Gain-of-function *HCN2* variants in genetic epilepsy (62 characters)

Melody Li ^{1*}, Snezana Maljevic ^{1*}, A. Marie Phillips ^{1,8*}, Slave Petrovski ², Michael Hildebrand ², Rosemary Burgess ², Therese Mount ¹, Federico Zara ³, Pasquale Striano ⁴, Julian Schubert ⁵, Holger Thiele ⁶, Peter Nürnberg ⁶, Michael Wong ⁷, Judith L. Weisenberg ⁷, Liu Lin Thio ⁷, Holger Lerche ⁵, Ingrid E. Scheffer ², Samuel F. Berkovic ², Steven Petrou ¹, Christopher A. Reid ^{1#}

1. Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria 3010, Australia.

2. Epilepsy Research Centre, Department of Medicine, The University of Melbourne, Austin Health, Heidelberg, Victoria, Australia

3. Laboratory of Neurogenetics, Department of Neuroscience, Institute "G. Gaslini", Genoa, Italy

4. Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Institute "G. Gaslini", Genoa, Italy.

5. University of Tübingen, Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, Germany

6. Cologne Centre for Genomics, University of Cologne, Germany

7. Department of Neurology, Washington University School of Medicine and St. Louis Children's Hospital, St Louis, MO, USA.

8. School of Biosciences, The University of Melbourne, Parkville, Victoria 3010, Australia.

* Equally contributing first authors

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#Corresponding author: Christopher A. Reid

Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria 3010, Australia, Tel: 61-3-8344 1957; Fax: 61-3-9347 0446

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E-mail: careid@unimelb.edu.au

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Abstract (203 words)

Genetic generalized epilepsy (GGE) is a common epilepsy syndrome that encompasses seizure disorders characterized by spike-and-wave discharges (SWDs). Pacemaker hyperpolarization-activated cyclic nucleotide-gated channels (HCN) are considered integral to SWD genesis, making them an ideal gene candidate for GGE. We identified *HCN2* missense variants from a large cohort of 585 GGE patients, recruited by the Epilepsy Phenome-Genome Project (EPGP), and performed functional analysis using two-electrode voltage clamp recordings from *Xenopus* oocytes. The p.S632W variant was identified in a patient with idiopathic photosensitive occipital epilepsy (IPOE) and segregated in the family. This variant was also independently identified in an unrelated patient with childhood absence seizures from a European cohort of 238 familial GGE cases. The p.V246M variant was identified in a patient with photo-sensitive GGE and his father diagnosed with juvenile myoclonic epilepsy (JME). Functional studies revealed that both p.S632W and p.V246M had an identical functional impact including a depolarizing shift in the voltage dependence of activation that is consistent with a gain-of-function. In contrast, no biophysical changes resulted from the introduction of common population variants, p.E280K and p.A705T, and the p.R756C variant from EPCP that didn't segregate with disease. Our data suggest that *HCN2* variants can confer susceptibility to GGE via a gain-of-function mechanism.

Key words: spike-and-wave discharges, thalamo-cortical networks, HCN channels, febrile seizures

Introduction

Genetic Generalized Epilepsy (GGE) is the most common form of inherited epilepsy; it has a number of subtypes, all with the signature of generalized spike-wave discharges on electroencephalography (EEG) (Loiseau et al., 1990). Clinical genetic evidence suggests that inheritance of GGE is complex (Helbig et al., 2008). Various chromosomal microdeletions, found in a small proportion of cases, increase risk of GGE (de Kovel et al., 2010; Helbig et al., 2009; Scheffer et al., 2009). Loss-of-function mutations in *SLC2A1*, a gene encoding the glucose transporter GLUT1, responsible for the transport of glucose across the blood brain barrier, account for 0.5-1% of GGE (Arsov et al., 2012; Striano et al., 2012). Genome-wide association studies have also implicated *PCDH7* and *SCN1A* in GGE, although the absolute increase in risk these variants confer is likely to be modest (International League Against Epilepsy Consortium on Complex Epilepsies, 2014). Single gene mutations, in particular in GABA_A receptors (Cossette et al., 2002; Kananura et al., 2002; Maljevic et al., 2006; Wallace et al., 2001) and T-type Ca²⁺ channels (Chen et al., 2003; Heron et al., 2004) have also been described in GGE and related syndromes. These genetic studies and the recent report from the Epi4K Consortium suggest high locus heterogeneity contributing to GGE susceptibility (Epi4K consortium; Epilepsy Phenome/Genome Project, 2017). The hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are strongly implicated in the pathology of SWD-related disorders making them good risk candidates for GGE (Reid et al., 2012).

