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Neurons Are GIRKed in GNB1 Encephalopathy: Unraveling Pathogenic Mechanisms in a Complex Neurodevelopmental Disorder

Epilepsy in a Mouse Model of GNB1 Encephalopathy Arises From Altered Potassium (GIRK) Channel Signaling and Is Alleviated by a GIRK Inhibitor

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De novo mutations in *GNB1*, encoding the $G\beta_1$ subunit of G proteins, cause a neurodevelopmental disorder with global developmental delay and epilepsy, *GNB1* encephalopathy. Here, we show that mice carrying a pathogenic mutation, K78R, recapitulate aspects of the disorder, including developmental delay and generalized seizures. Cultured mutant cortical neurons also display aberrant bursting activity on multi-electrode arrays. Strikingly, the antiepileptic drug ethosuximide (ETX) restores normal neuronal network behavior *in vitro* and suppresses spike-and-wave discharges (SWD) *in vivo*. ETX is a known blocker of T-type voltage-gated Ca^{2+} channels and G protein-coupled potassium (GIRK) channels. Accordingly, we present evidence that K78R results in a gain-of-function (GoF) effect by increasing the activation of GIRK channels in cultured neurons and a heterologous model (*Xenopus* oocytes)—an effect we show can be potently inhibited by ETX. This work implicates a GoF mechanism for GIRK channels in epilepsy, identifies a new mechanism of action for ETX in preventing seizures, and establishes this mouse model as a pre-clinical tool for translational research with predicative value for *GNB1* encephalopathy.

Commentary

Over the past decade, much progress has been made in identifying genetic causes of epilepsy. Genetic etiologies include genes associated with primary epilepsy and genes associated with neurological disorders, such as developmental and epileptic encephalopathies, in which epilepsy may be one of the symptoms.[1] However, determining the variants in a gene underlying a specific type of epilepsy or syndrome is only the first step toward understanding the mechanisms leading to seizures. Identifying the pathogenic molecular mechanism is critical for determining the best treatment options and for developing new treatments. In an ideal case, identification of the causal gene would reveal a clear type of dysregulation and that dysregulation could be directly targeted by a drug. For example, dysregulation of molecular target of rapamycin (mTOR) signaling in genetic "mTORopathies" such as tuberous sclerosis caused by variants in *TSC1* and *TSC2* may respond well to treatment with rapamycin.[2] However, determining the molecular mechanisms underlying genetic epilepsies can be complicated with many gene variants exhibiting multiple downstream effects. In these cases, cellular and molecular experiments combined with animal models may help researchers pinpoint mechanisms with the largest effect on seizure pathogenesis.

In the case of GNB1 encephalopathy, this combined strategy of molecular work and animal modeling proved useful in determining the primary pathogenic mechanism underlying seizures. Individuals with *GNB1* encephalopathy experience seizures, developmental delay, abnormal muscle tone, and structural brain abnormalities.[3] *GNB1* encodes the $G\beta_1$ subunit of heterotrimeric $G\beta\gamma$ proteins, which mediate G protein-coupled receptor signaling. $G\beta\gamma$ modulates activity of many downstream targets that affect neuronal excitability, including G protein-gated inwardly rectifying potassium channels (GIRKs), voltage-gated calcium channels, synaptic SNARE proteins that mediate synaptic vesicle release, and enzymes such as adenylyl cyclases, phospholipases, and kinases. Thus, it was not immediately clear which pathway or pathways may be most perturbed in *GNB1* encephalopathy. Diverse experimental approaches were needed to determine which functions of $G\beta\gamma$ proteins were altered by *GNB1* mutations and how these alterations lead to abnormal neuronal excitability. Detailed in vitro molecular studies revealed altered binding to GIRKs and changes in GIRK activity, while excluding changes in calcium channel activity.[4] Thus, the investigators chose to focus on regulation of GIRKs in *GNB1* encephalopathy.

To determine whether altered GIRK activity was indeed the pathogenic mechanism underlying *GNB1* epileptic encephalopathy, the authors of the highlighted study developed a

mouse model harboring the pathogenic p.Lys78Arg (K78R) mutation in *GNB1*.[5] Heterozygous *Gnb1*$^{K78R/+}$ mice exhibit similar aspects of *GNB1* encephalopathy, including developmental delay, motor and cognitive impairment, and experience absence-like generalized seizures. *Gnb1*$^{K78R/+}$ mice also exhibited a lower electroconvulsive seizure threshold compared to wild-type littermates. Multi-electrode arrays measuring local field potentials of neurons cultured from cortical tissue revealed abnormal bursting of *Gnb1*$^{K78R/+}$ neurons. Having established the *Gnb1*$^{K78R/+}$ mouse as a viable model of *GNB1* encephalopathy, the investigators studied GIRK channel activity in the mutant mice to determine whether there was aberrant activity of GIRK channels in *Gnb1*$^{K78R/+}$ neurons. The amplitude of evoked currents was larger in inhibitory cortical and hippocampal neurons in *Gnb1*$^{K78R/+}$ mice compared to wildtype. Expression of the *Gnb1*^{K78R} mutation together with GIRK channels in *Xenopus* oocytes demonstrated that the K78R mutation modulated activity of the GIRK channels. Interestingly, lower amounts of K78R activated GIRKs whereas high expression of K78R inhibited GIRKs.

