COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

De novo missense variant in *GRIA2* in a patient with global developmental delay, autism spectrum disorder, and epileptic encephalopathy

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Abstract De novo variants are increasingly recognized as a common cause of early infantile epileptic encephalopathies. We present a 4-yr-old male with epileptic encephalopathy characterized by seizures, autism spectrum disorder, and global developmental delay. Whole-genome sequencing of the proband and his unaffected parents revealed a novel de novo missense variant in *GRIA2* (c.1589A > T; p.Lys530Met; ENST00000264426.14). Variants in the *GRIA2* gene were recently reported to cause an autosomal dominant neurodevelopmental disorder with language impairments and behavioral abnormalities (OMIM; MIM #618917), a condition characterized by intellectual disability and developmental delay in which seizures are a common feature. The de novo variant identified in our patient maps to the edge of a key ligand binding domain of the AMPA receptor and has not been previously reported in gnomAD or other public databases, making it novel. Our findings provided a long-sought diagnosis for this patient and support the link between *GRIA2* and a dominant neurodevelopmental disorder.

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Ontology terms: autism; epileptic encephalopathy; severe global developmental delay

Published by Cold Spring Harbor Laboratory Press

doi:10.1101/mcs.a006172

[Supplemental material is available for this article.]

CASE PRESENTATION

The index proband in our study is a 4-yr-old male with a history of global developmental delay, autism spectrum disorder, and early infantile epileptic encephalopathy with status epilepticus. He presented with seizures at 9 mo with focal tonic seizures and generalized tonic–clonic seizures; at 17 mo he was hospitalized with his first episode of refractory status epilepticus. Serial electroencephalograms (EEGs) demonstrated multifocal epileptiform discharges with a poorly organized and generally slow EEG background consistent with an epileptic encephalopathy. Biochemical testing for infantile-onset epilepsies was nondiagnostic (see supplement for further details). A brain magnetic resonance image (MRI) at 20 mo showed mild diffuse cerebral volume loss with normal spectroscopy. Single-nucleotide polymorphism (SNP) microarray testing revealed 3.32% homozygosity across the genome but was otherwise nondiagnostic.

Table 1. Clinical features

HPO	Features	Proband	Sister	Previously reported
HP:0000717	Autism spectrum disorder	+	-	+
HP:0200134	Epileptic encephalopathy	+	-	+
HP:0001263	Global developmental delay	+	+	+
HP:0002133	Status epilepticus	+	-	+
	Large occipitofrontal circumference	+	+	+
HP:0001249	Intellectual disability	+	-	+
	Rett-like features	-	-	+
HP:0005484	Deceleration of head growth	-	-	+
HP:0002376	Developmental regression	-	-	+
HP:0000750	Poor or delayed speech	+	-	+
HP:0001344	Absent speech	+	-	+
HP:0031936	Delayed walking	+	+	+
HP:0002540	Inability to walk	-	-	+
HP:0001288	Gait disturbances	+	-	+
HP:0001332	Dystonia	-	-	+
HP:0001251	Ataxia	+	-	+
HP:0001250	Seizures	+	-	+
HP:0002059	Cerebral atrophy	+	-	+
HP:0001272	Cerebellar atrophy	-	-	+
HP:0000733	Stereotypic behavior	+	-	+
HP:0100023	Recurrent hand flapping	+	-	+
HP:0000722	Obsessive–compulsive behavior	-	-	+
HP:0000735	Impaired social interaction	+	-	+
HP:0003593	Infantile onset	+	-	+
HP:0025352	Autosomal dominant germline de novo variant	+	-	+
HP:0002463	Language impairment	+	-	+
HP:0100753	Schizophrenia	-	-	+

Observed Human Phenotype Ontology (HPO) terms are listed below for proband and sister compared to other *GRIA2* patients previously described in the literature.
(+) Present, (–) absent.

