Supplementary data

Analysis of *SCN1A* (NM_001165963. 1).

Genomic DNA of the patient was extracted from peripheral leukocytes, using QIAsymphony S (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA 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.). A multiplexed 150-bp paired-end-read exome sequencing was carried out on 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 as belonging to the specific 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 the 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 analysed 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. Classification of identified variants was made according to guidelines (Matthijs *et al*.; Guidelines for the diagnosis of next-generation sequencing EJHV v.24 2016): (1) benign, (2) probably benign, (3) of uncertain meaning (VUS), (4) probably pathogenic, and (5) pathogenic. Interpretation of variants was carried out according to the guidelines (Richards *et al*.; Standards and guidelines for the interpretation of sequence variants.) ACMG - Genetics in Medicine 2015).

*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).

Analysis of *ZEB2* (NM_014795.3)

Clinical whole-exome sequencing (WES) was performed for the proband and her unaffected parents. The Nextera Rapid Capture Enrichment kit (Illumina) was used according to the manufacturer's instructions. Libraries were sequenced with an Illumina HiSeq3000 using a 100-bp paired-end protocol. Sequence alignment with the human reference genome (UCSC hg19) and variants call and annotation were performed using an in-house pipeline, as described elsewhere (Mencacci *et al*., 2016). The raw data of single nucleotide variants (SNVs) and indels was then filtered. Only exonic

and donor/acceptor splicing variants were considered. In accordance with the pedigree and phenotype, priority was given to rare variants (<1% in public databases, including the1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium [ExAC v0.2]) that fit a recessive or a *de novo* model and are located within genes previously associated with EOEE. The *de novo ZEB2* variant identified by WES in the proband (c.2264dupA; p.Asp755fs) was confirmed by traditional Sanger sequencing. The detailed conditions for sequencing analysis are available upon request.