De novo mutations in *SLC1A2* and *CACNA1A* are important causes of epileptic encephalopathies

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ABSTRACT

Epileptic encephalopathies (EEs) are the most clinically important group of severe early-onset epilepsies. Next-generation sequencing has highlighted the important contribution of *de novo* mutations to the genetic architecture of EEs in addition to their underlying genetic heterogeneity. Our previous whole-exome sequencing study of 264 parent-child trios revealed more than 290 candidate genes in which only a single individual had a *de novo* variant. We sought to identify additional pathogenic variants in a subset (n=27) of these genes via targeted sequencing in an unsolved cohort of 531 individuals with a diverse range of EEs. We report 17 individuals with pathogenic variants in 7 of the 27 genes, defining a genetic etiology in 3.2% of this unsolved cohort. Our results provide definitive evidence that *de novo* mutations in *SLC1A2*, *CACNA1A* cause specific EEs and expand the compendium of clinically relevant genotypes for *GABRB3*. We also identified EEs caused by genetic variants in *ALG13*, *DNM1*, and *GNAO1* and report a novel mutation in *IQSEC2*. Notably, recurrent mutations accounted for 7/17 of the pathogenic variants identified. As a result of high-depth coverage, parental mosaicism was identified in 2 out of 14 cases tested with mutant allelic fractions of 5-6% in the unaffected parents, carrying significant reproductive counseling implications. These results confirm that dysregulation in diverse cellular neuronal pathways causes EEs and will inform the diagnosis and management of individuals with these devastating disorders.

INTRODUCTION

Epileptic encephalopathies (EEs) frequently begin in infancy or childhood and often carry a poor prognosis. They are typically characterized by multiple seizure types and abundant epileptiform activity and are associated with developmental delay or regression. Genetic studies in recent years have established that *de novo* mutations are responsible for many EE phenotypes. These studies have also highlighted the marked genetic heterogeneity of the EEs, emphasizing the value of sequencing large cohorts to identify multiple individuals with mutations in the same gene to establish a specific gene's role in disease causation. Identification of multiple individuals affected by a novel disease gene allows us to define the phenotypic spectrum of that disease and enables recognition of co-morbidities, prognosis and therapeutic response.

Our initial whole-exome sequencing (WES) study of 264 parent-child trios focused on the specific EEs of infantile spasms (IS) and Lennox-Gastaut syndrome and revealed 329 *de novo* variants in 305 genes [1]. While 19 individuals had *de novo* mutations in previously described epilepsy genes, we also identified a statistically significant enrichment of mutations in *GABRB3* (MIM: 137192) and *ALG13* (MIM: 300776), establishing their role in the etiology of EEs [1]. Similarly, *DNM1* (MIM: 602377) was confirmed as an EE gene in our subsequent study [2]. For over 290 genes, a *de novo* variant was identified in only a single individual with insufficient evidence to establish association with disease causation. Identifying a significant enrichment of cases with *de novo* variants in an individual gene is required to establish that gene's role in EEs, ideally in combination with functional validation to support pathogenicity.

In this study, we employ massively parallel targeted sequencing of 27 candidate and recently described EE genes in a large cohort of 531 individuals with a range of EEs. We hypothesized that if the genes in which a *de novo* variant in the original study was identified were causative, then we may identify additional *de novo* variants in a

large cohort of individuals with EE. The identification of an enrichment of cases with the same genetic etiology would confirm the role of a specific gene in causing EE. We have identified individuals with a *de novo* mutation in 7 of the 27 genes, including several recurrent mutations. In particular, we define a new entity of *SLC1A2* encephalopathy and highlight its importance as a novel glutamatergic gene for EE. We also emphasize the emerging importance and diagnostic value of *CACNA1A* (MIM: 601011) and *GABRB3* as recurring genes causing EEs.

