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GRIN2B encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects

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Abstract

Background—We aimed for a comprehensive delineation of genetic, functional and phenotypic aspects of *GRIN2B* encephalopathy and explored potential prospects of personalised medicine.

Methods—Data of 48 individuals with de novo *GRIN2B* variants were collected from several diagnostic and research cohorts, as well as from 43 patients from the literature. Functional consequences and response to memantine treatment were investigated in vitro and eventually translated into patient care.

Results—Overall, de novo variants in 86 patients were classified as pathogenic/likely pathogenic. Patients presented with neurodevelopmental disorders and a spectrum of hypotonia, movement disorder, cortical visual impairment, cerebral volume loss and epilepsy. Six patients presented with a consistent malformation of cortical development (MCD) intermediate between tubulinopathies and polymicrogyria. Missense variants cluster in transmembrane segments and ligand-binding sites. Functional consequences of variants were diverse, revealing various potential gain-of-function and loss-of-function mechanisms and a retained sensitivity to the use-dependent blocker memantine. However, an objectifiable beneficial treatment response in the respective patients still remains to be demonstrated.

Conclusions—In addition to previously known features of intellectual disability, epilepsy and autism, we found evidence that *GRIN2B* encephalopathy is also frequently associated with movement disorder, cortical visual impairment and MCD revealing novel phenotypic consequences of channelopathies.

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Web resource

For the ExAC database, see: http://exac.broadinstitute.org

Contributors KP, HOY, BIL, SFT, JRL conceived the project. KP, KLH, ST, MCW, BTT, DJA, CD, BK, CM, EF, SB, DD, TMS, HCM, CTM, AMM, AL, LS, IES, EB, LAB, RSM, UBJ, JJM, ATB, EMG, IDB, SF, PM, JRJ, EHZ, RAJ, AR, RJL, JL, TR, FEJ, ER, CMK, MMvH, JJvdS, AEL, CC, TL, DRS, CS, MM, DM, AD, WHT, MAT, BIL, MW, LD, SEP, KLJ, ADP, DNF, RV, EM, JDR, ND, WBD, SFT, JRL recruited and phenotyped patients. HY, HS, AW, WC, CH, HK, BIL, SFT performed in vitro experiments. KP, HY, HS, AW, WC, CH, HK, HOH, ND, WBD, BL, SFT, JRL performed data analysis and statistics. KP, HY, SFT, JRL wrote the manuscript. All authors edited the manuscript.

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INTRODUCTION

N-methyl-D-aspartate receptors (NMDAR) are ligand-gated ion channels expressed throughout the brain mediating excitatory neurotransmission. Signalling via NMDAR plays an important role in brain development, learning, memory and other higher cognitive functions. NMDAR are di-heterotetramers or triheterotetramers composed of two glycine-bindingGluN1(encodedby*GRIN1*)andtwo glutamate-binding GluN2 subunits (*GRIN2A-D*).[1] Simultaneous binding of both agonists activates the NMDAR, which opens a cation-selective pore leading to an influx of Ca^{2+} and depolarisation. Compared with the ubiquitously expressed GluN1 subunit, the GluN2 subunits show specific spatiotemporal expression profiles throughout the central nervous system.[2] GluN2B and GluN2D subunits are expressed prenatally, whereas expression of GluN2A and GluN2C significantly increases shortly after birth. Over time, postnatal expression of GluN2B becomes progressively restricted to the forebrain.

Pathogenic de novo variants in four genes encoding NMDAR subunits (*GRIN1*, *GRIN2A*, *GRIN2B* and *GRIN2D*) have been identified in patients with neurodevelopmental disorders comprising developmental delay (DD), intellectual disability (ID), autism spectrum disorders (ASD), epilepsy and epileptic encephalopathy, as well as movement disorders such as choreoathetosis or dystonia.[3–13]

To delineate the phenotypic spectrum of *GRIN2B* encephalopathy, we reviewed previously published and newly diagnosed patients with pathogenic/likely pathogenic de novo variants in *GRIN2B*. We evaluated the functional consequences of 16 variants in *Xenopus laevis* oocytes, investigated in vitro responses to memantine for six potential gain-of-function variants and aimed for translation of these results into personalised therapeutic approaches.

MATERIALS AND METHODS

Patients

We reviewed the clinical and genetic data of patients in whom *GRIN2B* de novo variants were detected within diagnostic or research settings focusing on neurodevelopmental disorders. Referring physicians provided detailed clinical information via a standardised clinical questionnaire. Molecular diagnostics were performed by targeted panel sequencing,[14] whole exome sequencing (WES) or array comparative genomic hybridisation. All legal guardians provided informed written consent for genetic testing in accordance with the respective national ethics guidelines and with approval of the local ethics committees in the participating study centres.

