**Supplements**

e-1) Sequencing Methods

The following next generation sequencing methods were used by the involved institutes to identify the variants we report in this paper.

Variants c.277A>T; p.K93\* and c.782G>A; p.R261Q have been detected as part of a larger whole genome sequencing (WGS) study on epilepsy led by Sanjay Sisodiya at the UCL, London, UK. Within this study, 99 unrelated adults, with a range of epileptic phenotypes, were sequenced at the Oxford Genomics Centre (www.well.ox.ac.uk/ogc) using the HiSeq2500 platform and v.3 chemistry (Illumina, San Diego, CA). 100bp paired-end reads were mapped to hs37d5 using BWA and duplicate reads were removed using the MarkDuplicates option from the Picard toolkit (see https://broadinstitute.github.io/picard/). The two patients reported here, F12 and F22 both derive from this cohort. Sequencing was performed across 2.3 lanes per sample and this yielded a mean sequence depth of 27.8x and 26.8x for F12 and F22 respectively, as assessed by extrapolating from a 100Mb non-centromeric segment of chromosome 1p. Variants were called with Platypus2 (version 0.7.9.3) and multi-nucleotide polymorphisms were subsequently split. VCF files were interrogated using Ingenuity Variant Analysis (Qiagen, Redwood City, CA). High confidence, potentially deleterious variants that were also rare (i.e. population allele frequency <0.2% in public and in-house databases) were filtered using a hand-curated set of 154 established epilepsy genes. The c.277A>T; p.K93\* variant in *STX1B*, present in a heterozygous state in individual F12, stood out as it was a predicted loss of function (LoF) allele, with a CADD score of 36, in a gene that has been shown to be intolerant to LoF variants. Another heterozygous variant detected in this gene (c.782G>A; p.R261Q in F22) was predicted to change the charge of the encoded protein and was calculated to have a CADD score of 34. Visual inspection of the read alignments at both loci using the Integrative Genomics Viewer (version 2.3.5) was consistent with the variant calls and each SNV was supported by +ve and –ve strand reads. No other convincing causative variants were identified in these two cases.

The variant c.23\_26dupTGCG; p.S10Afs\*7 and c.563dupA; p.N189Afs\*5 were found by a diagnostic next-generation sequencing panel consisting of 85 epilepsy genes by Amplexa Genetics, Odense, Denmark. Genomic DNA from blood was extracted using standard methods. Libraries were prepared using KAPA Fragmentase and SureSelect target enrichment (Agilent Technologies) with KAPA Library preparation kit. Clonal amplification was performed using the Ion PGM OneTouch 2 system and Ion PGM Template OT2 200 kit followed by sequencing on the Ion Torrent PGM system with the Ion PGM 200 kit v2 according to manufacturer’s instructions (ThermoFisher®). Sequences were aligned to hg19 using the Torrent Suite (ThermoFisher®) and SNPs with a read depth ≥20 and variant allele frequency of ≥0.25 were called using the Strand NGS software.

For detection of the variant c.628T >C; p.E210K (performed at Swansea University by Mark Rees, Rhys Thomas in the frame of GRCh37), DNA was extracted from a peripheral blood sample. To capture the protein-coding regions, we used the Agilent 65MB Exome Enrichment Kit. All sequencing was performed on the Illumina HiSeq 2000 platform by the Sequencing Core of the Institute for Genomic Medicine (Columbia University), formerly the Center for Human Genome Variation (Duke University). Using Burrows-Wheeler Alignment Tool (BWA-0.5.10), sequencing reads were mapped to a Genome Reference Consortium Human Genome Build 37 (GRCh37)-derived alignment set including decoy sequences; the same reference genome is used in the 1000 Genomes Project (http://www.1000genomes.org/). Single-nucleotide variants were called using the Unified Genotyper of the Genome Analysis Toolkit (GATK-1.6–11) and annotated using SnpEff-3.3 (Ensembl 73 database).