HCN channels carry a non-selective cationic conductance that is activated by membrane hyperpolarization. There are four HCN channels encoded by *HCN1*, *HCN2*, *HCN3* and *HCN4*, each having distinct kinetic and voltage characteristics, as well as differences in cAMP sensitivity (Wahl-Schott and Biel, 2009). Several experimental studies in animal

models have mechanistically linked changes in the function and expression of HCNs to focal and generalized epilepsy. This includes transcriptional changes in both acquired and genetic models of epilepsy (Benarroch, 2013, DiFrancesco and DiFrancesco, 2015, Reid et al., 2012). Recent genetic screening efforts have also described epilepsy variants in genes encoding HCN channels. Nava *et al.*, (2014) describe several *de novo* mutations in *HCN1* associated with early onset epileptic encephalopathy (Nava et al., 2014). A number of *HCN2* (MIM# 602781) variants associated with epilepsy have also been reported (Dibbens et al., 2010; DiFrancesco et al., 2011; Nakamura et al., 2013; Tang et al., 2008). Our own study found a small deletion in *HCN2* (p.719-721ΔP; *HCN2*_{delPPP}) that was enriched in patients with febrile seizure syndromes with functional analysis revealing an increased current density (Dibbens et al., 2010). The p.S126L variant associated with febrile seizures (FS) and increased current density was described in two unrelated patients (Nakamura et al., 2013). Di Francesco *et al.*, (2011) described a homozygous *HCN2* mutation, p.E515K in a patient with sporadic severe GGE that caused a dramatic loss-of-function (DiFrancesco et al., 2011).

Here, we investigate the functional impact of rare *HCN2* missense variants found among a sample of 585 EPGP patients with GGE. Gain-of-function biophysical changes were observed for two *HCN2* missense variants suggesting that they may act as GGE risk alleles.

Material and Methods

Patient Sample and Clinical Evaluation

The study was prospectively reviewed and approved by the Human Research Ethics Committee (Project No. H2007/02961) of Austin Health, Melbourne, Australia. Informed consent was obtained from all subjects or their parents or legal guardians. The EPGP base population was comprised of 585 patients with familial GGE that were exome sequenced by

the Epi4K consortium. Four rare variants were identified in *HCN2* (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017). Three of these variants were single base substitutions, p.V246M (NM_001194.3(HCN2_v001):c.736G>A), p.S632W (NM_001194.3(HCN2_v001):c.1895C>G) and p.R756C (NM_001194.3(HCN2_v001):c.2266C>T), and one, p. P720Rfs*17 (NM_001194.3(HCN2_v001):c.2159del), was a frameshift variant resulting in a truncated protein with an added arginine rich C-terminal domain. The variants have been submitted to the public LOVD database (www.lovd.nl). Variants p.S632W, p.V246M and p.R756C were successfully introduced into vectors for study *in vitro*.

An additional case with the p.S632W variant was identified in an independent cohort of the EuroEPINOMICS-CoGIE project that sequenced 238 exomes of index cases with familial GGE, as described previously (Galizia et al., 2015). Pedigrees of these cases were reviewed and extended when it was possible to obtain DNA from relatives. Source clinical and EEG data were reviewed and classification of epilepsy syndromes assigned (Berg et al., 2010).

