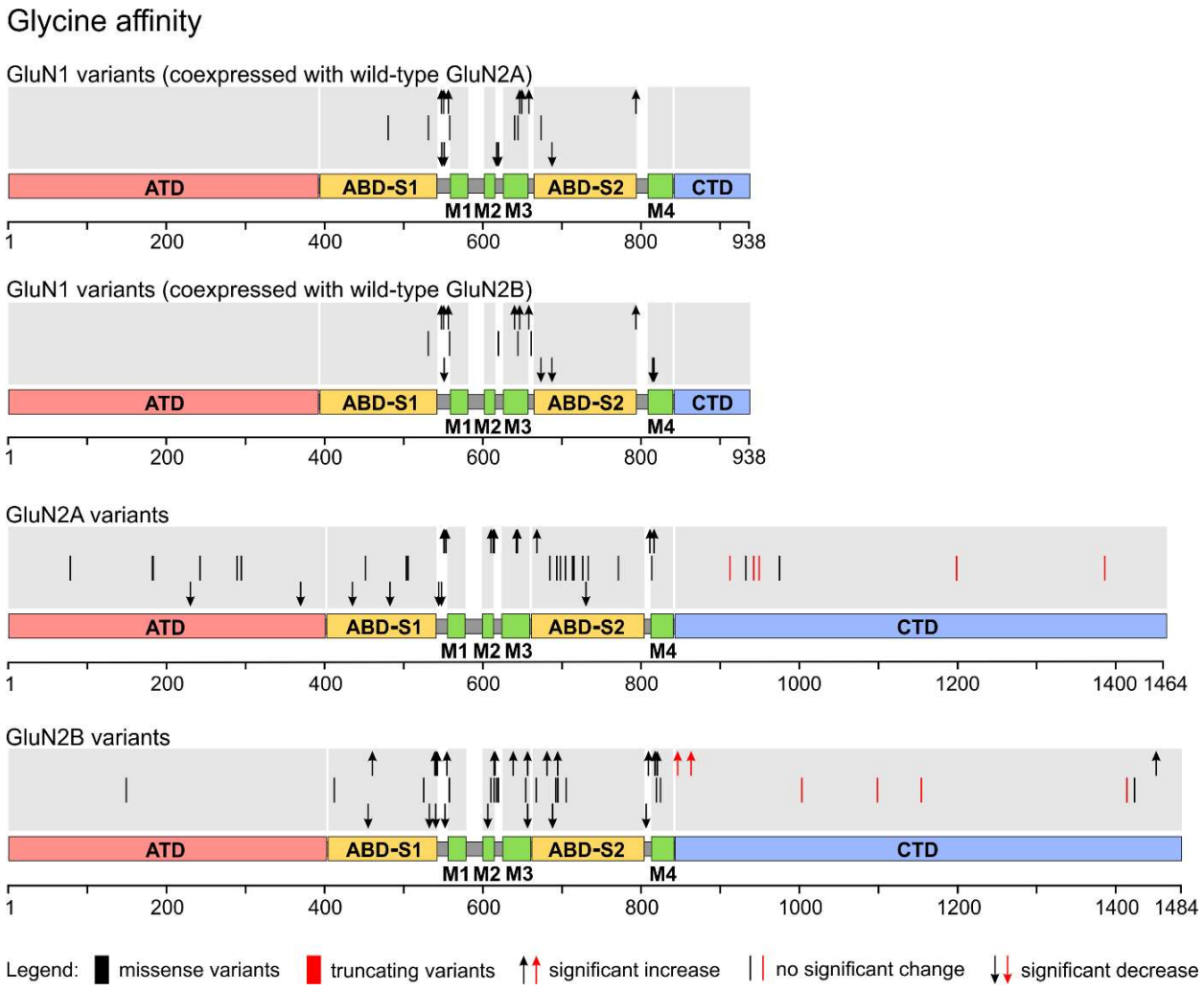

Fig. 2. Effects of disease-associated *GRIN* gene variants on glycine affinity. Relationship between the amino acid positions of individual variants (scale shown below the schematic of the domain structure of each subunit) and the observed effect on glycine affinity (increase, decrease, no change), as determined for diheteromeric recombinant receptors in non-neuronal cells. Abbreviations as in Figure 1.

Glutamate and glycine affinity thus represent key functional parameters influencing NMDAR signalling. In whole-cell patch-clamp measurements of agonist-evoked currents, a change in the receptor affinity for glutamate or glycine is quantified as a change in the concentration of glutamate or glycine that evokes a half-maximal current response (EC_{50}), with a higher affinity associated with a lower EC_{50} value and *vice versa*.

For disease-associated variants for which agonist/co-agonist affinity was reported we summarize the consensus regarding the observed changes (increase, decrease, or no change), or indicate if different studies reached different conclusions (Tables 1,2,3). We plot the qualitative change in glutamate or glycine affinity as a function of the amino acid position of the variant (Figs. 1,2). Missense variants causing significant changes in glutamate or glycine affinity tend to be localized in the ABDs, ABD-TMD linkers, and in the TMDs [34,41,42,61,32]. Variants in the ATDs and the CTDs are considerably understudied, but those that have been evaluated tend to leave the agonist/co-agonist affinity intact, with some exceptions [62,63]. It is difficult to predict the direction of the change based on the location of the variant in the subunit amino acid sequence. This could be related to the nature of the amino acid substitution in the variant, as illustrated by two different disease-associated variants affecting serine S541 in GluN2B: a substitution of this serine by glycine (S541G) results in a significant increase of both glutamate and glycine affinities but a substitution of the same serine by arginine (S541R) results in a significant decrease of glutamate and glycine affinities [45]. Cases where the same residue is altered by more than one disease-associated variant (Tables 1, 2, 3) need further study.

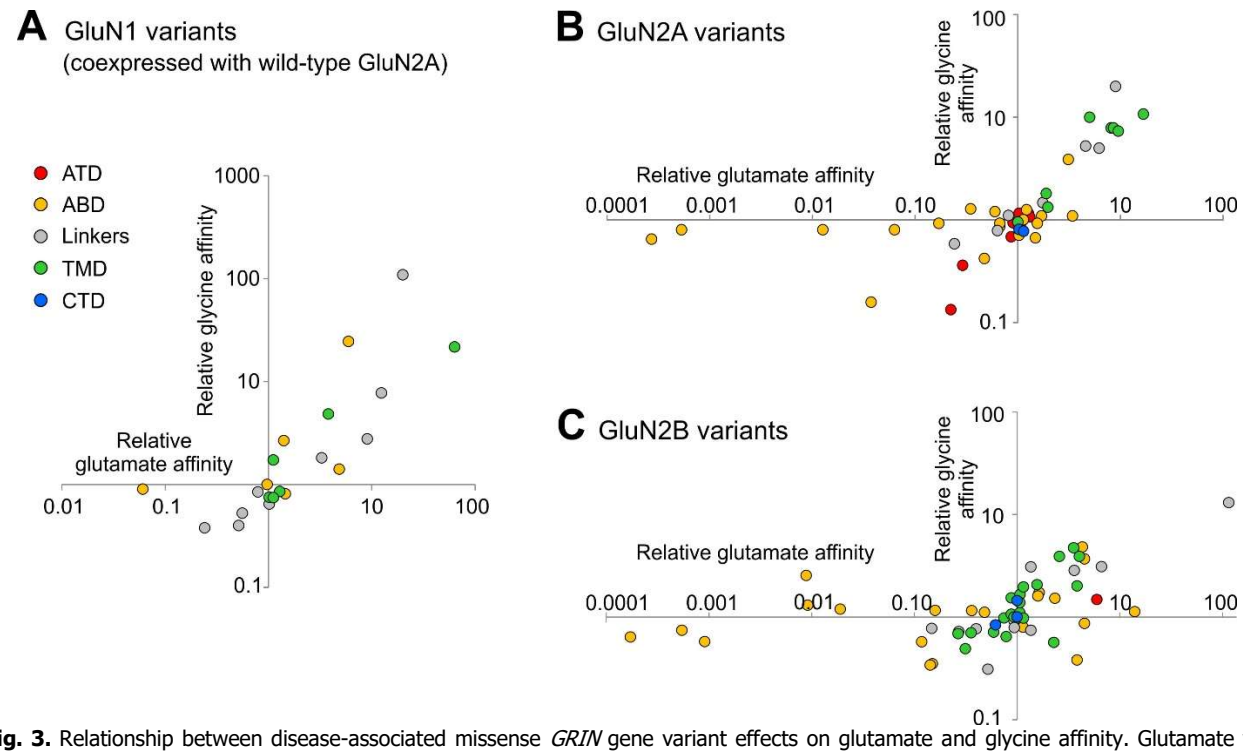

Fig. 3. Relationship between disease-associated missense *GRIN* gene variant effects on glutamate and glycine affinity. Glutamate vs. glycine affinity for individual missense *GRIN* gene variants relative to wild-type as determined for diheteromeric recombinant receptors in non-neuronal cells. The position of each variant within the domain structure of the subunit is indicated by color.

Interestingly, it is very common that variants in GluN1 change not only the affinity for glycine that binds to the GluN1 ABD, but also the affinity for glutamate that binds to the ABDs of the unaffected GluN2 subunits. Similarly, GluN2 variants are often associated with a change in the affinity not only for glutamate but also for glycine. This observation may be related to the fact that glutamate and glycine binding sites in the NMDAR complex are allosterically coupled [64,1]. Indeed, Figure 3 shows that the changes in glutamate and glycine affinity observed for individual variants are correlated. Several variants show a very large (>10-fold) change in glutamate affinity, with variants associated with the strongest decrease in glutamate affinity located in the GluN2A or GluN2B ABDs. For glycine, >10-fold increase in affinity can be seen for several variants but none show >10-fold decrease in affinity. The transient concentration of glutamate released into the synaptic cleft is supersaturating for the glutamate binding sites at the GluN2 subunits [65,1], thus even a substantial decrease in glutamate affinity may be relatively well tolerated. On the other hand, the binding sites for glycine/D-serine at the GluN1 subunits are likely not saturated by ambient co-agonist concentrations [66,67], so a substantial decrease in glycine affinity may be too damaging.

Channel open probability

Upon agonist/co-agonist binding, the channel may undergo the transition from the closed to the open state. Based on kinetic models of NMDAR activation [59,68], channel open probability (P_o) can be expressed in terms of the rate constants of transitions to and from the open state. The P_o is influenced by the combination of GluN2 subunits in the NMDAR complex, with GluN2A associated with a higher P_o than GluN2B [1]. In single-channel experiments the P_o typically refers to the proportion of the total recording time that the channel spends in the open state under conditions of steady-state activation by saturating agonist/co-agonist concentrations [38]. In whole-cell patch-clamp experiments, the P_o can be estimated from the time course of receptor inhibition by an open-channel blocker MK-801 [42], or from the degree of potentiation induced in receptors containing GluN1-A652C (or GluN2A-A650C) by a cysteine-modifying reagent MTSEA that locks receptors in the open state [34,40].