The variant c.852dup; p.T285Dfs\*75 was identified by a gene panel sequencing approach in Florence by Carla Marini at Children’s Hospital A. Meyer. First, genomic DNA (gDNA) was extracted from whole blood to perform next generation sequencing using a panel targeting 95 genes associated to epilepsy. The Haloplex panel was designed using the Agilent SureDesign tool (https://earray.chem.agilent.com/suredesign/index.htm) to capture the 95 epilepsy genes. Probes were generated to cover all coding exons and their flanking intronic sequences (10 base pairs padding). Detailed methodology is available upon request. In silico prediction of the variant pathogenicity were performed using ANNOVAR and the dbNSFP database (v3.0a), which provides functional prediction, scores on more than 20 different algorithms (REF; https://sites.google.com/site/jpopgen/dbNSFP). A mean coverage of 1222X was achieved for the targeted genes with 99.2% of the target sequenced at >40X.

The variant c.662T>C; p.L221P was identified by exome sequencing of 40 trios with myoclonic astatic epilepsy by the EuroEPINOMICS-RES Consortium. The methods for variant discovery are described here 1.

The variant c.845T>C; p.I282T was discovered by a gene panel sequencing approach as previously described 2. This patient was identified by routine diagnostics at the Cegat GmbH, Tübingen. The patient was recruited by Gerhard Kluger at the Schön Klinik, Vogtareuth, Germany

The underlying discovery methods that lead to the identification of c.155delA; p.Q52Rfs2 are described here 3. The patient was included to this study by Daniel Lowenstein and recruited at the UCSF Medical Center.

The variant c.262G>T; p.V88F was identified by whole exome sequencing at Baylor Genetics Laboratory, Houston, TX. The test was initiated by Charu Kaiwar at the Center for Individualized Medicine, Mayo Clinic, Scottsdale, AZ.

All variants were validated by Sanger sequencing.

Diagnostic whole exome sequencing was performed on patient F18 (c.431G>T; p.C144F) and his unaffected parents and in patient F15 (c.773G>A; p.S258N) and her mother at Ambry Genetics (Aliso Viejo, CA). Genomic DNA extraction, exome library preparation, sequencing, bioinformatics, and data analyses were performed as previously described4. 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 and included nucleotide and amino acid conservation, biochemical nature of the amino acid substitutions, population frequencies, and in-silico predictions of impact on protein function4. Identified candidate variants were confirmed using Sanger sequencing in all available family members.

A 115-gene panel for the diagnosis of Mendelian epileptic disorders was performed in patient F20 and F21 on a NextQeq500 (Illumina, San Diego, CA, USA) after sonication of genomic DNA (Covaris, Woburn, MA, USA) and library-building with SeqCap EZ (Roche, Madison, WI, USA), in the Next-generation sequencing platform of the Lyon University Hospital (France). CNV analysis, performed using the DeCovA in-house software, showed a heterozygous microdeletion in F20 involving the whole coding sequence of *STX1B*, c.(?\_242)\_(\*3565\_?)del. It was confirmed by qPCR and was also found in the affected father.

References Supplement

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2. Lemke JR, Riesch E, Scheurenbrand T, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. Epilepsia. 2012;53:1387–1398.

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4. Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med Off J Am Coll Med Genet. 2015;17:578–586.

