

A gain-of-function mutation in the *GRIK2* gene causes neurodevelopmental deficits

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ABSTRACT

Objective: To identify inherited or de novo mutations associated with a suite of neurodevelopmental abnormalities in a 10-year-old patient displaying ataxia, motor and speech delay, and intellectual disability.

Methods: We performed whole-exome sequencing of the proband and her parents. A pathogenic gene variant was identified as damaging based on sequence conservation, gene function, and association with disorders having similar phenotypic profiles. Functional characterization of the mutated protein was performed in vitro using a heterologous expression system.

Results: A single de novo point mutation in the *GRIK2* gene was identified as causative for the neurologic symptoms of the proband. The mutation is predicted to change a codon for alanine to that of a threonine at position 657 (A657T) in the GluK2 kainate receptor (KAR) subunit, a member of the ionotropic glutamate receptor gene family. Whole-cell voltage-clamp recordings revealed that KARs incorporating the GluK2(A657T) subunits show profoundly altered channel gating and are constitutively active in nominally glutamate-free extracellular media.

Conclusions: In this study, we associate a de novo gain-of-function mutation in the *GRIK2* gene with deficits in motor and higher order cognitive function. These results suggest that disruption of physiologic KAR function precludes appropriate development of the nervous system. *Neurol Genet* 2017;3:e129; doi: 10.1212/NXG.000000000000129

GLOSSARY

ACSF = artificial cerebrospinal fluid; **cDNA** = complementary DNA; **eGFP** = enhanced green fluorescent protein; **iGluR** = ionotropic glutamate receptor; **NASPM** = 1-naphthyl acetyl spermine trihydrochloride; **PolyPhen2** = Polymorphism Phenotyping v2; **WIRB** = Western Institutional Review Board.

Neurodevelopmental disorders manifest as a diverse array of neurologic and neuropsychiatric symptoms that can be challenging to link to a discrete genetic basis. Identification of causative genetic variants for a growing number of these neurodevelopmental disorders can reduce complexity by uncovering common biological pathways affected in the disease state.¹ The objective of this study was to elucidate pathologic genetic alterations underlying the neurologic symptoms of a 10-year-old child displaying congenital gait ataxia, developmental delay, and learning deficits.

Genetic testing detected a missense variant in the *GRIK2* gene, which encodes the kainate receptor (KAR) subunit GluK2. KARs are channels activated by the neurotransmitter L-glutamate that play complex roles in neural development and CNS function.² Loss-of-function mutations in *GRIK2* have been previously associated with intellectual disability.^{3–5} In this study, we show that the GluK2 mutation identified in the proband yields GluK2-containing KARs with altered gating kinetics and constitutive activity. These findings associate a gain-of-function mutation in a *GRIK* gene with neurologic disease in humans.

Supplemental data
at Neurology.org/ng

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METHODS Participants. The proband is a 10-year-old Caucasian female born without complications to healthy nonconsanguineous parents. Clinical and behavioral assessments were performed at the Center for Rare Childhood Disorders at the Translational Research Institute in Arizona, United States. MRI was performed to identify possible neuroanatomic abnormalities.

Whole-exome sequencing. Genomic DNA was extracted from blood taken from the proband and both parents. Whole-exome sequencing was performed at the Translational Genomics Research Institute in Arizona when the patient was aged 6 years. Exomic libraries were prepared with the Illumina TruSeq Exome Enrichment Kit v2. Sequencing was performed by 101 bp paired-end sequencing on a HiSeq2000 instrument (Illumina Inc., San Diego, CA). Filtered reads were aligned to the human genome (Hg19/GRC37) using the Burrows-Wheeler transform. Reads were sorted, and PCR duplicates were removed using Picard, and base-quality recalibration and indel realignment were performed using the Genome Analysis Toolkit. Variants were called jointly with UnifiedGenotyper and annotated with dbNSFP and SnpEff for protein-coding events. Prediction scores were loaded from dbNSFP and used for filtering. An annotated variant file containing variants in 3 family members was filtered to include novel, private, or rare variants according to the Exome Aggregation Consortium (ExAC) database and the Genome Aggregation Database (gnomAD). Variants predicted to be damaging by the Combined Annotation Dependent Depletion (CADD) tool or

to disrupt protein structure or function by Polymorphism Phenotyping v2 (PolyPhen2) algorithms were considered as candidate genes responsible for the child's phenotype.

Cell culture and electrophysiology. Rat GluK2a(A657T) and Neto2 complementary DNAs (cDNAs) were provided by Michisuke Yuzaki (Keio University) and Susumu Tomita (Yale University), respectively. HEK293T/17 cells were maintained and transfected as described previously.⁶ After transfection, the cell media were supplemented with 1-naphthyl acetyl spermine trihydrochloride (NASPM, 20 μ M) to prevent cell death by excitotoxicity. Plasmid DNAs were cotransfected at ratios of 1:4 for enhanced green fluorescent protein (eGFP) and GluK2 (A657T); 1:3:0.5 for GluK2(A657T), Neto2, and eGFP, and 1:3:0.5 for GluK2(A657T), GluK5, and eGFP cDNAs, respectively.