Variant classification

Variants in *GRIN2B* (NM_000834.3) were determined to be pathogenic, likely pathogenic or of unknown significance according to established guidelines of the American College of Medical Genetics (ACMG) (tables 1 and 2).[15] All variants classified as (likely) pathogenic constitute de novo variants. The database of the Exome Aggregation Consortium (ExAC) served as the control population.[16]

GRIN2B physiology

We used cDNA for wild-type (WT) human NMDA GluN1-1a (hereafter GluN1; NP_015566) and GluN2B subunits in pCI-neo (NP_000827.1).[17] The mutant GluN2B constructs were generated by site-directed mutagenesis using the Quik-Change protocol (Stratagene). Synthesis and injection of cRNA into *Xenopus laevis* oocytes (Ecocyte Inc.) and two-electrode voltage-clamp current (TEVC) recordings were performed at −40 mV and 23°C (unless otherwise stated), as previously described.[18] Recording electrodes were filled with 0.3 M KCl for voltage electrode and 3 M KCl for the current electrode. The recording solution contained (in mM) 90 NaCl, 1 KCl, 10 HEPES, 0.5 $BaCl_2$ and 0.01 EDTA (pH 7.4). The concentration-response curves were fitted with the following equations:

$$\text{Response}\,(\%)=100/(1+(\text{EC}_{50}/(\text{agonist}))^{nH}) \quad \text{Equation 1}$$

$$\text{Response}\,(\%)=(100-minimum)/(1+((\text{antagonist})/\text{IC}_{50})^{nH})+minimum \quad \text{Equation 2}$$

where *nH* is the Hill slope, EC_{50} is the concentration of the agonist that produces a half-maximal effect, IC_{50} is the concentration of the antagonist that produces a half-maximal effect, and *minimum* is the degree of residual inhibition at a saturating concentration of the antagonist. The effects of co-application of increasing concentrations of memantine and maximally effective concentrations of glutamate (100 μM) and glycine (30 μM) on the response of NMDAR were determined using TEVC recordings from oocytes co-expressing GluN1 with the WT or the mutant GluN2B. The concentration-effect curves were recorded at a holding potential of −40 mV and fitted with equation 2.

Patient treatment

Patients were offered memantine treatment after functional confirmation of a gain-of-function variant retaining memantine sensitivity in vitro. In four patients, oral memantine treatment was initiated aiming for doses of 0.5–0.6 mg/kg body weight per day referring to the dosage of a treatment trial in *GRIN2A* encephalopathy.[19] Patients were neurologically assessed prior and during memantine treatment. Individual treatment trials were approved by the local ethics boards of the participating centres.

RESULTS

We evaluated 48 novel and all 43 previously published individuals with de novo *GRIN2B* variants. Overall, variants in 86 out of these 91 individuals were classified as pathogenic/likely pathogenic. For 58 patients (39 novel and 19 published), detailed clinical data were available to comprehensively delineate the phenotypic spectrum of *GRIN2B* encephalopathy. The novel variant p.(Ser810Arg) was identified in monozygotic twin brothers with identical phenotype who were thus regarded as only one index case.

Phenotypic spectrum

All patients carrying a (likely) pathogenic *GRIN2B* variant presented with DD, ID and/or ASD (86/86; 100%, table 1, table 2, detailed phenotypic data in online supplementary table 3). The level of ID could be specified in 52 of 58 patients, with a majority displaying severe ID (31/52; 60%), whereas smaller proportions had moderate (13/52; 25%) or mild ID (8/52; 15%). Autistic features were seen in 28% of patients (16/58). At the time of data ascertainment, about half of the patients have had seizures (30/58; 52%), with a variable age of onset ranging from birth to 9 years and the frequency of seizures varying from multiple per day to a few seizures per year. Patients presented with generalised seizures (18/30; 60%, mostly tonic or tonic-clonic), focal seizures (14/30; 47%) and/or epileptic spasms (11/30; 37%). EEG patterns comprised hypsarrhythmia, focal, multifocal and/or generalised epileptiform activity. Follow-up data concerning seizure outcome were available for 22 patients, with one half becoming seizure-free (11/22; 50%) and the other half remaining refractory to therapy. No obvious treatment strategy using conventional antiepileptic drugs (AED) was associated with a higher likelihood of seizure freedom. At least 31 patients displayed hypotonia (31/58; 53%), which required tube feeding in five patients (5/31; 16%). Spasticity was documented in 14 patients (14/58; 24%). In addition, six patients presented with dystonic, dyskinetic or choreiform movement disorders (6/58; 10%). Four patients (4/58; 7%) showed signs of developmental regression, two of whom were temporary and one case had recurrent regression. Microcephaly was seen in multiple patients (11/58; 19%) and cortical visual impairment (CVI) was reported in four (4/58; 7%), three of whom also had a malformation of cortical development (MCD).

Neuroimaging data

Neuroimaging was performed in 44 of 58 patients: six patients (6/44, 14%) showed a consistent MCD intermediate between typical polymicrogyria (PMG) and the cortical appearance of tubulinopathies, consisting of mixed large and small gyri separated by shallow sulci, a smooth grey-white border and little infolding (figure 1). These patients also had hypoplastic corpus callosum of varying degrees, enlarged and mildly dysplastic basal ganglia, hippocampal dysplasia with thick leaves and open hilus as well as enlarged tecta (figure 1A, E and M). One patient had no septum pellucidum (figure 1C). Generalised cerebral volume loss, compatible with cerebral atrophy, was described in four additional patients (4/44; 9%).

