**RESEARCH HIGHLIGHT**

**KCNT1** gain-of-function mutations linked to human epilepsy are modulated by quinidine

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Epilepsy of infancy with migrating focal seizures (EIMFS) and a severe form of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) are strikingly different epilepsy syndromes, which have very recently been associated with mutations in the same gene. EIMFS is a rare early infantile epileptic encephalopathy characterized by heterogeneous migrating focal seizures, and is associated with developmental deterioration leading to severe disability. In contrast, ADNFLE begins in mid-childhood and is characterized by clusters of motor seizures arising from sleep and is associated with significantly impaired cognitive function and psychiatric deficits. Whole exome sequencing of patients with EIMFS and severe ADNFLE has revealed a number of KCNT1 mutations with 100% penetrance, many of which are de novo. KCNT1, widely expressed in the mammalian central nervous system, encodes a potassium channel that is activated by increases in cytoplasmic sodium concentrations, which occur during normal neuronal signaling. While KCNT1 channels are thought to play important roles in regulating neuronal excitability, their precise contribution to electrical activity differs in different neuronal cell types. KCNT1 disorders are resistant to standard anti-epileptic drugs and have a severe prognosis, creating an urgent need for novel therapies. Several studies have demonstrated that antiarrhythmic compounds, such as quinidine, bepridil and clofilium are effective blockers of KCNT1 channels in vitro and despite their known adverse effect profile, our recent study suggested that they may hold promise as effective therapies. Recently, the first results of a clinical trial in a 3 year old patient with EIMFS demonstrated that quinidine is effective in reducing seizure frequency and improving psychomotor function. This research highlight describes our study, evaluating the electrophysiological and pharmacological gain-of-function phenotype of KCNT1 mutations in vitro and examining the potential neurodevelopmental impact of this channel to central nervous system excitability in vivo.

Potassium channels contribute to the many different types of action potential firing patterns observed in neurons. Sodium activated potassium channels, such as KCNT1, are positioned to sense the large increases in intracellular sodium concentrations that occur during the action potential upstroke and can profoundly alter subsequent firing of the neurons in which they reside. High expression levels of KCNT1 in the central nervous system position the channel to impact on neuronal firing patterns by contributing to the hyperpolarization that follows repetitive firing. Furthermore, KCNT1 is also expressed highly in electrocytes of the electric fish,
Eigenmannia virescens, where it is suggested to be vital for facilitation of extremely rapid action potential firing frequencies (500Hz)\textsuperscript{[3-4]}. The channels expression pattern and its potential to contribute to elevated firing rates are consistent with a role in the pathogenesis of epilepsy. In the last decade research has also shown that these channels have additional modulatory functions, which are regulated by a number of signaling pathways\textsuperscript{[5-6]} The majority of the recently identified mutations\textsuperscript{[7-10]} are situated in the c-terminus of the alpha subunit of KCNT1, where modulatory regions, such as the NAD\textsuperscript{+} binding domain\textsuperscript{[5]} and several putative consensus sites for protein kinase C (PKC)\textsuperscript{[6]} are located. Emerging evidence indicates that subtle changes in the function of this channel have a deleterious impact on the fragile developing brain\textsuperscript{[7-10]}. Our latest study sought to examine the functional phenotype of KCNT1 mutations associated with these early-onset epileptic syndromes. In addition, we analysed the expression levels of mKcnt1 mRNA in mouse brain to elucidate a possible neurodevelopmental correlation\textsuperscript{[11]}.

To assess the functional phenotype of the pathogenic mutations, we analyzed human KCNT1 wild-type (WT) and R398Q, R428Q, Y796H, M896I, R928C, A934T, and P924L mutated channels using a Xenopus oocyte expression system. We found that, without exception, all the mutations displayed large increases in current magnitude indicative of a profound gain-of-function phenotype when compared to the WT channel. Group data revealed a threefold gain-of-function with mutations associated with ADNFLE and a fivefold gain-of-function with those identified in patients with EIMFS. Importantly, the magnitude of this gain-of-function correlates with the severity of the clinical presentation, albeit confirmation with more samples will strengthen this finding. KCNT1 linked early-onset epileptic syndromes are refractory with devastating clinical severity\textsuperscript{[7-10]}, generating a serious need for effective therapies. This prompted us to investigate the effects of quinidine; an FDA approved antiarrhythmic compound and an earlier proven blocker of rodent KCNT1 channels\textsuperscript{[12-13]}. We found that quinidine resulted in inhibition of the current amplitude of human WT and all pathogenic mutations and in some cases reversed the gain-of-function towards a WT phenotype. The correlation of phenotype and severity observed with these KCNT1 mutations supports the notion that gain-of-function is directly pathogenic and, consequently, its reversal presents a promising therapeutic target. Our \textit{in vitro} demonstration that quinidine can inhibit mutation specific pathology, provides a strong indication that targeted therapy may be the way forward for patients with activating KCNT1 mutations. A previous study postulated that the pathogenic mutations identified in patients with EIMFS, which they analyzed in an expression system using rodent DNA, are in a constitutive PKC phosphorylation-like state\textsuperscript{[8]}. We examined this possibility using our human pathogenic clones. Our results did not support an involvement of channel phosphorylation as a potential candidate underlying the mechanism of action of the gain-of-function, because we actually observed suppression of activity when the channels were exposed to a PKC activator. Therefore, a phosphorylation-like state of the channel may not fully explain the mechanism underlying increased neuronal excitability.

The difference in age of onset and clinical severity between ADNFLE and EIMFS is marked. This motivated us to examine whether neurodevelopmental expression profiles of the channel correlate with enhanced neuronal excitability. To examine this possibility we looked at the expression levels of mKcnt1 mRNA in four different regions of mouse brain throughout early development. Our data revealed that although expression levels at birth were similar throughout the brain, cortical expression increased three fold over the first two weeks of life. These findings demonstrate that delayed onset in patients may result from the delay in the cortical expression and that because the extreme fivefold gain-of-function is observed with EIMFS associated mutations, this would explain the early onset as compared to ADNFLE. Furthermore, the cortical expression profile positions the channel to contribute to nocturnal frontal lobe seizures. This delay in expression could lead to early intervention strategies in patients with confirmed activating mutations to reduce both seizures and co-morbidities.

Thus, our findings suggest a direct genotype-phenotype correlation between KCNT1 mutations and early-onset epileptic syndromes. Although the underlying mechanism of gain-of-function with these mutations is unclear, this work provides insights into the pathophysiology of these disorders. Moreover, we have established that quinidine reversibly inhibits all human WT and mutant KCNT1 channels studied here. This, together with the demonstration that quinidine effectively reduces seizure frequency for a patient with EIMFS\textsuperscript{[14]}, suggests that quinidine holds great promise as a new treatment paradigm for patients with early-onset epileptic encephalopathies that are associated with KCNT1 mutations. A better understanding of how these mutations affect the biophysical properties of the channel could lead to the development of quinidine analogues with greater specificity for KCNT1 and improved safety.
References


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