Further investigation revealed that the parents were first cousins once removed. Clinical exome testing was nondiagnostic; at the time of that report, OMIM had not yet recognized the disease association for *GRIA2*. The proband's younger sister was noted to have some similar features (some developmental delay, hypotonia, macrocephaly) but was considered less severe; at 3 yr, 3 mo she could speak in full sentences and parents understood 90% of her speech. She did not have seizures (Table 1). Although it was unclear if the proband and sister had a shared etiology of disease, both were enrolled along with their parents for research genome sequencing under an IRB-approved protocol (pedigree in Fig. 1A).

TECHNICAL ANALYSIS AND METHODS

The proband, his parents, and his sibling underwent whole-genome sequencing (WGS; 2 × 100 bp). Library preparation was done using NEBNext Ultra II (New England Biolabs) and sequencing was performed using the NovaSeq6000 instrument (Illumina Inc.). Reads

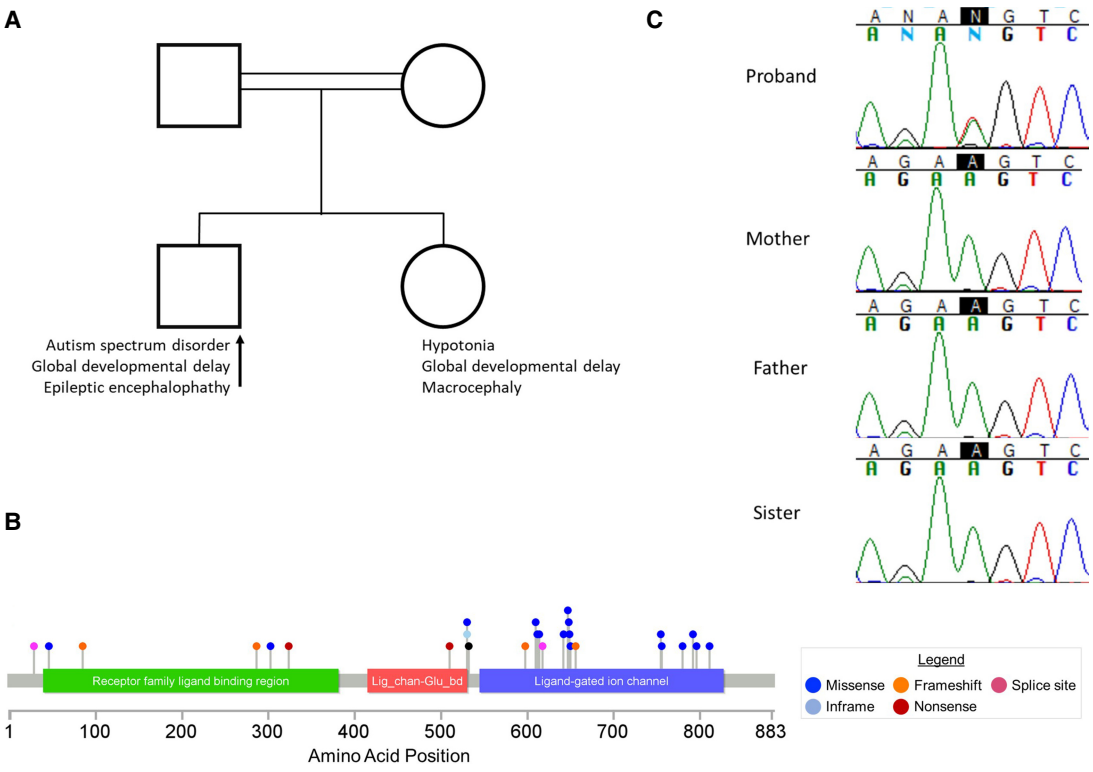

Figure 1. (*A*) The pedigree of the family shows that the parents are first cousins and the phenotypic outcomes for proband and sister. (*B*) Disease-causing variants reported in *GRIA2*. Variants from the literature (Salpietro et al. 2019; Zhou et al. 2021; Coombs et al. 2022) and the ClinVar database (Pathogenic/Likely Pathogenic as of 2022-04-28) are plotted on the GRIA2 protein structure (UniProt ID: P42262) and using lollipops (https://github.com/pbnjay/lollipops) v1.5.3 using domain information from PFAM. The full name of the middle domain is "Ligated ion channel L-glutamate- and glycine-binding site." Variants are shown at their predicted protein position as colored circles reflecting the effect type, with the missense variant reported here shown in black. (*C*) Sanger sequencing confirmed the de novo variant in *GRIA2* (c.1589A > T; p.Lys530Met) in the proband.