Genetic spectrum

The 86 (likely) pathogenic variants in *GRIN2B* comprise 52 distinct missense variants. Variants cluster within or in very close proximity to the ligand-binding domains S1 and S2, as well as transmembrane domains M1–M4 (figure 2A, table 1). The only variant escaping this clustering pattern is p.(Ile150Val) in the amino-terminal domain (ATD). Although most variants were unique, five variants occurred multiple times: p.(Arg540His), p.(Gly689Ser), p.(Arg696His), p.(Ile751Thr) and p.(Gly820Ala). The 21 distinct pathogenic variants presumably leading to truncation/haploinsufficiency comprised nonsense and frameshift variants (n=11), splice site variants (n=3), chromosomal rearrangements (n=3) as well as gross deletions encompassing only *GRIN2B* (n=4) (figure 2B, table 2). According to the

ACMG criteria, five de novo variants were classified as variants of unknown significance (see online supplement 2).[15]

Frequency of GRIN2B encephalopathy

Several of our collaborators (RSM, DS, CS and SB) performed different diagnostic panel sequencing approaches in 3136 independent epilepsy patients revealing seven (likely) pathogenic *GRIN2B* variants and a diagnostic frequency of 0.22%. Three diagnostic WES trio cohorts reported 8051 patients with neurodevelopmental disorders (defined as human phenotype ontology terms (1) abnormality of the nervous system, (2) multiple congenital anomalies, (3) seizures or (4) ASD.[20–22] Overall, 15 pathogenic/likely pathogenic *GRIN2B* variants (14 missense, 1 frameshift) were identified, equalling a similar frequency of 0.19%. Expanding the data of the Deciphering Developmental Disorders Study,[23] 14 de novo missense variants in *GRIN2B* are significantly enriched (p value 2×10^{-17}) in a combined cohort of WES trio data (n=8051) of patients with neurodevelopmental disorders (see online supplement 3). Among 209 independent individuals with PMG, we identified two patients with de novo *GRIN2B* variants.

Functional investigation

Seven of 17 variants evaluated showed small apparent currents (less than 15 nA for 1000 μM glutamate and 100 μM glycine) (see online supplementary table 1). Because it was unclear whether these small currents were agonist evoked, the properties of these variants could not be studied further. We assessed the pharmacological properties of NMDAR containing the remaining 10 GluN2B mutants. Three variants (p.(Ser541Arg), p.(Val558Ile), and p.(Ile655Phe)) increased glutamate EC_{50} values (ie, decreased glutamate potency) by 6.7-fold, 2.8-fold and 3.7-fold (table 3), indicating that higher concentrations of glutamate are needed to activate these receptors. The variant p.(Ser541Arg) increased glycine EC_{50} values by 2.9-fold compared with WT receptors. Three variants (p.(Ser810Arg), p.(Met818Thr), and p.(Al-a819Thr)) decreased both the glutamate and glycine EC_{50} values (ie, increased glutamate and glycine potency) (table 3), allowing these mutant receptors to be activated by lower concentration of agonists, suggesting a potential gain-of-function, provided trafficking and other features of receptor function are unchanged.

Voltage-dependent inhibition by extracellular Mg^{2+} and negative modulation by extracellular protons are two important features of NMDAR function. The voltage-dependent potency of Mg^{2+} inhibition showed that p.(Gly611Val) and p.(Ile655Phe) increased the Mg^{2+} IC_{50} value from 25 μM for WT NMDAR to over 1000 μM and 220 μM for NMDAR containing GluN2B-Gly611Val and GluN2B-Ile655Phe, respectively (−60 mV holding potential; $p<0.05$, one-way analysis of variance) (table 3). Proton sensitivity was evaluated by comparing the current amplitude at two different extracellular pH values (6.8 vs. 7.6). Four mutant GluN2B subunits (p.(Ile655Phe), p.(Ser810Arg), p.(Met818Thr) and p.(Ala819Thr)) show significantly larger current response at pH 6.8 compared with pH 7.6 than WT receptors (table 3), suggesting that these variants significantly reduce tonic proton inhibition. This could result in more current flowing through the channel when the receptor is bound by agonists. Taken together, these data suggest that three variants (p.(Ser810Arg), p.(Met818Thr) and p.(Ala819Thr)) are likely overactive under resting conditions as a result of

both the increased activation at low concentrations of agonists, reduced voltage-dependent Mg^{2+} block and reduced proton inhibition. The combination of these effects could potentially contribute to hyperexcitability and thus to patients' epileptic phenotype. Further data are needed to determine how receptors with mixed functional changes (decreased agonist potency, decreased regulation by Mg^{2+} or protons) will alter, for example, charge transfer during synaptic transmission.

Evaluation of NMDAR antagonist memantine

The Food and Drug Administration-approved NMDAR antagonist memantine[24] has been suggested to have anticonvulsant effects in some epilepsy animal models[25] and was previously used off-label to effectively treat one patient with a gain-of-function GluN2A variant (p.(Leu812Met)).[19] Thus, we evaluated its ability to inhibit six potential gain-of-function GluN2B variants (p.(Gly611Val), p.(Asn615Ile), p.(Val618Gly), p.(Ser810Arg), p.(Met818Thr) and p.(Ala819Thr))[9] (see online supplementary figure 1 and supplementary table 2). The data indicated GluN2B-Gly611Val and GluN2B-Asn615Ile decreased memantine IC_{50} by 1.7-fold and 3-fold, respectively (ie, increased potency; 1.0 μM for p.(Gly611Val) and 0.44 μM for p.(Asn615Il)e compared with 1.7 μM of WT). The other four variants (p.(Val618Gly), p.(Ser810Arg), p.(Met818Thr) and p.(Ala819Thr)) increased the IC_{50} values (ie, decreased potency) compared with WT (see online supplementary figure 1 and supplementary table 2), suggesting that memantine can reduce NMDAR hyperactivity caused by these variants, although several mutants showed a reduced potency compared with WT receptors.

