Supplemental material

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Table S1. **Missense SNPs and mutations in the *KCNMA1* gene reported by population sequencing databases**

Database	Missense SNPs	Mutations
ClinVar	46	**7/16** - G354S, G375R (rs1554829003), D434G (rs137853333), Y676Lfs*7 (rs762705295), E884K (rs1554966197), N995S/N999S/N1053S (rs886039469), N1159S (rs563967757)
ExAC	253	**2/16** - Y676Lfs*7 (rs762705295), N1159S (rs563967757)
gnomAD	311	**7/16** - D434G (rs137853333), K518N (rs770007121), E656A (rs149000684), Y676Lfs*7 (rs762705295), E884K (rs1554966197), N995S/N999S/N1053S (rs886039469), N1159S (rs563967757)

The number of missense SNPs and mutations in the *KCNMA1* gene reported by three population sequencing databases differs (Lek et al., 2016; Landrum et al., 2018). The denominator of 16 in the mutation column reflects the total number of mutations identified in symptomatic patients reported in this review. No reference SNP cluster ID was reported for G354S. *ClinVar* (https://www.ncbi.nlm.nih.gov/clinvar/); *ExAC* Exome Aggregation Consortium (http://exac.broadinstitute.org); *gnomAD* Genome Aggregation Database (https://gnomad.broadinstitute.org). The authors acknowledge the Genome Aggregation Database (gnomAD), Exome Aggregation Consortium, and the groups that provided exome and genome variant data to these resources. A full list of contributing groups can be found at https://gnomad.broadinstitute.org/about and http://exac.broadinstitute.org/about.

Table S2. **Neurological gene panels which include the *KCNMA1* gene**

Gene panel name and lab	Genes	Methods
All Neuro panel; Centogene AG - the Rare Disease Company, Germany	1,205	Sequence analysis of the entire coding region
Generalized epilepsy and paroxysmal dyskinesia; Centogene AG - the Rare Disease Company, Germany	1	Deletion/duplication analysis; sequence analysis of the entire coding region
Childhood Epilepsy; Amplexa Genetics, Amplexa Genetics A/S, Denmark	125	Mutation scanning of the entire coding region
Epilepsy, Intellectual Disability, and Autism Spectrum Disorder; Amplexa Genetics, Amplexa Genetics A/S, Denmark	569	Mutation scanning of the entire coding region
Epilepsy and Seizure Plus Sequencing Panel with CNV Detection; Prevention Genetics, US	222	Deletion/duplication analysis; sequence analysis of the entire coding region; targeted variant analysis
Childhood Epilepsy NGS Panel; Fulgent Genetics, US	209	Deletion/duplication analysis; sequence analysis of the entire coding region
Neonatal Epilepsy NGS Panel; Fulgent Genetics, US	275	Deletion/duplication analysis; sequence analysis of the entire coding region
Epilepsy Advanced Sequencing and CNV Evaluation; Athena Diagnostics Inc, US	234	Deletion/duplication analysis; sequence analysis of the entire coding region
Epilepsy Advanced Sequencing and CNV Evaluation - Generalized, Absence, Focal, Febrile and Myoclonic Epilepsies; Athena Diagnostics Inc, US	84	Deletion/duplication analysis; sequence analysis of the entire coding region
Epilepsy and Seizure Disorders: Deletion/Duplication Panel; EGL Genetic Diagnostics Eurofins Clinical Diagnostics, US	107	Deletion/duplication analysis
Neurogenetic Disorders – panels; MGZ Medical Genetics Center, Germany	597	Deletion/duplication analysis; sequence analysis of the entire coding region
Epilepsy/Seizure; Knight Diagnostic Laboratories - Molecular Diagnostic Center Oregon Health & Science University, US	98	Sequence analysis of the entire coding region
Epilepsy; Asper Biogene Asper Biogene LLC, Estonia	175	Deletion/duplication analysis; sequence analysis of the entire coding region
Epilepsy Comprehensive NGS Panel; Fulgent Genetics, US	398	Deletion/duplication analysis; sequence analysis of the entire coding region
Epilepsy Hereditary Panel; GENETAQ Molecular Genetics Centre and Diagnosis of Rare Diseases, Spain	37	Sequence analysis of the entire coding region
Dystonia; Asper Biogene Asper Biogene LLC, Estonia	38	Deletion/duplication analysis; sequence analysis of the entire coding region
Autism Spectrum Disorders 53-Gene Panel; Center for Human Genetics, Inc, US	53	Sequence analysis of the entire coding region
Dystonia (NGS panel for 43 genes); CGC Genetics, Portugal	43	Sequence analysis of the entire coding region
Single gene testing KCNMA1; CeGaT GmbH, Germany	1	Sequence analysis of the entire coding region
Generalized epilepsy and paroxysmal dyskinesia (sequence analysis of KCNMA1 gene); CGC Genetics, Portugal	1	Sequence analysis of the entire coding region
Dystonia All Panel; CeGaT GmbH, Germany	54	Sequence analysis of the entire coding region
Paroxysmal Movement Disorders Panel; CeGaT GmbH, Germany	4	Sequence analysis of the entire coding region
Paroxysmal Dyskinesia Panel; CeGaT GmbH, Germany	6	Sequence analysis of the entire coding region
Idiopathic Generalized and Focal Epilepsy Panel; CeGaT GmbH, Germany	40	Sequence analysis of the entire coding region
KCNMA1 Single Gene; Fulgent Genetics, US	1	Deletion/duplication analysis; sequence analysis of the entire coding region
Neurology: Sequencing Panel; EGL Genetic Diagnostics Eurofins Clinical Diagnostics, US	164	Sequence analysis of the entire coding region
Epilepsy and Seizure Disorders: Sequencing Panel; EGL Genetic Diagnostics Eurofins Clinical Diagnostics, US	110	Sequence analysis of the entire coding region
Clinical Exome; Fulgent Genetics, US	4,673	Deletion/duplication analysis; sequence analysis of the entire coding region
Epilepsy with paroxysmal disorders panel; Genome Diagnostics Laboratory University Medical Center, Utrecht, Netherlands	5	Sequence analysis of select exons; sequence analysis of the entire coding region