NMDAR P_o has been evaluated for only a small proportion of disease-associated variants (Fig. 4). Given that channel gating fundamentally involves the TMDs, the P_o is particularly influenced by missense variants in the ABD-TMD linkers [34,45] and the TMD helices

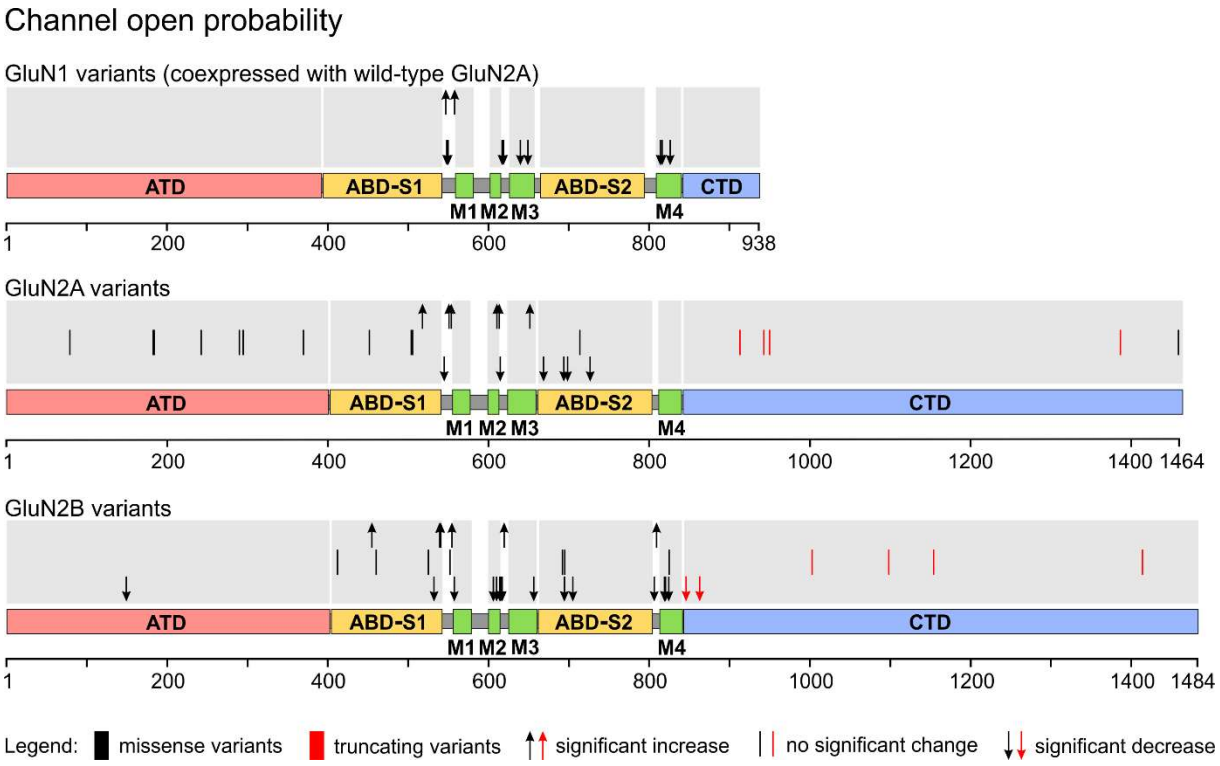

Fig. 4. Effects of disease-associated *GRIN* gene variants on NMDAR P_o. Relationship between the amino acid positions of individual variants and the observed qualitative effect on NMDAR P_o (increase, decrease, no change), as determined for diheteromeric recombinant receptors in non-neuronal cells. Abbreviations as in Figure 1.

[42,38,40]. Membrane regions determine additional aspects of NMDAR channel function, such as Ca^{2+} permeability and Mg^{2+} block, so disease-associated variants located in the TMDs often have a complex receptor-level functional phenotype [38,40,69,48]. Similar to the analysis of agonist/co-agonist affinities, missense variants in the ATDs or the CTDs generally do not change the P_o, but additional variants in these domains should be tested.

Receptor cell surface expression

The assembly of NMDAR complexes and their delivery to the cell surface is a key prerequisite for proper receptor signalling, therefore receptor surface expression is an essential parameter influencing the functional outcome associated with individual disease-associated variants. Heterotetrameric NMDAR complex is assembled in the ER [60], with the ATDs, ABDs, and TMDs all participating in proper protein folding and subunit assembly. In addition, several different polypeptide motifs promoting ER retention or ER export have been described, most located in the CTDs of GluN1 or GluN2A/GluN2B subunits [70]. In neurons, the abundance of NMDAR complexes in the postsynaptic membrane is regulated by the interplay between receptor internalization driven by endocytic signal sequences in the CTDs and receptor anchoring in the synapse via interactions between the CTDs and synaptic scaffolding proteins [27]. To analyze receptor surface expression, most studies have used the endogenous trafficking system of stable cell lines (HEK293T or COS-7). A commonly used approach is to express variant or wild-type GluN subunit with a tag fused to its extracellular end. Surface and intracellular expression of the studied subunit can then be assessed by sequential immunofluorescent labeling performed under non-permeabilizing and permeabilizing conditions, respectively [41,42,43,53,71]. Some studies have used surface protein biotinylation assays [37,72], or expressed β-lactamase fused to the ATD of the studied GluN subunit and performed a colorimetric measurement of β-lactamase activity on the surface of transfected cells [34,40,45].

Disease-associated missense variants in the ABDs and the TMDs in all subunits can influence receptor surface expression (Fig. 5). Interestingly, while many ABD or TMD variants decrease receptor surface expression [34,37,41,42,53], only a few have been found to have the opposite effect [42,73,71,45]. This suggests

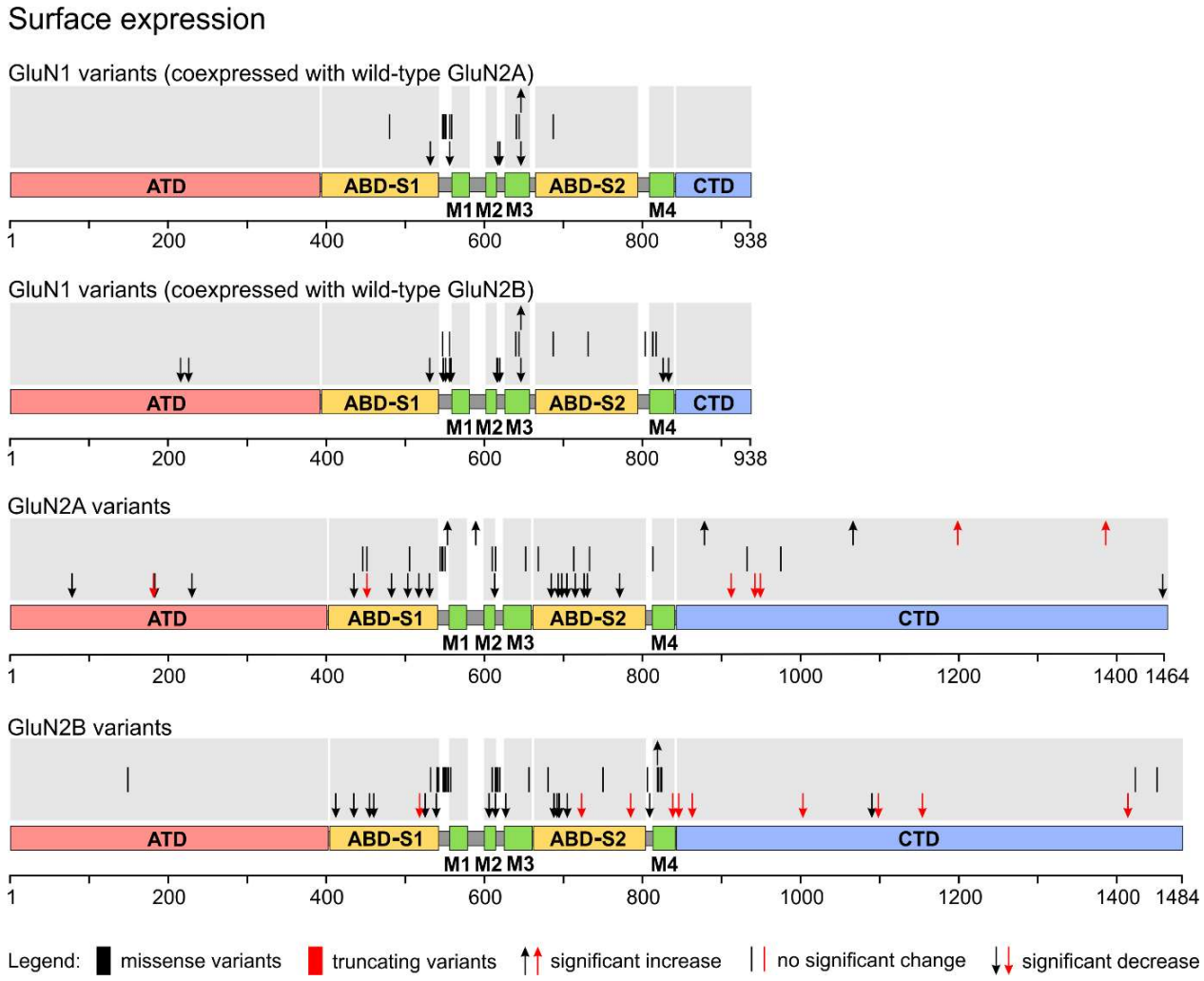

Fig. 5. Effects of disease-associated *GRIN* gene variants on receptor surface expression. Relationship between the amino acid positions of individual variants and the observed effect (increase, decrease, no change) on the variant-containing subunit surface expression, as determined for recombinant receptors in non-neuronal cells. Abbreviations as in Figure 1.

that the nascent receptor quality control is very strict, and any conformational changes that may affect tetramer stability or impair agonist binding or receptor gating prevent receptor trafficking to the cell surface. In contrast to the agonist/co-agonist affinity and the P_o that tend to be relatively unaffected by missense variants in the ATDs and CTDs, receptor surface expression is sensitive to variants in these domains, possibly because disease-associated variants may impair receptor assembly regulated by the ATDs, or disrupt the trafficking and sorting signal sequences present in the CTDs [43,73,74]. Non-neuronal cell lines used for surface expression analyses express only some scaffolding proteins, but biochemical assays can be used to show that certain disease-associated variants in the GluN2A or GluN2B CTDs disrupt the binding of the affected subunits to synaptic scaffolding proteins such as PSD-95 [62,75,63], predicting a deficit in synaptic targeting of these variant-containing subunits.

