BRIEF COMMUNICATION

Compound-heterozygous GRIN2A null variants associated with severe developmental and epileptic encephalopathy

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Abstract
We report on an 8-year-old girl with severe developmental and epileptic encephalopathy due to the compound heterozygous null variants p.(Gln661*) and p.(Leu830Profs*2) in GRIN2A resulting in a knockout of the human GluN2A subunit of the N-methyl-D-aspartate receptor. Both parents had less severe GRIN2A-related phenotypes and were heterozygous carriers of the respective null variant. Functional investigations of both variants suggested a loss-of-function effect. This is the first description of an autosomal recessive, biallelic type of GRIN2A-related disorder. Nonetheless, there are marked parallels to two previously published families with severe epileptic encephalopathy due to homozygous null variants in GRIN1 as well as various knockout animal models. Compared to heterozygous null variants, biallelic knockout of either GluN1 or GluN2A is associated with markedly more severe phenotypes in both humans and mice. Furthermore, recent findings enable a potential precision medicine approach targeting GRIN-related disorders due to null variants.

KEYWORDS
epilepsy, genetics, recessive

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1 | INTRODUCTION

N-Methyl-D-aspartate receptors (NMDARs) are expressed throughout the brain, mediating excitatory neurotransmission important for development, learning, memory, and other higher cognitive functions. NMDARs are di- or triheterotetrameric ligand-gated ion channels composed of two glycine-binding GluN1 (encoded by GRIN1) and two glutamate-binding GluN2 subunits (encoded by GRIN2A–D). For their data. This study has been approved by the ethics committee of the University of Leipzig (224/16-ek, 402/16-ek, 465/19-ek).

2.1 | Clinical data

We collected phenotypic information on affected family members following a standard clinical questionnaire, which is used in the GRIN Registry (http://grin-portal.broadinstitute.org/).

2.2 | Genetic data

A venous blood sample of the index girl was analyzed by panel sequencing (TruSight One Sequencing Panel, Illumina) within routine diagnostic settings. Parental samples have been tested by conventional Sanger sequencing. The detected GRIN2A variants were classified according to the recommendations of the American College of Medical Genetics and Genomics.

2.3 | Functional data

Introduction of the genetic variants (Quikchange, Stratagene) into the human GluN2A cDNA (NM_000833) and analysis of expression were essentially as described. Briefly, after confirmation by Sanger sequencing (Eurofins), the variant cRNAs were synthesized (Ambion), and separately combined with GluN1 cRNA and injected into Xenopus laevis oocytes prepared as described. After incubation for 3–4 days at 15–19°C in Barth’s solution, two electrode voltage clamp recordings were performed at Vhold −40 mV at room temperature in solution containing (in mmol·L−1) 90 NaCl, 1 KCl, 10 hydroxyethylpiperazine ethane sulfonic acid, .5 BaCl2, and .01 EDTA (pH 7.4). Current and voltage electrodes were filled with 3.0 mol·L−1 and .3 mol·L−1 KCl, respectively. After stable baseline recordings were achieved, oocytes were challenged with 100 μmol·L−1 L-glutamate and 100 μmol·L−1 glycine for 1 min to determine maximal GluN2A NMDAR responses. In separate studies, the same variants were introduced into cDNAs with β-lactamase fused in frame to the amino terminus of the GluN2A subunit, combined with GluN1 cDNA and cotransfected into mammalian human embryonic kidney (HEK) cells, and surface expression of these variant receptors was determined 24 h later by measuring β-lactamase hydrolysis of nitrocefin as described. Statistical comparisons were made using a one-way analysis of variance (ANOVA) and Dunnett multiple comparison test for maximum current in oocytes or an unpaired t-test for receptor expression in HEK cells.

2 | MATERIALS AND METHODS

We reviewed clinical and genetic data of a family with GRIN2A-related disorder. Respective family members have agreed to genetic testing and publication of their data. This study has been approved by the ethics
3  |  RESULTS

3.1  |  Clinical description

We describe an 8-year-old girl born to nonrelated parents. At the age of 9 months, she was diagnosed with delayed motor development and muscular hypotonia (free sitting at 12 months, crawling at 30 months). At the age of 3 years, there was a stagnation of development followed by epilepsy with focal impaired awareness seizures of unknown frequency (including deviation of eyes as well as apathy) at 4 years. Electroencephalography (EEG) at 4 years old revealed generalized slowing and multifocal discharges including bilateral centrotegmental spikes. At age 8 years, electrical status epilepticus during slow-wave sleep (ESES) was diagnosed. Additionally, the girl was diagnosed with mild ataxic movement disorder and muscular hypotonia of the trunk. Lamotrigine improved epileptic symptoms and slightly the symptoms of the ataxic movement disorder. After adding valproate at 8 years, the EEG normalized. Magnetic resonance imaging at 4 years was normal. At the age of 8 years, she is nonverbal and walks only a few steps unaided. Body measures at 6.5 years were normal: head circumference = 53.5 cm (percentile [P]90–97), weight = 23 kg (P75), length = 116 cm (P50–75).