e-2: Detailed phenotype table

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| **Family ID** | **Patient ID** | **Gender** | **Year of Birth** | **Variant** | **Febrile Seizures, AoO/AoE (years), Seizure Type** | **Non-Febrile Seizures, AoO/AoE (years), Seizure Type** | **Intellectual Disability** | **Neurological exam** | **EEG** | **Imaging** | **Treatment Current/** Past | **Outcome** |
| ***Generalized epilepsy with febrile seizures + (GEFS+)*** |
| **F1** | 1 | F | uk | c.166C>T; p.Q56\* | n.a. | 69/n.a., TS | no | no | n.a. | large hemispheric stroke | PHT/- | sf |
| 2 | F | uk | n.a. | 5/40, GTCS | no | global apasia, right hemiparesis | n.a. | na | n.a. | sf |
| 3 | F | 1935 | childh., US | childh., GTCS | no | no | n.a. | na | n.a. | sf |
| 4 | F | 1944 | childh., US | childh., US | no | no | no | na | n.a. | sf |
| 5 | F | 1951 | n.a. | 24/38, GTCS, TS | no | no | 4Hz gsw | na | **VPA, PHT,CBZ**/- | sf |
| 6 | F | 1962 | 3.5/3.5, 1xGTCS | n.a. | no | no | n.a. | na | n.a. | sf |
| 7 | F | 1966 | n.a. | 3/3.5, 1xAS | no | no | n.a. | na | n.a. | sf |
| 8 | F | 1968 | 3/3, US | 3/3, 1xAS | no | no | n.a. | na | n.a. | sf |
| 9 | M | 1964 | 4/4, 1xTS | n.a. | no | no | no | na | n.a. | sf |
| 10 | M | 1973 | 2/2, GTCS, cluster | 2/2, GTCS, Abs | no | no | gsw | no | -/ESM, PRM, VPA | sf |
| 11 | F | 1980 | 2/2, GTCS | 1.5/uk, AS | no | no | 4Hz gsw | no | -/PB, VPA | sf |
| 12 | F | 1975 | n.a. | 6/10, Abs | no | no | 4Hz gsw | no | **ESX, VPA**/- | sf |
| 13 | M | 1995 | 1.5/1.5, GTCS | 1.8/2.5, GTCS, Abs, AS | no | no | no | na | **PB**/- | sf |
| 14 | M | 1991 | 1.8/1.8, GTCS | 2/6.5, Abs, US | no | no | 3-4Hz gsw | no | **LTG**/PRM, CLZ | sf |
| 15 | M | 1995 | 2/2, TS | 2/2, Abs, TS | no | no | 3-4Hz gsw | no | -/ CLB, PB, ESX, LTG | sf |
| 16 | M | 1995 | 7/7, GTCS | 1.7/16, GTCS, AS | speech retardation, Asperger syndrome | no | fsw | no | **STM**/PB | sf |
| 17 | M | 1997 | 4/4, GTCS | n.a. | Asperger syndrome | no | n.a. | na | -/- | sf |
| **F2** | 1 | F | 2000 | c.133\_134insGGATGTGCATTG; p.K45delinsRCMIE and c.135\_136AC>GA; p.L46M  | 0.8/4, GTCS, cluster | 1.4/5.5, GTCS, FIAS | dyslexia, dyscalculia | no | fsw, gsw | no | **STM, TPM**/- | sf |
| 2 | F | 1998 | 1.6/.16, CPS, GTCS | 1.7/5, GTCS, FIAS | mild learning disability | no | fsw, gsw | no | **STM**/- | sf |
| 3 | F | 2002 | n.a. | 1.2/5, GTCS, FIAS | no | no | fsw, gsw | no | **STM, OXC**/- | sf |
| 4 | F | 1980 | 1.5/2,8, GTCS | 1.7/2.8, GTCS | no | no | fsw, gsw | no | -/PRM | sf |
| 5 | M | 1975 | 1.2/1.2, 1xGTCS cluster | n.a. | no | no | fsw, gsw | na | -/PRM | sf |
| 6 | F | 1960 | n.a. | 1.2/2 AS | no | no | n.a. | na | -/PRM | sf |
| 7 | F | 1996 | 1.2/4, GTCS | 1.2/5 AS | no | macrocephaly | fsw, gsw | na | -/STM | sf |
| 8 | F | 1998 | 3/3, GTCS | 1.5/9 GTCS, AS | speech retardation | no | fsw, gsw | no | **STM**/VPA | sf |
| **F7** |   | M | 2013 | c.23\_26dupTGCG; p.S10A*fs*\*7 | 0.8/n.a., GTCS | 1.3/n.a., GTCS | no | mild hypotonia | n.a. | no | **VPA**/LEV, CLB | os |