Targeted treatment with memantine

In four out of these six patients, we added memantine to the AED regimen (p.(Gly611Val), p.(Asn615Ile), p.(Val618Gly) and p.(Met818Thr)) (see online supplement 1). Parents and physicians initially observed beneficial effects such as improvements in awareness, behaviour and sleep. However, there were no changes in seizure frequencies and none of the potential benefits could be sufficiently objectivised. Long-term follow-up data were available in only one patient (p.(Val618Gly)) and did not suggest significant improvement.

DISCUSSION

We present the largest series of patients with (likely) pathogenic *GRIN2B* variants and provide a comprehensive review of the different aspects of *GRIN2B* encephalopathy. We strictly applied the ACMG criteria for variant classification to lessen the impact of potential biases caused by the heterogeneous collection of patients.

Phenotypic spectrum

In agreement with the known *GRIN2B*-associated features, our data confirm that all patients had DD and a majority developed severe ID, with autistic features in a quarter of patients. Half of the patients developed seizures with a broad heterogeneity with respect to age of onset, seizure semiology, EEG features and outcome. Expanding our previous observation, 37% of patients presented with epileptic spasms.[9] Less frequent findings of *GRIN2B* encephalopathy include generalised cerebral volume loss, CVI, hyperkinetic movement

disorders (dystonia, dyskinesia, chorea) and developmental regression. These features have been repeatedly observed in other GRIN-associated encephalopathies,[4510121926][27] suggesting a shared phenotypic spectrum, with differences likely reflecting variant class, location and effect in the various NMDAR subunits. However, we did not observe EEG patterns of continuous spikes and waves during sleep or centrotemporal spikes in our *GRIN2B* cohort, which appears to reflect a milder end of the GRIN spectrum that is so far predominantly associated with *GRIN2A*.[28] As systematic genetic testing is more likely performed in patients with severe early-onset disorders, the mentioned frequencies of, for example, severe ID and epilepsy in *GRIN2B* encephalopathy are probably overestimated due to ascertainment bias.

Malformation of cortical development

Six patients presented with a consistent MCD intermediate between typical PMG and tubulinopathies. This has also been referred to as tubulinopathy-related dysgyria.[29] Whereas tubulinopathies are classified as disorders of neuronal migration leading to cortical dysgenesis,[30] PMG is considered to be due to postmigrational disruption of cortical development with fusion of cortical laminae.[31] Knockdown of *Grin2b* in rat has been shown to disturb proper neuronal migration[32] and glutamate has been implemented in its regulation.[33] As GluN2B is predominantly expressed prenatally, disruption of neuronal migration seems to be the possible cause of *GRIN2B*-associated MCD. All six individuals had a very similar degree of severity and we did not observe less pronounced patterns of dysgyria in others. We can largely exclude the co-occurrence of, for example, known genetic tubulinopathies in all six cases, as individuals were screened by (trio)WES (n=4) or a panel targeting MCD genes (n=2). Other than in *GRIN2B*, we did not identify any other putatively causative variants in all six cases. Deficient ion channels are known to cause a plethora of human neurodevelopmental disorders, with the exception of abnormal cell migration. Despite the observation of six patients with pathogenic *SCN1A* variants who also had focal cortical dysplasia or bilateral periventricular nodular heterotopia,[34] the identification of six unrelated individuals with (likely) pathogenic variants in *GRIN2B* and a consistent MCD expands the phenotypic spectrum and reveals novel phenotypic consequences of channelopathies.

Genetic spectrum

GRIN2B is a gene with a significantly reduced number of missense variants in controls, indicating a selective constraint.[16] All (likely) pathogenic missense variants cluster within or in direct proximity to ligand-binding sites and transmembrane domains. These regions are largely spared by (likely) benign single nucleotide variants (SNV) in ExAC controls, suggesting that missense variants within these functionally important and conserved domains are only scarcely tolerated.[3536] In contrast, the ATD and carboxy-terminal domain (CTD), as well as two small segments within S1 and M1, are markedly enriched for (likely) benign SNV in controls. The likely pathogenic missense variant p.(I-le150Val) escapes this clustering pattern, as it is located within a stretch of ~300 bp that contains very few missense variants within the ATD. It remains unclear whether or not this variant alters subunit assembly, ligand-binding or allosteric regulation by extracellular Zn^{2+}.[12]

The ratio of expected versus observed numbers of truncating variants of 33.9:0 in controls and the probability of loss-of-function intolerant score of 1.00 suggest that *GRIN2B* haploinsufficiency is most likely not tolerated.[16] We observed seven truncating variants in the CTD establishing a premature termination codon in the last exon and therefore likely escaping nonsense-mediated mRNA decay and possibly not significantly altering protein function[1537]: two de novo truncating variants in patients with ID,[20] one de novo frameshift variant in a healthy control individual[38] and four frameshift variants reported in ExAC. The ExAC-annotated frameshift variant c.99dupC, p.(Ser34Gln*fs**25) occurring in 27 controls conflicts with the hypothesis of haploinsufficiency. However, this variant is part of a homopolymer stretch of seven recurrent C nucleotides, raising the suspicion of a technical artefact despite having been described as pathogenic de novo variant via different sequencing approaches in an individual with ASD.[39]

Genotype-phenotype correlations

MCD-associated variants are located in the transmembrane domain M3 (p.(Ala636Val), p.(Ala639Val) and p.(Ile655Phe)), in the ligand-binding domain S2 (p.(Arg693Ser)) or within the linker domain connecting S2 and M4 (p.(Ser810Arg) and p.(Ser810Asn)). Both missense variants at position 810 (p.(Ser810Arg) and p. (Ser810Asn)) were associated with MCD, whereas position 636 also showed two different (likely) pathogenic missense variants, with only p.(Ala636Val) being seen in MCD.[40] Thus, the specific functional mechanisms mediating MCD still remain to be elucidated. In contrast to our previous hypothesis,[9] we did not find a significant correlation between variant class (missense vs truncation) and occurrence of seizures (Fisher's exact test, p=0.1187). However, there was a significant correlation between variant class and intellectual outcome (mild-moderate vs severe ID) (Fisher's exact test, p=0.0079), with truncation carriers tending to present with mild or moderate instead of severe ID.