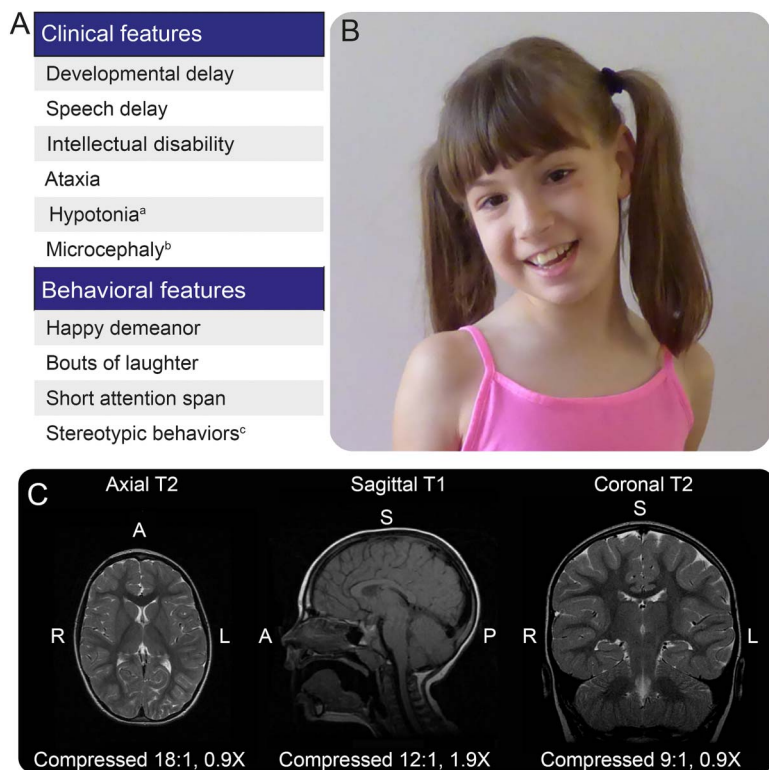
Whole-cell voltage-clamp recordings were performed 1–2 days after transfection as described previously.⁶ The external solution contained the following: 150 mM NaCl, 2.8 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES, pH 7.3. The internal solution was composed of the following: 110 mM CsF, 30 mM CsCl, 10 mM Cs-HEPES, 5 mM EGTA, 4 mM NaCl, and 0.5 mM CaCl₂, pH 7.3. Cells were held at –70 mV while in voltage clamp, and glutamate (10 mM) was fast-applied with a piezo-bimorph system. This recording configuration elicited currents with 10%–90% rise times of 1–3 milliseconds (ms). Desensitization rates were analyzed with Clampfit 10 (Molecular Devices, Sunnyvale, CA) by fitting the current decay during a 1-second application using single or biexponential parameters.

Statistical analysis. Data are represented as mean \pm SEM. Unpaired Student *t* test group analysis was performed using Prism 6.0 (GraphPad Software, La Jolla, CA) on receptor biophysical parameters with statistical significance denoted as follows: **p* < 0.05, ***p* < 0.01, and ****p* < 0.001.

Standard protocol approvals, registrations, and patient consents. Written consent for the acquisition and disclosure of data generated by the study was obtained from the guardians, as verbal assent from the proband was not possible. The participating family enrolled into a research protocol sponsored by the Translational Genomics Research Institute, which was approved by Western Institutional Review Board (WIRB Protocol No. 20120789).

RESULTS Identification of a de novo single-point mutation in the *GRIK2* gene in a patient with neurodevelopmental delays and intellectual disability. Neurologic evaluations of a 10-year-old female yielded a general diagnosis of severe neurodevelopmental delays and intellectual disability (figure 1A). She had congenital gait ataxia accompanied by impairments in motor and speech development (figure 1B, video at Neurology.org/ng). At 1 month of age, her parents reported generalized hypotonia and later noted significant delays in her fine and gross motor skill development. The patient underwent physical therapy beginning at 12 months of age. She was able to sit up on her own at 16 months of age and started walking short distances at 5.5 years, albeit with an ataxic gait and poor balance. Her muscle tone and bulk are currently within the normal

Figure 1 Summary of the clinical picture of the proband



(A) Clinical and behavioral characteristics of the proband: ^aonly during the perinatal period; ^bat 22 months, her head circumference was 43 cm (<second percentile), and at 7 years, it was 47 1/2 cm (<second percentile); ^ctongue thrusting. (B) Facial appearance of the proband. (C) Selected MRIs of the proband (sagittal T1, axial T2, and coronal T2) demonstrating normal anatomy of the cerebrum, cerebellum, and brainstem.

range, and her fine motor coordination skills have improved slowly. She started using single words (up to 4 words) by 2–3 years of age, currently understands a limited set of words, and can identify colors and animals with 80% accuracy. She also communicates nonverbally using gestures. Neurologic evaluations revealed that her speech impairments were a result of intellectual disability rather than dysarthria. The proband also exhibits sialorrhea and sensory alterations such as aversion to loud sounds and light touch. Approximately 3 times a year, as reported by the parents, she experiences episodes of constant tongue protrusion that last for 2 weeks.