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| **F8** | 1 | M | 2012 | c.852dup; p.T285D*fs*\*75 | 1.4/3, GTCS, clusters | n.a. | no | no | fsw | no | **VPA**/ PHT, MDZ | sf |
| 2 | M | 1984 | 0.9/7, GTCS | 5/7, 1xGTCS, TS | mild learning disability | no | no | no | **PB, VPA**/- | sf |
| 3 | F | 1954 | infancy/childhood, GTCS | n.a. | no | no | n.a. | n.a. | n.a. | sf |
| **F9** | 1 | F | 1995 | c.733C>T; p.R245\* | 2; FIAS, GTCS | 3; FIAS | no | no | fsw | no | **OXC**/ VPA, CBZ, LTG, ZNS, PB, CLB | sf |
| **F10** | 1 | M | 2005 | c.420C>G; p.Y140\* | 2/8; AS, GTCS | n.a. | Mild, IQ=59 (9y) | minor facial dysmorphic features; micrognathia; blepharoptosis; scoliosis; | fsw | no | **VPA, CLB**/ -  | sf |
| ***Geneteic Generalized Epilepsy (GGE)*** |
| **F11** |   | F | 1983 | c.628G>A; p.E210K | n.a. | 20/n.a., GTCS clusters, Myo, Abs | Impaired executive functions, normal IQ and good working memory | no | gsw, photosensitive | no | **LEV, CLB**/VPA | os |
| **F12** |   | M | 1968 | c.277A>T; p.K93\*  | n.a. | 11/n.a., GTCS, Myo | no | no | gsw | no | VPA, LEV, TPM/ CBZ, PHT, CLB LTG, PIR, GBP | sf |

