Supplementary material

Methods

The genomic DNA of the patient was extracted from peripheral leukocytes using QIAsymphony S (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA of the patient was enriched using HaloPlex Target Enrichment System (Agilent Technologies Inc., 2013). Genomic DNA samples were digested with eight pairs of restriction enzymes to create a library of gDNA restriction fragments. Restricted fragments were pooled and hybridized with customized probes hybridizing to exons and 10-bp flanking regions of the selected genes*. Circularized target DNA-HaloPlex probe hybrids, containing biotin, were captured with streptavidin-coated magnetic beads and DNA ligase was added to the capture reaction to close nicks in the circularized probe-target DNA hybrids. Subsequently, we amplified the samples by PCR and purified using AMPure XPbeads (Beckman Coulter, Inc.) and a magnetic plate. Prior to sample pooling, the quality of the library was inspected using Tapestation (Agilent Technologies Inc.). Multiplexed 150-bp paired-end-read exome sequencing was carried out on an Illumina MiSeq (Illumina Inc.) sequencer running on MiSeq Control Software (HCS). Raw data from MiSeq sequencing runs were processed using two software pipelines; SureCall 4.0 (Agilent Technologies Inc) and the Biomedical Genomics Workbench software version 3.5.2 (Qiagen). Sequencing reads were filtered for low-quality reads, trimmed for adapter sequences, and tagged to the patient according to the barcode. Using the spectrum of the expected mutations in the training set, the parameters for variant calling were established to minimize the number of false-positive results and guarantee the characterization of all true-positive calls; the following filter thresholds were considered: minimum allele frequency for single-nucleotide polymorphism (SNP) and indel (SNP% ≥20), phred-like quality score of the called variant (Qcall ≥20) and depth of coverage (Depth ≥20). Using Sanger sequencing, we analyzed the exons classified as uncovered in order to reach the percentage of target region correctly covered; moreover, the new non-synonymous nucleotide variants identified were also confirmed by Sanger sequencing. Variants were classified according to guidelines of Matthijs *et al*. (2016): (1) benign; (2) probably benign; (3) of uncertain meaning (VUS); (4) probably pathogenic; and (5) pathogenic. Variants were interpreted according to the guidelines of Richards *et al*. (2015).

MLPA analysis was carried out using SALSA P197 and P166 probe mix (MRC-Holland) containing probes specific for *KCNQ2* and *KCNQ3* genes, according to the recommendations of the manufactures.

*list of genes: *ALDH7A1* (NM_001182. 2), *PNPO* (NM_018129. 3), *ARHGEF9* (NM_015185. 2), *SLC25A22* (NM1191060. 1), *PLCB1* (NM_015192. 3), *TBC1D24* (NM_001199107. 1), *PNKP* (NM_007254. 3), *KCNT1* (NM_020822. 2), *KCNQ2* (NM_172107. 2), *SCN2A* (NM_021007. 2), *SCN8A* (NM_014191. 3), *STXBP1* (NM_003165. 3), *SCN1A* (NM_001165963. 1), *PCDH19* (NM_001184880. 1), *CDKL5* (NM_003159. 2), *SPTAN1* (NM_001130438. 2), *SLC2A1* (NM_006516. 1), *ST3GAL3* (NM_174963. 3), *GRIN2A* (NM_001134407. 2) *CHD2* (NM_001271. 3), *HCN1* (NM_021072. 3), *SYNGAP1* (NM_006772.2), *SLC35A3* (NM_012243. 2), *KCNQ3* (NM_004519.3)

Matthijs G, Souche E, Alders M, *et al*. Guidelines for diagnostic next-generation sequencing. *Eur J Hum Genet* 2016; 24: 2-5.

Richards S, Aziz N, Bale S, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405-24.

Epileptic Disorders