Functional variants in HCN4 and CACNA1H may contribute to genetic generalized epilepsy

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SUMMARY

Objective: Genetic generalized epilepsy (GGE) encompasses seizure disorders characterized by spike-and-wave discharges (SWD) originating within thalamo-cortical circuits. Hyperpolarization-activated (HCN) and T-type Ca\(^+\) channels are key modulators of rhythmic activity in these brain regions. Here, we screened HCN4 and CACNA1H genes for potentially contributory variants and provide their functional analysis.

Methods: Targeted gene sequencing was performed in 20 unrelated familial cases with different subtypes of GGE, and the results confirmed in 230 ethnically matching controls. Selected variants in CACNA1H and HCN4 were functionally assessed in tsA201 cells and Xenopus laevis oocytes, respectively.

Results: We discovered a novel CACNA1H (p.G1158S) variant in two affected members of a single family. One of them also carried an HCN4 (p.P1117L) variant inherited from the unaffected mother. In a separate family, an HCN4 variant (p.E153G) was identified in one of several affected members. Voltage-clamp analysis of CACNA1H (p.G1158S) revealed a small but significant gain-of-function, including increased current density and a depolarizing shift of steady-state inactivation. HCN4 p.P1117L and p.G153E both caused a hyperpolarizing shift in activation and reduced current amplitudes, resulting in a loss-of-function.

Significance: Our results are consistent with a model suggesting cumulative contributions of subtle functional variations in ion channels to seizure susceptibility and GGE.

KEY WORDS: HCN4, T-type Ca\(^+\) channels, Thalamo-cortical circuits, Generalized epilepsy.

Epilepsy is a prevalent neurological disorder with a lifetime incidence of up to 3%.\(^1\) The most common inherited form of epilepsy is genetic generalized epilepsy (GGE), which encompasses four major subtypes: childhood and juvenile absence epilepsy (CAE/JAE), juvenile myoclonic epilepsy (JME), and epilepsy with generalized tonic-clonic

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Four HCN channel subtypes, HCN1–HCN4 are encoded by HCN1, HCN2, HCN3, and HCN4, respectively. Several epilepsy-associated variants in HCN genes have been recently described. Di Francesco et al. reported a homozygous HCN2 mutation p.E515K in a patient with sporadic GGE, and a deletion (719–721ΔP) and p.S126L mutation in HCN2 have been associated with febrile seizure syndromes. Nava and colleagues detected several de novo HCN1 mutations in patients with early-onset epileptic encephalopathy.

In this study, we embarked on the genetic analysis of the GGE-associated CACNA1H gene as well as the HCN4 gene, which are among the channels expressed at high levels in the thalamus. The sequencing was done in 20 independent patients with GGE core phenotypes, including patients from the GEFS+ (genetic epilepsy with febrile seizures) spectrum. We also provide functional analysis of the detected variants to assess their potential impact on the biophysical properties of affected channels and understand how they may contribute to seizure genesis in these GGE families.

Materials and Methods

Clinical and genetic analysis

Twenty patients with core GGE phenotypes, including some with febrile seizures, were recruited. All patients and relatives or their legal representatives gave written informed consent to participate in this study. Ethical approval was obtained from the responsible local authorities. The clinical information for the two families in which the mutations have been detected is presented in the Appendix S1.

Genomic DNA was extracted from peripheral blood leukocytes using a salting-out method. Polymerase chain reaction (PCR) was performed with 50-ng genomic DNA, 10 pmol of each primer, 200 μM dNTP, 50 mM Tris-HCl, 15 mM ammoniumsulfate, 2.5 mM MgCl₂, 5% dimethyl sulfoxide (DMSO), 0.75 U AccuTaq Polymerase (Sigma-Aldrich) in a total volume of 25 μl. Primers were designed to amplify the entire coding region and adjacent intron sequences of the candidate genes. PCR was performed in an MJ Research thermocycler with the following conditions: 35 cycles of denaturation at 95°C for 30 s, annealing temperatures ranging from 62°C to 90°C for 30 s, and extension at 68°C degree for 90 s. The amplicons were purified and subsequently sequenced.