Sanger Sequencing

The *HCN2* gene was amplified using gene-specific primers (oligonucleotides available on request) designed to the full-length reference human gene transcript (RefSeq NM_001194.3). Amplification reactions were setup using high fidelity PrimeSTAR GXL DNA polymerase (Takara Bio USA Inc., Mountain View, CA) and cycled using a standard protocol on a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA) at 58°C annealing temperature for 1 minute. Bidirectional sequencing of all exons and flanking regions was completed with a BigDyeTM v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. Sequencing products and microsatellite markers were resolved using a 3730xl DNA Analyzer (Applied Biosystems). All sequencing chromatograms were

compared to published cDNA sequence; nucleotide changes were detected using Codon Code Aligner (Codon Code Corporation, Dedham, MA).

HCN2 Mutagenesis and In Vitro Transcription

Human *HCN2* cDNA was subcloned from a pcDNA3 clone, gift from M. Biel, into the pGEMHE-mcs vector for *in vitro* transcription. The p.S632W and p.V246M variants were generated using site-directed mutagenesis. The hHCN2 sequence (NM_001194.3) between the EcoRI site at position c.2050 and the XbaI site in the pcDNA3 clone, and containing the c.2266C>T exchange (p.R756C) was sent to GenScript for synthesis (GenScript, Piscataway, NJ 08854, USA). The mutagenized fragment in pUC57 was sub-cloned into pcDNA3 hHCN2 and then into the pGEMHE-mcs vector. All clones were verified by Sanger sequencing. *In vitro* synthesis of cRNA was performed using linearized cDNA template and the mMessage mMachine® T7 transcription kit (Ambion, USA). RNA integrity was assessed both spectrophotometrically (Nanodrop) and by denaturing gel electrophoresis.

HCN2 two-electrode voltage clamp analysis and statistical design

Oocytes from *Xenopus laevis* were prepared as previously described (Dibbens et al., 2010). Briefly, 40 nl of cRNA encoding WT or mutant HCN2 subunits (12.5 ng/μl; concentration confirmed spectrophotometrically and by gel analysis) was injected into stage 5/6 oocytes using the Roboinject (Multi Channel Systems, Reutlingen, Germany). In co-expression experiments, oocytes were injected with WT and a mutant HCN2 subunit, both at a concentration of 12.5 ng/μl, or as a single subunit at 25 ng/μl. Injected oocytes were incubated for 2 days at 18°C prior to experimentation. Two-electrode voltage clamp recordings were performed on a Roboocyte2 (Multi Channel Systems). Oocytes were perfused with a bath solution (in mM): 96 KCl, 2 NaCl, 2 MgCl₂ and 10 HEPES (pH 7.5

using KOH) and impaled with two glass electrodes containing 1.5 M potassium acetate and 0.5 M KCl. Holding potential was -30 mV. A current-voltage (I-V) relationship was analyzed by incrementing voltage in 5 mV steps from -130 mV to -30 mV for 10 s, with a 1 s test potential of -100 mV at the end of each pulse. Tail currents were measured over a 50 ms epoch at the peak. To obtain a normalized GV relationship, peak tail current amplitudes recorded at -100 mV were normalized to the maximum peak tail current, plotted against voltage and fit with a Boltzmann curve (GraphPad Prism, USA). The time to half the peak steady-state amplitude was used as a measure of the rate of HCN2 channel activation at various voltages. Cyclic adenosine monophosphate (cAMP) modulation was investigated by incubating oocytes in 15 μ M forskolin (Sigma, Castle Hill, Australia) for a period of 7 minutes.

Statistics

Statistical analysis for the functional study used one way ANOVA or two way ANOVA with post-hoc multiple comparison as appropriate, see Figure legends (GraphPad). Multiple-testing corrected $p < 0.05$ was set as significant. Data are presented as mean $\pm$ standard error of the mean.