Behavioral assessments revealed that the patient had a short attention span, stereotyped behaviors, and a happy demeanor, which, together with the early-age hypotonia and ataxia, bore some similarity to children with Angelman syndrome. In testing her muscle tone, she did not display rigidity, spasticity, or dystonia, although she displayed adventitious (choreoathetoid) movements while walking (video). No seizures have been documented in the patient. She has not regressed in her development and does not display features suggestive of neurodegeneration. In accordance, MRI showed normal gross anatomy of the brain, including the cerebellum (figure 1C). Her head circumference was 43 and 47 ½ cm at 22 months and 7 years of age, respectively, which in both cases was below the second percentile. Neither parent exhibited microcephaly (father: 59 cm; mother: 58.4 cm).

Genetic testing ruled out several neurodevelopmental and neuromuscular diseases including Angelman syndrome, Prader-Willi syndrome, fragile X syndrome, Rett syndrome, spinal muscular atrophy, and myotonic dystrophy. A chromosomal microarray

(Lineagen, Salt Lake City, UT) test performed at age 7 years revealed an interstitial deletion in chromosome 6q22.33: 129,669,542–129,734,572 (hg build 19), which includes the *LAMA2* gene associated with merosin-deficient congenital muscular dystrophy (table). This deletion in chromosome 6q22.33 was subsequently identified in the paternal genome during analysis of exome sequencing data.

Whole-exome sequencing was performed, and highly deleterious or damaging gene variations were identified by CADD and PolyPhen2 analysis and cross-referenced with the ExAC database and gnomAD to determine the prevalence of these mutations. Three heterozygous inherited or de novo mutations were classified as detrimental by 1 or both of the algorithms used (table). All other variants were seen at high frequencies in public databases, had poor coverage, or were predicted to be benign. Four inherited variants in 2 genes, *NEB* and *ORIS2*, showed compound heterozygosity. The *NEB* variants were excluded because loss-of-function mutations in this gene lead to congenital nemaline myopathies,⁷ characterized by hypotonia and hypoventilation, symptoms not present in the proband. The *ORIS2* (olfactory receptor 1 subfamily S2) variant was excluded because no pathogenic mutations have been identified for this gene and there is no evidence of olfactory deficits in the proband. In addition, while the CADD scores for the 2 *ORIS2* compound heterozygous mutations were quite high, both parents were asymptomatic and heterozygous carriers were reported in the ExAC database and gnomAD. *GRIK2* was the only gene that contained a heterozygous de novo mutation (table).

The elimination of other variants and the fact that ionotropic glutamate receptor (iGluR) genes have

Table		Potential causative missense or nonsense variation							
Gene	Variant	Protein	Allele	Inheritance	CADD	PP2	gnomAD		
							Het	Homo	
<i>GRIK2</i>	A657T	GluK2	Heterozygous	De novo	32	D	0	0	
<i>GRIK2</i>	M867I	GluK2	Heterozygous	Paternal	19	B	4910	52	
<i>NEB</i>	K8488R	Nemaline myopathy	Compound heterozygous	Paternal	17.5	B	87	0	
<i>NEB</i>	H6144R	Nemaline myopathy	Compound heterozygous	Maternal	19	B	156	0	
<i>ORIS2</i>	I302N	Olfactory receptor	Compound heterozygous	Paternal	36.5	D	43	0	
<i>ORIS2</i>	S243F	Olfactory receptor	Compound heterozygous	Maternal	23	D	347	0	
6q22.33 deletion Inc. <i>LAMA2</i>		Merosin	Heterozygous	Paternal	NA	NA	NA	NA	

Abbreviations: CADD = Combined Annotation Dependent Depletion; gnomAD = Genome Aggregation Database; NA = not applicable; PP2 = Polymorphism Phenotyping v2.