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| ***Developmental and Epileptic Encephalopathy (DEE)*** |
| **F3** |   | F | uk | c.140C>A; p.S47\*, *de novo* | uk | uk | uk | uk | uk | uk | uk | uk |
| **F4** |   | M | 1957 | c.657T>A; p.V216E | n.a. | 3.5/n.a., GTCS, TS, Myo, Abs, clusters | moderate cognitive impairment | ataxia, dysarthria, speech delay, macrocephaly | fsw, gsw | cerebellar atrophy | **VPA, LTG**/ PB, PRM, ETS, CLN, PHT, OXC, Acetazolamid | os |
| **F5** |   | F | 2005 | c.678G>C; p.G226R; *de novo* | 1.1/1.7, GTCS | 1.7/7, GTCS, Myo, Abs, AS, TS | global developm. delay; onset 3y | ataxia  | 3.5 Hz gsw, fsw | no | **LEV, STP, VPA**/TPM, ESX, LTG, RUF, CLB, ZON, LCM, Steroids; VNS, KD | sf |
| **F6** |   | F | 2012 | arr[hg19] 16p11.2 (30,332,532-31,104,012)x1; *de novo* | n.a., but fever sensitivity | 1.1/n.a., Myo, AS, GTCS, clusters | global developm. delay | severe speech delay, mild dysmorphic features | fsw | mild cortical atrophy, bilateral hippocampal malrotation | **CLB, STP/** VPA | os |
| **F13** | 1 | M | 1995 | c.563dupA; p.N189Afs\*5 | n.a. | 1.3/n.a., GTCS clusters, atypical Abs, Myo, AS | developm. regression after onset, severe learning disability; only single words/ phrases | ataxia, gait disorder, initially with hemineglect / dystonia. Severe behavioural disturbance. | fsw, gsw | no | **PHT, VPA, CBZ**/LEV, CBZ, B6, TPM, ESM, PB, LTG, VGB; VNS | os |
| 2 | M | 1997 | n.a. | 0.8/n.a., GTCS, atypical Abs, Myo, AS | Learning disability – moderate. Talks in sentences. | no | fsw, gsw | no | **PHT, CBZ, VGB**/ VPA, CLB, CBZ, LTG; VNS | os |
| **F14** |   | M | 2011 | c.845T>C; p.I282T; *de novo* | 1.3/1.3, GTCS | 2/n.a., Abs, Myo, AS, TS | developm. regression after onset | ataxia, aphasia | gsw, gpsw | no | **VPA, BR, ESM**/ LEV, CBZ, TPM, LCM, B6, ZNS, RUF, PMP, ACTH, steroids; KD | os |
| **F15** |   | F | uk | c.773G>A; p.S258N | uk | uk | uk | uk | uk | uk | uk | uk |
| **F16** |   | F | 1993 | c.662T>C; p.L221P | 2/2, GTCS | 3/n.a., GTCS, Abs, Myo, AS, hypermotor seizures | developm. stagnation with onset of seizures | mild ataxia | gsw, gpsw | no | **VPA, LTG, LEV, CLB**/ ESM, GBP, RFN, , PB, CNZ, FBM, TPM, CS; VNS  | os |
| **F17** |   | M | 1994 | c.155delA; p.Q52R*fs*\*2; *de novo* | 1.3/1.7, GTCS | 4/n.a., GTCS, Myo, Abs, AS | developm. regression after onset, hyperactivity | dysarthria, relative less movement on right, ataxia | gsw | no | **LEV, VPA**/PB, CBZ, CLZ, TPR, PHT, LTG, Acetazolamid; VNS, KD | os |
| **F18** |   | M | 1993 | c.431G>T; p.C144F; *de novo* | n.a. | 0.8/16, Abs | developm. delay after onset of seizures | ataxia, tremor, dysarthria | Abnormal, not further specified | Mega cisterna magna, otherwise normal | -/LTG | sf |
| **F19** |  | F | 2015 | c.736 G>C; p.A246P; *de novo* | n.a. | Since birth/n.a.; IS | severely impaired | Severe motor delay, wheel-chair bound, only head control, no expressive language | hypsarrhythmia | no | **CBD**/ ACTH, VGB, TPR | os |
| **F20** | 1 | M | 1976 | c.(?\_242)\_(\*3565\_?)del | 2/5, GTCS | n.a. | n.a. | n.a. | n.a. | n.a. | **PB/ -**  | sf |
| 2 | F | 2014 | 2, GTCS | 2/n.a.; AS, Abs, Myo, GTCS | intellectual impairment | ataxia | gsw, gpsw | No | **VPA, LEV/** LTG, CLB | os |
| **F21** |  | M | 2017 | c.383del; p.Q128Gfs\*2 | n.a. | 0.2/n.a.; Myo, US with apnea and cyanosis | developmental stagnation | hypotonia | gsw, gpsw | no | **CLB/** VPA, VGB, TPM | sf |

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| ***Focal Epilepsy*** |
| **F22** |   | F | 1984 | c.782G>A; p.R261Q | 6/6, GTCS | 6/n.a., GTCS, FIAS | no | no | fsw (right temporal) | unspecific white matter lesions | **OXC, LCM, ZNS**/ CBZ, VPA, VGB, LTG, TPR, LEV, TGB, CLB | os |
| **F23** |   | F | 1967 | c.262G>T; p.V88F | n.a. | 12/47, GTCS, FIAS | no | no | fsw (left temporal) | no | **LTG**/ CBZ, PHT | sf |

n=number, w/o=without, AoO=Age of onset; AED=antiepileptic drug, FS=febrile seizures, GTCS=generalized tonic-clonic seizure, FIAS=focal impaired awareness seizure, Abs=absence seizure, Myo=generalized myoclonic seizure, AS=atonic seizure, TS=generalized tonic seizures, IS=infantile spasms, US=unclassified seizures, VPA=valproic acid, PB=phenobarbitone, LEV=levetiracetam, CLB=clobazam, TPM=topiramate, LTG=lamotrigine, STP=stiripentole, BR=bromide, VGB=vigabatrin, OXC, oxcarbazepine, LCM=lacosamide, ZNS=zonisamide, CBD=cannabidiol, Os=ongoing seiuzures, Sf=seizure free, uk=unknown, no=normal, gsw=generalized sharp-waves, gpsw= generalized polyspike sharp-wave, fsw= focal sharp waves, developm.=developmental