**Table e-1:** Investigation of 33 patients with early onset epilepsy for *KCNT1* mutations.

| Patient | Phenotype | Screening Method | | | Diagnostic chromosomal microarray | Karyotype |
|---|---|---|---|---|---|---|
| | | Sanger sequencing | NGS Diagnostic Panel | WES | | |
| **1** | EIMFS | + | - | + | - | N |
| **2** | EIMFS | + | + | - | - | - |
| **3** | NFLE | - | + | - | 2.4Mb heterozygous duplication Chr 15q14 Inherited from mother | - |
| **4** | EIMFS | + | - | - | - | N |
| **5** | EIMFS | + | - | - | N | - |
| **6** | EIMFS | - | + | - | N | - |
| **7** | EIMFS | + | - | - | N | - |
| **8** | EIMFS | + | - | - | N | - |
| **9** | EIMFS | + | - | + | - | N |
| **10** | EIMFS | - | + | - | N | - |
| **11** | NFLE | - | + | - | N | - |
| **12** | EIMFS | - | + | - | N | - |
| **13** | EIMFS | + | - | - | N | - |
| **14** | EIMFS | + | - | - | N | - |
| **15** | EIMFS | + | - | - | N | - |
| **16** | EIMFS | + | - | - | N | - |
| **17** | EIMFS | + | - | - | - | N |
| **18** | EIMFS | + | - | - | N | - |
| **19** | EIMFS | + | - | - | N | - |
| **20** | EIMFS | + | - | - | N | - |
| **21** | EIMFS | + | - | - | N | - |
| **22** | EIMFS | + | - | - | N | - |
| **23** | EIMFS | + | - | - | N | - |
| **24** | EIMFS | + | - | - | N | - |
| **25** | EIMFS | + | - | - | 1.1Mb heterozygous deletion Chr 20p13 Inherited from father. | - |
| **26** | EIMFS | + | - | - | N | - |
| **27** | EIMFS | + | - | - | N | - |
| **28** | EIMFS | + | - | - | N | - |
| **29** | EIMFS | + | + | - | 11.8 Mb heterozygous deletion | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | Chr 20q11 copy number variant of uncertain significance | |
| **30** | EIMFS | - | + | - | N | - |
| **31** | EIMFS | - | + | - | N | - |
| **32** | EIMFS | - | + | - | N | - |
| **33** | EIMFS | - | + | - | N | - |

EIMFS epilepsy of infancy with migrating focal seizures, N normal, NFLE nocturnal frontal lobe epilepsy, NGS next generation sequencing, WES whole exome sequencing.

**Supplementary table e-2:** List of genes which cause early onset epileptic encephalopathy used for interrogation of whole exome data in 2 patients.

| Gene name | Inheritance | Phenotype(s) |
|---|---|---|
| *AARS* | AR | NS-EOEE |
| *ALG1* | AR | EIMFS |
| *ALG3* | AR | EIMFS |
| *ALG13* | AD *(de novo)* | EIMFS, WS, LGS, NS-EOEE |
| *ARHGEF9* | X-linked | NS-EOEE |
| *ARX* | X-linked (males may be affected) | OS, WS, NS-EOEE |
| *BRAT1* | AR | OS, NS-EOEE |
| *CACNA1A* | AR | EIMFS, NS-EOEE |
| *CDKL5* | X-linked (females and males affected) | WS |
| *CHD2* | AD *(de novo)* | NS-EOEE |
| *DNM1* | AD *(de novo)* | WS, LGS |
| *DOCK7* | AR | WS, NS-EOEE |
| *FOXG1* | AD *(de novo)* | WS, LGS, NS-EOEE |
| *GABRA1* | AD *(de novo)* | OS, WS, DS |
| *GABRB3* | AD *(de novo)* | WS, NS-EOEE |
| *GABRG2* | AD *(de novo)* | DS |
| *GNAO1* | AD *(de novo)* | WS, NS-EOEE |
| *GRIN1* | AD *(de novo)* | NS-EOEE |
| *GRIN2A* | AD *(de novo)* | WS, EAS |
| *GRIN2B* | AD *(de novo)* | WS, LGS |
| *HCN1* | AD *(de novo)* | DS-like |
| *IQSEC2* | X-linked (both males and females affected) | WS, LGS, NS-EOEE |
| *KCNA2* | AD *(de novo)* | DS-like, EMA-like |
| *KCNB1* | AD *(de novo)* | NS-EOEE |
| *KCNQ2* | AD *(de novo in EOEE, often inherited in 'benign' familial epilepsies)* | OS, WS |
| *KCNT1* | AD *(de novo)* | EIMFS, OS, WS |
| *MEF2C* | AD *(de novo)* | WS, NS-EOEE |
| *PCDH19* | X-linked (with unaffected males unless mosaic) | DS-like, EFMR |
| *PIGA* | X-linked | OS, EME, WS, NS-EOEE |
| *PIGO* | AR | NS-EOEE |
| *PLCB1* | AR | WS, EIMFS, NS-EOEE |
| *PNKP* | AR | EOEE and microcephaly |
| *PURA* | AD *(de novo)* | NS-EOEE, LGS |
| *QARS* | AR | OS, EIMFS, NS-EOEE |
| *RARS2* | AR | WS, NS-EOEE with PCH |
| *RFT1* | AR | EIMFS with CDG |
| *SCN1A* | AD *(de novo or inherited)* | DS, WS, EIMFS, NS-EOEE |