Key Points

- HCN4 and CACNA1H are highly expressed in the thalamo-cortical loop involved in generation of generalized seizures
- Both genes were sequenced in a cohort of patients with generalized epilepsy
- Functional analysis of one CACNA1H revealed gain-of-function, and two HCN4 variants showed an overall loss-of-function
- These subtle but distinctive functional changes may contribute to seizure susceptibility in the affected individuals

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For all missense variants, we assessed genotype and allele frequencies in a total of 230 ethnically matched controls using specific restriction digestion assays. All available members of the families were genotyped for the cosegregation analysis.

**Functional analysis**

Full-length cDNA encoding the HCN4 channel (NM_005477) was derived from a human cDNA library (Life Technologies) and subcloned into the pGEMHE vector for in vitro cRNA transcription. Cloning of the human Cav3.2 has been previously described.\(^{32}\) Site-directed mutagenesis in HCN4 and Cav3.2 was performed using overlap PCR strategy or Quick Change kit (Stratagene). In vitro synthesis of HCN4 wild type (WT) and mutant cRNAs was performed using the mMessage mMachine T7 transcription kit (Ambion).

**Patch clamp recordings**

For transient expression of Cav3.2, we used tsA201 cells grown in DMEM/F-12 (Life Technologies) supplemented with 10% fetal bovine serum. Cells were transfected with either WT or mutant channel using the calcium phosphate method. Cotransfection with CD8 marker plasmid was used to control for transfection efficiency and selection of cells for electrophysiological analysis.

Whole cell patch-clamp recordings were performed at room temperature using Axopatch 200 A amplifier. Data were sampled at 10 kHz and filtered at 3–5 kHz. Whole cell Ca\(^{2+}\) currents were recorded in extracellular solution containing (in mM): 10 CaCl\(_2\), 10 HEPES, 6 CsCl, 140 TEA-Cl; pH was adjusted to 7.4 with CsOH. The intracellular solution (in mM): 1 MgCl\(_2\), 10 HEPES, 10 EGTA, 135 CsCl, with pH adjusted to 7.4 using CsOH. Patch pipettes were pulled from borosilicate glass (DMZ-Universal Puller) and had a resistance of 2–5 M\(\Omega\) when filled with intracellular solution. Series resistance was compensated to 65–80%, resulting in maximal residual voltage error below 5 mV during measurements.

**HCN4 two-electrode voltage clamp recordings**

Oocytes from *Xenopus laevis* were prepared as previously described.\(^{28}\) Briefly, 50 nl of cRNA-encoding HCN4 subunit (12.5 ng/µl; concentration confirmed spectrophotometrically and by gel analysis) was injected into stage 5/6 oocytes using the Roboocyte (Multi Channel Systems) and incubated for 2 days at 15°C prior to experimentation. Oocytes were perfused with a bath solution containing (in mM): 96 KCl, 2 NaCl, 2 MgCl\(_2\), and 10 HEPES (pH 7.5 using KOH). For voltage clamp recordings, oocytes were impaled with two glass electrodes containing 1.5 m potassium acetate (I) and 0.5 m KCl (V) and clamped at a holding potential of –30 mV. All experiments were performed at room temperature.

**Data analysis**

Detailed electrophysiological protocols and data analysis are presented in the Appendix S2. Data are presented as mean ± SEM. Statistical differences were obtained using unpaired t test with post hoc test for multiple comparisons or one-way ANOVA (Prism 6, GraphPad Prism Software, La Jolla, CA, U.S.A.).

**Results**

**New variants detected in GGE**

A systematic search for variants in CACNA1H and HCN4 genes was performed in a sample of 20 unrelated patients with GGE. Seizure subtypes represented among the patients included CAE, JAE, JME, and epilepsy with generalized tonic-clonic seizures (EGTCS). Detected nonsynonymous missense variations are presented in Table 1. Out of the 13 variants detected in CACNA1H, one novel variant, p.G1158S, appeared only in two GGE patients of Family 1 (Figs 1A, S1A) and not in 230 controls. This variant was also found in another sample of 80 GGE cases, in a patient with EGTCS phenotype and is present at low frequency (1/13,148 alleles) in the ExAC database.\(^{33}\) Furthermore, of the five newly detected HCN4 gene variants in our sample, two were not detected in the 230 tested controls, but one (p.P1117L) appeared in ExAC in 63/19,948 alleles (Table 1). The p.P1117L variant was detected in one of the two affected members of Family 1 carrying the CACNA1H p.G1158S variant and was inherited from the unaffected mother (Figs 1A, S1A). The second variant, p.E153G, was identified in only one of several affected members of Family 2 (Figs 1B, S1B). Alignments of sequences among different species revealed high amino acid conservation at positions 1158 and 153 within the Cav3.2 and HCN4 protein, respectively (Fig. 2A). Predicted localization of these variants within the affected channel proteins is shown in Fig. 2B.