Functional investigation

We evaluated several different aspects of receptor function, as it is possible that variants could have opposing effects on receptor activity (eg, Ile655Phe). We found that several other variants (Ser810Arg, Met818Thr, Ala819Thr) showed multiple differences in their properties that each enhanced NMDAR function, suggesting a potential gain-of-function of NMDAR assuming there is no change in the fraction of receptors that reach the cell surface or synapse. Such functional changes could lead to excito-toxic cell death, circuit rewiring, changes in neuronal migration or persistent increases in excitatory synaptic and non-synaptic drive through surface receptors. Thus there are multiple ways these variant receptors could contribute to clinical symptoms. Extensive functional analyses of rare variants in the GluN2B agonist binding domain recently showed the multifaceted and sometimes conflicting consequences that these variants can have on NMDAR activity.[36]

Targeted treatment with memantine

Initial subjective improvements in awareness, behaviour and sleep could not be sufficiently objectivised, and seizure frequencies showed no significant changes. In three cases, the missense variant led to significant loss of Mg^{2+} block engaging a different mechanism compared with the multiple means by which channel function was enhanced in a patient

with p.(Leu812Met) in *GRIN2A*, which responded favourably to memantine treatment.[19] In comparison, the variant of the fourth individual (p.(Met818Thr)) displayed similarities with respect to, for example, the increased glycine and glutamate potency of the NMDAR; however this does not hold true for patients' treatment response to memantine. At present, it remains entirely unclear whether memantine is effective at all in this patient population and what factors (eg, variant location, mechanism of gain-of-function, patient age, memantine dose, differences in blood and brain concentrations) might influence memantine treatment response. Although some variants retain sensitivity to channel blockers like memantine in vitro, translating these potential benefits into patient care still remains elusive. Options for personalised therapy in *GRIN2B* encephalopathy still require more systematic and thorough evidence best through double-blinded prospective trials with a homogeneous patient population in terms of variant class.

Summary

Given the frequency of about 0.2% among individuals with neurodevelopmental disorders and/or childhood-onset epilepsy, *GRIN2B* encephalopathy appears to be a recurrent and distinct diagnosis. Our observations of novel features expand the phenotypic spectrum and suggest novel consequences of channelopathies resulting in disturbed neuronal migration. We found phenotypic similarities and a marked clustering of missense variants in ligand-binding and transmembrane domains paralleling other GRIN-associated disorders. Despite the limited conclusions regarding treatment with memantine in four patients with potential gain-of-function variants, the diverse functional consequences of (likely) pathogenic variants possibly enable future personalised therapeutic approaches in patients with *GRIN2B* encephalopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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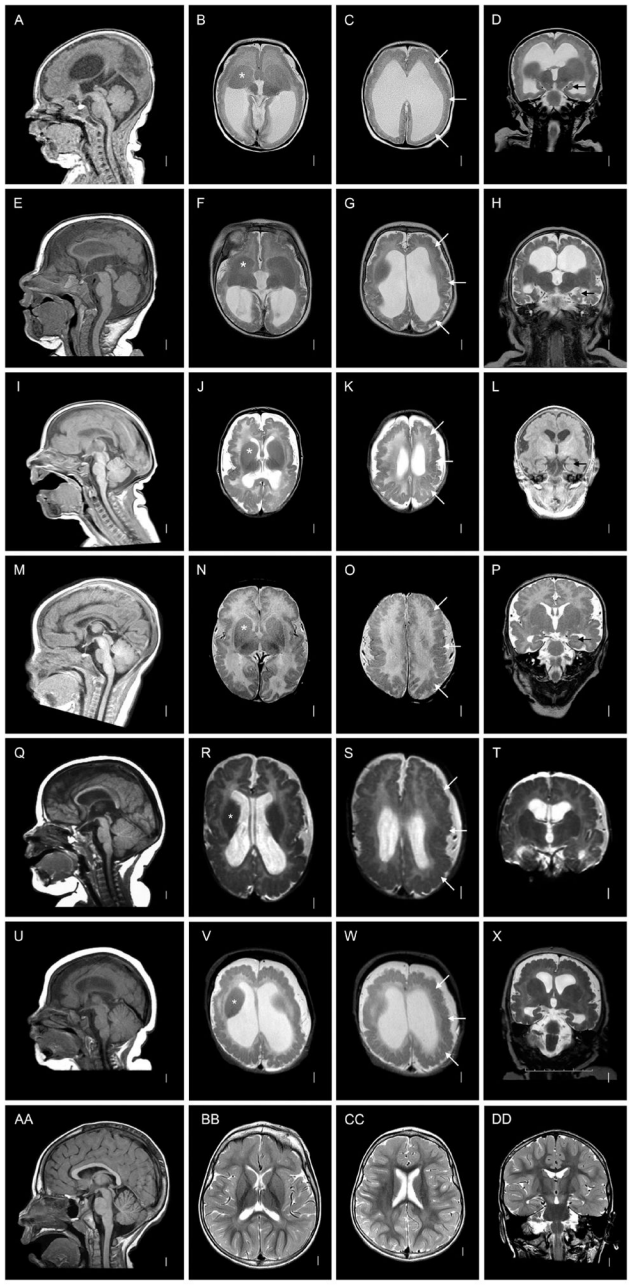

Figure 1.