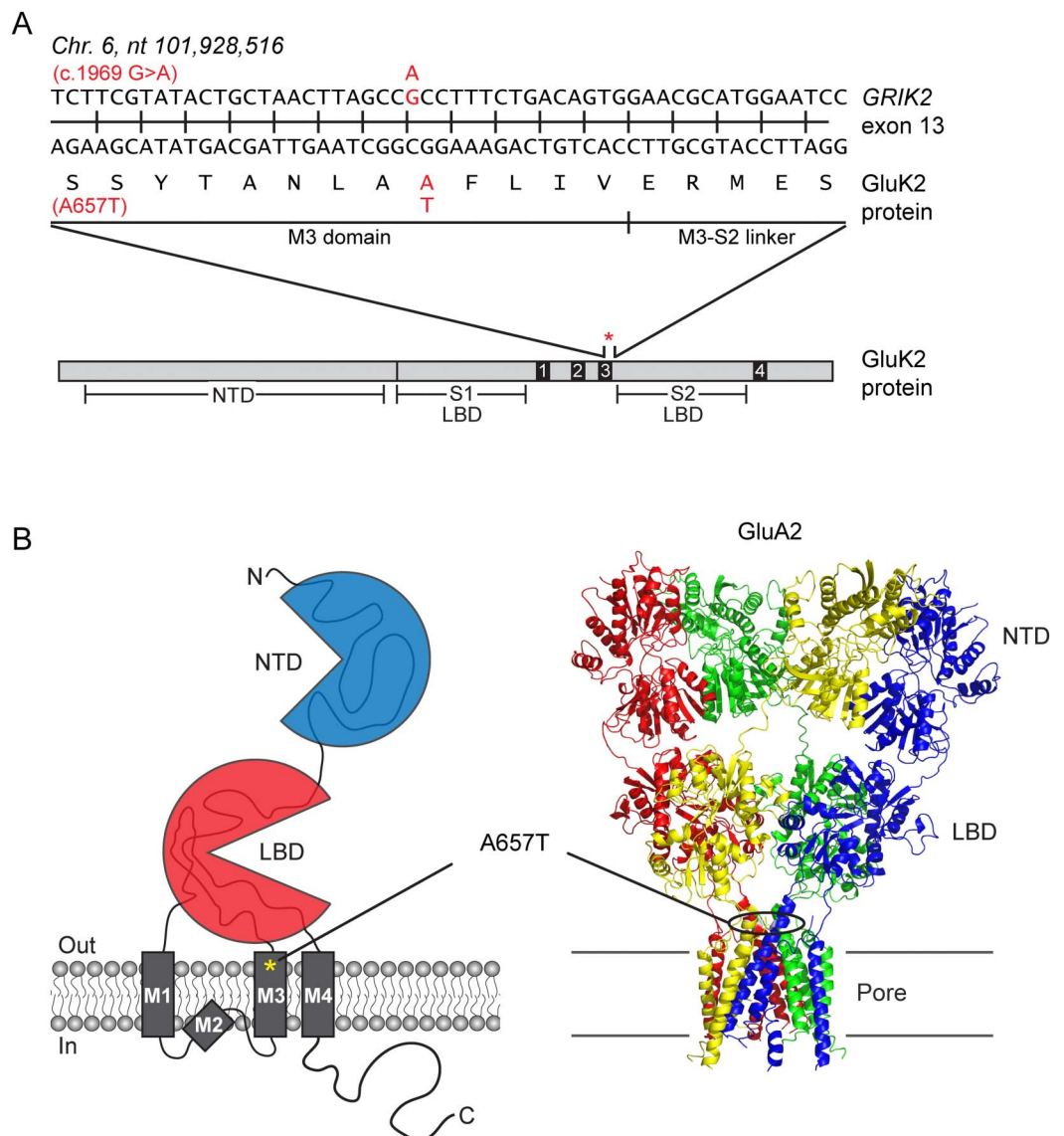
Potential causative missense or nonsense mutations identified in the proband or her parents. CADD scores are presented as scaled values. For PP2 scores, the letter B stands for “benign” and the letter D stands for “damaging.” Definitions of databases and algorithms are as given in the Methods section.

been linked to neurodevelopmental disorders makes the *GRIK2* mutations the most probable to be causative for the child's symptoms. Two heterozygous variants in *GRIK2* were identified. The first is a paternally inherited c.2601G>A variant that alters methionine 867 to an isoleucine (M867I) within a highly conserved region in the cytoplasmic C-terminal region. This variant has been associated with autism spectrum disorders.⁸ We eliminated it as a candidate gene, however, because it was found in 52 homozygotes in the gnomAD, it was present in the asymptomatic father of the proband, and it has minimal effect on receptor function.⁹ The second de novo

GRIK2 variant, c.1969G>A, is predicted to change alanine 657, a highly conserved amino acid within the pore-forming M3 transmembrane domain, to a threonine (A657T; figure 2, A and B). This variant had a CADD score of 32, placing it among the top 0.1% most deleterious mutations in the human genome, and it was not found in the ExAC database or gnomAD (table).

The A657T mutation in other iGluRs greatly affects channel kinetics and function.^{10–12} Rodents expressing delta receptors bearing the A657T mutation, also known as Lurcher mutant mice, show ataxia and cerebellar neurodegeneration,¹³

Figure 2 Description of the *GRIK2* variant



(A) Location of the single point mutation in the *GRIK2* gene responsible for the alanine to threonine amino acid substitution at position 657 within the highly conserved region of the GluK2 protein. (B) Cartoon depiction of a kainate receptor and crystal structure of an α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) showing the location of the (A657T) mutation within the M3 domain of ionotropic glutamate receptors. NTD and LBD stand for N-terminal domain and ligand-binding domain, respectively. Crystal structure for GluA2 AMPAR was obtained from the Protein Data Bank code 3H5V.

symptoms which are phenocopied in a human patient with the equivalent mutation in the *GRID2* gene.¹⁴ Together, these data support the identification of the *GRIK2* A657T variant as causative for some or all of the deficits in the proband.