In Family 1, the CACNA1H p.G1158S variant is inherited by both affected children from their father, who experienced syncope of unknown etiology. The child carrying only the CACNA1H mutation had generalized tonic-clonic seizures on awakening (GTCA) and absence seizures from 12 years of age. His sister, carrying both the CACNA1H and the HCN4 variant, experienced GTCA starting from 26 years of age. The clinical phenotype in Family 2 included febrile, generalized tonic-clonic, and absence seizures, representative of GEFS*. The HCN4 mutation carrier had only one seizure at the age of 17. There were no cardiac pathologies or arrhythmias reported for the patient with syncope or any of the carriers of HCN4 variants.

**CACNA1H variant causes a gain-of-function**

Whole cell patch-clamp recordings in tsA201 cells revealed that, compared to the WT Cav3.2, the p.G1158S channels showed a significant increase in the current density
at more depolarized potentials (Figs. 3A,B). Whereas no significant changes were found in the voltage dependence of activation, the steady-state inactivation curve of the mutant was shifted by +5 mV to more depolarized potentials in the presence of Ca2+ as charge carrier (Figs. 3A–C).

There were no significant differences in the recovery from inactivation (Fig. 3D), or activation, inactivation, and deactivation kinetics between mutant and WT channels (Table S1). Thus, the p.G1158S mutation causes a small but significant gain-of-function.

HCN4 variants result in a loss-of-function

Robust currents could be recorded in X. laevis oocytes expressing WT channels and the p.E153G and p.P1117L epilepsy variants (Fig. 4A). Current amplitudes were not significantly different between the variants and the WT channel (Fig. 4B). However, conductance-voltage relationship revealed a left shift in activation for both the p.E153G and p.P117L relative to WT, consistent with a loss-of-function (Fig. 4C). Boltzmann functions fit to the data points confirm this, revealing a significant shift in $V_{1/2}$ for both GGE variants (Fig. 4C inset). Wild type and the p.E153G variant had a similar slope (WT $= 7.3 \pm 0.2$, n = 21 vs. p.E153G $= 7.5 \pm 0.2$, n = 19; $p = 0.56$), but the slope of the p.P1117L variant curve was significantly different from that of the WT ($= 7.3 \pm 0.2$, n = 21 vs. p.P1117L $= 8.1 \pm 0.2$, n = 30, $p = 0.02$). Time to half maximal activation of the two epilepsy variants was not different from the WT across a range of voltages (Fig. S2).

**Discussion**

We report here three functionally relevant novel variants in CACNA1H and HCN4 that were found in four individuals with GGE, one of them carrying one variant in both genes. As expected from previous studies in mouse and

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**Table 1. Overview of CACNA1H and HCN4 gene variants detected in this study, with highlighted analyzed variants**