MRI of patients with malformation of cortical development. MRI scans of patients p.(Ala639Val) (A–D), p.(Ser810Arg) (E–H), p.(Ile655Phe) (I–L), p.(Ala636Val) (M–P), p.(Arg693Ser) (Q–T), p.(Ser810Asn) (U–X), and a normal control (AA-DD) showing T1-weighted mid-sagittal images (first column), T2-weighted axial images through the basal ganglia (second column) and higher lateral ventricles (third column), and T2-weighted coronal images through the hippocampus (fourth column). The mid-sagittal images are normal except for mildly low forehead in several subjects (A, I, U), although several are slightly off the midline. The lower axial images show relatively large and mildly dysplastic basal ganglia compared with normal (asterisks in B, (F, J, N, R and V)). All axial images

(middle two rows) show a diffuse irregular gyral pattern with small gyri and limited intracortical microgyri (white arrows on the right side of images that point to the left hemispheres), an appearance intermediate between typical polymicrogyria and the cortical appearance of tubulinopathies. The coronal images show hippocampal dysplasia with thick leaves and open hilus, which varies from severe (D, H, X) to moderate (L, P).

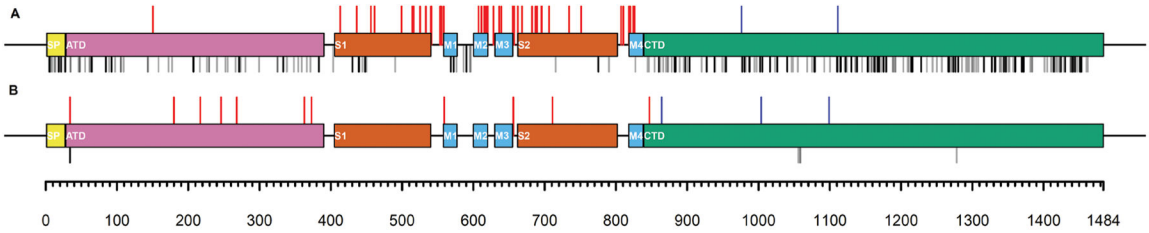

Figure 2.

De novo variants in *GRIN2B*. (A) Clustering of (likely) pathogenic missense variants in *GRIN2B*. All but one of the (likely) pathogenic missense variants cluster in the ligand-binding or transmembrane domains of GluN2B, which are regions with little to no benign missense variation in the control population. The de novo missense p.(Arg1111His) and the inframe deletion p.(Lys976del) in the CTD were classified as VUS. (B) Location of pathogenic truncating variants in *GRIN2B*. The recurrent variant c.99dupC, p.(Ser34Gln*fs**25) listed in ExAC is part of a homopolymer stretch of seven recurrent C nucleotides suggesting a technical artefact. Variants classified as VUS establish a premature termination codon in the last exon of *GRIN2B*, including a de novo truncating variant in a control individual p.(Arg1099Ala*fs**51).[38] Red bars indicate pathogenic/likely pathogenic de novo variants. Blue bars indicate de novo VUS. Grey bars indicate single nucleotide variants (SNV) listed in one ExAC individual each. Black bars indicate SNV seen in more than one ExAC individual. ATD, amino-terminal domain; CTD, carboxy-terminal domain; ExAC, Exome Aggregation Consortium; M1–M4, transmembrane domain; S1, S2, ligand-binding domain; VUS, variants of unknown significance.

Table 1

Heterozygous de novo missense variants and inframe deletions in *GRIN2B*

DNA	Protein	Domain	Classification[15]	Phenotype	DPI
c.448A>G	p.(Ile150Val)	ATD	LP	DD/ID	NA
c.1238A>G[41]	p.(Glu413Gly)	S1	P	Severe ID	+
c.1306T>C	p.(Cys436Arg)	S1	LP	Moderate ID, ASD, fSz, gSz	+
c.1367G>A[39]	p.(Cys456Tyr)	S1	LP	ASD/ID	NA
c.1382G>T[42]	p.(Cys461Phe)	S1	LP	ID, ASD, gSz	+
c.1495G>A	p.(Gly499Arg)	S1	LP	Moderate ID, ASD	+
c.1540A>G	p.(Thr514Ala)	S1	LP	Severe ID, gSz, MC	+
c.1547A>G	p.(Asn516Ser)	S1	LP	Mild ID	+
c.1573T>G[43]	p.(Phe525Val)	S1	LP	ASD/ID	NA
c.1619G>A[9]	p.(Arg540His)	S1	LP	Mild ID, fSz	+
c.1619G>A	p.(Arg540His)	S1	LP	Severe ID, ASD, fSz, gSz	+
c.1623C>G	p.(Ser541Arg)	L2	P	Severe ID, ASD, gSz, dystMD	+
c.1658C>T[44]	p.(Pro553Leu)	L2	LP	Severe ID	+
c.1664G>T	p.(Ser555Ile)	L2	LP	Severe ID	+
c.1672G>A	p.(Val558Ile)	M1	P	Moderate ID	+
c.1821G>T[45]	p.(Trp607Cys)	M2	LP	DD	+
c.1832G>T	p.(Gly611Val)	M2	LP	Severe ID, gSz, MC	+
c.1844A>T[9]	p.(Asn615Ile)	M2	LP	Severe ID, ASD, ES, gSz, MC	+
c.1845C>G[46]	p.(Asn615Lys)	M2	LP	DD/ID	NA
c.1848C>G	p.(Asn616Lys)	M2	LP	Severe ID, ES, gSz, GVL, MC	+
c.1853T>G[9]	p.(Val618Gly)	M2	LP	Severe ID, ES, dystMD	+
c.1858G>A[20]	p.(Val620Met)	M2	LP	DD/ID	NA
c.1883C>G	p.(Ser628Cys)	L4	LP	Severe ID, ASD, gSz, R	+
c.1883C>T[46]	p.(Ser628Phe)	L4	LP	DD/ID	NA
c.1906G>C[40]	p.(Ala636Pro)	M3	LP	Mild ID	+
c.1907C>T	p.(Ala636Val)	M3	LP	Severe ID, ES, fSz, MCD, MC, CVI	+