Results

The p.S632W variant was identified in an individual diagnosed with photosensitive seizures and generalized spike-wave on EEG with some seizures having a focal component; this syndrome is known as Idiopathic Photosensitive Occipital Epilepsy (IPOE) and has features of both generalized and focal epilepsy (Taylor et al., 2003). The mutation segregated in the family, with both the mother with IPOE and a brother with FS carrying the variant, while an

unaffected father did not (Fig. 1). The p.S632W variant was also found in an unrelated male from Italy who experienced absence seizures from age of three and generalized tonic-clonic seizures in adolescence with generalized spike-wave discharges on EEG. He had an affected brother with a similar history of early-onset absence epilepsy, who had some seizures only while watching television, suggestive of photosensitivity. Since a DNA sample was unavailable from the brother and the proband's parents, it is not known whether the variant is inherited or *de novo*.

The variant p.V246M was identified in an individual diagnosed with photosensitive myoclonic seizures at age six and generalized tonic-clonic seizures in adolescence. She had mild intellectual disability and EEG revealed generalized SWDs with prominent photosensitivity. Her father, who also carries the p.V246M variant, had adolescent onset myoclonic jerks with photosensitive generalized tonic-clonic seizures consistent with juvenile myoclonic epilepsy. Her brother, who also carries the variant, had two seizures; one probable focal seizure at 10 years and a tonic-clonic seizure at 12 years. EEG revealed both centro-temporal spikes and generalized spike-wave discharges, and it was unclear if he had generalized or focal epilepsy or both. The brother had attention deficit hyperactivity disorder and has had a hospital admission for severe behavioural problems. No intellectual disability was reported in either the father or brother of the proband (Fig. 1).

The HCN2 p.R756C variant was identified in a 33 year old female with unclassified epilepsy (Fig. 1). She had approximately 20 tonic-clonic seizures between early childhood and adolescence; MRI was normal, EEG was unavailable and it is uncertain whether she had generalized or focal epilepsy. She had learning difficulties and poor speech, however, details of both are limited. She gave birth to two daughters. One of them developed febrile seizures and the other one had febrile seizures plus, experiencing both febrile and afebrile seizures

between 12 months and 4 years. The second daughter also had some learning difficulties. Her MRI was normal and EEG showed some posterior epileptiform discharges but it was unclear if the seizures had a focal onset. The HCN2 p.R756C variant was not carried by the daughter suffering febrile seizures plus.

The p.P720Rfs*17 variant was identified in a 13 year old male, with generalized epilepsy. He had seizures from 16 months and these were ongoing at 13 years. They all occurred during sleep, his MRI was normal and his EEG showed generalized epileptiform activity with hyperventilation. He had learning disability and attention deficit disorder. His family history was positive for unconfirmed seizures in a maternal aunt and a younger brother with epilepsy with myoclonic-atonic seizures. The p.P720R*fs17 deletion did not segregate within the limited available pedigree (Fig. 1).

In silico prediction scores indicated that both the p.V246M and p.S632W variants are likely to be detrimental to channel function. Serine 632 is located in the cytoplasmic, CNBD domain and is a highly conserved amino acid (Fig. 1). The p.S632W variant is not found in the gnomAD database of over 240000 exomes or genomes (<http://gnomad.broadinstitute.org>), had a Grantham score of 177, and maximal PolyPhen (HumDiv) score of 1.0 suggesting that it was probably damaging to the HCN2 protein. Valine 246 is located in the second transmembrane domain of the HCN2 protein and is relatively highly conserved (Fig. 1). The p.V246M variant is present in the gnomAD server four times with a minor allele frequency of 1.631e-5. Each gnomAD observation was reported among a distinct genetic ancestry. The p.V246M variant has a Grantham score of 21 and PolyPhen score of 0.994 (HumDiv) suggesting that it was probably damaging. Arginine 756 is also located in the cytoplasmic domain and is highly conserved (Fig. 1). The p.R756C variant is not found in the gnomAD database, has a high Grantham score of 180, but has a maximal PolyPhen

(HumDiv) score of only 0.013. Finally, the novel p.P720Rfs*17 deletion leads to a frameshift resulting in the addition of 15 new amino acids before a premature stop codon leading to the loss of the last 154 amino acids of the cytoplasmic domain and was not studied further.

