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Keeping up with KCNQ2: A New Model of Epileptic Encephalopathy

Keywords
animal model, KCNQ2, m-channel, human mutation, comorbidities

Spontaneous Seizure and Memory Loss in Mice Expressing an Epileptic Encephalopathy Variant in the Calmodulin-Binding Domain of Kv7.2

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Epileptic encephalopathy (EE) is characterized by seizures that respond poorly to antiseizure drugs, psychomotor delay, and cognitive and behavioral impairments. One of the frequently mutated genes in EE is KCNQ2, which encodes the Kv7.2 subunit of voltage-gated Kv7 potassium channels. Kv7 channels composed of Kv7.2 and Kv7.3 are enriched at the axonal surface, where they potently suppress neuronal excitability. Previously, we reported that the de novo dominant EE mutation M546V in human Kv7.2 blocks calmodulin binding to Kv7.2 and axonal surface expression of Kv7 channels via their intracellular retention. However, whether these pathogenic mechanisms underlie epileptic seizures and behavioral comorbidities remains unknown. Here, we report conditional transgenic cKcnq2+/M547V mice, in which expression of mouse Kv7.2-M547V (equivalent to human Kv7.2-M546V) is induced in forebrain excitatory pyramidal neurons and astrocytes. These mice display early mortality, spontaneous seizures, enhanced seizure susceptibility, memory impairment, and repetitive behaviors. Furthermore, hippocampal pathology shows widespread neurodegeneration and reactive astrocytes. This study demonstrates that the impairment in axonal surface expression of Kv7 channels is associated with epileptic seizures, cognitive and behavioral deficits, and neuronal loss in KCNQ2-related EE.

Commentary

Epileptic encephalopathies (EEs) encompass a range of seizure disorders associated with severe neurodevelopmental impairment.[1] Several gene variants are associated with EE, many of which result in drug-refractory seizures soon after birth. The treatment options available often provide inadequate seizure control and do not target the underlying disease mechanism.[2] KCNQ2 and KCNQ3 are genes commonly implicated in EE. They encode potassium channel subunits $K_V7.2$ and $K_V7.3$ which together form heterotetrameric "M-channels".[3] These are low-threshold voltage-gated K^+ channels that act to stabilize neuron membrane potential and suppress neuronal excitability.[4] $K_V7.2$ and $K_V7.3$ are highly expressed in the brain and are enriched on the cell surface at the axonal initial segment (AIS), where action potentials are generated, via ankyrin-G-mediated localization.[5] Many of the KCNQ mutations resulting in EE are dominant-negative, meaning assembly of a mutant subunit with wild-type (WT) subunits can hinder function of any resulting tetrameric channel.[6] Mutations in KCNQ2 are also commonly localized to 2 mutation hotspots: a functional domain responsible for voltage-sensing and a helix that interacts with the kinase calmodulin (CaM).[7] Mutations that involve the CaM binding site decrease axonal surface enrichment because CaM regulates trafficking from the endoplasmic reticulum to the cell surface. Elucidating the mechanisms by which diverse mutations lead to unique molecular, cellular, and circuit dysfunction will enable us to develop personalized therapies targeting the mechanism of action for each specific mutation.

In this study, Kim et al used this approach to better understand a KCNQ2 mutation from a human patient with EE.[8] This patient presented with neonatal tonic-clonic seizures, cognitive disability, autism spectrum disorder, and spasticity. The mutation, M546V, is located in the CaM binding domain of KCNQ2 and results in impaired CaM binding, decreased plasma membrane expression of K_V7 channels, and increased degradation of $K_V7.2$. The authors generated a mouse line containing this mutation that closely recapitulates the human mutation. In brief, the authors used a CRISPR-based knock-in strategy to express one copy of the M546V KCNQ2 mutation in excitatory forebrain neurons in mice that have either one normal copy of KCNQ2 or completely lack KCNQ2. Consistent with

disrupted potassium channel function, mutant mice had decreased survival and a variety of severe seizure phenotypes. The authors also performed behavioral tests to assess cognitive and behavioral function. Mutant mice demonstrated decreased anxiety-like behavior, impaired spatial memory and object-recognition, and repetitive grooming behaviors relevant to autism spectrum disorder phenotypes. Several sex differences were found, with males showing increased hyperactivity and obsessive-like behaviors and potentially more severe epilepsy compared to females. Unlike previous work examining KCNQ2 mutations using in vitro expression to identify differences in surface expression and potassium currents,[6,7] Kim et al identify in vivo phenotypes consistent with many of the symptoms observed in the human patient.