The GluK2(A657T) mutation alters the biophysical properties of KARs. KARs are glutamate-gated ion channels that play diverse roles in neuronal development, synaptic plasticity, and modulation of network excitability.² It is unknown how the GluK2(A657T) mutation alters one or more of these functions to produce the developmental and behavioral deficits in the proband. Preliminary biophysical studies suggested that GluK2(A657T)-containing channels exhibited constitutive activation in the absence of agonist when analyzed in heterologous expression systems.¹⁰ Neuronal KARs are heteromeric combinations of distinct subunits together with auxiliary proteins that differ in stoichiometry in distinct neuronal populations. Thus, a critical first step toward relating the patient's symptoms to dysregulated receptor function is to more fully describe the biophysical properties of KARs that incorporate GluK2(A657T) subunits. We therefore performed whole-cell voltage-clamp recordings of HEK293T/17-expressing GluK2(A657T)-containing channels in various stoichiometries.

Homomeric receptors containing either the wild-type GluK2 or the GluK2(A657T) subunit responded to 10 mM glutamate applied for 1 second with robust inward currents (mean peak amplitudes: GluK2, 7.1 ± 1.1 nA, $n = 8$; GluK2(A657T), 1.7 ± 0.4 nA, $n = 12$; $p < 0.001$; figure 3A). During the glutamate application, GluK2(A657T) receptors desensitized less than the wild-type GluK2 (percent desensitization: GluK2, $99.8\% \pm 0.1\%$, $n = 8$; GluK2(A657T), $86.8\% \pm 2.1\%$, $n = 12$; $p < 0.001$; figure 3A), and the entry into the desensitized state was significantly slower than wild-type receptor currents (mean weighted tau for desensitization: GluK2, 4.4 ± 0.3 ms, $n = 8$; GluK2(A657T), 30.1 ± 7.9 ms, $n = 11$; $p < 0.01$; figure 3A).

The auxiliary protein neuropilin and tolloid-like protein-2 (Neto2) alters KAR function and is a likely component of native KAR complexes.¹⁵ Glutamate-evoked currents from GluK2(A657T)/Neto2 KARs also had smaller amplitude currents and desensitized ~10-fold more slowly than GluK2/Neto2 currents (amplitudes: GluK2/Neto2, 7.1 ± 1.0 nA, $n = 7$; GluK2(A657T)/Neto2, 1.7 ± 0.5 nA, $n = 8$; $p < 0.001$; tau desensitization: GluK2/Neto2, 14.5 ± 2.1 ms, $n = 7$; GluK2(A657T)/Neto2, 192.8 ± 24.9 ms, $n = 8$; $p < 0.001$; figure 3B). GluK2

