Newly Identified *KCNA3* Gene Variants Put the "Excite"-ment Back in Kv1.3 Channelopathy

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De Novo Variants in KCNA3 Cause Developmental and Epileptic Encephalopathy

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Objective: Variants in several potassium channel genes, including KCNA1 and KCNA2, cause Developmental and Epileptic Encephalopathies (DEEs). We investigated whether variants in KCNA3, another mammalian homologue of the Drosophila shaker family and encoding for Kv1.3 subunits, can cause DEE. Methods: Genetic analysis of study individuals was performed by routine exome or genome sequencing, usually of parent-offspring trios. Phenotyping was performed via a standard clinical questionnaire. Currents from wild-type and/or mutant Kv1.3 subunits were investigated by whole-cell patch-clamp upon their heterologous expression. Results: Fourteen individuals, each carrying a de novo heterozygous missense variant in KCNA3, were identified. Most (12/14; 86%) had DEE with marked speech delay with or without motor delay, intellectual disability, epilepsy, and autism spectrum disorder. Functional analysis of Kv1.3 channels carrying each variant revealed heterogeneous functional changes, ranging from "pure" loss-of-function (LoF) effects due to faster inactivation kinetics, depolarized voltage-dependence of activation, slower activation kinetics, increased current inactivation, reduced or absent currents with or without dominant-negative effects, to "mixed" loss- and gain-of-function (GoF) effects. Compared to controls, Kv1.3 currents in lymphoblasts from 1 of the proband displayed functional changes similar to those observed upon heterologous expression of channels carrying the same variant. The antidepressant drug fluoxetine inhibited with similar potency the currents from wild-type and 1 of the Kv1.3 GoF variant. Interpretation: We describe a novel association of de novo missense variants in KCNA3 with a human DEE and provide evidence that fluoxetine might represent a potential targeted treatment for individuals carrying variants with significant GoF effects.

Commentary

When Kv1.3 was discovered in T lymphocytes more than 3 decades ago, it became the first K^+ channel identified in non-excitable cells.[1] Encoded by the *KCNA3* gene, Kv1.3 is a voltage-gated pore-forming K^+ channel α-subunit belonging to the *Shaker* family of Kv1 channels. In addition to T lymphocytes, Kv1.3 is also highly expressed in many other immune-related cells, including macrophages, neutrophils, and microglia, emphasizing its importance in immune function.[2] Kv1.3 channels prevent membrane depolarization by allowing K^+ efflux, thereby sustaining the large driving force for calcium entry that is needed for immune cell activation and proliferation.[2] Due to its critical role in mediating immune responses, Kv1.3 is an important therapeutic target for immunosuppression in neuroinflammatory diseases like multiple sclerosis and Alzheimer's disease.[3]

Recent findings by Soldovieri et al are reshaping Kv1.3's reputation as a primarily immune-related channel. Their recent work reveals the first link between genetic variants in *KCNA3* and developmental and epileptic encephalopathy (DEE).[4] Developmental and epileptic encephalopathy is a neurodevelopmental disorder marked by early-onset epileptic seizures coupled with developmental impairment.[5] About 100 genes are implicated in DEE, with approximately one-third encoding ion channels or transporters, including *KCNA1* and *KCNA2*, which are closely related to *KCNA3*.[6,7]

To identify people carrying *KCNA3* variants, Soldovieri et al screened exome and genome sequence data from parent-offspring trios linked to developmental disorders. They discovered 14 individuals with 13 different heterozygous *de novo* missense mutations in *KCNA3*. Comprehensive phenotypic assessment revealed that two-thirds of these individuals had early onset epilepsy with seizures beginning in infancy. Seizure types varied, including tonic, tonic–clonic, clonic, myoclonic, atonic, and absence seizures. Most seizures were drug-resistant, and one person died at 3 years of age after status epilepticus. All individuals with epilepsy were classified as having DEE due to concomitant developmental impairments,

such as global delay, delayed speech, and intellectual disability. Despite the importance of Kv1.3 in immune function, no individuals showed evidence of autoimmune disorders. These findings convincingly establish an association between *KCNA3* mutations and DEE.

Soldovieri et al proceeded to map the identified genetic variants onto the Kv1.3 protein. Kv1 channels share a similar structure with 6 transmembrane segments (S1-S6) that contain critical voltage-sensing (S1-S4) and pore domains (S5-S6).[7] The transmembrane regions are flanked by N- and C-terminal intracellular domains that have less understood functions. Among the 13 de novo Kv1.3 variants identified, 9 mapped to the S6 transmembrane segment or S5-S6 linker of the pore domain, which allows K^+ flux across the membrane. These pore domain-associated variants were highly associated with epilepsy, with all 5 located in S6 exhibiting DEE. One of the DEE-associated variants in S6 (P477 H) affected the second proline of the highly conserved proline-valine-proline (PVP) motif crucial for forming the pore activation gate. Missense mutations affecting the corresponding amino acid in Kv1.1 and Kv1.2 also cause DEE, reinforcing the association between the PVP motif in Kv1 channels and severe epilepsy.[7]