METHODS

Gene selection for targeted sequencing

We used various selection criteria and expert panel review to select ~10% of the 290 genes in which at least a single *de novo* variant was identified in the original Epi4K cohort[1] for targeted sequencing. Selection criteria included: a gene in which more than one proband in the original study had a *de novo* variant (*ALG13, GABRB3, DNM1, HDAC4*); a gene recently described as causing EE (*GNAO1*); gene or a closely related gene family member in which mutations cause epilepsy or related disease (*CACNA1A, GABRB1*, *GRIN1*, *MTOR, PIK3AP1*, *SLC1A2*, *IQSEC2*, *SLC35A2*); gene predicted to have protein-protein interaction with a known epilepsy gene (*HNRNPH1*, *NEDD4L*); genomic location is in an epilepsy-related CNV (*YWHAG*); or intolerance to functional variation using the residual variation intolerance score (RVIS)[3] <25% and biological plausibility based on predicted protein function (*DNAJC6, EMILIN3, TRIM32, RALGAPB, VSP37A, EPHB1, NFASC, PLXNA1, SMURF1, THOC2, TRIM32, ZFHX3*).

Cohort

The cohort comprised 531 individuals with EEs of unknown cause. Most of these individuals had been tested for mutations of known and novel candidate genes as well as pathogenic copy number variants [5-8], so the cohort represented a highly selected group of individuals without mutations of known epilepsy genes. Detailed phenotypic characterization was performed using a validated seizure questionnaire

[9] together with review of medical records, EEG, imaging and investigation results. Each individual underwent phenotypic analysis and was classified into a specific epilepsy syndrome where possible [10].

All individuals, or their parents or legal guardians in the case of minors or those with intellectual disability, gave consent for participation in the study. The study was approved by the Human Research Ethics Committee of Austin Health, Melbourne, Australia and by the Institutional Review Board at the University of Washington.

Molecular inversion probe design and library preparation

Molecular inversion probes (MIPs) were designed as previously described [5] to capture the coding exons plus a minimum of 5 base pairs of flanking sequence of intronic/exonic boundaries in each of the 27 genes. Hybridization and amplification was performed as previously described [5; 6; 8].

Variant calling

Libraries were sequenced on an Illumina HiSeq following the manufacturer's instructions to generate 100 base pair paired-end reads. Raw reads were mapped to the human genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA v0.7.8) and variant calling was performed by the Genome Analysis Toolkit (GATK v3.1.1). Duplicate reads were marked using the Picard toolkit (v.1.113). SeattleSeq (v137) was used for annotating variants. Only variants that were predicted to affect protein sequence (missense, nonsense, frameshift, or splice variants) were assayed further. CADD, PolyPhen and GERP scores were evaluated, but no variants were excluded due to *in silico* predictions. Either a second independent MIP capture or Sanger sequencing was used to validate and test segregation of variants of interest. A read-depth of 50X was required for accurate variant calling.

Criteria for pathogenicity

Variants in recently identified EE genes were considered pathogenic if they arose *de novo* (or from a somatic mosaic parent) and had never been observed in the publically available control datasets (ExAC v0.3, ESP6500, 1000 Genomes). For novel genes, the number of *de novo* changes observed versus predicted was assessed to determine if it reached statistical significance on a per-gene basis [11].

In cases where the inheritance was not known because of the lack of availability of parental DNA in an established EE gene, the significance of a variant was based on whether it was a recurrent mutation or convergent information provided by the *in silico* tools CADD, PolyPhen-2 and GERP. The *in silico* tools had to be in agreement (CADD >25, PolyPhen-2 >0.9 and GERP >5) to classify a variant as 'likely pathogenic'. We took a more conservative approach to novel variants with limited inheritance information for genes not yet established as causative and classified these as variants of unknown significance regardless of *in silico* predictions. Microsatellite markers were used to test the relatedness within families in which the proband had a '*de novo*' mutation and to rule out, when possible, sample duplication in the case of recurrent mutations (n=5/7 recurrent mutations tested). For one inherited GABRB3 variant (NM_000814.4:c.470C>T[p.Thr157Met]), review of the pedigree showed that the variant segregated in a family with genetic epilepsy and febrile seizures plus (GEFS+).

Statistical Analysis

Mutation enrichment was assayed in the new EE genes *SLC1A2* and *CACNA1A* using denovolyzeR [11]. Briefly, using weighted per-gene mutation rates, the number of expected *de novo* mutations in our cohort of 531 individuals was calculated [11]. The probability of observing greater than or equal *de novo* missense mutations than those expected by chance was calculated using denovolyzeR correcting for 27 genes examined.