The 29 gene panels were identified using NCBI Genetic Testing Registry (GTR) database (https://www.ncbi.nlm.nih.gov/gtr/).

GOF Mutations

D434G

The D434 residue is located in the AC region of the RCK1 domain (Fig. 1), which contributes to the calcium gating of BK channels (Du et al., 2005). Patch-clamp recordings from Chinese hamster ovary (CHO) cells and *Xenopus laevis* oocytes expressing D434G channels demonstrated increased BK current. The increased current was primarily due to a three- to fivefold increase in Ca^{2+} sensitivity and faster activation compared to WT BK channels. The voltage of half-maximal activation ($V_{1/2}$) for D434G channels was shifted to more negative potentials by 26 mV and 56 mV at 0.1 and 2 μM Ca^{2+}, respectively. These experiments revealed that D434G makes the channels easier to open, and the mutation was identified as the first human GOF *KCNMA*1 allele. The conclusions were confirmed in two independent studies that characterized the effect of D434G on BK/β4 channels and further probed the molecular mechanism behind the aspartate-to-glycine substitution (Wang et al., 2009; Yang et al., 2010). Several putative neurophysiological mechanisms consistent with either hyperexcitation in brain areas such as thalamocortical circuits and the basal ganglia, or disinhibition of GABAergic circuits, were hypothesized to explain the symptoms experienced by patients (Du et al., 2005), but the specific neuronal circuit alterations caused by the D434G mutation remain unknown.

N995S/N999S/N1053S

A second GOF mutation is reported in the literature using three different reference sequence numbering schemes but constitutes the same residue substitution (Fig. 1 and Table 1; Zhang et al., 2015; Wang et al., 2017; Li et al., 2018; Heim et al., 2019; Plante et al., 2019). In this review, this mutation will be referred to by the numbering scheme in the original publication for the data being discussed. Patch-clamp recordings from HEK293 cells expressing N995S or N999S channels exhibited increased BK current compared to WT (Li et al., 2018; Plante et al., 2019). This increased current was due to a >40-mV hyperpolarizing shift in the $V_{1/2}$ (Li et al., 2018; Plante et al., 2019). The mechanism of this shift was proposed to be independent of Ca^{2+}, as the N995S mutation increased BK current when the intracellular Ca^{2+} binding sites were mutated (Li et al., 2018). Additionally, activation of the mutant N995S (N999S) channels was faster and deactivation was slower than WT, correlated with increased mean open times in single channel recordings (Li et al., 2018; Plante et al., 2019). Interestingly, the GOF BK current phenotypes from N999S channels were found to exceed the GOF alterations produced by D434G (Plante et al., 2019), suggesting that the relative alterations in BK channel properties exhibited by distinct GOF mutations could influence the clinical heterogeneity among patients.