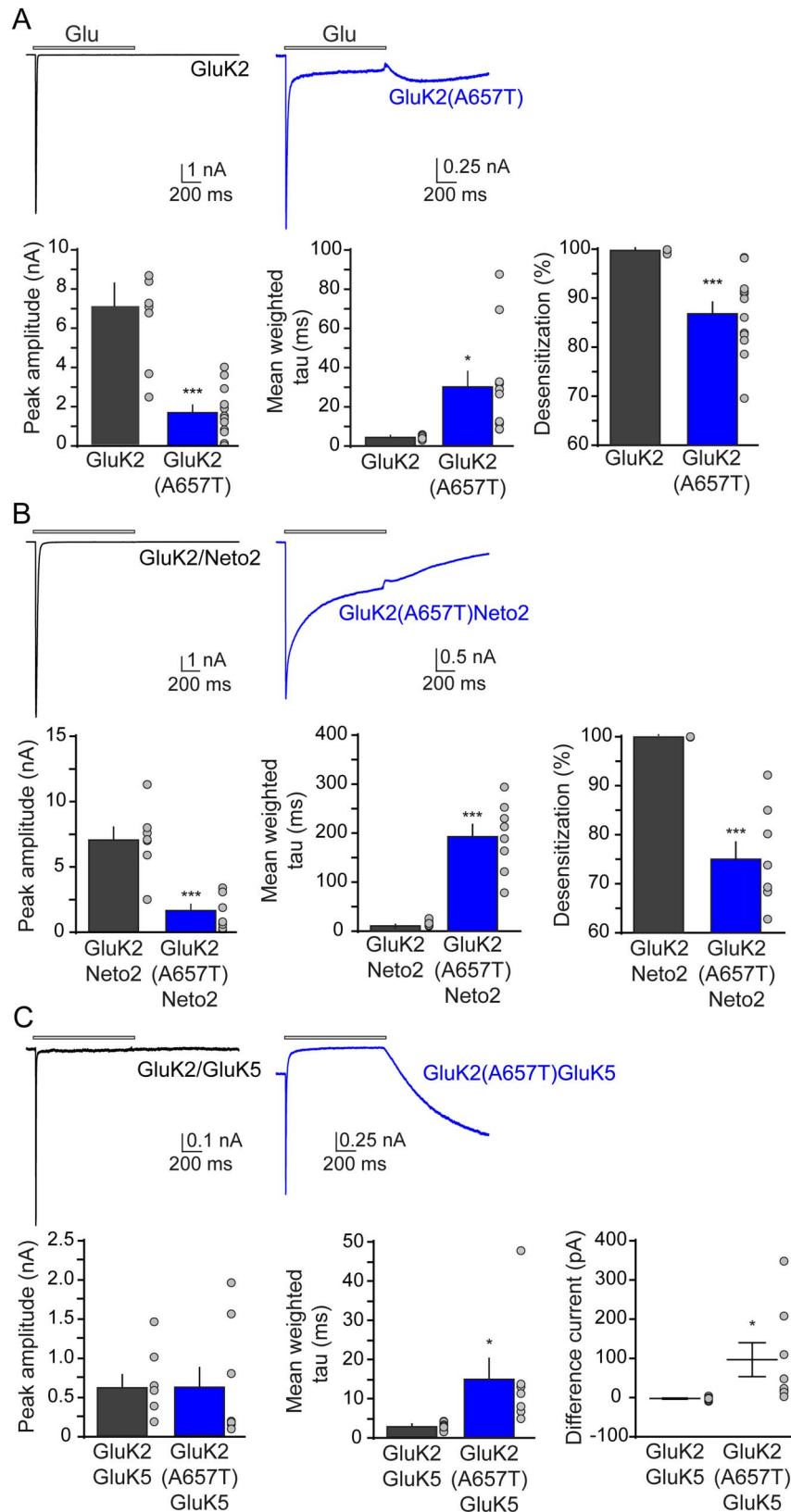
(A657T) reduced the percent desensitization of Neto2-containing receptors (GluK2/Neto2, $99.9 \pm 0.02\%$, $n = 7$; GluK2(A657T)/Neto2, $75.0 \pm 3.5\%$, $n = 8$; $p < 0.001$; figure 3B).

GluK2 and GluK5 subunits in part comprise post-synaptic KARs at the dentate granule cell to CA3 pyramidal cell synapse.^{16,17} We therefore compared the biophysical properties of heteromeric GluK2/GluK5 KARs-containing GluK2 wild-type or A657T subunits. Glutamate-evoked currents with unusual kinetic features from heteromeric GluK2(A657T)/GluK5 KARs (figure 3C). When glutamate was applied, the peak current from GluK2(A657T)/GluK5 receptors desensitized to a steady-state current that was more positive than the baseline holding current. The constitutive current amplitude, as measured by the difference between the holding current amplitude and the amplitude of the steady-state current, was 96.7 ± 43.3 pA ($n = 8$; figure 3C); by contrast, wild-type GluK2/GluK5 receptors did not exhibit a positive steady-state current and instead desensitized profoundly, by 99.4%, to steady-state current amplitudes similar to the baseline holding current. The atypical behavior of GluK2(A657T)/GluK5 KARs has been observed previously¹⁸ and results from partial activation of a subset of component subunits in nominally glutamate-free artificial cerebrospinal fluid (ACSF)-bathing solution. The peak amplitude of currents from wild-type and mutant GluK2/GluK5 receptors was similar (mean peak amplitude: GluK2/GluK5, 0.6 ± 0.2 nA, $n = 8$; GluK2(A657T)/GluK5, 0.6 ± 0.3 nA, $n = 8$, $p = 0.88$; figure 3C), but GluK2(A657T)/GluK5 receptors desensitized with a time course slower than that of GluK2/GluK5 receptors (mean weighted tau: GluK2/GluK5, 2.7 ± 0.3 , $n = 8$; GluK2(A657T)/GluK5, 14.8 ± 5.6 , $n = 7$, $p < 0.05$; figure 3C).

The unusual currents observed from GluK2(A657T)/GluK5 receptors strongly suggested that KARs incorporating the mutant subunit are constitutively activated or are acutely sensitive to trace levels of glutamate in nominally free ACSF.¹² This hypothesis was consistent with our observation that mean holding currents of recordings from GluK2(A657T)-containing receptors were greater than those of their wild-type counterparts (I_{hold} : GluK2, 108 ± 20 pA vs GluK2(A657T), 458 ± 66 , $n = 8$ and 12 ; $p < 0.001$; GluK2/Neto2, 36 ± 10 pA vs GluK2(A657T)/Neto2, 432 ± 126 pA, $n = 7$ and 8 ; $p < 0.05$; GluK2/GluK5, 117 ± 25 pA vs GluK2(A657T)/GluK5, 196 ± 57 pA, $n = 8$ and 8 ; $p = 0.2$; figure 4A).