RESULTS

We sequenced 27 candidate or newly described EE genes in a cohort of 531 individuals with unsolved EE representing a wide range of epilepsy syndromes (Table 1). On average, 88% of the target (27 genes) was covered at 50X or higher with a per-gene range of 69.65% (*GRIN1*) to 99.04% (*GABRB1*) (Fig. S1).

Pathogenic or likely pathogenic variants were discovered in 7 of the 27 genes (*ALG13*, *CACNA1A*, *DNM1*, *GABRB3*, *GNAO1*, *IQSEC2*, *SLC1A2*), accounting for 17/531 cases or 3.2% of the entire cohort (Table 2). Mutations in *GABRB3* (n=7) and *CACNA1A* (n=4) accounted for the largest proportion of individuals. Recurrent mutations were identified in six genes (*ALG13*, *CACNA1A*, *DNM1*, *GABRB3*, *GNAO1*, *SLC1A2*). An additional six variants of uncertain significance (VOUS) were detected (Table S1).

We identified two individuals with *de novo* mutations in *SLC1A2* (MIM: 600300*)*, establishing this as an EE gene (p=0.0052). Previously only a single individual with IS and a *de novo* missense mutation was reported [1]. *SLC1A*2 encodes Excitatory Amino Acid Transporter 2 (EAAT2), one of the major glutamate transporters expressed in astroglia that is responsible for clearing glutamate from the extracellular space at the synapse [12]. One individual from our study shares the same missense mutation (NM_004171.3:c.244G>C[p.Gly82Arg]) as the original IS individual while the other person has a novel mutation (NM_004171.3:c.254T>C[p.Leu85Pro]) (Figure S2). Microsatellite testing confirmed that the two cases with the p.Gly82Arg were different individuals. These mutations cluster in the first extracellular domain of this transmembrane protein.

Our individuals with *SLC1A2* mutations presented with an extremely severe phenotype with profound impairment from birth without detectable regression and seizure onset in the first week of life. The original IS individual had seizure onset in the neonatal period (Table 3). All 3 individuals had multiple seizure types with prominent myoclonic and tonic seizures as well as spasms. One (T23159) had profound growth failure with microcephaly (at age 17 years: weight 15 kg, height

and head circumference 49 cm), which was not observed in the remaining two cases.

We identified seven individuals with *GABRB3* mutations, a gene previously reported with *de novo* mutations in four individuals with LGS or IS [1]. The seven new individuals presented with somewhat heterogeneous phenotypes of LGS (1), epilepsy with myoclonic-atonic seizures (1), and unclassified epileptic encephalopathy (4). The seventh case had features reminiscent of Dravet syndrome but could be distinguished by the absence of typical Dravet features including generalized spike wave activity, hemiclonic and focal seizures, although it is recognized that these features are not universal in Dravet syndrome (Table 4). For this case, the variant segregated in a family with genetic epilepsy and febrile seizures plus (GEFS+). Collectively, ten of the eleven individuals we found to carry a GABRB3 *de novo* missense mutation had seizure onset by 1 year of age, occurring in the context of either preceding normal (3) or slow (6) development (details unknown for 1), whereas, one child with developmental delay had seizure onset at 12 years (Table 4). Five had severe to profound intellectual disability, three mild to moderate disability and the degree of cognitive impairment was unclear in the remaining three. Predominant seizure types were myoclonic, tonic, absence and generalized tonic-clonic seizures. One individual had epileptic spasms.

Five of the eleven pathogenic *GABRB3* variants clustered in the transmembrane domains at evolutionarily conserved sites (average GERP 5.54±0.4, nucleotide resolution). Six pathogenic variants, including the recurrent mutation, are dispersed throughout the N-terminal extracellular domain at well-conserved residues (average GERP 5.38±0.4, nucleotide resolution). One novel variant in *GABRB3* was classified as 'likely pathogenic' because inheritance information was limited but the amino acid change from a polar to a basic residue in the transmembrane domain (Figure S3), and a CADD score >25 and PolyPhen-2 of 0.992 (probably damaging) predicted a likely damaging effect on protein function.