Disease-associated *GRIN* gene variants resulting in protein truncation

Some disease-associated *GRIN* gene variants introduce a premature stop codon predicted to create a truncated protein. Protein-truncating variants (PTVs) account for 20-25 % of disease-associated *GRIN* gene variants and they tend to be associated with a less severe clinical presentation [29,76,1]. Interestingly, GluN1 PTVs in the heterozygous condition are non-pathogenic [76]. Some authors consider PTVs to be null variants [29], assuming that the variant-containing mRNA is degraded by nonsense-mediated decay, producing simple haploinsufficiency. However, it is likely that mRNA surveillance mechanisms may activate compensatory

changes in the expression of the unaffected allele or other functionally related genes [77]. Further, nonsense-mediated decay is less efficient in neurons, particularly early in development, leaving open the possibility that truncated proteins may be expressed to some extent [78].

Nevertheless, GluN1, GluN2A, or GluN2B subunits truncated in any domain other than the CTD are virtually absent from the cell surface [79,76] (Fig. 5); such PTVs can thus be considered functionally null. In contrast, for PTVs affecting the CTD, the truncated protein is expressed and can be analyzed functionally [22] (Figs. 1, 2, 4). All CTD truncating variants studied so far lead to altered subunit surface expression, but for the most part they do not affect the receptor functionally [22]. Only the variants causing the most extensive CTD truncation (e.g., GluN2B-R847X and GluN2B-I864SfsX20) show some effects on agonist/co-agonist affinity, receptor desensitization, and the P_o [22]. While more work is needed, data so far suggest that, compared to missense variants, *GRIN* gene PTVs in any domain other than the CTD form a more homogeneous group leading to haploinsufficiency, with relatively milder symptoms in carriers. These findings may open new possibilities for non-pharmacological (genetic) therapy for patients with more damaging *GRIN* variants that could possibly be silenced by a newly introduced premature stop codon in the aberrant *GRIN* allele.

Functional characterization of *GRIN* gene variants in neurons *in vitro*

Much useful functional information about disease-associated *GRIN* gene variants has been gained from studies in non-neuronal heterologous expression systems, but there are aspects of NMDAR function that are influenced by conditions specific to neurons. NMDARs operate in a highly specialized subcellular compartment – the synapse – where they interact with a specific microenvironment characterized by distinct membrane composition and a dense network of scaffolding and signaling proteins. Neuronal NMDARs assemble as various combinations of different subunits, and the effects of disease-associated variants should be characterized in this context. To study a disease-associated variant in neurons it is necessary to work out a strategy for expressing the variant-containing subunit in the presence or absence of native subunits expressed endogenously [80]. This is a challenge and an opportunity: with appropriate experimental design studies in neurons can elucidate effects of disease-associated variants under more physiological and clinically relevant conditions.

To study NMDARs with a defined subunit composition in neurons, one group focused on the obligatory GluN1 subunit, using shRNA knockdown of native GluN1 combined with the expression of variant-containing GluN1 subunits [61,53,71]. This strategy, assuming efficient knockdown, results in receptors containing two copies of the variant-containing GluN1 subunit, similar to non-neuronal heterologous expression of diheteromeric receptors. Indeed, in these studies, experiments in neurons largely confirmed effects of GluN1 variants observed in non-neuronal models.

It is more complicated to characterize effects of disease-associated GluN2 subunit variants, because the different GluN2 subunit types may partially substitute for each other. Since NMDARs in principal neurons in the cortex and hippocampus predominantly contain GluN2A and/or GluN2B subunits [4], Cre-Lox recombination in excitatory neurons prepared from *Grin2a*$^{fl/fl}$/*Grin2b*$^{fl/fl}$ mice effectively eliminates the majority of native GluN2 subunit expression [6]. In a series of elegant molecular replacement experiments, GluN2A or GluN2B subunits containing specific disease-associated variants were introduced together with Cre-recombinase in neurons from *Grin2a*$^{fl/fl}$/*Grin2b*$^{fl/fl}$ mice [62,81,82]. In the simplest case, native GluN2A and GluN2B are both eliminated and replaced only with wild-type or variant GluN2A [81] or only with wild-type or variant GluN2B [62,82]. The results of such experiments tend to confirm the LoF or GoF phenotypes of the variants, similar to results observed in non-neuronal systems. Most revealing, however, are experiments in *Grin2a*$^{fl/fl}$ mice where native GluN2A is replaced with variant-containing GluN2A, while the native GluN2B expression is left intact [81], or experiments in *Grin2b*$^{fl/fl}$ mice where native GluN2B is replaced with variant-containing GluN2B, while the native GluN2A expression is intact [82]. In the first case, surprisingly, the outcome of expressing either LoF or GoF GluN2A variants is similar, with excitatory postsynaptic currents (EPSCs) characterized by slower deactivation kinetics. This reflects either the GoF of the GluN2A variant or, in case of the LoF GluN2A variants, the increased relative contribution of native GluN2B subunits to synaptic NMDAR currents [81]. In the second case, the expression of either LoF or GoF GluN2B variants in the presence of native GluN2A results in EPSCs with faster deactivation kinetics, in part because the GoF phenotype of certain GluN2B variants is lost in triheteromeric receptors containing the GluN2B

variant together with wild-type GluN2A [82]. These results suggest that for some variants the functional phenotype at the receptor level may not accurately predict the functional phenotype at the synaptic level, underscoring the need for more work in neuronal preparations.

Many studies have expressed variant subunits in neurons on the background of endogenous expression of native subunits. This approach is technically relatively simple and can address cell surface or synaptic expression of subunits harboring disease-associated variants in the presence of neuronal trafficking and scaffolding machinery. Several studies have expressed tagged variant-containing subunits in neurons and used immunofluorescence microscopy to evaluate the levels of dendritic surface expression of the different variants [34,62,79,75,76,61,53,71,22]. Synaptic localization of variant subunits can be examined more specifically, for example by quantifying colocalization between the tagged subunit and endogenous PSD-95 [22]. A few reports include surface expression data for the same variants in a non-neuronal system and in neurons, in some cases finding comparable results [79,61,53,71], and in other cases finding qualitative or quantitative differences between variant subunit trafficking in neurons and in non-neuronal systems [34,76,22].

Functionally, expressing a disease-associated variant subunit in neurons on the background of endogenous expression of native subunits may approximate the situation in patients heterozygous for the variant, if the method used does not result in significant overexpression of the introduced subunit [51,79]. Such experiments tend to show that in the presence of native wild-type subunits, the functional consequences of the variant are more subtle than in non-neuronal heterologous expression systems, with wild-type subunits partially mitigating the effects of the pathogenic variant [51]. Studies of this type can also begin to address possible indirect effects of changes in NMDAR signaling due to disease-associated variants on synapse structure and function and the development of neural networks [79,75,63].

Functional characterization of *GRIN* gene variants in animal models

Methods used to introduce subunits containing disease-associated variants to neurons in culture or in acute slices only achieve transient expression of the target subunit in a subset of cells in the preparation. As a result, these experiments can only describe relatively acute and cell-autonomous effects of disease-associated variants [80]. However, in patients, the *GRIN* gene variant is permanently present in all cells, and patient symptoms may be related to circuit-level effects of the variant, possibly influenced by compensatory changes of gene expression, synapse structure or function, or circuit connectivity. Transgenic animal models can be used to reveal how changes in NMDAR signaling due to the widespread and chronic presence of the pathogenic *GRIN* gene variant may affect the nervous system function and development.

Mouse strains with targeted disruption of *Grin1* [83], *Grin2a* [84], or *Grin2b* [85] may serve as models of null variants in the corresponding human *GRIN* genes, with heterozygous animals representing the condition of patients. Mouse strains with truncated *Grin2a* or *Grin2b* gene leading to the expression of GluN2A or GluN2B subunits lacking the CTDs have also been created [86]. While homozygous animals of these strains have been characterized, data about heterozygous animals are limited. Heterozygous *Grin1*$^{-/+}$ mice are healthy and their neurons have normal NMDAR responses [83], consistent with the finding that truncating human *GRIN1* variants in the heterozygous condition are not pathogenic [76]. Recently, some studies have examined selected parameters in heterozygous *Grin2a*$^{-/+}$ mice [87,88,89] and *Grin2b*$^{-/+}$ mice [90], generally observing a milder phenotype in heterozygous compared to homozygous animals. Still, much more work is needed to understand the effects of *GRIN2A* or *GRIN2B* haploinsufficiency at the cellular, circuit, and system level. Additional useful data may emerge from using a zebrafish (*Danio rerio*) model to study the developmental roles of different NMDAR subunits [91,92].

Very few transgenic mouse models of specific patient-derived *GRIN* gene variants have been created to date. In 2010, a large-scale mutagenesis project at RIKEN generated a mouse strain expressing GluN1-R844C [93], a variant subsequently identified in two patients with severe intellectual disability, motor disorder, and seizures [94]. At the receptor level this variant has no apparent functional phenotype and in heterozygous mice it is associated with hyperactivity and increased novelty-seeking behavior [93].

Three mouse strains expressing patient-derived GluN2A variants, all associated with intellectual disability and epilepsy, have been described. The variant GluN2A-S644G [95] increases glutamate and glycine affinity and

prolongs NMDAR EPSC deactivation, and in heterozygous animals it is associated with abnormal network activity, hyperactive behaviors, and mixed effects on seizure susceptibility [95]. The variant GluN2A-K879R increases subunit surface expression [74], and in heterozygous animals it is associated with increased NMDAR EPSC amplitude but faster deactivation, altered levels of surface expression of NMDAR and AMPAR subunits, synaptic plasticity deficits, and impaired cognitive function [74]. The variant GluN2A-V685G reduces NMDAR glutamate affinity and surface expression [34], and reduces NMDAR EPSC amplitude, yet this LoF variant increases seizure susceptibility in heterozygous mice, possibly due to circuit-level effects on excitation/inhibition balance [96].

To our knowledge, two mouse models of disease-associated GluN2B variants, both found in patients with intellectual disability and autism, have been created to date. The variant GluN2B-C456Y [90] strongly reduces receptor surface expression [34] and in heterozygous animals it is associated with smaller and faster NMDAR EPSCs, mild deficits in long-term synaptic depression, and hypoactivity and reduced anxiety [90]. The variant GluN2B-L825V reduces receptor P_o [42] (but see [38]), and in heterozygous animals it leads to NMDAR EPSCs with faster deactivation, hypoactivity in the open field, impaired sensorimotor gating, and cognitive inflexibility [97].

Together, these examples illustrate how difficult it is to extrapolate the receptor-level functional phenotype of a given variant to its circuit-level or system-level effects that ultimately underlie symptoms in carriers. Many more disease-associated *GRIN* gene variants need to be studied in animal models and their impact should be examined thoroughly, not only in the canonical circuits (hippocampus, cortex), but in multiple brain regions, over the course of development, and in both sexes. A better understanding of how different *GRIN* gene variants lead to neurological and neuropsychiatric disease is a prerequisite for finding effective therapies for *GRIN* disorders.