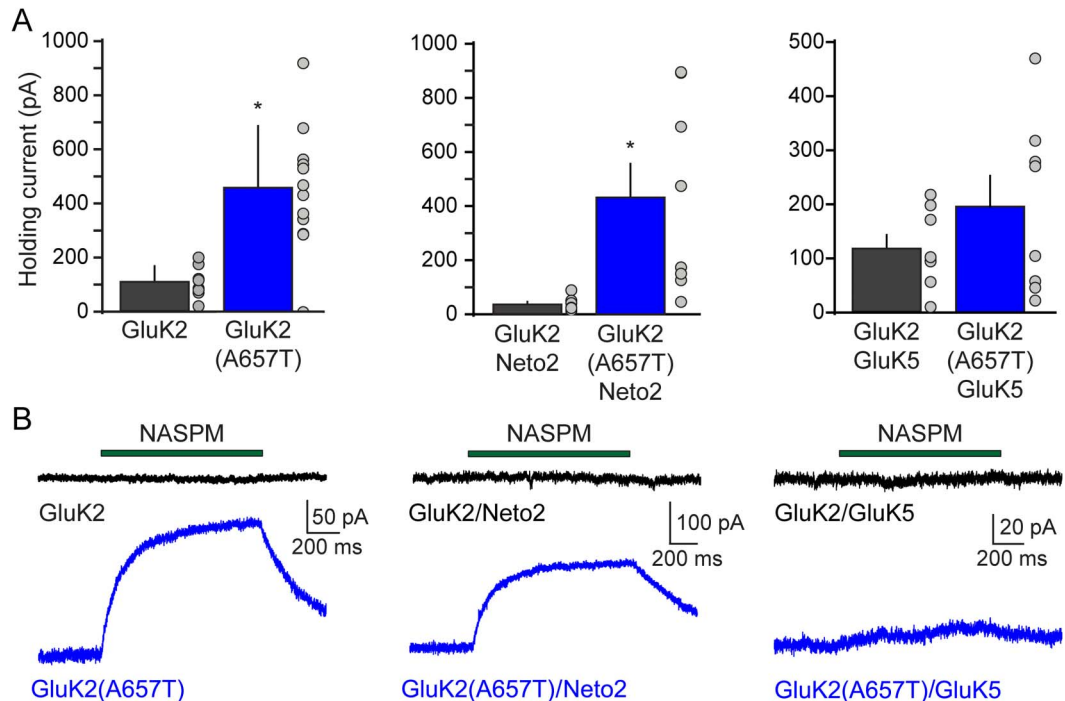
As more direct means of detecting constitutive activation, we measured the effects of the open-channel blocker, NASPM, on the holding current amplitude. For homomeric GluK2(A657T) KARs,

Figure 3 Biophysical properties of kainate receptors bearing the GluK2(A657T) subunit



Representative traces, peak amplitudes, tau of desensitization, and percent desensitization of inward currents evoked by a 1-second 10 mM glutamate (glu) application in (A) homomeric kainate receptors (KARs) bearing the GluK2 (A657T) subunit. (B) Similar traces and data for GluK2(A657T)/Neto2 KARs. (C) Representative traces, peak amplitudes, tau of desensitization, and difference current evoked by a 1-second 10 mM glutamate (glu) application in heteromeric KARs composed of the GluK2(A657T) and GluK5 subunits.

Figure 4 Kainate receptors bearing the GluK2 subunit are constitutively active



(A) Amplitude of holding current at -70 mV for HEK293T/17 cells expressing homomeric GluK2(A567T) kainate receptors (KARs) (left panel), GluK2(A657T)/Neto2 (middle panel), or heteromeric GluK2(A657T)/GluK5 KARs (right panel). (B) Representative traces demonstrating the change in holding current after a 1-second application of $20 \mu\text{M}$ NASPM in cells expressing homomeric GluK2(A657T) KARs (left panel, $n = 4$), GluK2(A657T)/Neto2 KARs (middle panel, $n = 4$), or heteromeric GluK2(A657T)/GluK5 KARs (right panel, $n = 4$). NASPM = 1-naphthyl acetyl spermine trihydrochloride.

NASPM application displaced the holding current amplitude by 105 ± 44 pA from baseline, which corresponded to a 35% decrease in holding current amplitude and was $7.3 \pm 1.8\%$ of the peak glutamate-evoked currents in the same recordings (figure 4B). Thus, the constitutive current arises from activation of a substantial fraction of the receptors. This was even more striking for GluK2(A657T)/Neto2 receptors, in which NASPM reduced the holding current by 266 ± 64 pA (a 62% decrease from the holding current amplitude). The constitutive current was $10.2 \pm 0.4\%$ of the peak glutamate currents in these cells. Finally, NASPM also reduced the holding currents of heteromeric GluK2(A657T)/GluK5 KARs by 11%, with a positive average displacement of 8.1 ± 3.3 pA. The NASPM response represented a $1.7 \pm 0.7\%$ of the glutamate-evoked peak amplitude. In sum, these results demonstrate that incorporation of the GluK2(A657T) subunit into KARs produces constitutively active channels with significantly altered gating kinetics.