Pathogenic mutations in *CACNA1A* were identified in four probands and one affected sibling. Deep sequencing of the parents with two affected children revealed that the unaffected mother was a mosaic carrier with a 6.3% mutant allelic fraction in lymphocyte-derived DNA. Two probands and the affected sibling have a recurrent mutation (NM_023035.2:c.2137G>A[p.Ala713Thr]) first identified in our original study [1]. This number of *de novo* missense mutations in our cohort represents a significant enrichment ($p= 8.0 \times 10^{-5}$), providing clear evidence that *de novo* mutations in *CACNA1A* cause epileptic encephalopathy.

For the six *CACNA1A* cases, seizures began on the first day of life in five with the siblings having *in utero* movements suggestive of seizures described as "butterfly wings rapidly flapping". Seizures were recognized by 2 hours of age in three infants (Table 5). For the remaining case, onset was at 4 weeks. Three individuals, including the sibling pair, initially had myoclonic seizures, while three presented with convulsive seizures with tonic or tonic clonic features. Multiple seizure types developed in each case and included focal, tonic and tonic-clonic seizures. Status epilepticus occurred in all, including at seizure onset in one case. A reflex component was observed in the siblings with their myoclonic seizures triggered by touch and noise and in the original case with LGS. EEG studies showed multifocal epileptiform activity in three individuals, generalized spike wave and polyspike wave in two individuals and multifocal slowing in one. Development was delayed in all with five individuals having severe ID and one moderate ID. Cerebral palsy was observed in all with variable features (Table 5). Ophthalmic findings included strabismus (1) and nystagmus (2). Motor features included ataxia (3), tremor (2) and athetosis (1).

We also identified one individual each with a recurrent mutation in *DNM1*, *GNAO1* and *ALG13* (Table S2). In the case with a *DNM1* mutation (NM_004408.2:c.709C>T[p.Arg237Trp]), one proband with an apparent *de novo* p.Arg237Trp mutation had a similarly affected sibling who carried the same

mutation. Deep sequencing of the unaffected parents confirmed somatic mosaicism in the father with a 5.5% mutant allelic fraction in blood-derived DNA. The siblings had onset of seizures at 1 month and 4 months of age with multiple seizure types, severe to profound delay and prominent movement disorder features.

A recurrent mutation in *GNAO1* (NM_020988.2:c.836T>A[p.Ile279Asn]) was identified in a 2 year old boy with EOEE. All 11 reported individuals with mutations in *GNAO1* are female despite *GNAO1* being located on chromosome 16. Seizures may have begun *in utero* with unusual movements from 32 weeks gestation. Apnoeic episodes began at 26 minutes of age with 20 episodes per day. At 9 days, focal clonic seizures of the face were noted. Unusual jerky movements, involving the eyes as well, began at 4 months followed by extensor spasms. Tonic seizures developed at 16 months. Development was never normal with regression with the onset of spasms and hypsarrhythmia on EEG, and subsequent profound impairment.

We also report the eighth female with a recurrent mutation (NM_001099922.2:c.320A>G[p.Asn107Ser]) in *ALG13*, which is located on the X chromosome. The ALG13 p.107Ser had previously arisen *de novo* in seven other reported females; the father of our case was not available [1; 13-15]. Microsatellite testing confirmed our case was not related to the two individuals we reported previously [1]. Our case showed minimal responsiveness prior to onset of tonic seizures at 1-2 months with spasms developing at 4 months. She had profound impairment with hypotonia and choreoathetoid movements. Seizures were refractory; vigabatrin increased spasms and her movement disorder. There is limited data on previous cases but common features include infantile spasms (7/7), severe to profound intellectual disability (6/7), visual impairment (2/7) and hypotonia (2/7).

Finally, we identified a 42 year old woman with a *de novo* nonsense mutation in *IQSEC2* (NM_001111125.1:c.2203C>T[p.Gln735*]), a known cause of X-linked intellectual disability [16]. She had preceding mild developmental delay when she

presented at 5 years with nonconvulsive status epilepticus with absence seizures, tonic-clonic seizures and drop attacks, associated with regression. She later developed myoclonic seizures.