Soldovieri et al extended their findings by performing functional characterization of the variants using whole-cell patch-clamp electrophysiology in Chinese hamster ovary cells. Most variants exhibited loss-of-function (LoF) effects due to reductions in current density, depolarized voltage-dependence of activation, or altered kinetics of activation and deactivation. The 7 DEE-associated variants generally displayed the most severe LoF with 6 showing nearly complete absence of currents, including the P477 H variant in the PVP motif. Co-expression of these severe LoF variants with wild-type (WT) subunits confirmed that most exert a dominant-negative effect, suppressing the current carried by WT subunits. Interestingly, 5 variants demonstrated mixed loss-of-function/gain-of-function (LoF/GoF) effects with augmented current density, less inactivation, slower inactivation kinetics, or hyperpolarized voltage-dependence of activation. Variants with mixed LoF/GoF effects were mostly associated with less severe pathology lacking the delayed motor development and epilepsy commonly observed in individuals with pure LoF variants.

Finally, Soldovieri et al examined whether the antidepressant drug fluoxetine could reverse the GoF effects associated with Kv1.3 variants, suggesting a potential therapy through drug repurposing. While fluoxetine is well-known as a clinically used selective serotonin reuptake inhibitor, it also blocks Kv1.3 channels. As anticipated, the drug blocked currents in both WT cells and those with the V478M variant, which causes GoF effects. However, a limitation of this experiment was that fluoxetine's effects were solely assessed on current density, which is not normally increased by V478M. A more comprehensive analysis, measuring parameters like voltage-dependences and kinetics of activation and deactivation, would have provided a more informative assessment of fluoxetine's potential to revert the GoF effects observed for V478M.

While fluoxetine may have promise for treating Kv1.3 channelopathy, it has notable limitations. First, being a Kv1.3 inhibitor, it could only treat variants exhibiting GoF effects, which excludes its use in the most severe DEE cases associated with Kv1.3 LoF. Second, fluoxetine lacks selectivity, inhibiting not only Kv1.3 but also Kv1.1, Kv1.4, and Kv3.1 channels.[8] Several natural venom peptides have been identified that block Kv1.3 with high potency and specificity and could therefore be explored as alternatives, but most cannot cross the blood–brain barrier.[3] A potentially better option is the small molecule PAP-1, which inhibits Kv1.3 with high specificity, crosses the blood–brain barrier, and has demonstrated preclinical efficacy for treating neuroinflammation in neurodegenerative disease.[9] Looking forward, the development of Kv1.3 activators holds the most promise for treating Kv1.3-related DEE, which is primarily caused by LoF variants.

The connection between *KCNA3* variants and DEE raises questions about how mutations in predominantly immune-related Kv1.3 channels can cause epilepsy. We now know that Kv1.3 is not restricted to immune-related cells, but it is also expressed in neurons in important epileptogenic brain regions like the hippocampus where LoF could cause seizure-inducing hyperexcitability. However, Kv1.3 knockout (KO) mouse models present a conundrum as they exhibit few overt neurological or immunological phenotypes.[2] Initial characterization of Kv1.3 KO mice revealed only reduced body weight due to increased metabolic rate. Later studies found that Kv1.1 KO mice also have anxiety-like behaviors, attention deficits, and a heightened sense of smell related to increased excitability of olfactory bulb mitral cells.[2] Thus, studies of Kv1.1 KO mice, which should theoretically mimic the pure LoF Kv1.3 variants, do not show evidence of epilepsy. Adding more complexity, treating WT mice with the Kv1.3-specific inhibitor PAP-1 significantly attenuates kainic acid (KA)-induced seizures.[9] Kv1.3 is upregulated in microglia following KA-induced seizures, but blockade of Kv1.3 suppresses proinflammatory microglial activation and reduced proinflammatory cytokine production, suggesting a neuroinflammatory mode of action involving microglia.

The complex interplay between microglia and neurons, both expressing Kv1.3, might hold the key to understanding how Kv1.3 contributes to epilepsy. Microglia interact with neurons in an activity-dependent fashion to control neuronal excitability.[10] Microglial synaptic pruning is also essential for development of healthy brain circuits and impaired pruning has been implicated in neuronal hyperexcitability and seizures.[10] Thus, the consequences of Kv1.3 variants may ultimately depend on the balance of neuronal and immune-related factors. To unravel this, conditional knockout animal models restricting Kv1.3 deletion to specific cell types or developmental time windows may be necessary. Thanks to the discovery of *KCNA3* variants underlying DEE, the focus of Kv1.3 research is now extending beyond non-excitable lymphocytes and microglia to include excitable cells, particularly neurons.

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Declaration of Conflicting Interests

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