Supplementary Methods:

Sanger Sequencing: Primers (available on request) were designed using Primer 3 (http://fokker.wi.mit.edu/primer3/input.html) based on NM_020822 from Ensembl (http://www.ensembl.org/index.html) to cover all 10 protein-coding transcripts. The exons were amplified by PCR using BioMix RedTM (Bioline Ltd). For some amplicons, GC rich solution of FastStart Taq DNA Polymerase (Roche Diagnostic) was added. PCR products were purified using MicroCLEAN (Web Scientific) and then directly sequenced by the BigDye Terminator Cycle Sequencing System (Applied Biosystems Inc., Life Technologies Corporation). Sequencing reactions were run on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems Inc) and analysed with Chromas software http://www.technelysium.com.au/chromas.html).

Next generation sequencing panel:

Custom TSCA method: SureSelect library was created using Illumina's Design Studio tool (http://designstudio.illumina.com/truseqca/project). Briefly, genomic coordinates of 48 genes were uploaded to Design Studio to create the targeted panel. Libraries were made following the TruSeq Custom Amplicon Kit protocol (Illumina Technologies) and sequenced in-house on an Illumina MiSeq.

Sure Select method: a custom SureSelect library was created using Agilent's SureDesign tool (https://earray.chem.agilent.com/suredesign/). Briefly, genomic coordinates of either 45 (3 patients) or 66 (1 patient) genes were uploaded to SureDesign to create the targeted panel. Libraries were made following the SureSelectXT Custom Capture protocol (Agilent Technologies) and sequenced in-house on an Illumina MiSeq. For both panel designs we analysed sequence data using an in-house pipeline. Regions of interest were defined in BED file format by uploading HGNC genes names to the UCSC table browser.[1] Sequence reads in FASTQ format were aligned to the reference human genome (hg19) using BWA (0.6.1-r104) and default settings.[2] Variant calling was performed across the entire region of interest using VarScan2 (v2.3.7) with the following settings: minimum 30X coverage, minimum 5 alternate reads, minimum phred-like base-quality of 20).[3,4] Variant calls in VCF format were then annotated using Ensembl Variant Effect Predictor (v73) and the output parsed using an in-house script, converting the annotated VCF file into Excel format for subsequent variant filtering and prioritization.[5] For each case, coverage was assessed across the coding exons of the target genes and their intron-exon boundaries (+6bp and -12bp) and expressed as the percentage of bases covered at ≥30X

Whole exome sequencing: DNA was sheared for paired-end Illumina library preparation then enriched for target capture according to the manufacturer's instructions. Enriched libraries were sequenced using the HiSeq platform. Variants were called on each sample individually and filtering was applied for quality variables. Calls were merged, then annotated with allele frequencies from 1000 Genomes and Exome Variant Server (EVS) and with dbSNP132 unique rs identification

numbers, if available. SIFT and PolyPhen scores for missense mutations were also included. All variants with a mean allele frequency of >0.01 in 1000 Genomes or EVS were excluded. A candidate gene approach was then taken to interrogate the filtered data for mutations in previously reported early onset epilepsy genes (**Table e-1**).

Diagnostic Chromosomal Microarray:

Where possible, microarray studies were performed by diagnostic laboratories, according to locally established protocols. Putative copy number variants (CNV) were further investigated by testing parental samples to determine whether the CNV was inherited or occurred *de novo.* For historical cases where microarray was not available, chromosomal karyotype results have been included (Table e-1).

Homology Modelling:

We modelled F346L (in the ion channel domain) and F502V in the gating ring containing RCK1 and RCK2 domains. HHPred[6] was used for template-based structure prediction for the individual domains

Modelling of F346L: For F346L, the final homology model of the four subunit ion-channel domain (in open conformation) was of reasonable quality with QMEAN Z-score of -3.97. The spatial position of the membrane is predicted using the PPM2.0 server.[7] The potassium-bound structure (open form) of calcium-gated potassium channel MthK from *Methanothermobacter thermaautotrophicus* (PDB ID: 1LNQ)[8] was used as a template for modelling the ion channel domain (residues 270-353). This template structure was identified upon sensitive sequence-based profile-profile comparison method-HHPred.[6] The probability score (significance of the alignment) of 99% and 50% sequence similarity asserted the homologous relationship between the selected template and human KCNT1 ion channel domain. Further to model the tetramer of the ion channel domain, the biological assembly from the Protein Data Bank (PDB: 3LDC)[9] was used. We also modeled the closed form of the ion-channel domain using the structure of potassium channel from *Streptomyces lividans* (PDBID: 2A9H), which shares 20% identity (31% similarity) with the kcnt1 ion-channel domain.[10]