Conclusions

Here we present a comprehensive review of the literature on the functional evaluation of human disease-associated variants in *GRIN1, GRIN2A* and *GRIN2B* genes. Most information so far comes from studies of receptor-level effects of *GRIN* gene variants in non-neuronal systems. These studies establish that variants in the core gating region comprised of the ABDs, the TMDs, and the ABD-TMD linkers frequently lead to profound changes of receptor function manifested as changes in agonist/co-agonist affinity, channel open probability, and/or receptor surface expression. Variants in the ATDs and the CTDs can significantly affect receptor surface expression, but have been relatively overlooked so far. An individual variant often influences multiple functional parameters, which may complicate variant classification as LoF or GoF, and the receptor-level functional impact may not accurately predict the consequences observed at the synaptic, cellular, or circuit level in neuronal preparations or animal models. Taking advantage of the more physiological experimental models in the future should lead to further advances in the understanding of the role of NMDAR signaling in healthy nervous system function, and in the etiology of *GRIN* disorders.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

ATD, amino-terminal domain; ABD-S1 and ABD-S2, agonist-binding domain segments S1 and S2; CTD, carboxy-terminal domain; del, deletion; dup, duplication; ins, insertion; fs, frame shift; M1–M4, transmembrane domain (TMD) helices; Ter, termination codon.

Table 1. Functional characterization of disease-associated variants in *GRIN1*/GluN1.

DNA	Protein	Coexpressed with GluN2A				Coexpressed with GluN2B			Functional characterization references	
		P_o	Glu[1]	Gly[2]	Surface Expression	Glu[1]	Gly[2]	Surface Expression	Non-neuronal cells	Neurons
c.649C>T	R217W					↓			[94], [43]	
c.679G>C	D227H							↓	[43]	
c.803G>A	W247Ter							↓	[76]	
c.977del	P305RfsTer21							↓	[76]	
c.1378A>G	I460V*		⇄	⇄	⇄				[98]	[98]
c.1567C>T	R523C								[99]	
c.1595C>A	P532H		↓	⇄	↓	↓	⇄	↓	[100]	
c.1643G>A	R548Q	↑	↑	↑	⇄	↑	↑	⇄	[45]	
c.1705C>T	R548W				⇄				[43]	
c.1645A>C	S549R	↓	↓	↓	⇄			↓	[94], [45]	
c.1652T>C	L551P	↓	↑	↑	⇄	↑	↑		[45]	
c.1656C>G	D552E		↓	↓	⇄	↓	↓	↓	[94], [37], [46]	
c.1666C>T	Q556Ter							↓	[94], [76]	
c.1670C>T	P557L				⇄			↓	[73]	
c.1670C>G	P557R		↑	↑	↓	↑	↑	⇄	[94], [37]	
c.1676A>G	Q559R	↑	↑	⇄	⇄	⇄	⇄	↓	[45]	
c.1679_1681dup	S560insS				⇄			↓	[94], [45], [101]	
c.1851C>A	S617C							↓	[43]	
c.1852G>C	G618R	↓	⇄	↓	↓	⇄		↓	[94]	
c.1854_1859dup	I619_G620dup							↓	[43]	
c.1858G>C	G620R	↓	↓	↓	⇄/↓	↓	⇄	⇄/↓	[94], [102], [40], [43]	
c.1923G>A	M641I	↓	⇄	⇄	↓	⇄	↑	⇄	[94], [56], [46], [53], [57]	[53]
c.1921A>G	M641V							⇄	[43]	
c.1933G>T	A645S*		⇄	⇄/↑	⇄	⇄	⇄	⇄	[94], [53], [98]	[53], [98]
c.1939T>C	Y647H*		↑	↑	↓				[98]	[98]
c.1940A>C	Y647S				↓			↓	[94], [53]	[53]
c.1940A>G	Y647C		↑	↑	↑	↑	↑	↑	[103]	
c.1950C>G	N650K	↓	↑	↑					[94], [71]	[71]
c.1975C>T	R659W		↑	↑		↑	↑		[103]	
c.1984G>A	E662K					↓	⇄		[94], [101]	
c.2021A>T	N674I		⇄	⇄		⇄	↓		[103]	
c.2063C>A	S688Y			↓	⇄		↓		[61]	[61]
c.2196T>G	D732E							⇄	[43]	
c.2365G>A	D789N								[103]	
c.2381G>A	R794Q		↑	↑		↑	↑		[103]	
c.2414C>T	P805L							⇄	[43]	
c.2413C>T	P805S							⇄	[43]	
c.2441C>A	A814D							⇄	[43]	
c.2443G>A	G815R	↓				↓	↓		[94], [38], [46]	
c.2444G>T	G815V	↓							[94], [38]	
c.2449T>C	F817L	↓				↓	↓		[94], [38]	
c.2515A>G	M818V							⇄	[43]	
c.2479G>A	G827R	↓						↓	[94], [38], [43]	
c.2500G>C	E834Q							↓	[43]	
c.2530C>T	R844C								[94]	[93]

[1]Affinity for glutamate [2]Affinity for glycine ↑ = increase compared to WT ↓ = decrease compared to WT ⇄ = no change compared to WT

Color denotes the domain: red = ATD; yellow = ABD; grey = linkers; green = TMD; blue = CTD

Abbreviations: del, deletion; dup, duplication; ins, insertion; fs, frame shift; Ter, termination codon. All variants refer to the sequence of GluN1-1a. *Variants GluN1-I460V, GluN1-A645S, and GluN1-Y647H were studied in GluN1-3b [96] where they correspond to I481V, A666S, and Y668H, respectively. In this and all other Tables, when the literature provides multiple measurements of the same parameter for the same variant, we consider this parameter to be significantly increased (decreased) if most studies report a significantly increased (decreased) value. Where no clear consensus exists, different reported outcomes are indicated.

Table 2. Functional characterization of disease-associated variants in *GRIN2A*/GluN2A.

DNA	Protein	P_o	Glu[1]	Gly[2]	Surface Expression	Functional characterization references	
						Non-neuronal cells	Neurons
c.172G>T	E58Ter					[104]	
c.236C>G	P79R	⇄	⇄	⇄	↓	[41], [35]	[35]
c.544del	E182NfsTer22				↓	[76]	
c.547T>A	F183I	⇄	⇄	⇄		[35]	
c.551T>G	I184S	⇄	⇄	⇄	↓	[35], [105]	
c.692G>A	C231Y		↓	↓	↓	[41], [35]	
c.728C>T	A243V	⇄	⇄	⇄		[35], [46]	
c.869C>T	A290V	⇄	⇄	⇄		[35]	
c.883G>A	G295S	⇄	⇄	⇄		[35]	
c.1108C>T	R370W	⇄	⇄	↓		[35]	[35]
c.1306T>C	C436R		↑	↓	↓	[41], [35], [34], [49]	[81], [82]
c.1341T>A	N447K		↑		⇄	[107]	
c.1354G>A	V452M	⇄	↑	⇄	⇄	[34]	
c.1354insT	V452CfsTer11				↓	[76]	
c.1447G>A	G483R		↓	↓	↓	[41], [34], [49], [108]	
c.1510C>T	R504W	⇄	⇄	⇄	↓	[34]	
c.1517T>C	V506A	⇄	↑	⇄	⇄	[34]	
c.1553G>A	R518H	↑			↓	[105], [34], [106]	[81]
c.1592C>T	T531M				↓	[34], [109]	[81]
c.1634C>T	S545L	↓	↓	↓	⇄	[45]	
c.1639_1641del	S547del				⇄	[45]	
c.1642G>A	A548T		↓	↓	⇄	[37], [49]	
c.1651G>A	E551K	↑	↑	↑	⇄	[47], [45]	
c.1655C>G	P552R		↑	↑	⇄	[47], [37], [110]	[37]
c.1661G>C	S554T	↑	↑	↑	↑	[45]	
c.1757G>A	R586K					[111]	[111]
c.1770A>C	K590N		⇄		↑	[73]	
c.1771G>A	G591R		⇄			[104]	
c.1815A>G	I605M		⇄			[104]	
c.1832T>A	L611Q	↑	↑	↑	⇄	[40], [48]	
c.1841A>G	N614S	↑	↑	↑	↓	[40], [48], [46]	
c.1845C>A	N615K	↓	⇄	↑	⇄	[112], [54], [40], [69], [113], [48], [114]	[69]
c.1844A>G	N615S*						[115]
c.1923G>A	M641I					[56]	
c.1928C>A	A643D		↑	↑		[116], [49]	
c.1930A>G	S644G		↑	↑		[47], [95]	[95]
c.1945C>G	L649V		↑			[47]	
c.1954T>G	F652V	↑	⇄			[110], [106]	
c.1959G>A	M653I					[104]	
c.2007G>T	K669N	↓	↑	↑	⇄	[34]	[81]
c.2054T>C	V685G		↓	⇄	↓	[34], [49]	
c.2081T>C	I694T	↓	↓	⇄	↓	[34]	
c.2093A>G	Y698C		↓			[104]	
c.2095C>T	P699S	⇄/↓	↑	⇄	↓	[34], [49], [108]	
c.2100C>G	Y700Ter					[104]	
c.2113A>G	M705V		↓	⇄	↓	[41], [34], [49], [46]	
c.2140G>A	E714K	⇄	⇄	⇄	⇄	[41], [34]	
c.2146G>A	A716T		↓	⇄	↓	[105], [34], [49], [108]	
c.2179G>A	A727T	↓	↓	⇄	↓	[34], [49], [108], [104]	
c.2191G>A	D731N		↓	↓	↓	[41], [39], [34], [49]	
c.2200G>C	V734L		↓	⇄	⇄	[34], [49], [108]	
c.2314A>G	K772E		↓	⇄	↓	[34], [49]	
c.2351G>C	G784A		⇄			[104]	
c.2364G>A	M788I		⇄			[104]	
c.2380C>A	L794M		⇄			[104]	
c.2427C>A	S809R					[104]	
c.2432A>C	Q811P		↓			[104]	
c.2434C>A	L812M		↑	↑		[47], [54], [33], [104]	
c.2441T>C	I814T		⇄	⇄	⇄	[41], [38], [46]	
c.2449A>G	M817V		↑	↑		[38], [47], [36]	
c.2636A>G	K879R		⇄		↑	[74], [73]	[74]
c.2738C>A	S913Ter	⇄	⇄	⇄	↓	[22]	[22]
c.2797G>A	D933N		⇄	⇄	⇄	[41]	
c.2829C>G	Y943Ter	⇄	⇄	⇄	↓	[22]	[22]
c.2848C>T	Q950Ter	⇄	⇄	⇄	↓	[22]	[22]
c.2927A>G	N976S		⇄	⇄	⇄	[41]	
c.3199C>T	R1067W		⇄		↑	[73]	
c.3596del	P1199RfsTer32		⇄			[117]	[117]
c.3884T>C	I1295T		⇄			[104]	
c.4128del	L1377fs		⇄			[104]	
c.4161C>G	Y1387Ter	⇄	⇄	⇄	↑	[22]	[22]
c.4375A>G	S1459G	⇄			↓	[75], [118]	[75], [118]

[1]Affinity for glutamate [2]Affinity for glycine ↑ = increase compared to WT ↓ = decrease compared to WT ⇄ = no change compared to WT

Color denotes the domain: red = ATD; yellow = ABD; grey = linkers; green = TMD; blue = CTD

Abbreviations as in Table 1. *The variant GluN2A-N615S has not been found in humans, but it is closely related to the patient variant GluN2A-N615K and has been characterized in a transgenic mouse model [112].