Genetic testing revealed two compound heterozygous pathogenic GRIN2A null variants: c.1981C>T, p.(Gln661*) and c.2488dupC, p.(Leu830Profs*2).

Family history proved to be challenging, as barely any clinical records were available. However, the mother (37 years old) was reported to have had epilepsy with prolonged generalized seizures and complex focal seizures since the age of 3.5 years. Furthermore, she had psychomotor developmental delay with dystonic movement disorder and learning difficulties in childhood. Speech appeared normal. Mood disorders had been treated with citalopram. It remains unclear when seizure freedom occurred. However, the EEG remained pathologic and showed mild intermittent slowing right temporal after treatment with lamotrigine. Segregation analysis revealed the heterogeneous GRIN2A variant c.1981C>T, p.(Gln661*).

The father (46 years old) is mildly intellectually disabled. He never had seizures. Segregation analysis revealed the heterogeneous GRIN2A variant c.2488dupC, p.(Leu830Profs*2).

Her brother (10 years old) has speech developmental disorder and attention-deficit/hyperactivity disorder. He spoke his first words at 3 years and has a less modulated, monotonous speech, with articulation errors and a conspicuous speech melody. Epilepsy is suspected, but an EEG at 10 years remained unremarkable. Genetic testing has not been performed.

A paternal cousin (son of the father’s brother) was reported to have had epileptic seizures of unknown semiology between 9 and 11 years of age and unknown EEG. Genetic testing has not been performed (Figure 1).

3.2  |  Functional analyses

For functional studies, we refer to p.(Leu830Profs*2) as h2A-L830PX2 and p.(Gln661*) as h2A-Q661X. Neither h2A-Q661X nor h2A-L830PX2 variants resulted in significant expression of functional NMDARs in Xenopus oocytes after 3–4 days of incubation (Figure 2A). For the h2A-Q661X variant, the mean current size measured after 1-min exposure to maximal glutamate and glycine application (100 μmol·L⁻¹ each) was 11.7 ± 3.7 nA (mean ± SEM, n = 18), compared to 428 ± 67 nA (n = 24) for h2A-WT GluN2A receptors. Similarly, for the h2A-L830PX2 variant, the mean current size measured was 2.9 ± 0.5 nA (n = 12), and for un.injected oocytes the current magnitude was 2.3 ± 0.4 nA (n = 8). Only the h2A-WT receptor current maximum tested significantly different from un.injected oocytes by one-way ANOVA and Dunnett multiple comparison test. To obtain measurable currents with these GRIN2A null variants, additional mRNA was injected, and when current maximums were normalized to the amount of mRNA injected per oocyte, the differences between h2A-WT (1836 ± 200 nA/ng RNA) and h2A-Q661X (1.2 ± 0.4) and h2A-L830PX2 (.5 ± .01) were further exemplified. Furthermore, we also tested higher concentrations of both glutamate and glycine (3 mmol·L⁻¹ each) on the two variants, and current magnitudes did not increase greater than those at 100/100 μmol·L⁻¹, indicating that low current sizes were not due to large shifts in glutamate or glycine potency (data not shown). Thus, current sizes for both variants were deemed too small to measure reliable pharmacological endpoints such as EC₅₀ of glutamate or glycine.

We also conducted β-lactamase-fusion protein studies in mammalian HEK cells (Figure 2B) on both the h2A-L830PX2 and h2A-Q661X variants, and although both variants made receptor subunit protein (total protein for h2A-L830PX2 was 74.2% ± 17.4% of wild type [WT], n = 4; total protein for h2A-Q661X was 44.9% ± 2.8% of WT, n = 4), neither variant was capable of driving significant cell surface expression, where h2A-L830PX2 was only 1.9% ± 1.4% that of WT (n = 4, p < .05 from WT control, t-test) and h2A-Q661X was not detectable above background (n = 4). Thus, lack of a current response was consistent with the truncation of the subunit and reduced expression shown by β-lactamase studies.

Thus, both human variants appear to be loss of function based on these results.
We describe a girl with biallelic, compound heterozygous \textit{GRIN2A} null variants p.(Gln661*) and p.(Leu830Profs*2) and severe developmental delay with ESES on EEG leading to the diagnosis of epileptic encephalopathy with continuous spike-and-wave during sleep. Additionally, ataxic movement disorder and muscular hypotonia of the trunk have been noted. Due to the lack of more detailed information and clinical descriptions, some of the clinical and epileptologic aspects still remain superficial and will need specification by follow-up reports of additional cases.