| Nr. | Exon | Nucleotide substitution | Amino acid substitution | Patients$^{\text{a}}$ wt:mut/wt:mut | Controls$^{\text{a}}$ wt:mut/wt:mut | ExAC$^{\text{b}}$ |
|-----|------|------------------------|-------------------------|----------------------------------------|----------------------------------------|----------------|
| CACNA1H | 1 | 4 | c.450G>T | p.E150D | 97:3:0 | 99:1:0 | No |
| | 2 | 7 | c.937A>G | p.M313V$^{48}$ | 70:28:2 | 61:30:9 | 14726/90236 homozyg |
| | 3 | 9 | c.1919C>T | p.P640L$^{21}$ | 45:49:6 | 47:36:17 | 10391/23896 homozyg |
| | 4 | 9 | c.1991T>C | p.V664A$^{21}$ | 50:40:10 | 49:44:7 | 1322/5608 homozyg |
| | 5 | 10 | c.2362C>T | p.R788C$^{21}$ | 85:15:0 | 83:12:5 | 9548/107658 homozyg |
| | 6 | 17 | c.3472G>A | p.G1158S | 98:2:0 | 230:0:0 | 1/13148 |
| | 7 | 33 | c.5612G>A | p.R1871Q$^{37}$ | 78:21:1 | 88:11:1 | 2525/25780 |
| | 8 | 34 | c.5897C>T | p.A1966V$^{37}$ | 94:6:0 | 96:4:0 | 380/17716 |
| | 9 | 34 | c.5921A>G | p.E1974G$^{37}$ | 96:4:0 | 97:3:0 | 74/18188 |
| | 10 | 34 | c.6013C>T | p.R2005C$^{21}$ | 76:23:1 | 86:13:1 | 1358/14960 |
| | 11 | 35 | c.6179G>A | p.R2060H$^{21}$ | 77:21:2 | 73:25:2 | 74/18188 |
| | 12 | 35 | c.6230G>A | p.R2077H$^{21}$ | 25:51:24 | 17:46:37 | 63097/95200 |
| | 13 | 35 | c.6322G>A | p.A2108T | 98:2:0 | 99:1:0 | 141/96230 |
| HCN4 | 1 | 1 | c.107G>A | p.G36E | 17:3:0 | 88:12:0 | 848/13832 |
| | 2 | 1 | c.458A>G | p.E153G | 19:1:0 | 250:0:0 | No |
| | 3 | 8 | c.2648C>G | p.P883R | 19:1:0 | 95:5:0 | 892/101748 |
| | 4 | 8 | c.3337A>G | p.M1113V | 19:1:0 | 97:3:0 | 467/18868 |
| | 5 | 8 | c.3350C>T | p.P1117L | 19:1:0 | 250:0:0 | 63/19948 |

$^a$Analyzed variants are highlighted in bold type.

$^b$Ratio of individuals carrying both WT, WT and mutant, and both mutant alleles.
humans and discussed further below, the \textit{CACNA1H} variant caused a gain-of-function, whereas both \textit{HCN4} variants caused a loss-of-function. The effects of variants were small, and they were found in only a single affected individual (\textit{HCN4} variants) or in a small family with only one affected sib-pair (\textit{CACNA1H} variant). Our results are consistent with the hypothesis of a polygenic disease model in which multiple variants that cause small-to-moderate...
effects on protein function cumulatively contribute to epileptogenesis rather than any particular variant being disease causing in isolation. We, therefore, suggest that both CACNA1H and HCN4 may act as susceptibility genes in GGE, in terms of additive contributions of subtle functional variations to overall seizure susceptibility.

The genetic architecture of GGE is yet to be fully explained. Although microdeletions present the most common genetic alteration predisposing to GGE,10,13 large-scale genetic efforts are only beginning to better resolve the contribution of common and rare variants to GGE.9,10,12,15 We acknowledge that small families tested here are insufficient to provide statistically valid genetic evidence and that the variants may have been discovered by chance alone. Once fully validated statistical models of GGE are developed, the implications of our results will become clearer.

Initial reports have described numerous CACNA1H variants associated with GGE,19–22,34 particularly CAE. CAE is also a feature of the family harboring the CACNA1H p.G1158S mutation. However, recent large-scale studies of common and rare variants in GGE have not revealed a major impact of CACNA1H variants,9,12,15 and de novo mutations in this gene have been linked to early-onset hypertension and primary aldosteronism.35,36 This has prompted the reassessment of the role of CACNA1H in epilepsy. In this regard, our retrospective analysis of 19 previously identified CACNA1H variants found in patients and families presenting with different GGE forms37 revealed the presence of all but two variants in the ExAC database. The allele frequency varied between 10/1000 and 10%, with the majority of variants (14/19) having frequency of <1%. No distinctive correlation between allele frequency and reported functional effects could be established.

The functional impact of variants in CACNA1H associated with GGE is variable, although a net gain-of-function, as found here, is generally considered to be the basis of increased excitability as observed in primary neuronal cultures recombinantly expressing a CACNA1H epilepsy-associated variant.35 This is consistent with pharmacological and physiological data implicating T-type Ca2+ channels in GGE: (1) the first-line anti-absence drug ethosuximide