Table 3. Functional characterization of disease-associated variants in *GRIN2B*/GluN2B.

DNA	Protein	P_o	Glu[1]	Gly[2]	Surface Expression	Functional characterization references	
						Non-neuronal cells	Neurons
c.448A>G	I150V	↓	↑	⇄	⇄	[44]	
c.1238A>G	E413G	⇄	↓	⇄	↓	[119], [50], [49], [108], [120], [34]	[34], [121]
c.1306T>C	C436R				↓	[49], [34]	[82]
c.1367G>A	C456Y	↑	↑	↓	↓	[49], [108], [34]	[82], [90]
c.1382G>T	C461F	⇄	↓	↑	↓	[49], [51], [34]	[51], [34], [82]
c.1555C>T	R519Ter				↓	[76]	[76]
c.1576T>C	S526P	⇄	↓	⇄	↓	[44]	
c.1598G>A	G533D	↑	↑	↑	↓	[44]	
c.1619G>A	R540H	↓	↓	↓	⇄	[50], [30], [122], [34], [51], [82]	[82]
c.1623C>G	S541R		↓	↓	⇄	[28], [49], [45]	
c.1621A>G	S541G	↑	↑	↑	⇄	[45]	
c.1627G>C	G543R		↑	↑	⇄	[47], [45]	
c.1646C>T	A549V				⇄	[45]	
c.1649T>C	F550S				⇄	[45]	
c.1652T>C	L551S				⇄	[45]	
c.1658C>T	P553L		↓			[37], [42], [51]	[51]
c.1657C>A	P553T	⇄	↓	↓	⇄	[45], [55]	[55]
c.1664G>A	S555N	↑	↑	↑	⇄	[45]	
c.1664G>T	S555I				⇄	[45]	
c.1672G>A	V558I	↓	↓	⇄	⇄	[50], [28], [42]	
c.1821G>T	W607C	↓	↓	↓	↓	[50], [40], [48], [42]	
c.1820G>C	W607S	↓	⇄	↓	↓	[44]	
c.1832G>T	G611V	↓	⇄	⇄	⇄	[40], [28], [48]	
c.1844A>T	N615I	↓	⇄	⇄	⇄	[50], [30], [40], [122], [48], [42], [51], [114]	[51]
c.1845C>G	N615K	↓	⇄	↑	↓	[40], [48]	
c.1848C>G	N616K	↓	⇄	↑	⇄	[40], [48]	
c.1853T>G	V618G	↓	⇄	⇄	⇄	[50], [94], [40], [122], [48], [42], [51], [114]	[51]
c.1858G>A	V620M	↑	⇄	⇄	⇄	[40], [48]	
c.1883C>T	S628F				↓	[50], [42]	
c.1906G>C	A636P					[50]	
c.1916C>T	A639V		↑	↑		[47]	
c.1963A>T	I655F		↓	⇄		[28]	
c.1970A>G	E657G		↑	↓	⇄	[50], [42]	
c.1971G>C	E657D	↓	↑	↑	⇄	[44]	
c.2002G>A	D668N		⇄	⇄		[50]	
c.2044C>T	R682C		↑	↑	⇄	[50], [34], [112]	
c.2045G>A	R682H		↑	↑	⇄	[44]	
c.2065G>T	G689C		↓	↓	↓	[52]	[52]
c.2065G>A	G689S		↓	↓	↓	[44], [52]	[52]
c.2079A>T	R693S	⇄	↑	⇄	↓	[44]	
c.2084T>G	I695S	↓	↓	↑	↓	[44]	
c.2084T>C	I695T	↓	↓	⇄	↓	[44]	
c.2087G>A	R696H	⇄	↑	⇄	↓	[34], [82]	[82]
c.2116A>G	M706V	↓	⇄	⇄	↓	[44], [28], [108]	
c.2172-2A>G	G724Ter				↓	[79]	[79], [123]
c.2252T>C	I751T				⇄	[58]	
c.2355del	D786MfsTer23				↓	[76]	[76]
c.2419G>A	E807K	↓	↓	↓	⇄	[50], [44]	
c.2430C>A	S810R		↑	↑		[28]	
c.2429G>A	S810N	↑	↑	↑	↓	[44]	
c.2443G>A	G815R					[38]	
c.2453T>C	M818T		↑	↑		[47], [28]	
c.2455G>A	A819T		↑	↑		[47], [28]	
c.2459G>C	G820A	↓	⇄	⇄	⇄	[38], [50], [28], [42]	
c.2459G>A	G820E	↓			↑	[38], [58], [42]	
c.2461G>T	V821F	↓	↑	↑	⇄	[44]	
c.2471T>G	M824R				⇄	[42]	
c.2473T>G	L825V	⇄/↓	⇄	⇄	⇄	[38], [50], [28], [42], [51]	
c.2477G>A	G826E	⇄				[38]	
c.2515G>T	E839Ter				↓	[76]	[76]
c.2539C>T, c.2539-2540CG>TA	R847Ter	↓	↓	↑	↓	[76], [22]	[76], [22]
c.2589del	I864SfsTer20	↓	⇄	↑	↓	[22]	[22]
c.3012C>G	Y1004Ter	⇄	⇄	⇄	↓	[22]	[22]
c.3272A>C	K1091T		↓		↓	[63]	[63]
c.3295del	R1099AfsTer51	⇄	⇄	⇄	↓	[22]	[22]
c.3465C>ACATCTA	Y1155Ter	⇄	⇄	⇄	↓	[22]	[22]
c.4244C>T	S1415L		⇄	⇄	↓	[62]	[62]
c.4244del	S1415Ter	⇄	⇄	⇄	↓	[22]	[22]
c.4270C>T	L1424F		↓	⇄	⇄	[62]	[62]
c.4355C>T	S1452F		⇄	↑	⇄	[62]	[62]

[1]Affinity for glutamate [2]Affinity for glycine ↑ = increase compared to WT ↓ = decrease compared to WT ⇄ = no change compared to WT

Color denotes the domain: red = ATD; yellow = ABD; grey = linkers; green = TMD; blue = CTD

Abbreviations as in Table 1.

References

1. Hansen KB, Wollmuth LP, Bowie D, Furukawa H, Menniti FS, Sobolevsky AI, Swanson GT, et al. Structure, function, and pharmacology of glutamate receptor ion channels. Pharmacol Rev 2021; 73:1469-658. https://doi.org/10.1124/pharmrev.120.000131
2. Korte M, Schmitz D. Cellular and system biology of memory: Timing, molecules, and beyond. Physiol Rev 2016; 96:647-93. https://doi.org/10.1152/physrev.00010.2015
3. Benke TA, Park K, Krey I, Camp CR, Song R, Ramsey AJ, Yuan H, et al. Clinical and therapeutic significance of genetic variation in the GRIN gene family encoding NMDARs. Neuropharmacology 2021; 199:108805. https://doi.org/10.1016/j.neuropharm.2021.108805
4. Hanson JE, Yuan H, Perszyk RE, Banke TG, Xing H, Tsai MC, Menniti FS, et al. Therapeutic potential of N-methyl-D-aspartate receptor modulators in psychiatry. Neuropsychopharmacology 2024; 49:51-66. https://doi.org/10.1038/s41386-023-01614-3
5. Hansen KB, Ogden KK, Yuan H, Traynelis SF. Distinct functional and pharmacological properties of triheteromeric GluN1/GluN2A/GluN2B NMDA receptors. Neuron 2014; 81:1084-96. https://doi.org/10.1016/j.neuron.2014.01.035
6. Gray JA, Shi Y, Usui H, During MJ, Sakimura K, Nicoll RA. Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: Single-Cell NMDA receptor subunit deletion *in vivo*. Neuron 2011; 71:1085-101. https://doi.org/10.1016/j.neuron.2011.08.007
7. Rauner C, Köhr G. Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampal synapses. J Biol Chem 2011; 286:7558-66. https://doi.org/10.1074/jbc.M110.182600
8. Tovar KR, McGinley MJ, Westbrook GL. Triheteromeric NMDA receptors at hippocampal synapses. J Neurosci 2013; 33:9150-60. https://doi.org/10.1523/JNEUROSCI.0829-13.2013
9. Kellermayer B, Ferreira JS, Dupuis J, Levet F, Grillo-Bosch D, Bard L, Linarès-Loyez J, et al. Differential nanoscale topography and functional role of GluN2-NMDA receptor subtypes at glutamatergic synapses. Neuron 2018; 100:106-119.e7. https://doi.org/10.1016/j.neuron.2018.09.012
10. Stroebel D, Carvalho S, Grand T, Zhu S, Paoletti P. Controlling NMDA receptor subunit composition using ectopic retention signals. J Neurosci 2014; 34:16630-6. https://doi.org/10.1523/JNEUROSCI.2736-14.2014
11. Lü W, Du J, Goehring A, Gouaux E. Cryo-EM structures of the triheteromeric NMDA receptor and its allosteric modulation. Science 2017; 355. https://doi.org/10.1126/science.aal3729
12. Zhou C, Tajima N. Structural insights into NMDA receptor pharmacology. Biochem Soc Trans 2023; 51:1713-31. https://doi.org/10.1042/BST20230122
13. Cerny J, Bozikova P, Balik A, Marques SM, Vyklicky L. NMDA Receptor opening and closing-transitions of a molecular machine revealed by molecular dynamics. Biomolecules 2019; 9. https://doi.org/10.3390/biom9100546
14. Durham RJ, Paudyal N, Carrillo E, Bhatia NK, Maclean DM, Berka V, Dolino DM, et al. Conformational spread and dynamics in allostery of NMDA receptors. Proc Natl Acad Sci U S A 2020; 117:3839-47. https://doi.org/10.1073/pnas.1910950117
15. Vyklicky V, Stanley C, Habrian C, Isacoff EY. Conformational rearrangement of the NMDA receptor amino-terminal domain during activation and allosteric modulation. Nat Commun 2021; 12. https://doi.org/10.1038/s41467-021-23024-z
16. Mony L, Paoletti P. Mechanisms of NMDA receptor regulation. Curr Opin Neurobiol 2023; 83:102815. https://doi.org/10.1016/j.conb.2023.102815
17. Jalali-Yazdi F, Chowdhury S, Yoshioka C, Gouaux E. Mechanisms for zinc and proton inhibition of the GluN1/GluN2A NMDA receptor. Cell 2018; 175:1520-1532.e15. https://doi.org/10.1016/j.cell.2018.10.043
18. Vyklicky V, Krausova B, Cerny J, Balik A, Zapotocky M, Novotny M, Lichnerova K, et al. Block of NMDA receptor channels by endogenous neurosteroids: Implications for the agonist induced conformational states of the channel vestibule. Sci Rep 2015; 5:1-15. https://doi.org/10.1038/srep10935