Ethosuximide rescued aberrant neuronal bursting, and acute treatment of *Gnb1*$^{K78R/+}$ mice with ethosuximide abolished the spike-wave discharges observed by electroencephalogram during absence seizures. Ethosuximide is thought to primarily act by inhibiting voltage-activated T-type calcium channels.[6] However, ethosuximide has also been shown to inhibit GIRK channels in vitro.[7] The research team used their Xenopus system to demonstrate that Gbg-evoked GIRK1/2 currents were inhibited in a dose-dependent manner by ethosuximide. Thus, inhibition of GIRK activity by ethosuximide or other compounds may be a beneficial therapeutic strategy for *GNB1* encephalopathy.

This study brings together the available genetic and molecular data to investigate pathogenic mechanisms of a complex epileptic encephalopathy in an animal model. Though Gβγ can modulate many pathways that regulate neuronal excitability, the initial data pointed to altered interactions of mutant Gβγ with GIRK channels. In this study, the researchers were able to expand and test the specific hypothesis that *GNB1* mutations result in aberrant regulation of GIRK channels leading to neuronal hyperexcitability and seizures in *GNB1* encephalopathy. Their results demonstrated that altered GIRK activity is a major contributor to the disease phenotype. Furthermore, treatment with ethosuximide could rescue the altered GIRK activity, the neuronal bursting, and the absence seizures. Thus, this study serves as a nice example of how researchers dissect disease pathology in a genetic epilepsy with complex pathology.

Elucidating the cellular and molecular mechanisms underlying genetic epilepsies is a challenge for basic and clinical research. Approximately 70% to 80% of epilepsy cases are thought to have a genetic cause.[8] With the increased utilization of next-generation sequencing techniques, such as whole exome sequencing and whole genome sequencing, hundreds of new genetic variants have been identified. These include single genes as well as copy number variants, which add to the complexity of identifying pathogenic molecular mechanisms. Researchers will need to integrate multiple modes of investigation to parse the underlying pathology in genetic epilepsies, including informatics to screen the genetic contributions and identify pathogenic variants, computation methods such as modeling of neurons and neural networks, in vitro experimentation in heterologous expression systems and neuronal cultures, and finally animal models to assess biological relevance of identified cellular and molecular dysregulation. Animal models also serve as important tools for preclinical development and testing of new treatment strategies. With emerging technologies and interdisciplinary teamwork, the future is bright for investigators interested in unraveling the underlying causes of complex genetic epilepsy syndromes.

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Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Dunn P, Albury CL, Maksemous N, et al. Next generation sequencing methods for diagnosis of epilepsy syndromes. *Front Genet*. 2018;9:20.
2. French JA, Lawson JA, Yapici Z, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. *Lancet*. 2016;388(10056):2153-2163.
3. Petrovski S, Küry S, Myers CT, et al. Germline de novo mutations in GNB1 cause severe neurodevelopmental disability, hypotonia, and seizures. *Am J Hum Genet*. 2016;98(5):1001-1010.
4. Reddy HP, Yakubovich D, Keren-Raifman T, et al. Encephalopathy-causing mutations in Gβ1 (GNB1) alter regulation of neuronal GIRK channels. *iScience*. 2021;24(9):103018.
5. Colombo S, Reddy HP, Petri S, et al. Epilepsy in a mouse model of GNB1 encephalopathy arises from altered potassium (GIRK) channel signaling and is alleviated by a GIRK inhibitor. *Front Cell Neurosci*. 2023;17:1175895. doi:10.3389/fncel.2023.1175895
6. Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol*. 1989; 25(6):582-593.
7. Kobayashi T, Hirai H, Iino M, et al. Inhibitory effects of the antiepileptic drug ethosuximide on G protein-activated inwardly rectifying K+ channels. *Neuropharmacology*. 2009;56(2): 499-506.
8. Myers CT, Mefford HC. Advancing epilepsy genetics in the genomic era. *Genome Med*. 2015;7(1):91.