Modelling of mutation F502V: F502V is a conserved residue among homologs (CONSURF).[11] The residue stretch (372-1044) which lies in the KCNT1 gating region (373-1174) was modelled using an almost identical (95%) recent cryo-EM structure of Slo2.2 channel from *Gallus gallus* in a closed form (without calcium) (PDB ID: 5A6F).[12] This appeared to be a suitable model (QMEAN aZ-Score = -1.82). Human KCNT1 gating region and the cryo-EM model share a sequence identity of ~95%. A template dimer was built from the cryoEM model by fitting a second copy (5A6F) in the EM density map (EMD: 3063), using the *fit_in_map* tool in Chimera[13] based on which a model of the dimer of KCNT1 gating ring was generated.

MODELLER-9v14[14] was used for homology modelling. For the modelling of the region possessing ion channel domain (residues 270-353), 20 models were built and assessed using DOPE (Discrete Optimized Protein Energy) score.[15] The best scoring model was then selected and examined for its quality using QMEAN.[16] The side chain conformations for the selected model were further refined using a method based on backbone-dependent rotamer library – SCWRL4.[17] The model of the gating region (residues 372-1044) was almost identical to the EM model except only seven and six residue substitutions in RCK1 and RCK2, respectively. This model has coordinates for both RCK1 and RCK2 domains but is missing a 163 amino acid linker (residues 633-796). A dimer model was generated by structure superimposition of individual models on the template dimer. This model was further subjected to 1000 steps (steepest descent) of energy minimization to reduce steric clashes and optimize the positioning of side chains. Mutations were inserted into the respective models (F502V in gating region and F346L in ion channel) using the *swapaa* command in Chimera[13] with Dunbrack rotamer library and the rotamer was selected based on lowest clashes, highest number of H-bonds and highest probability.

Electrophysiology:

Oocytes (Dumont stage V or VI) were surgically removed from *Xenopus laevis* frogs and prepared.[18] Oocytes were kept in ND96 solution and stored at 17°C. Fifty nanoliters (0.2 ng/nl) of capped cRNA was injected into each oocyte using the Roboocyte system (Multi Channel Systems, Reutlingen, Germany) and oocytes incubated at 17°C.

Electrodes containing1.5M K-acetate and 0.5M KCl were used to impale oocytes that were held at −90mV and perfused with bath solution containing 96mM NaCl, 2mM KCl, 1.8mM CaCl2, 1mM MgCl2, and 5mM HEPES, pH 7.5. Recording frequency was 1kHz, and temperature was maintained between 20 and 22°C. To record expressed membrane currents, oocytes were held at −90mV, and test depolarizations lasting for 600-milliseconds were applied in 10mV increments, from −80mV to +80mV, at 5 second intervals. Contemporaneous measurement of wild type (WT) currents was performed with the same batch of oocytes injected with mutant channels to maintain an internal control of possible variability in expression from different batches of oocytes. Peak currents were measured at the end of each sweep for all clones, however in many of the mutants, saturation of the amplifiers were observed at +80mV, due to large currents so current at +10mV was set as a comparison point across all mutants and WT. Representative current traces and current voltage relationship curves were obtained using recordings that did not saturate the amplifier at +80mV. Quinidine (Sigma, St Louis, MO) was dissolved in ethanol to a concentration of 300 µM and was perfused continuously to the oocytes for 1 minute, followed by a 5 minute incubation. Currents were recorded before and after the application of the compound.

AxoGraph (AxoGraph Scientific, Sydney, Australia) was used to analyse the electrophysiological data, which is presented as mean ± standard error of the mean. Student t test was used to test statistical significance, performed on Prism 6 (GraphPad Software, La Jolla, CA).

Supplementary References

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