19. Vyklicky V, Smejkalova T, Krausova B, Balik A, Korinek M, Borovska J, Horak M, et al. Preferential inhibition of tonically over phasically activated nmda receptors by pregnane derivatives. J Neurosci 2016; 36:2161-75. https://doi.org/10.1523/JNEUROSCI.3181-15.2016
20. Krausova BH, Kysilov B, Cerny J, Vyklicky V, Smejkalova T, Ladislav M, Balik A, et al. Site of action of brain neurosteroid pregnenolone sulfate at the N-methyl-D-aspartate receptor. J Neurosci 2020; 40:5922-36. https://doi.org/10.1523/JNEUROSCI.3010-19.2020
21. Hubalkova P, Ladislav M, Vyklicky V, Smejkalova T, Krausova BH, Kysilov B, Krusek J, et al. Palmitoylation controls nmda receptor function and steroid sensitivity. J Neurosci 2021; 41:2119-34. https://doi.org/10.1523/JNEUROSCI.2654-20.2021
22. Kysilov B, Kuchtiak V, Hrcka Krausova B, Balik A, Korinek M, Fili K, Dobrovolski M, et al. Disease-associated nonsense and frame-shift variants resulting in the truncation of the GluN2A or GluN2B C-terminal domain decrease NMDAR surface expression and reduce potentiating effects of neurosteroids. Cell Mol Life Sci 2024; 81:36. https://doi.org/10.1007/s00018-023-05062-6
23. Korinek M, Vyklicky V, Borovska J, Lichnerova K, Kaniakova M, Krausova B, et al. Cholesterol modulates open probability and desensitization of NMDA receptors. J Physiol 2015; 593:2279-93. https://doi.org/10.1113/jphysiol.2014.288209
24. Korinek M, Gonzalez-Gonzalez IM, Smejkalova T, Hajdukovic D, Skrenkova K, Krusek J, Horak M, et al. Cholesterol modulates presynaptic and postsynaptic properties of excitatory synaptic transmission. Sci Rep 2020; 10:1-18. https://doi.org/10.1038/s41598-020-69454-5
25. Iacobucci GJ, Popescu GK. Ca2+-Dependent Inactivation of GluN2A and GluN2B NMDA Receptors Occurs by a Common Kinetic Mechanism. Biophys J 2020; 118:798. https://doi.org/10.1016/j.bpj.2019.07.057
26. Bhatia NK, Carrillo E, Durham RJ, Berka V, Jayaraman V. Allosteric Changes in the NMDA Receptor Associated with Calcium-Dependent Inactivation. Biophys J 2020; 119:2349. https://doi.org/10.1016/j.bpj.2020.08.045
27. Warnet XL, Bakke Krog H, Sevillano-Quispe OG, Poulsen H, Kjaergaard M. The C-terminal domains of the NMDA receptor: How intrinsically disordered tails affect signalling, plasticity and disease. Eur J Neurosci 2021; 54:6713-39. https://doi.org/10.1111/ejn.14842
28. Platzer K, Yuan H, Schütz H, Winschel A, Chen W, Hu C, Kusumoto H, et al. GRIN2B encephalopathy: Novel findings on phenotype, variant clustering, functional consequences and treatment aspects. J Med Genet 2017; 54:460-70. https://doi.org/10.1136/jmedgenet-2016-104509
29. Strehlow V, Heyne HO, Vlaskamp DRM, Marwick KFM, Rudolf G, De Bellescize J, Biskup S, et al. GRIN2A -related disorders: Genotype and functional consequence predict phenotype. Brain 2019; 142:80-92. https://doi.org/10.1093/brain/awy304
30. Lemke JR, Hendrickx R, Geider K, Laube B, Schwake M, Harvey RJ, James V, et al. GRIN2B mutations in west syndrome and intellectual disability with focal epilepsy. Ann Neurol 2014; 75:147-54. https://doi.org/10.1002/ana.24073
31. Hirschfeldova K, Cerny J, Bozikova P, Kuchtiak V, Rausch T, Benes V, Spaniel F, et al. Evidence for the association between the intronic haplotypes of ionotropic glutamate receptors and first-episode schizophrenia. J Pers Med 2021; 11. https://doi.org/10.3390/jpm11121250
32. Amin JB, Moody GR, Wollmuth LP. From bedside-to-bench: What disease-associated variants are teaching us about the NMDA receptor. J Physiol 2021; 599:397-416. https://doi.org/10.1113/JP278705
32a. Xu Y, Song R, Perszyk RE, Chen W, Kim S, Park KL, Allen JP, et al. De novo GRIN variants in M3 helix associated with neurological disorders control channel gating of NMDA receptor. Cell Mol Life Sci 2024; 81(1):153. (Added in proof). https://doi.org/10.1007/s00018-023-05069-z
33. Yuan H, Hansen KB, Zhang J, Mark Pierson T, Markello TC, Fajardo KVF, Holloman CM, et al. Functional analysis of a de novo GRIN2A missense mutation associated with early-onset epileptic encephalopathy. Nat Commun 2014;5. https://doi.org/10.1038/ncomms4251
34. Swanger SA, Chen W, Wells G, Burger PB, Tankovic A, Bhattacharya S, Strong KL, et al. Mechanistic insight into nmda receptor dysregulation by rare variants in the GluN2A and GluN2B agonist binding domains. Am J Hum Genet 2016; 99:1261. https://doi.org/10.1016/j.ajhg.2016.10.002

35. Serraz B, Grand T, Paoletti P. Altered zinc sensitivity of NMDA receptors harboring clinically-relevant mutations. Neuropharmacology 2016; 109:196-204. https://doi.org/10.1016/j.neuropharm.2016.06.008
36. Chen W, Tankovic A, Burger PB, Kusumoto H, Traynelis SF, Yuan H. Functional evaluation of a de novo GRIN2A mutation identified in a patient with profound global developmental delay and refractory epilepsy. Mol Pharmacol 2017; 91:317-30. https://doi.org/10.1124/mol.116.106781
37. Ogden KK, Chen W, Swanger SA, McDaniel MJ, Fan LZ, Hu C, Tankovic A, et al. Molecular Mechanism of Disease-Associated Mutations in the Pre-M1 Helix of NMDA Receptors and Potential Rescue Pharmacology. PLoS Genetics 2017; 13: 1-35. https://doi.org/10.1371/journal.pgen.1006536
38. Amin JB, Leng X, Gochman A, Zhou HX, Wollmuth LP. A conserved glycine harboring disease-associated mutations permits NMDA receptor slow deactivation and high Ca^{2+} permeability. Nat Commun 2018; 9. https://doi.org/10.1038/s41467-018-06145-w
39. Gao K, Tankovic A, Zhang Y, Kusumoto H, Zhang J, Chen W, Xiangwei W, et al. A de novo loss-of-function GRIN2A mutation associated with childhood focal epilepsy and acquired epileptic aphasia. PLoS One 2017; 12:1-20. https://doi.org/10.1371/journal.pone.0170818
40. Li J, Zhang J, Tang W, Mizu RK, Kusumoto H, XiangWei W, Xu Y, et al. De novo GRIN variants in NMDA receptor M2 channel pore-forming loop are associated with neurological diseases. Hum Mutat 2019; 40:2393-413. https://doi.org/10.1002/humu.23895
41. Addis L, Virdee JK, Vidler LR, Collier DA, Pal DK, Ursu D. Epilepsy-associated GRIN2A mutations reduce NMDA receptor trafficking and agonist potency-molecular profiling and functional rescue. Sci Rep 2017;7:1-14. https://doi.org/10.1038/s41598-017-00115-w
42. Vyklicky V, Krausova B, Cerny J, Ladislav M, Smejkalova T, Kysilov B, Korinek M, et al. Surface expression, function, and pharmacology of disease-associated mutations in the membrane domain of the human GluN2B subunit. Front Mol Neurosci 2018;11:1-20. https://doi.org/10.3389/fnmol.2018.00110
43. Santos-gómez A, Miguez-cabello F, Juliá-palacios N, García-navas D, Soto-insuga V, García-peñas JJ, Fuentes P, et al. Paradigmatic de novo grin1 variants recapitulate pathophysiological mechanisms underlying grin1-related disorder clinical spectrum. Int J Mol Sci 2021; 22:1-19. https://doi.org/10.3390/ijms222312656
44. Myers SJ, Yuan H, Perszyk RE, Zhang J, Kim S, Nocilla KA, Allen JP, et al. Classification of missense variants in the N-methyl-d-aspartate receptor GRIN gene family as gain- or loss-of-function. Hum Mol Genet 2023; 32:2857-71. https://doi.org/10.1093/hmg/ddad104
45. Xie L, McDaniel MJ, Perszyk RE, Kim S, Cappuccio G, Shapiro KA, Muñoz-Cabello B, et al. Functional effects of disease-associated variants reveal that the S1-M1 linker of the NMDA receptor critically controls channel opening. Cell Mol Life Sci 2023; 80:1-19. https://doi.org/10.1007/s00018-023-04705-y
46. Moody G, Musco A, Bennett J, Wollmuth LP. An integrated approach to evaluate the functional effects of disease-associated NMDA receptor variants. Neuropharmacology 2023; 240:109703. https://doi.org/10.1016/j.neuropharm.2023.109703
47. Han W, Yuan H, Allen JP, Kim S, Shaulsky GH, Perszyk RE, Traynelis SF, et al. Opportunities for Precision Treatment of GRIN2A and GRIN2B Gain-of-Function Variants in Triheteromeric N-Methyl-D-Aspartate Receptors. J Pharmacol Exp Ther 2022; 381:54-66. https://doi.org/10.1124/jpet.121.001000
48. Song R, Zhang J, Perszyk RE, Camp CR, Tang W, Kannan V, Li J, et al. Differential responses of disease-related GRIN variants located in pore-forming M2 domain of N-methyl-D-aspartate receptor to FDA-approved inhibitors. J Neurochem 2023: 1-14. https://doi.org/10.1111/jnc.15942
49. Tang W, Liu D, Traynelis SF, Yuan H. Positive allosteric modulators that target NMDA receptors rectify loss-of-function GRIN variants associated with neurological and neuropsychiatric disorders. Neuropharmacology 2020; 177:108247. https://doi.org/10.1016/j.neuropharm.2020.108247
50. Kysilov B, Hrcka Krausova B, Vyklicky V, Smejkalova T, Korinek M, Horak M, Chodounska H, et al. Pregnane-based steroids are novel positive NMDA receptor modulators that may compensate for the effect of loss-of-function disease-associated GRIN mutations. Br J Pharmacol 2022; 179:3970-90. https://doi.org/10.1111/bph.15841
51. Fedele L, Newcombe J, Topf M, Gibb A, Harvey RJ, Smart TG. Disease-associated missense mutations in GluN2B subunit alter NMDA receptor ligand binding and ion channel properties. Nat Commun 2018; 9. https://doi.org/10.1038/s41467-018-02927-4