Kim et al next looked for a physiological etiology for the observed phenotypes. They noted decreased $K_V7.2$ and ankyrin-G at the AIS in mutant animals. Due to unexpected off-target Cre expression, $K_V7.2$ was also expressed in hippocampal and cortical astrocytes. Striking images of astrocytes with classical reactive morphologies of hypertrophy and process elongation leave no doubt of increased reactive astrocytosis in mutant mice at all timepoints and locations examined. Finally, they showed a significantly higher number of degenerating hippocampal neurons at all timepoints assessed. While KCNQ2 is not expressed in adult astrocytes, it is expressed in fetal astrocytes, raising questions regarding the significance of cell-specific expression during development and the effect this has on neuroinflammation and degeneration.[9] These exciting findings establish a mouse model for a KCNQ2 mutation that closely mirrors patient presentation of EE with regard to early spontaneous seizures, cognition, behavior, and brain pathology. Kim et al differentiate nicely between the effect of the mutant allele and simply losing a KCNQ2 allele by utilizing heterozygous $K_V7.2$ knockout mice.[10] These heterozygous mice exhibit decreased surface expression of $K_V7.2$ and increased seizure susceptibility; however, they had much less pronounced phenotypic responses including no spontaneous seizures.

While providing strong evidence that epileptic activity observed in the mutant is not solely due to the loss of a KCNQ2 allele, this begs the question, how much of an effect does a reduction in axonal surface expression play in the development of spontaneous seizures? The authors note a transient decrease in mutant KCNQ2 expression at postnatal day 30. Previous studies suggest differential KCNQ2 and KCNQ3 expression throughout development could regulate neuronal differentiation and affect EE phenotypes.[11] Additionally, $K_V7.2/K_V7.3$ ratio affects current density, and dominant-negative mutations like the one examined here perturb this ratio. Therefore, is there a developmental timepoint when loss of K_V7 current is most detrimental? Do different KCNQ2 mutations affect trafficking of other K_V7 subunits differently? The compelling model Kim et al develop here can be used to clarify temporal differences in K_V7 expression which in turn can be used to inform when to initiate therapy and which subunits to target. Several questions also remain surrounding associations between early postnatal presentation and phenotypic severity. What is different about mutant mice that go on to survive vs those that die early postnatally? Is postnatal death in mutant animals caused by seizures? If so, do early life seizures occur before cell death and astrocytosis?

This study also brings up several points to contemplate regarding mechanisms of epilepsy and strategies to develop novel therapies. First, like other studies examining KCNQ2 mutations, the authors find that the mutant protein fails to traffic to the cell membrane and AIS. From a therapeutic perspective, this situation may be more easily treatable because a largely functional protein may be present but simply located in the wrong place. Emerging approaches to modulate protein kinases and phosphatases can have powerful effects on protein surface targeting and retention, offering the possibility to properly traffic the mutant protein to its correct location. Once properly targeted, the existing mutant protein may be able to rescue functional deficits. Second, many neurological diseases result from improper protein processing. The authors suggest that disrupted protein ubiquitination, which targets proteins for degradation, may occur due to the complex trafficking and degradation of $K_V7.2/7.3$. Impaired protein degradation can lead to cellular stress, protein aggregation, and cell death. Whether any of those mechanisms drive the phenotypes observed here remain to be seen, but thinking of epilepsy as a protein-based neurodegenerative disease is an intriguing concept. Perhaps the combination of physiological and circuit effects of specific ion channel mutations, combined with disrupted protein homeostasis, combine to drive epilepsy. Further examination of how mutant $K_V7.2$ affects temporal and cell-specific expression throughout development as well as how it perturbs exportation and the subsequent effect on neurodegeneration will give additional insight into the mechanism behind epileptogenesis and developmental delays associated with EE.

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