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**Figure 3.**
Functional analysis of the CACNA1H variant in tsA201 cells. (A) Whole cell currents of WT Cav3.2 and G1158S channels elicited by depolarizing the membrane between −70 mV and 70 mV in 5-mV steps from a holding potential of −90 mV. (B) Current density for the Cav3.2 WT and G1158S mutant channels at different potentials. (C) Steady-state activation and inactivation for Cav3.2 WT and G1158S mutant channels obtained by standard protocols using a holding potential of −90 mV. Recordings were performed with Ca2+ as the charge carrier. Parameters for activation were as follows: WT − V0.5 = −41.4 ± 0.8 mV, k = −6.7 ± 0.3 mV (n = 13); G1158S − V0.5 = −40.8 ± 0.8 mV, k = −1.3 ± 0.3 (n = 10). For the inactivation (inset), parameters were: WT − V0.5 = −69.2 ± 1.1 mV, k = 4.3 ± 0.2 mV (n = 11); G1158S − V0.5 = −64.1 ± 1.1 mV, k = −4.4 ± 0.1 (n = 7), **p < 0.01, unpaired t test. (D) Recovery from inactivation for Cav3.2 WT and G1158S mutant channels. Curves represent monoexponential fits to the averaged data (n = 4–12). Inset: mean time constant for recovery from inactivation for Cav3.2 WT and G1158S mutant, obtained from monoexponential fits to the normalized current recovery curve (n = 4–12, n.s. = not significant).
blocks T-type Ca$^{2+}$ channels, suggesting that increased channel function leads to hyperexcitability; and (2) physiological experiments in rat models of GGE report increased T-type Ca$^{2+}$ currents in reticular thalamic neurons. Our results are therefore consistent with the idea that gain-of-function $CACNA1H$ variants may contribute to neuronal hyperexcitability in GGE. Taken together, these data corroborate that $CACNA1H$ is not a gene of major effect in GGE, but its variants might act as susceptibility factors contributing to epilepsy phenotypes.

Several studies have implicated HCN channels in epilepsy, but only limited evidence links HCN4 channels to increased neuronal network excitability. A seizure-associated up-regulation of HCN4 expression occurs in the rat pilocarpine model of temporal lobe epilepsy. Furthermore, Paz and colleagues have shown in a cortical stroke model that thalamo-cortical neurons switch from a predominant HCN2 to a predominant HCN4 channel expression. Whether these changes in HCN4 expression are part of the pathogenic mechanism is not clear. Here we describe two HCN4 variants that cause a hyperpolarizing shift in the voltage dependence of activation resulting in reduced function within a physiologically relevant voltage range. Investigations in the HCN2 knockout mouse, which displays SWDs, reveal that thalamo-cortical neurons are more hyperpolarized because of a reduction in $I_h$. The more-hyperpolarized resting membrane potential increases the availability of T-type Ca$^{2+}$ channels and subsequent burst firing, a hallmark of SWDs. Future experiments are required to determine whether HCN4 channels can contribute to excitability in a similar manner.

Unraveling the genetic architecture of GGE is possibly complicated by its polygenic nature. Computational studies have suggested that multiple small changes in the biophysical properties of ion channels that alone cannot alter network excitability can in concert cause aberrant network hyperexcitability. We assume that all variants studied here are contributing factors to GGE in individual patients and that even in larger families each affected member may present with a different combination of genetic alterations, which in sum could cause the disease.

Genetic alterations in $HCN4$ have been linked to cardiac disease. In all reported cases, genetic variants associated with dysrhythmia cause a loss-of-function. Our data demonstrate for both $HCN4$ variants a subtle hyperpolarizing shift in activation, resulting in loss-of-function, but...
cardiac disease was not observed in either family. The subtle effect of mutations on HCN4 function may not be sufficient to cause dysrhythmia in these families. In addition, the fact that epilepsy was not reported in patients with cardiac dysrhythmias\(^27\) suggests that HCN4 variant alone is unlikely to cause epilepsy, but rather presents a modifier.

In conclusion, we identified and characterized variants in CACNA1H and HCN4 as putative susceptibility factors in GGE. Alterations in these two genes may work in concert to increase neuronal network excitability in thalamo-cortical networks and contribute to seizure susceptibility in the affected individuals.

