Supplementary Methods on Next-generation sequencing techniques

95 gene panel targeted resequencing analysis (Patients 1, 15, 17, 19)

A Haloplex panel (Agilent Technologies, Santa Clara, CA, USA) to capture all coding exons and their flanking intronic sequences (10 base pairs padding) of 95 epilepsy genes (Supplementary Table 3) was performed according to the manufacturer instructions. Libraries containing unique identifiers were pooled in equimolar concentration and sequenced on a MiSeq sequencer using a MiSeq Reagent Kit v3 and a 150 bp paired-end chemistry (Illumina, San Diego, CA, USA). Putative causative variants were validated by Sanger sequencing using a 3500 genetic analyzer (Applied Biosystems, CA, USA) and investigated in the parents of probands to check their inheritance status.) For patients sequence reads were aligned to the NCBI37/hg19 reference genome using a pipeline based on BWA (Li and Durbin, 2009) and Picard (https://broadinstitute.github.io/picard/). Variants were called and annotated using the GATK (McKenna *et al.*, 2010) toolkit and the ANNOVAR tool (Yang and Wang, 2015). Variants in introns and in 5'- and 3'-UTR regions were excluded. Variants reported in the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/) and/or in the 1000 Genomes Project (http://www.1000genomes.org) and/or in the NHLBI Exome Sequencing Project (ESP, ESP6500 database, http://evs.gs.washington.edu/EVS), with a Minor Allele Frequency (MAF) > 0.01 (1%) were dropped out. In silico prediction of the mutation pathogenicity was performed using ANNOVAR and the dbNSFP database (v3.0a) (https://sites.google.com/site/jpopgen/dbNSFP).

Supplementary Table 3

ADGRV1	*GABRA1*	*MTHFR*	*SCN8A*
ALDH7A1	*GABRB3*	*NECAP1*	*SLC13A5*
ARHGEF9	*GABRD*	*NHLRC1*	*SLC19A3*
ARX	*GABRG2*	*NRXN1*	*SLC25A1*
ATP1A2	*GNAO1*	*PC*	*SLC25A12*
ATP1A3	*GRIN2A*	*PCDH19*	*SLC25A15*
CACNA1A	*GRIN2B*	*PDHA1*	*SLC25A20*
CACNA1H	*HCN1*	*PDHB*	*SLC25A22*
CACNB4	*IQSEC2*	*PDP1*	*SLC2A1*
CASR	*KCNA1*	*PIGA*	*SLC35A2*

CDKL5	*KCNA2*	*PIGN*	*SLC35A3*
CHD2	*KCNB1*	*PIGT*	*SLC6A8*
CHRNA2	*KCNC1*	*PLCB1*	*SLC9A6*
CHRNA4	*KCNJ10*	*PNKP*	*SPTAN1*
CHRNB2	*KCNMA1*	*PNPO*	*ST3GAL3*
CLCN2	*KCNQ2*	*POLG*	*STX1B*
CNTNAP2	*KCNQ3*	*PRICKLE1*	*STXBP1*
CSTB	*KCNT1*	*PRICKLE2*	*SYNGAP1*
DEPDC5	*KCTD7*	*PRRT2*	*TBC1D24*
DLAT	*LGI1*	*PURA*	*UBE3A*
DNM1	*LIAS*	*QARS*	
DOCK7	*MBD5*	*SCARB2*	
EFHC1	*MECP2*	*SCN1A*	
EPM2A	*MEF2C*	*SCN1B*	
FOXG1	*MPC1*	*SCN2A*	

Supplementary Table 3: Epilepsy Genes included in the 95 gene panel

Gene panel targeted resequencing analysis (Patients 4, 6, 7, 9, 13, 14)

Genetic testing was performed using a panel of 75 genes associated with epileptic encephalopathy or other severe neurodevelopmental disorders with infantile epilepsy. Methods were performed as previously described (Lemke *et al.*, 2012).

In brief, coding regions and exon-intron boundaries were enriched using Agilent SureSelect technology (Agilent Technologies, Santa Clara, CA, USA) followed by next-generation sequencing on the Illumina HiSeq2500 platform (Illumina, San Diego, CA, USA). Reads were aligned using Burrows Wheeler Aligner (BWA-mem 0.7.2) using hg19 as reference genome. Unambiguous reads were removed using Picard 1.14. Annotation was performed using SAMtools (v0.1.18) and VarScan (v2.3). Variants were selected with a minor allele frequency below 5% (according to 1000 Genomes, dbSNP, ESP, ExAC and in-house database). More than 98% of targets had at least 30x coverage. Validation of detected variants and segregation analysis were performed by standard Sanger sequencing. In silico prediction of the mutation pathogenicity was performed using ANNOVAR.