We next performed functional analysis on missense HCN2 variants associated with genetic epilepsy. HCN2 channel-mediated currents could be detected using two-electrode voltage clamp recordings in *Xenopus* oocytes (Fig. 2A). No hyperpolarization-mediated currents were seen in oocytes injected with water suggesting that there was no endogenous HCN channels (Fig. 2B). There was no difference between the steady-state current amplitudes for the p.S632W and p.V246M variants relative to WT (Fig. 2B) suggesting that they were transcribed and trafficked to the membrane. The conductance-voltage relationship revealed a depolarizing shift in activation for both the p.S632W and p.V246M variants, which is consistent with a gain-of-function (Fig. 2C). Fits of Boltzmann functions to the activation curves confirmed this, revealing a significant depolarizing shift in $V_{1/2}$ for the p.S632W and p.V246M variants compared to WT (Fig. 2D). Similarly, there was a significant difference in the slope of the curve for these two GGE variants ($p < 0.05$; Fig. 2E). A significant decrease in the rate of activation was also seen for both the p.S632W and p.V246M variants ($p < 0.05$; Fig. 2F). Consistent with a lack of segregation in the family, the p.R756C variant did not show any significant differences in its biophysical properties including steady-state current, $V_{1/2}$, Boltzmann slope and channel activation (Supp. Figure S1).

To mimic the heterozygous expression seen in patients, we co-expressed mutant and WT channels in equal amounts. Functional analysis revealed that changes in the key biophysical properties were still seen for WT/p.S632W co-expressed channels including; a depolarizing shift in $V_{1/2}$ (HCN2 WT = -78.6 ± 1.0 mV vs. HCN2 WT/p.S632W = -75.7 ± 1.0 mV, $n = 14-18$, $p = 0.053$); a greater slope (HCN2 WT = 6.8 ± 0.2 vs. HCN2 WT/p.S632W = 7.9 ± 0.3 ,

n= 14-18, $p = 0.003$) and significantly faster activation kinetics at -85 mV (HCN2WT = 1.9 ± 0.1 sec vs. HCN2 WT/p.S632W = 1.5 ± 0.1 sec, n= 14-18, $p = 0.01$). Similarly, changes in biophysical properties were still observed for WT/p.V246M co-expressed channels that included a depolarizing shift in $V_{1/2}$ (HCN2 WT = -84.1 ± 1.4 mV vs. HCN2 WT/p.V246M = -79.7 ± 1.4 mV, n= 13-17, $p = 0.04$), a greater slope (HCN2 WT = 7.2 ± 0.1 vs. HCN2 WT/p.V246M = 7.8 ± 0.2 , n= 13-17, $p = 0.056$) and significantly faster activation kinetics at -85 mV (HCN2 WT = 2.5 ± 0.1 sec vs. HCN2 WT/p.V246M = 1.8 ± 0.1 sec, n= 13-17, $p < 0.0001$).

Given the proximity of the p.S632W variant to the intracellular cyclic nucleotide-binding domain we investigated whether cAMP gating was altered by comparing channel sensitivity to forskolin, an activator of adenylate cyclase. There was no difference in the shift in $V_{1/2}$ in forskolin for the WT and p.S632W channels (Supp. Figure S2A). The change in slope of the activation curves in forskolin was also not significantly different (Supp. Figure S2B).

We also included two population missense variants drawn from the gnomAD database, p.E280K (NM_001194.3(HCN2_v001):c.838G>A) and p.A705T (NM_001194.3(HCN2_v001):c.2113G>A), as further controls in addition to the wildtype (WT) reference construct. These were chosen based on their high population prevalence suggesting that they were unlikely to be clinically relevant. p.E280K is a polymorphism found in around 3 % of the population with African ancestry and absent among European samples in gnomAD. It has an overall minor allele frequency of 2.546×10^{-3} . p.A705T is found in samples of both European and African ancestry, and has an overall minor allele frequency of 1.857×10^{-4} . The population variants, p.E280K and p.A705T, did not show significant differences in their biophysical properties relative to WT channels (Fig. 3).