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**Disclosure**

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

**REFERENCES**

1. Hauser WA, Annegers JF, Rocca WA. Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota. *Mayo Clin Proc* 1996;71:576–586.
2. Lerche H, Shah M, Beck H, et al. Ion channels in genetic and acquired forms of epilepsy. *J Physiol (Lond)* 2013;591:753–764.
3. Helbig I, Scheffer IE, Mulley JC, et al. Navigating the channels and beyond: unravelling the genetics of the epilepsies. *Lancet Neurol* 2008;7:231–245.
4. Baulac S, Huberfeld G, Gourfinkel-An I, et al. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nat Genet* 2001;28:46–48.
5. Wallace RH, Marini C, Petrou S, et al. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet* 2001;28:49–52.
6. Kanamura C, Haug K, Sander T, et al. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch Neurol* 2002;59:1137–1141.
7. Cossette P, Liu L, Brisebois K, et al. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet* 2002;31:184–189.
8. Maljevic S, Krampl K, Cobilasachi J, et al. A mutation in the GABA (A) receptor alpha1-subunit is associated with absence epilepsy. *Ann Neurol* 2006;59:983–987.
9. EPICURE Consortium, EMINet Consortium, Steffens M, et al. Genome-wide association analysis of genetic generalized epilepsies implicates susceptibility loci at 1q43, 2p16.1, 2q22.3 and 17q21.32. *Hum Mol Genet* 2012;21:5359–5372.
10. Leu C, Coppola A, Sisodiya SM. Progress from genome-wide association studies and copy number variant studies in epilepsy. *Curr Opin Neurol* 2016;29:158–167.
11. Helbig I, Hartmann C, Mefford HC. The unexpected role of copy number variations in juvenile myoclonic epilepsy. *Epilepsy Behav* 2013;28 (Suppl 1):S66–S68.
12. Epi4K Consortium, Epilepsy Phenome/Genome Project. Ultra-rare genetic variation in common epilepsies: a case-control sequencing study. *Lancet Neurol* 2017;16:135–143.
13. Helbig I, Mefford HC, Sharp AJ, et al. 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nat Genet* 2009;41:160–162.
14. De Kovel CGF, Trucks H, Helbig I, et al. Recurrent microdeletions at 15q11.2 and 16p11.1 predispose to idiopathic generalized epilepsies. *Brain* 2010;133:23–32.
15. International League Against Epilepsy Consortium on Complex Epilepsies. Genetic determinants of common epilepsies: a meta-analysis of genome-wide association studies. *Lancet Neurol* 2014;13:893–903.
16. McCormick DA, Contreras D. On the cellular and network bases of epileptic seizures. *Annu Rev Physiol* 2001;63:815–846.
17. Reid CA, Phillips AM, Petrou S. HCN channelopathies: pathophysiology in genetic epilepsy and therapeutic implications. *Br J Pharmacol* 2012;165:49–56.
18. Zamponi GW, Lory P, Perez-Reyes E. Role of voltage-gated calcium channels in epilepsy. *Pflugers Arch* 2010;460:393–403.
19. Chen Y, Lu J, Pan H, et al. Association between genetic variation of CACNA1H and childhood absence epilepsy. *Ann Neurol* 2003;54:239–243.
20. Heron SE, Phillips HA, Mulley JC, et al. Genetic variation of CACNA1H in idiopathic generalized epilepsy. *Ann Neurol* 2004;55:595–596.
21. Klink C, Davis C, Goldman A, et al. Exome sequencing of ion channel genes reveals complex profiles confounding personal risk assessment in epilepsy. *Cell* 2011;145:1036–1048.
22. Vitko I, Bidaud I, Arias JM, et al. The I-I loop controls plasma membrane expression and gating of Ca(v)3.2 T-type Ca\(^{2+}\) channels: a paradigm for childhood absence epilepsy mutations. *J Neurosci* 2007;27:322–330.
23. Singh B, Monteil A, Bidaud I, et al. Mutational analysis of CACNA1G in idiopathic generalized epilepsy. Mutation in brief #962. Online. *Hum Mutat* 2007;28:524–525.
24. Wang J, Zhang Y, Liang J, et al. CACNA11 is not associated with childhood absence epilepsy in the Chinese Han population. *Pediatr Neurol* 2006;35:187–190.
25. Heinein EL, Depondt C, Cavalleri GL, et al. Exome sequencing followed by large-scale genotyping fails to identify single rare variants of large effect in idiopathic generalized epilepsy. *Am J Hum Genet* 2012;91:293–302.
26. DiFrancesco JC, Barbuti A, Milanesi R, et al. Recessive loss-of-function mutation in the pacemaker HCN2 channel causing increased neuronal excitability in a patient with idiopathic generalized epilepsy. *J Neurosci* 2011;31:17327–17337.
27. Nakamura Y, Shi X, Numata T, et al. Novel HCN2 mutation contributes to febrile seizures by shifting the channel’s kinetics in a temperature-dependent manner. *PLoS One* 2013;8:e80376.
28. Dibbens LM, Reid CA, Hodgson B, et al. Augmented currents of an HCN2 variant in patients with febrile seizure syndromes. *Ann Neurol* 2010;67:542–546.
29. Nava C, Dalle C, Rastetter A, et al. De novo mutations in HCN1 cause early infantile epileptic encephalopathy. *Nat Genet* 2014;46:640–645.
30. Biel M, Wahl-Schott C, Michalak S, et al. Hyperpolarization-activated cation channels: from genes to function. *Physiol Rev* 2009;89:847–885.
31. Camfield P, Camfield C. Febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+). *Epileptic Disord* 2015;17:124–133.
32. Cribbs LL, Lee JH, Yang J, et al. Cloning and characterization of alpha1H from human heart, a member of the T-type Ca\(^{2+}\) channel gene family. *Circ Res* 1998;83:103–109.
33. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–291.
34. Powell KL, Cain SM, Ng C, et al. A Cav3.2 T-type calcium channel pore mutation has splice-variant-specific effects on function and segregates with seizure expression in a polygenic rat model of absence epilepsy. *J Neurosci* 2009;29:371–380.
35. Daniil G, Fernandes-Rosa FL, Chemin J, et al. CACNA1H mutations are associated with different forms of primary aldosteronism. *EBioMedicine* 2016;13:225–236.