DNA	Protein	Domain	Classification[15]	Phenotype	DPI
c.1916C>T	p.(Ala639Val)	M3	LP	Severe ID, fSz, gSz, MCD, CVI	+
c.1963A>T	p.(Ile655Phe)	M3	LP	Severe ID, ES, gSz, MCD, MC, CVI	+
c.1970A>G	p.(Glu657Gly)	L5	LP	DD/ID	NA
c.1985A>C[47]	p.(Gln662Pro)	S2	LP	Severe ID, ES, fSz, gSz	+
c.2002G>T	p.(Asp668Tyr)	S2	LP	Severe ID, Sz	+
c.2044C>T[4]	p.(Arg682Cys)	S2	LP	Moderate ID	+
c.2060C>G[46]	p.(Pro687Arg)	S2	LP	DD/ID	NA
c.2060C>T[20]	p.(Pro687Leu)	S2	LP	DD/ID	NA
c.2065G>A	p.(Gly689Ser)	S2	LP	Severe ID, fSz, gSz, GVL, MC	+
c.2065G>A	p.(Gly689Ser)	S2	LP	Severe ID, ES, dyskMD	+
c.2065G>A[46]	p.(Gly689Ser)	S2	LP	DD/ID	NA
c.2065G>A[46]	p.(Gly689Ser)	S2	LP	DD/ID	NA
c.2079A>T	p.(Arg693Ser)	S2	LP	Severe ID, ES, fSz, gSz, MCD	+
c.2084T>C	p.(Ile695Thr)	S2	LP	Moderate ID	+
c.2087G>A	p.(Arg696His)	S2	LP	Moderate ID, ASD	+
c.2087G>A	p.(Arg696His)	S2	LP	Moderate ID, ASD, gSz	+
c.2116A>G	p.(Met706Val)	S2	LP	Severe ID, fSz	+
c.2201C>T	p.(Ala734Val)	S2	LP	DD/ID	NA
c.2252T>C	p.(Ile751Thr)	S2	LP	Severe ID, ASD	+
c.2252T>C	p.(Ile751Thr)	S2	LP	Mild ID	+
c.2252T>C	p.(Ile751Thr)	S2	LP	DD/ID	NA
c.2419G>A[46]	p.(Glu807Lys)	L6	LP	DD/ID	NA
c.2430C>A	p.(Ser810Arg)	L6	P	Severe ID, fSz, MCD, MC	+
c.2429G>A	p.(Ser810Asn)	L6	LP	Severe ID, ES, gSz, MCD	+
c.2452A>C	p.(Met818Leu)	M4	LP	DD/ID	NA
c.2453T>C	p.(Met818Thr)	M4	LP	DD, ES, fSz, gSz, CVI	+
c.2455G>A	p.(Ala819Thr)	M4	LP	DD/ID, Sz	+
c.2459G>C	p.(Gly820Ala)	M4	LP	Severe ID, ES, dyskMD	+
c.2459G>C	p.(Gly820Ala)	M4	LP	DD	+

DNA	Protein	Domain	Classification[15]	Phenotype	DPI
c.2459G>C	p.(Gly820Ala)	M4	LP	Severe ID, ASD, GVL	+
c.2459G>C	p.(Gly820Ala)	M4	LP	Severe ID, ASD	+
c.2459G>C[46]	p.(Gly820Ala)	M4	LP	DD/ID	NA
c.2459G>T	p.(Gly820Val)	M4	LP	DD/ID	NA
c.2459G>A[26]	p.(Gly820Glu)	M4	LP	Severe ID, GVL, MC	+
c.2471T>G[48]	p.(Met824Arg)	M4	LP	Severe ID, chMD, MC	+
c.2473T>G[49]	p.(Leu825Val)	M4	LP	ASD/ID	NA
c.2477G>A	p.(Gly826Glu)	M4	LP	Moderate ID	+
c.2926_2928delAAG	p.(Lys976del)	CTD	VUS	DD/ID	NA
c.3332G>A	p.(Arg1111His)	CTD	VUS	DD/ID	NA

ASD, autism spectrum disorder; ATD, amino-terminal domain; CTD, carboxy-terminal domain; CVI, cortical visual impairment;chMD, choreiform movement disorder; DD, developmental delay; DPI, detailed phenotypic information; dyskMD, dyskinetic movement disorder; dystMD, dystonic movement disorder; ES, epileptic spasms; fSz, focal seizures; gSz, generalised seizures; GVL, generalised cerebral volume loss; ID, intellectual disability; L1–L6, linker; LP, likely pathogenic; MC, microcephaly; MCD, malformation of cortical development; M1–M4, transmembrane domain; NA, not available; P, pathogenic; R, developmental regression; Sz, seizures (not further classified);S1, S2, ligand-binding domain; VUS, variant of unknown significance.