Whereas the phenotypic spectrum of the girl’s parents, with learning disability and mild intellectual disability, was in perfect agreement with the known range of severity in individuals with heterozygous \textit{GRIN2A} null variants,\(^4\) the phenotype of their daughter with compound heterozygous \textit{GRIN2A} null variants is much more severe. It appeared to be rather comparable to the severe-to-profound clinical phenotypes of individuals with gain-of-function variants. However, functional analyses of both \textit{GRIN2A} null variants (p.[Gln661*] and p.[Leu830Profs*2]) revealed a loss-of-function effect due to strong reduction of surface expression of the GluN2A protein in both mammalian HEK cells and in Xenopus oocytes. These results are consistent with a \textit{GRIN2A} knockout.

Our report is the first case of autosomal recessive \textit{GRIN2A}-related disorder and only the third case where a biallelic knockout of a GluN subunit was associated with human disease, with the other cases affecting \textit{GRIN1} c.1666C>T, p.(Gln556*) and c.349-1G>C.\(^6,7\)

In the previously published \textit{GRIN1} families, the knockout phenotype was associated with severe and fatal neonatal epileptic encephalopathy or severe myoclonic epileptic encephalopathy. Similar to our novel \textit{GRIN2A} family, knockout phenotypes were much more severe than the phenotype

\section{Discussion}

Figure 1 Pedigree of a family with two pathogenic \textit{GRIN2A} null variants. The heterozygous parents had mild \textit{GRIN2A}-related phenotypes. The daughter with compound heterozygous \textit{GRIN2A} variants had a more severe phenotype. The brother remains untested but had a clinical phenotype compatible with (heterozygous) \textit{GRIN2A}-related disorder. ADHD, attention-deficit/hyperactivity disorder; DD, developmental delay; ESES, electrical status epilepticus during slow-wave sleep; ID, intellectual disability; MD, movement disorder; susp., suspected.
of the heterozygously affected parents in the GRIN1 families, who even appeared to be completely healthy.6,7 For GRIN1, this pattern is perfectly mirrored in rodents, where GRIN1 homozygous knockout mice also suffer neonatal lethality, whereas heterozygous mice are healthy.12,13 However, there is a notable difference, because GRIN1 knockout mice die from a failure to breathe and suckle milk, not from seizures.

For GRIN2B, biallelic null variants have so far not been observed in humans. We speculate that this is due mainly to two reasons. First, individuals with heterozygous GRIN2B null variants are so severely affected that so far none is known to have reproduced. Second, human knockout of GRIN2B may be pre- or perinatally lethal. In mice, GRIN2B knockouts die as neonates because they do not suckle.14

For GRIN2A, the situation appears to be slightly different. GRIN2A knockout mice have a much milder phenotype than GRIN1 or GRIN2B knockouts, exhibiting a moderate deficit in spatial learning,15 spontaneous discharges during sleep, and reduced pup vocalization.16 In humans, this is again potentially mirrored by the finding that the GRIN2A knockout phenotype comprises severe developmental delay and epilepsy but not fatal epileptic encephalopathy as seen in the human GRIN1 knockout phenotype.

Thus, all the above observations and speculations are supported and paralleled by animal models, where murine knockout models of GRIN1 and GRIN2B are lethal,14 whereas GRIN2A knockout mice are viable and have the mildest knockout phenotype, with “jumpy” behavior, moderate deficiency in learning, and epilepsy.15,16

A recent retrospective observational description of a series of individuals with null variants in either GRIN2A or GRIN2B revealed beneficial effects of a coagonistic treatment of the NMDAR with L-serine.17 It appears conceivable but currently remains unclear whether this treatment will also be beneficial in the context of biallelic GRIN2A null variants, resulting in a knockout of GluN2A as seen in the index case of this study.

Independently, we would like to encourage the report of individuals with biallelic null variants in any GRIN gene to further illuminate the phenotypic spectrum associated with knockouts of any GluN subunit in humans.

**AUTHOR CONTRIBUTIONS**

Study design: Vincent Strehlow, Johannes R. Lemke.

Acquisition of clinical data: Claudine Rieubland, Sabina Gallati.

Acquisition and analysis of functional data: Scott J. Myers, Sukhan Kim, Vincent Peterson, Stephen F. Traynelis.

Interpretation of clinical and functional data: Vincent Strehlow, Johannes R. Lemke.

Participated in drafting the manuscript: Vincent Strehlow, Claudine Rieubland, Sabina Gallati, Sukhan Kim, Scott J. Myers, Vincent Peterson, Amy J. Ramsey, Daniel D. Teuscher, Stephen F. Traynelis, Johannes R. Lemke.

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CONFLICT OF INTEREST
The authors report no competing interests.

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