| | | |
|---|---|---|
| *SCN2A* | AD *(de novo in EOEE, often inherited in 'benign' familial epilepsies)* | OS, EIMFS, WS, BFIS, NS-EOEE |
| *SCN8A* | AD *(de novo)* | EIMFS, WS, NS-EOEE |
| *SIK1* | AD *(de novo)* | OS, EME, WS, NS-EOEE |
| *SLC1A2* | AD *(de novo)* | WS, NS-EOEE |
| *SLC2A1* | AD *(de novo* or *inherited)* | EOAE, EMA |
| *SLC6A1* | AD *(de novo)* | EMA |
| *SLC13A5* | AR | NS-EOEE |
| *SLC25A22* | AR | EMEE, EIMFS, WS |
| *SPTAN1* | AD *(de novo)* | WS, NS-EOEE |
| *STXBP1* | AD *(de novo)* | OS, WS, DS |
| *SYNGAP1* | AD *(de novo)* | NS-EOEE |
| *TBC1D24* | AR | EIMFS, NS-EOEE |
| *WWOX* | AR | WS, NS-EOEE |

AD autosomal dominant, AR autosomal recessive, BFIS benign familial infantile seizures, CDG congenital disorder of glycosylation, DS Dravet syndrome, EAS epilepsy aphasia spectrum, EFMR epilepsy in females with mental retardation, EMA epilepsy with myoclonic atonic seizures, EME early myoclonic epileptic encephalopathy, EOAE early onset absence epilepsy EOEE early onset epileptic encephalopathy, LGS Lennox Gastaut syndrome, NS-EOEE non-specific early onset epileptic encephalopathy, OS Ohtahara syndrome, PCH pontocerebellar hypoplasia, WS West syndrome

**Supplementary table e-3:** Mitochondrial investigations in Patients 4 and 8:

| | **Patient 4** | **Patient 8** |
|---|---|---|
| **Muscle Biopsy** | 2014: slight prominence of intermyofibrillar mitochondria<br>2016: Coarse clumps of mitochondria suggesting mitochondrial abnormality | Normal |
| **Respiratory chain enzymes** | 2014: Complex I 68.6nmol/min/UCS<br>2016: Complex I 84.5 nmol/min/UCS (normal range>94.17) | Complex I ratio: 0.077 (normal range 0.104-0.268)<br>Complex II ratio: 0.029 (normal range 0.040-0.204) |
| ***POLG* sequencing** | Normal | Normal |
| **Mitochondrial common deletions** | Not done | Normal |
| **Whole Mitochondrial Genome sequencing** | Not done | Normal |