Table 2

Heterozygous de novo truncating variants in *GRIN2B*

DNA	Protein	Domain	Classification[15]	Phenotype	DPI
c.99dupC[39]	p.(Ser34Glnfs*25)	ATD	P	ASD/ID	NA
t(10;12) exon 2/intron 2[4]	p.?	ATD	P	Severe ID, MC	+
c.411+1G>A[4]	p.?	ATD	P	Moderate ID	+
c.538C>T	p.(Gln180*)	ATD	P	Moderate ID, fSz	+
c.649C>T[50]	p.(Gln217*)	ATD	P	DD/ID	NA
c.737C>A	p.(Ser246*)	ATD	P	Mild ID, R	+
c.803_804delCA[4]	p.(Thr268Serfs*15)	ATD	P	Moderate ID	+
c.1088del[50]	p.(Val363Glyfs*2)	ATD	P	DD/ID	NA
c.1119G>A	p.(Trp373*)	ATD	P	Severe ID, ASD, fSz, gSz, dystMD, R	+
t(9;12) intron 4[4]	p.?	ATD	P	Moderate ID	+
c.1677G>A[39]	p.(Trp559*)	M1	P	ASD/ID	NA
c.1966C>T	p.(Gln656*)	L5	P	Moderate ID, fSz	+
c.2131C>T[51]	p.(Gln711*)	S2	P	ASD/ID	NA
c.2172-2A>G[39]	p.?	S2	P	Mild ID, ASD, R	+
c.2360-2A>G[4]	p.?	S2	P	Mild ID	+
c.2539C>T	p.(Arg847*)	CTD	P	Severe ID, ASD	+
c.2539C>T	p.(Arg847*)	CTD	P	DD/ID	NA
c.2539C>T[46]	p.(Arg847*)	CTD	P	DD/ID	NA
(12:13986000–14040000)Del[46]	p.?	-	P	DD/ID	NA
		-			
(12:13656611–13749771)Del[46]	p.?	-	P	DD/ID	NA
		-			
(12:13724779–13828818)Del[46]	p.?	-	P	DD/ID	NA
(12:13595477–13814290)Del	p.?	-	P	No ID, DD	+
inv(12)(p13.1q21.31)[52]	p.?	-	P	Mild ID, ASD, Sz	+
c.2589delC[20]	p.(Ile864Serfs*20)	CTD	VUS	DD/ID	NA

DNA	Protein	Domain	Classification[15]	Phenotype	DPI
c.3012C>G	p.(Tyr1004*)	CTD	VUS	Moderate ID, ASD	+
c.3295delC[38]	p.(Arg1099Alafs*51)	CTD	VUS	Control	NA

Genomic positions of deletions refer to genome build GRCh37/hg19.

ASD, autism spectrum disorder; ATD, amino-terminal domain; CTD, carboxy-terminal domain; DD, developmental delay; DPI, detailed phenotypic information; dystMD, dystonic movement disorder; ES, epileptic spasms; fSz, focal seizures; gSz, generalised seizures; GVL, generalised cerebral volume loss; ID: intellectual disability; LP, likely pathogenic; L1–L6, linker; MC, microcephaly; MCD, malformation of cortical development; M1–M4, transmembrane domain; NA, not available; P, pathogenic; R, developmental regression; Sz, seizures (not further classified); S1, S2, ligand-binding domain; VUS, variant of unknown significance.

Table 3

Summary of pharmacological data

	Glu, EC_{50}, μM (n)	Gly, EC_{50}, μM (n)	Mg^{2+}, IC_{50}, μM (n)*	Proton, $I_{pH6.8}/I_{pH7.6}$% (n)
WT 2B	1.5±0.08 (44)	0.35±0.02 (41)	25±2.7 (33)	15%±0.5% (47)
S541R	10±1.7 (10)†	1.0±0.15 (9)†	18±1.8 (9)	17%±0.4% (13)
V558I	4.2±0.8 (6)†	0.50±0.03 (6)	26±6.2 (6)	13%±0.2% (6)
G611V	1.7±0.1 (8)	0.33±0.02 (6)	>1000 (8)†	11%±0.4% (8)
I655F	5.6±0.60 (7)†	0.51±0.07 (6)	220±36 (6)†	67%±2.6% (10)†
M706V	1.5±0.18 (7)	0.24±0.02 (6)	26±4.8 (5)	16%±1.2% (6)
S810R	0.013±0.003 (8)†	0.027±0.03 (7)†	24±3.3 (5)	35%±4.2% (8)†
M818T	0.37±0.07 (10)†	0.09±0.02 (6)†	29±8.0 (8)	47%±4.1% (8)†
A819T	0.58±0.07 (9)†	0.09±0.02 (6)†	36±4.9 (9)	51%±1.4% (6)†
G820A	1.6±0.23 (6)	0.35±0.04 (8)	21±4.8 (7)	10%±0.21% (6)
L825V	1.3±0.2 (9)	0.36±0.07 (5)	35±3.6 (10)	14%±0.7% (8)

Mean±SEM (n).

The log of the EC_{50} and IC_{50} are normally distributed and were used in all statistical tests.

*Holding at −60 mV.

†$p<0.05$ compared with corresponding wild-type (WT): one-way analysis of variance, Tukey post-hoc.