Discussion

Although the genetic architecture of GGE is yet to be fully elucidated, the current view is that there is no major individual gene accounting for a high proportion of cases (Epi4K consortium; Epilepsy Phenome/Genome Project, 2017). Therefore, GGE is likely to arise due to numerous risk alleles with some having major effects. Here, we identify two novel HCN2 variants, p.S632W and p.V246M, that are likely to contribute to pathogenicity. Both segregate with the epilepsy phenotype in affected families and *in silico* analysis suggests they are ‘probably damaging’ to the HCN2 protein. Moreover, voltage-clamp analysis shows altered biophysical properties of the variants. Remarkably, both p.S632W and p.V246M have an identical impact on the biophysical properties of the channel including a depolarizing shift in the voltage dependence of activation, an increase in the slope of the conductance-voltage relationship and a quicker time to activation. These biophysical changes are consistent with a gain-of-function. The p.R756C mutation did not segregate within the family studied and functional analysis revealed no change in channel properties. Importantly, we also showed that two population variants did not affect the biophysical properties of the channel. Thus, there is a strong correlation between the clinical and *in silico* markers of pathogenicity and the functional impact of the variants we have investigated. Interestingly, based on the ExAC reference cohort, the *HCN2* gene has a very low tolerance to variation (<http://genic-intolerance.org/>) providing further support that functional changes in the HCN2 protein are likely to be deleterious (Petrovski et al., 2013).

The position of previously published HCN2 variants associated with epilepsy and all six variants studied here are shown in Figure 4. A homozygous p.E515K mutation was discovered in a single patient who had a severe form of GGE (DiFrancesco et al., 2011).

Analysis indicated a dramatic hyperpolarizing shift in the voltage dependence of activation consistent with a loss-of-function, a finding we replicated (data not shown). However, this is an unusual case given the mutation is homozygous in the patient and neither heterozygous parent exhibited a seizure phenotype (DiFrancesco et al., 2011). Both the HCN2-null and the homozygous *apathetic* mouse (harbouring a spontaneous *HCN2* truncation) display bilateral synchronous SWDs (Chung et al., 2009; Ludwig et al., 2003). This is consistent with the above clinical finding and suggests that significant reductions in HCN2 function can lead to aberrant SWD activity. In contrast, our results argue that gain-of-function heterozygous variants in HCN2 can contribute to generalized epilepsy, and are present in patients with phenotypes that span a spectrum of GGE phenotypes, often including photosensitivity. HCN2 gain-of-function variants have also been identified in FS syndromes (Dibbens et al., 2010; Nakamura et al., 2013). Interestingly, a brother of proband 1 harbouring the p.S632W variation has a diagnosis of simple FS. This suggests that gain-of-function HCN2 variation may also constitute a risk factor for febrile seizure syndromes. A familial GGE and FS association is frequently observed clinically (Scheffer and Berkovic, 1997).

I_h plays a complex role in neurons making it difficult to speculate on the precise impact that changes in function will have on neuronal excitability. Tonic activation of I_h contributes a depolarizing current that helps determine resting membrane potential (Maccaferri et al., 1993). The gain-of-function changes in HCN2 properties described here will likely result in a depolarized resting membrane potential taking the neuron closer to threshold for action potential firing. Computer simulations have suggested this is the major impact of increased I_h in epileptic states (Dyhrfeld-Johnsen et al., 2008). Additionally, I_h also acts as a ‘pacemaking’ current in thalamus with gain-of-function HCN2 variants potentially increasing the ability of neurons to drive rhythmicity in thalamo-cortical networks resulting in aberrant

generalized spike-wave activity that is a hallmark of generalized seizures (Chen et al., 2001; Huguenard, 2001). Determining the precise underlying cellular basis of excitability for both loss- and gain-of-function *HCN2* mutations and particularly their distinctive role to promote generalized spike-wave activity will require further functional investigation.