UCS units of citrate synthase

**Supplementary table e-4**: EEG features of patients with *KCNT1* mutations.

| Patient | **Age at first EEG** | **Features on initial EEG** | | **Suppression (age)** | **Further EEG evolution** | **Atypical EEG features (age)** |
|---|---|---|---|---|---|---|
| | | **Ictal** | **Interictal** | | | |
| 1 | 4 weeks | Migrating ictal focus | Slow background, multifocal sharp/slow waves | Inter-ictal suppression (6 weeks) | - | Brief ED (4 weeks) Atypical hypsarrhythmia (6 months) |
| 2 | 1 month | Migrating ictal focus | Multifocal sharp/slow waves | Brief periods of suppression (1 month) | - | - |
| 3 | 6 months | Onset right anterior quadrant | Asymmetric right sided slowing | - | Multifocal sharp/slow waves. Left frontal or posterior ictal onset (12 months) | - |
| 4 | 5 months | Onset left OL but also independently within the right OL, spreading anteriorly/to the CL hemisphere | Frequent bilateral independent spike wave discharges in posterior temporal regions | Post-ictal suppression (6 months) | Migrating ictal focus at 6 months | - |
| 5 | 4 months | Continuous focal epileptiform activity (rhythmic theta) arising from either hemisphere | Diffuse background slowing | - | Migrating ictal focus at 4.5 months | ED at 7 months Bilateral asymmetric tonic posturing with frontal ictal theta activity at 15 months |
| 6 | 2 months | Rhythmic delta left parietal | Slow background, multifocal sharp/slow waves | Asynchronous burst suppression (2 months) | Migrating ictal focus at 3 months | ED at 2 months |
| 7 | 5 months | Right mid-temporal region spike-slow wave complexes, subsequent seizure arising from left temporal region | Runs of discrete spikes/sharp waves right temporo-parietal regions, and independently left parieto-occipital region | Brief ictal suppression (15 months) | Migrating ictal focus noted at 14 months | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 2 months | Left or right temporal ictal foci with theta/alpha discharges | Independent multifocal sharp waves over the post-central regions | Asynchronous burst suppression (7 months) | Migrating ictal focus (7 months) | ED (7.5 months) |
| 9 | 3 weeks | Left temporal ictal focus | Multifocal spikes/sharp waves and slowing | - | Migrating ictal focus (4 months) | - |
| 10 | 1 month | Initial suppression followed by focal seizure | Multifocal spikes/sharp waves and slow waves | Ictal suppression (1 month and 7 months with tonic seizure) | Migrating ictal focus (7 months) | - |
| 11 | 2 months | Information unavailable | - | - | Multifocal independent asymmetric sharp waves with anterior predominance<br>Ictal focus right mid-temporal at 3 years and left mid-temporal at 11 years | - |
| 12 | 1 month | No ictal features | Multifocal spikes/sharp waves and slow waves | Inter-ictal suppression (from 1 month, still seen at 7 months) | Migrating ictal focus (5 months) | ED (2 months) |

CL contralateral, ED electrodecrement, OL occipital lobe

**Supplementary table e-5**: Radiological features of patients with *KCNT1* mutations.

| Patient | Age at imaging | MRI | MRS |
|---|---|---|---|
| 1 | 31 days | WNL | |
| | 4 months | Dolicocephaly; DM | Relative choline elevation and NAA reduction |
| 2 | 10 weeks | DM already apparent, underdeveloped FL, slim CC | - |
| | 10 months | Plagio and dolicocephaly, severe volume loss/CA, fronto-temporal worse than parieto-occipital, thin corpus callosum | |
| 3 | 5 months | WNL | - |
| | 18 months | DM | - |
| | 3 years | DM (18/12 stage), CA, cerebellar atrophy | - |
| 4 | 4 months | Underdevelopment of frontal and temporal lobes and cerebellum | Normal |
| | 5 months | Abnormally open operculum, CA/lack of brain development | - |
| 5 | 3 months | WNL | - |
| 6 | 10 weeks | WNL | - |
| | 10 months | Delayed myelination, cerebral and cerebellar atrophy | |
| 7 | 5 months | Delayed myelination | - |
| 8 | 9 weeks | DM | - |
| | 4 months | DM | - |
| | 2 years | Very little myelination maturation after 6-8 months with decreased white matter volume, volume loss/CA | |
| 9 | 11 weeks | DM | Low NAA |
| | 3 years | DM and global CA | |
| 10 | 23 days | WNL | - |
| 11 | 2 years | WNL | - |
| | 5 years | Global CA and cerebellar atrophy | - |
| | 8 years | Progression of CA, cerebellar atrophy, delayed myelination | - |
| 12 | 7 weeks | WNL | Relative choline elevation and NAA reduction |

| | | | |
|---|---|---|---|
| | 5 months | WNL | |
| | 6 months | Marked CA /volume loss, cerebellar atrophy | |
| | 8 months | Worsened CA & DM | |
| | 1 year | Further atrophy with left subdural effusion, cerebellar atrophy & DM | |