were mapped to the GRCh38/UCSC hg38 reference sequence. Secondary data analysis was performed using Churchill (Kelly et al. 2015), which implements the Genome Analysis Toolkit (GATK) "best practices" workflow to allow for a computationally efficient analysis of whole-genome sequencing (WGS) data. We generated ~104.7 Gbp of uniquely mapped reads per individual, achieving ~35× haploid coverage on average. Sequencing metrics are provided in Supplemental Table 1. SnpEff, ANNOVAR, and custom in-house scripts were used to annotate SNPs/indels with gene, transcript, function class, damaging scores, and population allele frequencies.

Our approach to variant annotation and prioritization has been described previously (Koboldt et al. 2018). Briefly, after removing common variants (minor allele frequency [MAF] > 0.01 in gnomAD), we selected all splice site, frameshift, and nonsense variants, as well as missense variants predicted to be damaging by SIFT (score < 0.05), PolyPhen (score > 0.453), GERP (score > 2.0), or CADD (Phred score > 15), for further analysis as previously described (Koboldt et al. 2018). We use several additional in silico tools to help predict whether a variant is benign or pathogenic, including pathogenicity scores on Varsome, ClinVar significance, gnomAD population frequencies, constraint, and conservation metrics. Given the lack of clinical presentation in the parents, we considered dominant (i.e., de novo

Table 2. Genomic findings and variant interpretation

Genomic location	HGVS cDNA	HGVS protein	Zygosity	Origin	Interpretation
Chr 4: 157336492	NM_000826.6: c.1589A > T	*GRIA2*: p.Lys530Met	Heterozygous	De novo	Likely pathogenic (PS2, PM2, PP3)

The patient was found to have a de novo variant in *GRIA2*. Genomic coordinates reflect build GRCh38.

variants), recessive, and X-linked inheritance models. No compelling recessive or X-linked variants were identified. The variant diagram in Figure 1B was generated with Lollipops v1.3.2 (Jay and Brouwer 2016) using information from UniProt (entry #P42262), the ClinVar (Landrum et al. 2018) database, and a previous report of de novo variants in *GRIA2* (Salpietro et al. 2019; Zhou et al. 2021; Coombs et al. 2022).

VARIANT INTERPRETATION

WGS of the family quad (proband, parents, sibling) revealed a de novo variant in *GRIA2*, a gene that encodes for a subunit of the ionotropic glutamate receptor: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). This c.1589A > T variant results in a substitution of lysine to methionine (denoted as p.Lys530Met; ENST00000264426.14; Chr 4: 157336492; Table 2) at the edge of the ligand-gated ion channel domain (Fig. 1B). This variant is novel to public databases including gnomAD, TopMed, and dbSNP. After submitting this variant to GeneMatcher (Sobreira et al. 2015), we connected with other investigators who had similar patients with *GRIA2* de novo variants; most of them were part of a cohort study of 28 patients with neurodevelopmental disorders, autism spectrum disorder, Rett syndrome–like features, and seizures or developmental epileptic encephalopathy that had been accepted for publication (Salpietro et al. 2019). We have since identified subsequent reports of patients with similar features (Zhou et al. 2021; Coombs et al. 2022). All of these patients harbored de novo variants in *GRIA2*, most of which were missense changes that mapped to key protein domains (Fig. 1C). Our proband's variant has not been reported to our knowledge but lies adjacent to two reported variants (p.P528T and p.528_530del) shown to decrease the agonist-evoked current compared to the wild-type channel and results in a similar syndromic presentation in the carriers as our proband. OMIM now recognizes variants in *GRIA2* as the cause of autosomal dominant neurodevelopmental disorder with language impairments and behavioral abnormalities (MIM #618917).