LOF mutations

Liang et al. (2019) reported nine unrelated patients affected by eight distinct *KCNMA1* mutations spanning from the pore domain to end of the intracellular C-terminal gating ring of the BK protein (Fig. 1). Five mutations abolished BK current in HEK293T patch-clamp recordings: S351Y and G356R in the pore domain, G375R in the S6 domain, N449fs* in the AC domain of RCK1, and I663V in the loop between RCK1 and RCK2, suggesting these mutations comprise LOF alleles of *KCNMA1* (Liang et al., 2019). Of these five mutations, only I663V was evaluated by western blot for protein expression levels. I663V channels had higher molecular weight compared to WT, but additional experiments would be needed to determine whether the size shift was due to changes in post translational modifications and how this relates to loss of BK current. The mechanisms for current abrogation of the other four mutations has not yet been addressed.

The other three mutations, C413Y, P805L, and D984N, reduced the mean amplitude of BK current compared to WT in patch clamp recordings, suggesting a mechanistically distinct LOF phenotype from the prior group. C413Y in the AC region of RCK1 and P805L located in the loop between S9 and S10 of the gating ring (Fig. 1) showed shifts in the $V_{1/2}$ values to more positive potentials, with a slope change suggestive of alterations in the voltage and Ca^{2+} sensitivity of the channels (Liang et al., 2019). Both mutations produced smaller current amplitudes compared to WT channels, and the expression level of P805L was decreased in western blot analysis. Interestingly, the patient harboring the C413Y mutation inherited this mutation from his asymptomatic mother, and the N449fs* from his asymptomatic father (Liang et al., 2019). This raises two possibilities, either that each mutation is akin to an autosomal recessive allele, or that co-expression with WT in the heterozygous parents may preclude a pathological phenotype. Finally, the mutation D984N located in the loop between S9 and S10 in RCK2 showed no shift in the $V_{1/2}$ at 10μM Ca^{2+}. Other Ca^{2+} concentrations and expression levels were not evaluated, leaving the mechanism for this LOF mutation unresolved.

G354S

Voltage-clamp recordings from *Xenopus* oocytes expressing the G354S mutant BK channels demonstrated a 10-fold reduction in BK current due to slower activation kinetics (Carvalho-de-Souza et al., 2016).

R458Ter and Tyr676Lfs*7

These mutations were predicated to be LOF allele based on the early termination of the BK channel protein (Tabarki et al., 2016; Yeşil et al., 2018). Tyr676Lfs*7 is an autosomal recessive *KCNMA1* duplication mutation (Tabarki et al., 2016). Due to the retention of the tetramerization domain in the C-terminus of the channel, Tyr676Lfs*7 could potentially reduce current through a dominant negative action, but the functional properties for both mutations remains to be tested.

K518N, E656A, and N1159S

Patch-clamp recordings in HEK293 cells for each of these mutant channels showed no differences in activation kinetics or BK current density compared to WT BK channels suggesting they are benign genetic variants (no change in BK channel properties under the tested conditions) under the tested conditions (Li et al., 2018). Patients with these mutations exhibit a range of epilepsy phenotypes that is also observed among the *KCNMA1* patient population with pathogenic variants (Fig. 4).

E884K

The functional effect for this mutation has not been shown, making it a VUS. A VUS polymorphism is classified as a mutation by genetic testing, but for which there is not enough information to conclude its causative relationship to the patient symptoms. However, the patient shares similar symptoms to other GOF and LOF patients including PNKD, developmental delay, and visual impairment (Zhang et al., 2015), and pathogenicity prediction algorithms reported this mutation to be possibly deleterious.

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