52. Kellner S, Abbasi A, Carmi I, Heinrich R, Garin-Shkolnik T, Hershkovitz T, Giladi M, et al. Two de novo GluN2B mutations affect multiple NMDAR-functions and instigate severe pediatric encephalopathy. Elife. 2021; 10. https://doi.org/10.7554/eLife.67555
53. Kolcheva M, Kortus S, Krausova BH, Barackova P, Misiachna A, Danacikova S, Kaniakova M, et al. Specific pathogenic mutations in the M3 domain of the GluN1 subunit regulate the surface delivery and pharmacological sensitivity of NMDA receptors. Neuropharmacology 2021; 189:108528. https://doi.org/10.1016/j.neuropharm.2021.108528
54. Pierson TM, Yuan H, Marsh ED, Fuentes-Fajardo K, Adams DR, Markello T, Golas G, et al. GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine. Ann Clin Transl Neurol 2014; 1:190-8. https://doi.org/10.1002/acn3.39
55. Soto D, Olivella M, Grau C, Armstrong J, Alcon C, Gasull X, Santos-Gómez A, et al. L-Serine dietary supplementation is associated with clinical improvement of loss-of-function GRIN2B-related pediatric encephalopathy. Sci Signal 2019; 12:1-16. https://doi.org/10.1126/scisignal.aaw0936
56. Xu Y, Song R, Chen W, Strong K, Shrey D, Gedela S, Traynelis SF, et al. Recurrent seizure-related GRIN1 variant: Molecular mechanism and targeted therapy. Ann Clin Transl Neurol 2021;8:1480-94. https://doi.org/10.1002/acn3.51406
57. Lewis SA, Shetty S, Gamble S, Heim J, Zhao N, Stitt G, Pankratz M, et al. Intrathecal magnesium delivery for Mg^{++}-insensitive NMDA receptor activity due to GRIN1 mutation. Orphanet J Rare Dis 2023; 18:1-5. https://doi.org/10.1186/s13023-023-02756-9
58. den Hollander B, Veenvliet ARJ, Rothuizen-Lindenschot M, van Essen P, Peters G, Santos-Gómez A, Olivella M, et al. Evidence for effect of L-serine, a novel therapy for GRIN2B-related neurodevelopmental disorder. Mol Genet Metab 2023; 138. https://doi.org/10.1016/j.ymgme.2023.107523
59. Lester RAJ, Jahr CE. NMDA channel behavior depends on agonist affinity. J Neurosci 1992; 12:635-43. https://doi.org/10.1523/JNEUROSCI.12-02-00635.1992
60. Benske TM, Mu TW, Wang YJ. Protein quality control of N-methyl-D-aspartate receptors. Front Cell Neurosci 2022; 16:1-25. https://doi.org/10.3389/fncel.2022.907560
61. Skrenkova K, Song J man, Kortus S, Kolcheva M, Netolicky J, Hemelikova K, Kaniakova M, et al. The pathogenic S688Y mutation in the ligand-binding domain of the GluN1 subunit regulates the properties of NMDA receptors. Sci Rep 2020; 10:1-15. https://doi.org/10.1038/s41598-020-75646-w
62. Liu S, Zhou L, Yuan H, Vieira M, Sanz-Clemente A, Badger JD, Lu W, et al. A rare variant identified within the GluN2B C-terminus in a patient with autism affects NMDA receptor surface expression and spine density. J Neurosci 2017; 37:4093-102. https://doi.org/10.1523/JNEUROSCI.0827-16.2017
63. Wang X, Mei D, Gou L, Zhao S, Gao C, Guo J, Luo S, et al. Functional evaluation of a novel GRIN2B missense variant associated with epilepsy and intellectual disability. Neuroscience 2023; 526:107-20. https://doi.org/10.1016/j.neuroscience.2023.06.018
64. Mayer ML, Vyklicky L, Clements J. Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine. Nat 1989; 338:425-7. https://doi.org/10.1038/338425a0
65. Clements JD, Lester RAJ, Tong G, Jahr CE, Westbrook GL. The time course of glutamate in the synaptic cleft. Science 1992; 258:1498-501. https://doi.org/10.1126/science.1359647
66. Bergeron R, Meyer TM, Coyle JT, Greene RW. Modulation of N-methyl-D-aspartate receptor function by glycine transport. Proc Natl Acad Sci U S A 1998; 95:15730-4. https://doi.org/10.1073/pnas.95.26.15730
67. Stroebel D, Mony L, Paoletti P. Glycine agonism in ionotropic glutamate receptors. Neuropharmacology 2021; 193:108631. https://doi.org/10.1016/j.neuropharm.2021.108631
68. Jahr CE. High probability opening of NMDA receptor channels by L-glutamate. Science 1992; 255:470-2. https://doi.org/10.1126/science.1346477
69. Marwick KFM, Hansen KB, Skehel PA, Hardingham GE, Wyllie DJA. Functional assessment of triheteromeric NMDA receptors containing a human variant associated with epilepsy. J Physiol 2019; 597:1691-704. https://doi.org/10.1113/JP277292
70. Lau CG, Zukin RS. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat Rev Neurosci 2007; 8:413-26. https://doi.org/10.1038/nrn2153

71. Kolcheva M, Ladislav M, Netolicky J, Kortus S, Rehakova K, Krausova BH, Hemelikova K, et al. The pathogenic N650K variant in the GluN1 subunit regulates the trafficking, conductance, and pharmacological properties of NMDA receptors. Neuropharmacology 2023; 222:109297. https://doi.org/10.1016/j.neuropharm.2022.109297
72. Wang CC, Held RG, Chang SC, Yang L, Delpire E, Ghosh A, Hall B, et al. A critical role for gluN2B-containing NMDA receptors in cortical development and function. Neuron 2011; 72:789-805. https://doi.org/10.1016/j.neuron.2011.09.023
73. Liu XR, Xu XX, Lin SM, Fan CY, Ye TT, Tang B, Shi YW, et al. GRIN2A Variants Associated With Idiopathic Generalized Epilepsies. Front Mol Neurosci 2021; 14:1-15. https://doi.org/10.3389/fnmol.2021.720984
74. Li QQ, Chen J, Hu P, Jia M, Sun JH, Feng HY, Qiao FC, et al. Enhancing GluN2A-type NMDA receptors impairs long-term synaptic plasticity and learning and memory. Mol Psychiatry 2022; 27:3468-78. https://doi.org/10.1038/s41380-022-01579-7
75. Mota Vieira M, Nguyen TA, Wu K, Badger JD, Collins BM, Anggono V, Lu W, et al. An Epilepsy-Associated GRIN2A Rare Variant Disrupts CaMKIIα Phosphorylation of GluN2A and NMDA Receptor Trafficking. Cell Rep 2020; 32:108104. https://doi.org/10.1016/j.celrep.2020.108104
76. Santos-Gómez A, Miguez-Cabello F, Garciá-Recio A, Locubiche-Serra S, Garciá-Diáz R, Soto-Insuga V, Guerrero-López R, et al. Disease-associated GRIN protein truncating variants trigger NMDA receptor loss-of-function. Hum Mol Genet 2020; 29:3859-71. https://doi.org/10.1093/hmg/ddaa220
77. El-Brolosy MA, Kontarakis Z, Rossi A, Kuenne C, Günther S, Fukuda N, Kikhi K, et al. Genetic compensation triggered by mutant mRNA degradation Europe PMC Funders Group. Nature 2019; 568:193-7. https://doi.org/10.1038/s41586-019-1064-z
78. Lee PJ, Yang S, Sun Y, Guo JU. Regulation of nonsense-mediated mRNA decay in neural development and disease. J Mol Cell Biol 2021; 13:269-81. https://doi.org/10.1093/jmcb/mjab022
79. Sceniak MP, Fedder KN, Wang Q, Droubi S, Babcock K, Patwardhan S, Wright-Zornes J, et al. An autism-associated mutation in GluN2B prevents NMDA receptor trafficking and interferes with dendrite growth. J Cell Sci 2019; 132:1-14. https://doi.org/10.1242/jcs.232892
80. Sabo SL, Lahr JM, Offer M, Weekes ALA, Sceniak MP. GRIN2B-related neurodevelopmental disorder: current understanding of pathophysiological mechanisms. Front Synaptic Neurosci 2023; 14:1-17. https://doi.org/10.3389/fnsyn.2022.1090865
81. Elmasri M, Hunter DW, Winchester G, Bates EE, Aziz W, Van Der Does DM, Karachaliou E, et al. Common synaptic phenotypes arising from diverse mutations in the human NMDA receptor subunit GluN2A. Commun Biol 2022; 5:1-17. https://doi.org/10.1038/s42003-022-03115-3
82. Elmasri M, Lotti JS, Aziz W, Steele OG, Karachaliou E, Sakimura K, Hansen KB, et al. Synaptic Dysfunction by Mutations in GRIN2B: Influence of Triheteromeric NMDA Receptors on Gain-Of-Function and Loss-Of-Function Mutant Classification. Brain Sci 2022;12. https://doi.org/10.3390/brainsci12060789
83. Forrest D, Yuzaki M, Soares HD, Ng L, Luk DC, Sheng M, Stewart CL, et al. Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. Neuron 1994; 13:325-38. https://doi.org/10.1016/0896-6273(94)90350-6
84. Sakimura K, Kutsuwada T, Ito I, Manabe T, Takayama C, Kushiya E, Yagi T, et al. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor ε1 subunit. Nat 1995; 373:151-5. https://doi.org/10.1038/373151a0
85. Kutsuwada T, Sakimura K, Manabe T, Takayama C, Katakura N, Kushiya E, Natsume R, et al. Impairment of Suckling Response, Trigeminal Neuronal Pattern Formation, and Hippocampal LTD in NMDA Receptor ε2 Subunit Mutant Mice. Neuron 1996; 16:333-44. https://doi.org/10.1016/S0896-6273(00)80051-3
86. Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, Rozov A, Hvalby O, et al. Importance of the Intracellular Domain of NR2 Subunits for NMDA Receptor Function In Vivo. Cell 1998; 92:279-89. https://doi.org/10.1016/S0092-8674(00)80921-6
87. Herzog LE, Wang L, Yu E, Choi S, Farsi Z, Song BJ, Pan JQ, et al. Mouse mutants in schizophrenia risk genes GRIN2A and AKAP11 show EEG abnormalities in common with schizophrenia patients. Transl Psychiatry 2023; 13. https://doi.org/10.1038/s41398-023-02393-7