36. Scholl U, Stöltzing G, Nelson-Williams C, et al. Recurrent gain of function mutation in calcium channel CACNA1H causes early-onset hypertension with primary aldosteronism. *Elife* 2015;4:e06315.

37. Heron SE, Khosravani H, Varela D, et al. Extended spectrum of idiopathic generalized epilepsies associated with CACNA1H functional variants. *Ann Neurol* 2007;62:560–568.

38. Eckle V-S, Shcheglovitov A, Vitko I, et al. Mechanisms by which a CACNA1H mutation in epilepsy patients increases seizure susceptibility. *J Physiol (Lond)* 2014;592:795–809.

39. Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol* 1989;25:582–593.

40. Tsakiridou E, Bertollini L, de Curtis M, et al. Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. *J Neurosci* 1995;15:3110–3117.

41. Ernst WL, Zhang Y, Yoo JW, et al. Genetic enhancement of thalamocortical network activity by elevating alpha 1 g-mediated low-voltage-activated calcium current induces pure absence epilepsy. *J Neurosci* 2009;29:1615–1625.

42. Surges R, Kukley M, Brewster A, et al. Hyperpolarization-activated cation current Ih of dentate gyrus granule cells is upregulated in human and rat temporal lobe epilepsy. *Biochem Biophys Res Commun* 2012;420:156–160.

43. Paz JT, Davidson TJ, Frechette ES, et al. Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. *Nat Neurosci* 2013;16:64–70.

44. Ludwig A, Budde T, Stieber J, et al. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *EMBO J* 2003;22:216–224.

45. Crunelli V, Leresche N. Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci* 2002;3:371–382.

46. Thomas EA, Reid CA, Berkovic SF, et al. Prediction by modeling that epilepsy may be caused by very small functional changes in ion channels. *Arch Neurol* 2009;66:1225–1232.

47. DiFrancesco D. Funny channel gene mutations associated with arrhythmias. *J Physiol (Lond)* 2013;591:4117–4124.

48. Liang J, Zhang Y, Wang J, et al. New variants in the CACNA1H gene identified in childhood absence epilepsy. *Neurosci Lett* 2006;406:27–32.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Clinical picture.

**Appendix S2.** Supplementary Methods.

**Table S1.** Time constants of activation, inactivation, deactivation, and recovery from inactivation at indicated membrane potentials.

**Figure S1.** Chromatograms presenting the Sanger sequencing results for the affected genes in Family 1 (A) and Family 2 (B).

**Figure S2.** Time to half maximal activation for WT and the two *HCN4* epilepsy variant channels across a range of voltages.
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