HCN4 and *HCN2* channel underlie I_h in the sinoatrial node with this current important for maintaining stable cardiac rhythm (Biel et al., 2009). The *HCN2* knock-out mouse has a sinus dysrhythmia suggesting the variants in this channel could potentially alter cardiac function (Ludwig et al., 2003). There were no reports of any cardiac abnormalities in the families carrying the p.S632W and p.V246M. However, family collections were done as part of retrospective studies so we cannot rule out that cardiac function was subtly altered by *HCN2* variants. This is an important aspect to test prospectively in future studies.

In summary, we provide strong genetic and functional evidence for two rare variants in *HCN2* as risk alleles of GGE. We identify a right-shift in activation as a mechanism common to both the p.S632W and p.V246M variants, which is consistent with previous reports of increased *HCN2* function for variants identified in FS syndromes (Dibbens et al., 2010, Nakamura et al., 2013). *HCN2* may therefore be a risk allele for both GGE and FS.

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Potential Conflicts of Interest:

Dr. Petrou reports commercial interests as Scientific Co-Founder of Praxis Precision Medicine, Scientific Founder of RogCon, and Advisory Board Member of Paimomix, outside the submitted work. Other authors have no disclosures relating to data published in this manuscript.

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Figure legends

Figure 1. Genetic generalized HCN2 variants present in the Epilepsy Phenome-Genome Project database. A. Pedigrees of families harbouring each of the variants. Individuals with a variant are identified by m/+ and individuals negative for the variant are indicated with +/+. B. The three identified missense variants occur at highly conserved sites within the HCN2 protein.

Figure 2. Electrophysiological characterization of the p.S632W and p.V246M HCN2 GGE variants. A. Examples of HCN2-mediated currents recorded from oocytes expressing reference wild-type (WT), p.S632W and p.V246M. B. Average current-voltage (I-V) relationship of water injected negative control (n = 6), WT (n = 15), p.S632W (n=15) and p.V246M (n = 18). C. Activation curve constructed from average normalized tail currents and fit with the Boltzmann equation. D. Bar graph of the average half activation voltage ($V_{1/2}$) for each situation. **** $p < 0.0001$ using one-way ANOVA with Dunnett's test. E. Slope factor derived from the Boltzmann fit in each situation. * $p < 0.05$ using one-way ANOVA with Dunnett's test. F. Time to half maximal activation at voltages between -120 and -80 mV. # $p < 0.05$ for p.V246M, * $p < 0.05$ for p.S632W using two-way ANOVA with multiple comparisons.

A

Reference WT

p.S632W

p.V246M

B

mV

-110 -90 -70 -50 -30

I Steady-state (μ A)

0 -1 -2 -3

— H₂O injected

— WT

— p.V246M

— p.S632W

C

g/g_{max}

1.0 0.5 0.0

-110 -75 -40

mV

D

p.S632W

p.V246M

WT

-80 -60 -40

mV

E

Slope (k)

8 6 4 2 0

WT p.V246M p.S632W

F

Time to half peak (s)

3 2 1 0

-120 -100 -80

mV

#

#

#

Figure 3. Electrophysiological characterization of HCN2 population variants. A. Examples of HCN2-mediated currents recorded from oocytes expressing reference wild-type (WT), p.E280K and p.A705T. B. Average current-voltage (I-V) relationship of WT (n = 15), p.E280K (n=17) and p.A705T (n = 11). C. Activation curve constructed from average normalized tail currents and fit with the Boltzmann equation. D. Bar graph of the average half activation voltage ($V_{1/2}$) for each situation. E. Slope factor derived from the Boltzmann fit in each situation. F. Time to half maximal activation at voltages between -120 and -80 mV.

Figure 3

Figure 4. Cartoon of the HCN2 channel illustrating the position of epilepsy-associated amino acid variations that have been previously studied as well as those studied here. The two population variants used as controls in this study are also included.

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Figure 2

Figure 3

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Author/s:

Li, M;Maljevic, S;Phillips, AM;Petrovski, S;Hildebrand, MS;Burgess, R;Mount, T;Zara, F;Striano, P;Schubert, J;Thiele, H;Nuernberg, P;Wong, M;Weisenberg, JL;Thio, LL;Lerche, H;Scheffer, IE;Berkovic, SF;Petrou, S;Reid, CA

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