DISCUSSION The genetic underpinnings of neurodevelopmental disorders and intellectual disability are varied.¹ Genes coding for KAR subunits have been associated with intellectual disability,^{3–5,19–21} but these were all predicted to be loss-of-function mutations.

By contrast, here we identified a *de novo* variant in the *GRIK2* gene predicted to result in KARs with a gain of function; we propose that the aberrant KAR activity results in the congenital gait ataxia, developmental delays, and intellectual disability displayed by the child. No other *de novo* genetic variation plausibly accounted for the spectrum of neurodevelopmental deficits.

The single nucleotide mutation in *GRIK2* is predicted to alter the naturally encoded A657 to a threonine. A657 is contained within the third transmembrane (M3) domain, a key determinant of receptor function¹⁵ and a highly conserved motif in the primary protein sequence of all members of the iGluR gene family. An analogous A657T “Lurcher” mutation in the GluD2 subunit of iGluRs in the delta family causes congenital gait ataxia in the eponymous Lurcher mouse model and in humans.^{14,22} In our study, we extended earlier biophysical analyses of GluK2(A657T)-containing receptors^{10,11} to show that the gating kinetics of KARs incorporating either Neto2 or GluK5 were altered by the incorporation of the mutated subunit. In addition, we showed that the constitutive activation of iGluRs bearing the GluK2 (A657T) mutation is also affected by the stoichiometry of KAR complexes, as demonstrated by the varying efficacy of NASPM in reducing the basal

constitutive current. Constitutive activation of GluK2(A657T)-containing KARs might be due to increased sensitivity to glutamate rather than by opening of the channel in the absence of ligand, as has been shown for α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors.¹² Regardless of the precise mechanisms, our findings suggest that the GluK2(A657T) mutation identified in the proband is a putative gain-of-function mutation and that the effects of this mutation on KAR function in vivo depend on the precise subunit composition of the receptor complex.

The specific makeup of iGluR complexes in the CNS is tightly regulated; as a consequence, the stoichiometry, subunit composition, and association with different auxiliary proteins can vary between different brain regions and neuronal populations. GluK2-containing KARs are found throughout the nervous system including regions associated with cognition and motor coordination, such as hippocampal and cortical principal neurons, striatal projection neurons, and cerebellar granule cells.^{23,24} Thus, we expect that incorporation of the GluK2(A657T) subunit will cause aberrant KAR signaling to varying degrees in the CNS. The broad distribution of GluK2 contrasts with the neuroanatomic specificity identified for the GluD2 Lurcher subunit; in that, the constitutive activation induced by the A657T variant caused abnormal development and increased neuronal death only in the cerebellum²⁵ because GluD2 expression is high and nearly exclusive to cerebellar Purkinje neurons. Because the proband in our study has mild microcephaly with grossly normal brain anatomy, we postulate that the GluK2(A657T) mutation causes subtle alterations in neuronal development and synaptic connectivity rather than excessive cell death by excitotoxicity. Genetic deletion of the mouse *Grik2* gene delays the functional maturation of the hippocampal mossy fiber to CA3 pyramidal cell synapse,^{26,27} and pharmacologic activation of KARs modulates excitatory and inhibitory transmission in the developing brain.^{28–30} GluK2 knockout mice also show decreased associative learning of fear-inducing stimuli³¹ and an autistic-like phenotype characterized by deficits in social behaviors and reduced behavioral flexibility.³² Excitatory-inhibitory imbalances can result in improper network connectivity, profound deficits in synaptic plasticity, and cognitive impairments.³³ Thus, the neurodevelopmental and learning disabilities identified in the proband might be explained by subtle alterations in neuronal development induced by faulty KAR function.

We report here that a gain-of-function mutation in a *GRIK* gene likely underlies developmental and learning disabilities. Future work should characterize the effects of this mutation on KAR function in vivo,

as well as its effect on neuronal development and learning. Together, these studies might shed some insight into mechanisms underlying the human pathology.

AUTHOR CONTRIBUTIONS

Yomayra F. Guzmán performed the in vitro experiments that characterized kainate receptors containing the GluK2(A657T) variant, analyzed the data, performed the statistical analysis for all in vitro experiments, and wrote and edited the manuscript. Jacob R. Stolz performed in vitro experiments characterizing kainate receptors containing the GluK2(A657T) variant and edited the manuscript. Keri Ramsey, David W. Craig, Mathew J. Huentelman, and C4RCDC Research Group identified the *GRIK2* gene variant, coordinated clinical research efforts, and edited the manuscript. Vinodh Narayanan performed clinical evaluation of the proband, identified the *GRIK2* variant, and edited the manuscript. Geoffrey T. Swanson designed in vitro experiments and wrote and edited the manuscript.

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DISCLOSURE

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