Sanger sequencing of the proband, sister, and both parents confirmed that the *GRIA2* variant was de novo (American College of Medical Genetics and Genomics [ACMG] evidence code PS2) (Fig. 1C) in the proband and absent from the sister, suggesting a different etiology for her syndromic features (detailed analysis of WGS in sister yielded no diagnostic variants; Supplemental Table 2). The c.1589A > T p.(Lys530Met) variant in *GRIA2* is absent from gnomAD and other public databases (PM2) and predicted to be damaging by 17/25 in silico tools according to VarSome (Kopanos et al. 2019) (PP3). Missense variants are a known mechanism of disease, and this gene shows strong constraint for missense variation ($Z = 4.56$ according to the gnomAD database) (PP2). Therefore, we interpret the variant to be likely pathogenic according to ACMG 2015 guidelines (Table 2; Richards et al. 2015).

SUMMARY

Here we report a patient with early infantile epileptic encephalopathy characterized by seizures, global developmental delay, and autism spectrum disorder who harbored a de novo

variant in the *GRIA2* gene associated with neurodevelopmental disorder with language impairment and behavioral abnormalities. *GRIA2* encodes Glutamate Ionotropic Receptor AMPA Type Subunit 2 (iGluR2), one of four related subunits that compose the AMPA receptor. The iGluR2 protein accounts for the majority of AMPA receptors in the central nervous system and thus are a vital component of fast-excitatory glutamatergic transmission in the mammalian brain (Isaac et al. 2007). The c.1589A > T (p.Lys530Met) variant in our proband is located within the ligand binding domain of the AMPA receptor and has not been previously reported. Adjacent variants within the ligand domain suggest that variants in this region may lead to altered backbone conformation of the neighboring residues that, in turn, allows hydrogen bonding formation between the two chains forming the ligand binding domain (Salpietro et al. 2019). Variants in this region destabilize the closed conformation of the domain, decreasing the relative efficacy of AMPA receptor activity (Zhang et al. 2008).

Salpietro et al. (2019) reported 25 unrelated patients with de novo variants in this gene, including 15 missense, two splice site, one nonsense, one in-frame deletion, and two frameshift variants. Functional expression studies demonstrated that these variants decreased ligand binding efficiency and current amplitude. Zhou et al. (2021) and Coombs et al. (2022) subsequently reported novel de novo missense variants in patients presenting with similar features as our proband.

Compared to the published cohort, our proband shared many clinical features including global developmental delay, impaired intellectual development with poor speech, autism spectrum disorder, and seizures. However, he did not exhibit some other reported features: obsessive–compulsive traits and hyperactivity, growth deceleration, and dystonia. Our work adds a novel missense variant to the growing catalog of disease-causing variants in *GRIA2* and adds further support to the association of this gene and autosomal dominant neurodevelopmental disorder with language impairment and behavioral abnormalities.

ADDITIONAL INFORMATION

Data Deposition and Access

The *GRIA2* variant and our interpretation evidence have been uploaded to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number SCV002505394.1. The informed consent for our current study does not permit deposition of sequence data.

Ethics Statement

Written informed consent was obtained for all participants in this study under a research protocol approved by the Institutional Review Board at Nationwide Children's Hospital (IRB11-00215 Study: Using Genome Sequencing to Identify Causes of Rare Birth Defects and Rare Disorders).

Competing Interest Statement

The authors have declared no competing interest.

Referees

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Received December 3, 2021; accepted in revised form May 2, 2022.

Acknowledgments

We thank the family for participation in this research. We are also grateful to Greg Wheeler, Peter Chang, Kyle Voytovich, HuaChun Zhong, and Amy Wetzel.

Author Contributions

All authors contributed to scientific discussion, variant interpretation, and manuscript review.

Funding

This work was supported by The Research Institute at Nationwide Children's Hospital.

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