CA cerebral atrophy, CC corpus callosum, DM delayed myelination, FL frontal lobe, MRI magnetic resonance imaging, MRS magnetic resonance spectroscopy, NAA N-acetyl aspartate, OL occipital lobe, WM white matter, WNL within normal limits

**Table e-6: *In silico* assessment of *KCNT1* mutations identified in patient cohort.**

| | CDS/ Protein change | PolyPhen-2 | SIFT (<0.05) | Provean (<-2.5) | Present in ExAC, 1000G, EVS |
|---|---|---|---|---|---|
| 1 | c.811G>T V271F | 0.773 Possibly damaging | 0.092 Tolerated | -3.29 Deleterious | No |
| 2 | c.820C>A L274I | 0.947 Possibly damaging | 0.002 Damaging | -1.76 Neutral | No |
| 3 | c.862G>A G288S | 0.978 Probably damaging | 0.086 Tolerated | -5.27 Deleterious | No |
| 4 | c.1038C>G F346L | 0.043 Benign | 0.341 Tolerated | -3.62 Deleterious | No |
| 5 | c.1504T>G F502V | 0.051 Benign | 0.004 Damaging | -6.29 Deleterious | No |
| 6 | c.2687T>A M896L | 0.978 Probably damaging | 0.001 Damaging | -4.82 Deleterious | No |
| 7 | c.2849G>A R950Q | 0.941 Possibly damaging | 0.012 Damaging | -2.95 Deleterious | No |
| 8 | c.2800G>A A934T | 0.602 Possibly damaging | 0.064 Tolerated | -2.51 Deleterious | No |
| 9 | c.2800G>A A934T | 0.602 Possibly damaging | 0.064 Tolerated | -2.51 Deleterious | No |
| 10 | c.2800G>A A934T | 0.602 Possibly damaging | 0.064 Tolerated | -2.51 Deleterious | No |
| 11 | c.2800G>A A934T | 0.602 Possibly damaging | 0.064 Tolerated | -2.51 Deleterious | No |
| 12 | c.2800G>A A934T | 0.602 Possibly damaging | 0.064 Tolerated | -2.51 Deleterious | No |

1000G 1000 genomes database, CDS coding sequence, EVS exome variant server, ExAC exome aggregation consortium.