DISCUSSION

Our study of 531 individuals with unsolved EEs highlights the value of large-scale studies to secure the role of novel disease-associated genes, given the marked genetic heterogeneity underlying the EEs [17]. We performed targeted sequencing of 27 genes drawn from more than 290 genes identified with a *de novo* variant in a cohort of 264 individuals with two specific EEs: infantile spasms and Lennox-Gastaut syndrome. We identified 17 (3.2%) cases with a causative mutation. By finding additional cases with *de novo*, and sometimes recurrent, mutations in a novel gene, we strengthen the case for pathogenicity of that gene. Our results provide evidence for *SLC1A2* as a novel cause of EE, and we refine the phenotypes of EEs associated with mutations in *GABRB3* (7/531, 1.3%) and *CACNA1A* (4/531, 0.8%), which accounted for the largest proportion of cases with mutations identified in our cohort. As larger cohorts of a new genetic EE are assembled, the efficacy (or exacerbation) of specific anti-epileptic therapies can be established. Moreover, understanding the functional consequences of specific mutations will pave the way to precision medicine.

We report conclusive evidence that mutations in *SLC1A2* cause an EE; the phenotype is extremely severe with seizure onset in the first week of life, multiple seizure types including tonic and myoclonic seizures, and profound impairment. *SLC1A*2 encodes one of the major glutamate transporters, EAAT2. Mutations in *Slc1a2* in mice lead to impaired glutamate uptake causing excess glutamate and subsequent excitotoxicity [18]. Null mice exhibit lethal spontaneous epileptic seizures with very few animals surviving beyond 13 weeks [18]. Functional studies are required to determine if our mutations cause haploinsufficiency; if so, recently identified translational enhancers of EAAT2 may offer a novel therapeutic approach [19; 20].

For *GABRB3*, our studies have now identified a total of 11 cases with a wide phenotypic spectrum, accounting for more than 1% of unsolved EEs. Seizure onset in 10/11 cases occurred in the first year of life with myoclonic, tonic, absence and generalized tonic-clonic seizures; spasms were rare. Early development could be normal or delayed; intellectual outcome varied from severe to mild. Recent case reports include two boys with an EOEE attributed to an in-frame insertion in *GABRB3* in one case and a *de novo* p.Thr287Ile substitution in the second case[21; 22].

CACNA1A encodes the alpha1 subunit of the $Ca_v2.1$ P/Q-type calcium channel and was first reported as a gene for episodic ataxia type 2 (OMIM: 18500), spinocerebellar ataxia 6 (OMIM: 18036) and familial hemiplegic migraine type 1 (OMIM: 145100). *CACNA1A* is amongst the 2% most intolerant genes in the human genome [3]. Although deletions that include *CACNA1A* and a single truncating mutation have been associated with EE [23-25], we show that *de novo* missense mutations can also cause a severe EE in six individuals. Seizures began on the first day of life in 5/6 cases typically including focal, tonic and tonic-clonic seizures. Myoclonic seizures were observed sometimes with a prominent reflex component. Severe ID was usual and a range of motor features were observed. All individuals had missense mutations that clustered in the transmembrane domains of this multi-pass transmembrane protein (Figure S4).

Seven of our 17 (41%) mutations were recurrent, having been reported in at least one affected individual. The *DNM1* p.Arg237Trp mutation is located in a CpG dinucleotide, a known mutation hotspot [26]. The recurrence of the *SLC1A2* p.Gly82Arg mutation may be accounted for by a homopolymer stretch of guanines. Sequence context does not appear to explain the recurrence of the other mutations. The most notable example of recurrence is the p.Asn107Ser mutation in *ALG13*, which has now been reported in seven affected females. Although loss of ALG13 function causes glycosylation defects in boys, two female individuals had normal glycosylation levels on biochemical testing [13; 14], invoking an alternative mechanism.

In 2/14 families in which we identified a mutation and were able to test both parents, we found the apparently '*de novo*' mutation in a similarly affected sibling. Deep sequencing in both sets of parents confirmed low-level somatic mosaicism of around 6% in one parent. This finding mirrors a recent Dravet syndrome study in which one parent of a child with an apparently *de novo SCN1A* mutation had low, but detectable, levels of mosaicism in ~10% of cases [27]. This has critical implications for recurrence risk calculations, reproductive choices and prenatal counseling.