Gene panel targeted resequencing analysis (Patients 2, 5, 20)

Patients 2 and 5 were tested with a targeted gene panel, as previously described (Lemke *et al.*, 2012; Cellini *et al.*, 2016; de Kovel *et al.*, 2016).

Whole-exome sequencing and sequence data analysis (Patient 3)

Diagnostic whole-exome sequencing was performed on the patient and his unaffected parents at Ambry Genetics (Aliso Viejo, CA). Genomic DNA extraction, exome library preparation, sequencing, bioinformatics, and data analyses were formed as previously described (Farwell *et al.*, 2015). Briefly, samples were prepared and sequenced using paired-end, 100 cycle chemistry on the Illumina HiSeq 2500 sequencer. Exome enrichment was performed using the IDT xGen Exome Research Panel V1.0. Data were annotated with the Ambry Variant Analyzer tool (Farwell *et al.*, 2015). Approximately 96% of the proband's exome was covered at 20x or higher. Identified candidate alterations were confirmed using Sanger sequencing in all available family members.

Whole-exome sequencing and sequence data analysis (Patients 10, 11)

Whole-exome sequencing and data analysis for patient 10 were performed as previously described (Kortum *et al.*, 2015). Briefly, targeted enrichment and massively parallel sequencing were performed on genomic DNA extracted from circulating leukocytes. Enrichment of the whole exome was performed according to the manufacturer's protocols using the Nextera Enrichment Kit (62 Mb) (Illumina) for subject 10 and her parents. Captured libraries were sequenced on a HiSeq 2500 platform (Illumina) in 2x100bp paired-end mode. Trimmomatic (Bolger *et al.*, 2014) was used to trim sequences of sequencing adapters and suffixes of low quality (Phred quality score below 5). Further processing was performed following the Genome Analysis Toolkit's (GATK) best practice recommendations. Briefly, the trimmed reads were first aligned to the human reference genome (UCSC GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA mem v0.7.12). Then GATK (v3.3) was used for the realignment of sequences encompassing indels, base quality score recalibration, calling variants using the HaplotypeCaller, joint

genotyping, and variant quality score recalibration. AnnoVar (v2015-03-22) was employed to annotate alterations using information from public databases. Exonic variants and intronic alterations at exon-intron boundaries ranging from -10 to +10, which were clinically associated and unknown in public databases, were retained.

SPTAN1 variant validation and segregation analysis in patient 10 and her parents we well as mutation scanning of exons 44-53 of *SPTAN1* (NM_001130438.2) in a cohort of 34 individuals suggestive of PEHO syndrome, including patient 11, were performed by Sanger sequencing. Primer pairs designed to amplify *SPTAN1* exons 44-53 (NC_000009.12, 128,552,558-128,633,665) and their intron boundaries and PCR conditions are available upon request. Amplicons were directly sequenced using the ABI BigDye Terminator Sequencing kit (Applied Biosystems) and an automated capillary sequencer (ABI 3500, Applied Biosystems). Sequence electropherograms were analyzed using the Sequence Pilot software (JSI Medical Systems).

Whole-exome sequencing and sequence data analysis (Patients 12, 16)

Patients 12 and 16, originating from Japan, were diagnosed through whole-exome sequencing. DNA was captured with the SureSelect Human All Exon V5 kit (Agilent Technologies, Santa Clara, CA) and sequenced on an Illumina HiSeq 2500 (Illumina, San Diego, CA) with 101 bp paired-end reads. Exome data processing, variant calling and variant annotation were performed as described previously (Saitsu *et al.*, 2013).

Whole-exome sequencing and sequence data analysis (Patient 18)

Diagnostic whole-exome sequencing was performed on the patient and his unaffected parents according to standard procedures at GeneDx. Briefly, samples were prepared and sequenced using 100 bp paired-end on an Illumina HiSeq sequencer. Bi-directional sequence was assembled, aligned to the human reference genome (GRCh37/UCSC hg19) and analysed using Xome Analyzer toll. Approximately 97% of the

proband's exome was covered at 117x or higher. Identified candidate variants were confirmed using Sanger sequencing.

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