**Supplementary table e-7:** Published mutations in *KCNT1* leading to epilepsy and cognitive impairment.

| Genomic mutation | Effect on protein | Age of onset | Phenotype | Additional features | Inheritance | Functional validation/ references |
|---|---|---|---|---|---|---|
| c.769C>G | p.His257Asp | 2 weeks | EIMFS | - | *De novo* | No[19] |
| c.785G>A | p.Arg262Gln | 8 weeks | EIMFS | - | *De novo* | No[19] |
| c.808C>G | p.Gln270Glu | 2 days | EIMFS | Brief EEG suppression<br>Spasticity | *De novo* | No[20] |
| c.811G>T | p.Val271Phe | 2 weeks | EIMFS | Subtle BS (6 weeks)<br>Hypsarrhythmia (6 months) | Unknown (donor egg IVF pregnancy) | Gain of function[21,22] |
| c.862G>A | p.Gly288Ser | 2 months | EIMFS | - | *De novo* | Gain of function[19,20,22-26] |
| c.862G>A | p.Gly288Ser | 2 months | EIMFS | - | *De novo* | |
| c.862G>A | p.Gly288Ser | 3 years | ADNFLE | - | Unknown | |
| c.862G>A | p.Gly288Ser | 4 months | EIMFS | Chorea (left finger)<br>Spasticity | *De novo* | |
| c.862G>A | p.Gly288Ser | 2 months | EIMFS | - | *De novo* | |
| c.862G>A | p.Gly288Ser | 3 months | EIMFS | Spasms<br>Choreiform movements | Unknown | |
| c.862G>A | p.Gly288Ser | 5 weeks | EIMFS | - | Unknown | |
| c.862G>A | p.Gly288Ser | 3 months | EOEE | Severe DM (HL) | Unknown | |
| c.862G>A | p.Gly288Ser | 2 months | EIMFS | - | | |
| c.1018G>A | p.Val340Met | 3 years | Multifocal epilepsy | Limbic encephalitis | *De novo* | No[19] |
| c.1193G>A | p.Arg398Gln | Median 5.5 years (range 5–18) | ADNFLE | Psychiatric/behavioural problems in 2/4<br>No ID | Familial (AD) | Gain of function[19,24,27-29] |
| c.1193G>A | p.Arg398Gln | | ADNFLE | | | |
| c.1193G>A | p.Arg398Gln | | ADNFLE | | | |
| c.1193G>A | p.Arg398Gln | | ADNFLE | | | |
| c.1193G>A | p.Arg398Gln | 10 days | EIMFS | - | Unknown | |
| c.1193G>A | p.Arg398Gln | Unknown | Likely ADNFLE | - | Familial (AD) | |
| c.1193G>A | p.Arg398Gln | 8-14 months | ADNFLE | - | | |
| c.1193G>A | p.Arg398Gln | 3 months | EIMFS | - | | |
| c.1193G>A | p.Arg398Gln | 6 months | Focal epilepsy | - | | |
| c.1193G>A | p.Arg398Gln | 5 months | EIMFS | - | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| c.1193G>A | p.Arg398Gln | 10 weeks | EIFMS | | *De novo* | |
| c.1225C>T | p.Pro409Ser | 2 months | EIMFS | - | *De novo* | No[20] |
| c.1283G>A | p.Arg428Gln | 2 months | EIMFS | - | *De novo* | Gain of function[19,20,30,31] |
| c.1283G>A | p.Arg428Gln | 17 hours | EIMFS | - | *De novo* | |
| c.1283G>A | p.Arg428Gln | 2 hours | EIMFS | - | *De novo* | |
| c.1283G>A | p.Arg428Gln | 10 weeks | EIMFS | Seizure-associated apnoeas (2 years) | Unknown | |
| c.1283G>A | p.Arg428Gln | 1 month | EOEE | Brief period of EEG suppression | *De novo* | |
| c.1283G>A | p.Arg428Gln | 3 weeks | EIMFS | Spasms | Unknown | |
| c.1420C>T | p.Arg474Cys | Day 1 | EIMFS | Tremor, hypsarrhythmia-like when awake BS in sleep, spasticity | *De novo* | No[20,32] |
| c.1421G>A | p.Arg474His | 2 weeks | EIMFS | - | *De novo* | Gain of function[20,22,30] |
| c.1421G>A | p.Arg474His | 4 days | EIMFS | - | *De novo* | |
| c.1421G>A | p.Arg474His | 15 days | EIMFS | - | *De novo* | |
| c.1421G>A | p.Arg474His | 2 months | WS | Infantile spasms | *De novo* | |
| c.1429G>A | p.Ala477Thr | 1-2 weeks | EIMFS | - | *De novo* | No[20] |
| c.1546A>G | p.Met516Val | Day 2 | EIFMS | - | *De novo* | Gain of function[26] |
| c.1887G>C | p.Lys629Asn | 4 months | EIMFS | | | Gain of function[33] |