88. Camp CR, Vlachos A, Klöckner C, Krey I, Banke TG, Shariatzadeh N, Ruggiero SM, et al. Loss of Grin2a causes a transient delay in the electrophysiological maturation of hippocampal parvalbumin interneurons. Commun Biol 2023; 6. https://doi.org/10.1038/s42003-023-05298-9
89. Farsi Z, Nicolella A, Simmons SK, Aryal S, Shepard N, Brenner K, Lin S, et al. Brain-region-specific changes in neurons and glia and dysregulation of dopamine signaling in Grin2a mutant mice. Neuron 2023; 111:3378-3396.e9. https://doi.org/10.1016/j.neuron.2023.08.004
90. Shin W, Kim K, Serraz B, Cho YS, Kim D, Kang M, Lee EJ, et al. Early correction of synaptic long-term depression improves abnormal anxiety-like behavior in adult GluN2B-C456Y-mutant mice. PLoS Biol 2020;18:1-28. https://doi.org/10.1371/journal.pbio.3000717
91. Zoodsma JD, Keegan EJ, Moody GR, Bhandiwad AA, Napoli AJ, Burgess HA, Wollmuth LP, et al. Disruption of grin2B, an ASD-associated gene, produces social deficits in zebrafish. Mol Autism 2022;13:1-17. https://doi.org/10.1186/s13229-022-00516-3
92. Zoodsma JD, Chan K, Bhandiwad AA, Golann DR, Liu G, Syed SA, Napoli AJ, et al. A Model to Study NMDA Receptors in Early Nervous System Development. J Neurosci 2020; 40:3631-45. https://doi.org/10.1523/JNEUROSCI.3025-19.2020
93. Furuse T, Wada Y, Hattori K, Yamada I, Kushida T, Shibukawa Y, Masuya H, et al. Phenotypic characterization of a new Grin1 mutant mouse generated by ENU mutagenesis. Eur J Neurosci 2010; 31:1281-91. https://doi.org/10.1111/j.1460-9568.2010.07164.x
94. Lemke JR, Geider K, Helbig KL, Heyne HO, Schütz H, Hentschel J, Courage C, et al. Delineating the GRIN1 phenotypic spectrum: A distinct genetic NMDA receptor encephalopathy. Neurology 2016; 86:2171-8. https://doi.org/10.1212/WNL.0000000000002740
95. Amador A, Bostick CD, Olson H, Peters J, Camp CR, Krizay D, Chen W, et al. Modelling and treating GRIN2A developmental and epileptic encephalopathy in mice. Brain 2020; 143:2039. https://doi.org/10.1093/brain/awaa147
96. Zhao T, Zhong R, Zhang X, Li G, Zhou C, Fang S, Ding Y, et al. Efavirenz restored NMDA receptor dysfunction and inhibited epileptic seizures in GluN2A/Grin2a mutant mice. Front Neurosci 2023; 17. https://doi.org/10.3389/fnins.2023.1086462
97. Candelas Serra M, Kuchtiak V, Kubik-Zahorodna A, Kysilov B, Fili K, Abramova V, Dobrovolski M, et al. Characterization of mice carrying a neurodevelopmental disease-associated GluN2B(L825V) variant. J Neurosci (accepted). https://doi.org/10.1523/JNEUROSCI.2291-23.2024
98. Ragnarsson L, Zhang Z, Das SS, Tran P, Andersson Å, des Portes V, Desmettre Altuzarra C, et al. GRIN1 variants associated with neurodevelopmental disorders reveal channel gating pathomechanisms. Epilepsia 2023; 1:0-3. https://doi.org/10.1111/epi.17776
99. Kalbaugh TL, VanDongen HMA, VanDongen AMJ. Ligand-binding residues integrate affinity and efficacy in the NMDA receptor. Mol Pharmacol 2004;66:209-19. https://doi.org/10.1124/mol.66.2.209
100. Zhang J, Tang W, Bhatia NK, Xu Y, Paudyal N, Liu D, Kim S, et al. A *de novo* GRIN1 variant associated with myoclonus and developmental delay: from molecular mechanism to rescue pharmacology. Front Genet 2021; 12:1-16. https://doi.org/10.3389/fgene.2021.694312
101. Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, Park AR, et al. Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. Am J Hum Genet 2011; 88:306-16. https://doi.org/10.1016/j.ajhg.2011.02.001, https://doi.org/10.1016/j.ajhg.2011.03.011
102. Chen W, Shieh C, Swanger SA, Tankovic A, Au M, McGuire M, Tagliati M, et al. GRIN1 mutation associated with intellectual disability alters NMDA receptor trafficking and function. J Hum Genet 2017; 62:589-97. https://doi.org/10.1038/jhg.2017.19
103. Fry AE, Fawcett KA, Zelnik N, Yuan H, Thompson BAN, Shemer-Meiri L, Cushion TD,et al. De novo mutations in GRIN1 cause extensive bilateral polymicrogyria. Brain 2018; 141:698-712. https://doi.org/10.1093/brain/awx358
104. Shepard N, Baez-Nieto D, Iqbal S, Kurganov E, Budnik N, Campbell AJ, Pan JQ, et al. Differential functional consequences of GRIN2A mutations associated with schizophrenia and neurodevelopmental disorders. Sci Rep 2024; 14:2798. https://doi.org/10.1038/s41598-024-53102-3

105. Sibarov DA, Bruneau N, Antonov SM, Szepetowski P, Burnashev N, Giniatullin R. Functional properties of human NMDA receptors associated with epilepsy-related mutations of GluN2A subunit. Front Cell Neurosci 2017; 11:1-10. https://doi.org/10.3389/fncel.2017.00155
106. Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, Salmi M, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat Genet 2013; 45:1061-6. https://doi.org/10.1038/ng.2726
107. Xu XX, Liu XR, Fan CY, Lai JX, Shi YW, Yang W, Su T, et al. Functional Investigation of a GRIN2A Variant Associated with Rolandic Epilepsy. Neurosci Bull 2018; 34:237-46. https://doi.org/10.1007/s12264-017-0182-6
108. Tang W, Beckley JT, Zhang J, Song R, Xu Y, Kim S, Quirk MC, et al. Novel neuroactive steroids as positive allosteric modulators of NMDA receptors: mechanism, site of action, and rescue pharmacology on GRIN variants associated with neurological conditions. Cell Mol Life Sci 2023; 80. https://doi.org/10.1007/s00018-022-04667-7
109. Carvill GL, Regan BM, Yendle SC, O'Roak BJ, Lozovaya N, Bruneau N, Burnashev N, et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. Nat Genet 2013; 45:1073-6. https://doi.org/10.1038/ng.2727
110. Iacobucci GJ, Liu B, Wen H, Sincox B, Zheng W, Popescu GK. Complex functional phenotypes of NMDA receptor disease variants. Mol Psychiatry 2022; 27:5113-23. https://doi.org/10.1038/s41380-022-01774-6
111. Marwick KFM, Parker P, Skehel P, Hardingham G, Wyllie DJA. Functional assessment of the NMDA receptor variant GluN2AR586K. Wellcome Open Res 2017; 2:1-16. https://doi.org/10.12688/wellcomeopenres.10985.1, https://doi.org/10.12688/wellcomeopenres.10985.2
112. Endele S, Rosenberger G, Geider K, Popp B, Tamer C, Stefanova I, Milh M, et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet 2010; 42:1021-6. https://doi.org/10.1038/ng.677
113. Marwick KFM, Skehel PA, Hardingham GE, Wyllie DJA. The human NMDA receptor GluN2AN615K variant influences channel blocker potency. Pharmacol Res Perspect 2019; 7:1-7. https://doi.org/10.1002/prp2.495
114. Chen X, Keramidas A, Harvey RJ, Lynch JW. Effects of GluN2A and GluN2B gain-of-function epilepsy mutations on synaptic currents mediated by diheteromeric and triheteromeric NMDA receptors. Neurobiol Dis 2020; 140:104850. https://doi.org/10.1016/j.nbd.2020.104850
115. Bertocchi I, Eltokhi A, Rozov A, Chi VN, Jensen V, Bus T, Pawlak V, et al. Voltage-independent GluN2A-type NMDA receptor Ca2+ signaling promotes audiogenic seizures, attentional and cognitive deficits in mice. Commun Biol 2021; 4:1-21. https://doi.org/10.1038/s42003-021-01717-x, https://doi.org/10.1038/s42003-020-01538-4
116. Fernández-Marmiesse A, Kusumoto H, Rekarte S, Roca I, Zhang J, Myers SJ, Traynelis SF, et al. A novel missense mutation in GRIN2A causes a non-epileptic neurodevelopmental disorder. Mov Disord 2018;33:992. https://doi.org/10.1002/mds.27315
117. Vieira MM, Peng S, Won S, Hong E, Inati SK, Thurm A, Thiam AH, et al. A Frameshift Variant of GluN2A Identified in an Epilepsy Patient Results in NMDA Receptor Mistargeting. J Neurosci 2024; 44:JN-RM-0557-23. https://doi.org/10.1523/JNEUROSCI.0557-23.2023
118. Yong XLH, Zhang L, Yang L, Chen X, Tan JZA, Yu X, Chandra M, et al. Regulation of NMDA receptor trafficking and gating by activity-dependent CaMKIIα phosphorylation of the GluN2A subunit. Cell Rep 2021;36:109338. https://doi.org/10.1016/j.celrep.2021.109338
119. Adams DR, Yuan H, Holyoak T, Arajs KH, Hakimi P, Markello TC, Wolfe LA, et al. Three rare diseases in one Sib pair: RAI1, PCK1, GRIN2B mutations associated with Smith-Magenis Syndrome, cytosolic PEPCK deficiency and NMDA receptor glutamate insensitivity. Mol Genet Metab 2014; 113:161-70. https://doi.org/10.1016/j.ymgme.2014.04.001
120. Wells G, Yuan H, McDaniel MJ, Kusumoto H, Snyder JP, Liotta DC, Traynelis SF, et al. The GluN2B-Glu413Gly NMDA receptor variant arising from a de novo GRIN2B mutation promotes ligand-unbinding and domain opening. Proteins Struct Funct Bioinforma 2018; 86:1265-76. https://doi.org/10.1002/prot.25595
121. Bell S, Maussion G, Jefri M, Peng H, Theroux JF, Silveira H, Soubannier V, et al. Disruption of GRIN2B Impairs Differentiation in Human Neurons. Stem Cell Reports 2018;11:183-96. https://doi.org/10.1016/j.stemcr.2018.05.018

122. Mullier B, Wolff C, Sands ZA, Ghisdal P, Muglia P, Kaminski RM, André VM, et al. GRIN2B gain of function mutations are sensitive to radiprodil, a negative allosteric modulator of GluN2B-containing NMDA receptors. Neuropharmacology 2017; 123:322-31. https://doi.org/10.1016/j.neuropharm.2017.05.017
123. Bahry JA, Fedder-Semmes KN, Sceniak MP, Sabo SL. An Autism-Associated de novo Mutation in GluN2B Destabilizes Growing Dendrites by Promoting Retraction and Pruning. Front Cell Neurosci 2021; 15:1-11. https://doi.org/10.3389/fncel.2021.692232