| c.1955G>T | p.Gly652Val | 5 months | West syndrome | Infantile spasms | Unknown | No[34] |
| c.2280C>G | p.Ile760Met | 3 days | EIMFS | - | *De novo* | Gain of function[30] |
| c.2386T>C | p.Tyr796His | Median 5.5 years (range 3–8) | ADNFLE | Psychiatric/ behavioural problems in 2/4 | Familial AD | Gain of function[22,28,29,33] |
| c.2386T>C | p.Tyr796His | | ADNFLE | | | |
| c.2386T>C | p.Tyr796His | | ADNFLE | | | |
| c.2386T>C | p.Tyr796His | | ADNFLE | | | |
| c.2386T>C | p.Tyr796His | 18 months | Sporadic NFLE | - | *De novo* | Gain of function[33] |
| c.2688G>A | p.Met896Ile | 9 years | Sporadic FLE | No ID | *De novo* | Gain of function[24,28,29] |
| c.2718G>T | p.Gln906His | 3 months | EOEE | Severe DM (HL) | *De novo* | No[35] |
| c.2771C>T | p.Pro924Leu | 1 month | EIMFS | Infantile spasms | *De novo* | Gain of function[20,27,28] |
| c.2771C>T | p.Pro924Leu | 1.5 months | EIMFS | - | Inherited (maternal somatic mosaicism) | |
| c.2782C>T | p.Arg928Cys | Median 2 years | ADNFLE | | Familial AD | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | (range 1-15) | | Psychiatric/behavioural problems in 5/6 | | Gain of function[19,22,24,28,29] |
| c.2782C>T | p.Arg928Cys | | ADNFLE | | | |
| c.2782C>T | p.Arg928Cys | | ADNFLE | | | |
| c.2782C>T | p.Arg928Cys | | ADNFLE | | | |
| c.2782C>T | p.Arg928Cys | | ADNFLE | | | |
| c.2782C>T | p.Arg928Cys | | ADNFLE | | | |
| c.2782C>T | p.Arg928Cys | - | Unaffected | - | Familial (AD)- reduced penetrance | |
| c.2782C>T | p.Arg928Cys | 1 year | ADNFLE | - | Familial (AD) | |
| c.2782C>T | p.Arg928Cys | 12 years | ADNFLE | Family history of SUDEP<br><br>Cardiac arrhythmia | Familial (AD) | |
| c.2782C>T | p.Arg928Cys | 15 years | ADNFLE | | Familial (AD) | |
| c.2782C>T | p.Arg928Cys | 7 years | ADNFLE | | Familial (AD) | |
| c.2782C>T | p.Arg928Cys | 6 years | ADNFLE | | Familial (AD) | |
| c.2782C>T | p.Arg928Cys | 5 years | NFLE | - | Unknown | |
| c.2794T>A | p.Phe932Ile | 1 month | EOEE with myoclonic seizures | Choreoathetosis (2 years). Severe DM and HL | *De novo* | Gain of function[22,36] |
| c.2800G>A | p.Ala934Thr | 2 weeks | EIMFS | GI dysmotility | *De novo* | Gain of function[20-23,24,30] |
| c.2800G>A | p.Ala934Thr | 1 month | EIMFS | - | *De novo* | |
| c.2800G>A | p.Ala934Thr | 2.5 months | EIMFS | - | Unknown | |
| c.2800G>A | p.Ala934Thr | 1 month | EIMFS | Spasticity | *De novo* | |
| c.2800G>A | p.Ala934Thr | 8 weeks | EIMFS | Spasticity, hyperreflexia | Inherited (maternal somatic mosaicism) | |
| c.2800G>A | p.Ala934Thr | 2 months | EIMFS | - | *De novo* | |
| c.2849G>A | p.Arg950Gln | 5 months | EIMFS | Hyperreflexia, autistic traits. | *De novo* | No[20] |
| c.2849G>A | p.Arg950Gln | 3 years | NFLE | Psychosis, depression | *De novo* | Gain of function[37] |
| c.G2896G>A | p.Ala966Thr | Day 1 | Ohtahara syndrome | BS | Paternal isodisomy (autosomal recessive mutation) | Marked gain of function[38] |

Recurrent mutations are highlighted in blue. AD autosomal dominant, ADNFLE autosomal dominant frontal lobe epilepsy, BS burst suppression, DM delayed myelination, EIMFS epilepsy of infancy with migrating focal seizures, EOEE early onset epileptic encephalopathy, FLE frontal lobe epilepsy, GI gastrointestinal, HL hypomyelinating leucoencephalopathy, ID intellectual disability, NFLE nocturnal frontal lobe epilepsy, SUDEP sudden unexplained death in epilepsy, WS West syndrome.