There are several possible limitations to our study. First, from the phenotypic viewpoint, the discovery cohort included individuals with IS or LGS, while this cohort included much more diverse EEs (Table 1). Of the 531 individuals screened, 90 had IS and 41 LGS, meaning >75% of the cohort had other EEs. If mutations in some of these genes specifically cause IS or LGS, our cohort may be too small to detect additional mutations. However, as the majority of individuals in our cohort with a mutation did not have IS or LGS, our findings highlight the importance of screening broader populations of EEs to define the phenotypic spectrum of new genetic diseases.

From the molecular standpoint, we acknowledge that mutations may remain undetected due to suboptimal coverage of a given gene or in a given individual. Some genomic regions are less amenable to sequencing with the molecular inversion probe technology [28]. Moreover, our conservative requirement of 50X coverage for variant calling could result in reduced sensitivity. Finally, it is important to note that not all 329 *de novo* variants identified in our original WES study are pathogenic. The lack of mutations in some candidate genes in our panel may be because these genes are not relevant to EE pathogenesis, or perhaps they explain a far smaller fraction of the EE population. Sequencing additional individuals and combining large datasets will facilitate identifying additional EE genes among the candidate genes from the original study.

In summary, we identified mutations in 17/531 probands, with pathogenic variants in 7/27 genes selected for targeted sequencing. There are 6 additional variants of unknown significance (Table S1) but further genetic and functional studies are required to determine if they are pathogenic. *GABRB3* and *CACNA1A* explained the largest proportion of cases in this study providing a molecular cause for 1.3% (7/531) and 0.8% (4/531) individuals respectively; we also confirm *SLC1A2* as an EE gene. Importantly, our study highlights the role of recurrent mutations, the relevance of parental mosaic transmission, and the genetic and phenotypic heterogeneity of the EEs.

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Web Resources

Burrows-Wheeler Aligner, http://bio-bwa.sourceforge.net/

DenovolyzeR, http://jamesware.github.io/denovolyzeR/

Exome Aggregate Consortium (ExAC) Browser, http://exac.broadinstitute.org/

GATK, https://www.broadinstitute.org/gatk/

OMIM, http://www.omim.org

Picard, http://broadinstitute.github.io/picard

SeattleSeq Annotation, http://snp.gs.washington.edu/SeattleSeqAnnotation137/

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Table 1. Epilepsy syndromes in unsolved cohort of individuals with EEs

Diagnosis	**No.**
Epileptic encephalopathy unclassified	205
Infantile spasms	90
Epilepsy with myoclonic-atonic seizures	80
Lennox-Gastaut syndrome	41
Early onset epileptic encephalopathy (seizure onset ≤3 months)	33
Epilepsy aphasia syndrome	20
Dravet syndrome (*SCN1A* negative)	15
Febrile infection-related epilepsy syndrome	14
Epileptic encephalopathy with continuous diffuse spike-waves during slow-wave sleep	13
Epilepsy of infancy with migrating focal seizures	8
Atypical benign partial epilepsy of childhood	7
Early myoclonic encephalopathy	4
Early infantile epileptic encephalopathy (Ohtahara syndrome)	1
Total individuals	**531**

Table 2. Pathogenic and likely pathogenic variants in EE genes identified in this study

Gene	Proband (Sex)	Inheritance	Inferred Effect	GRCh37/hg19 Genomic Coordinate	cDNA Change	Protein Cha
SLC1A2	EG1291 (F)	*De novo*	Pathogenic [a]	chr11:g.35336636	NM_004171.3:c.244G>C	p.Gly82Arg
	T23159 (F)	*De novo*	Pathogenic	chr11:g.35336626	NM_004171.3:c.254T>C	p.Leu85Pro
GABRB3	EG0254 (M)	*De novo*	Pathogenic [a]	chr15:g.26866564	NM_000814.4:c.358G>A	p.Asp120As
	EG0258 (M)	Segregates with GEFS+ family	Pathogenic	chr15:g.26828553	NM_000814.4:c.470C>T	p.Thr157Me
	T22598 (F)	*De novo*	Pathogenic [b]	chr15:g. 26825603	NM_000814.4:c.545A>T	p.Tyr182Ph
	T25111 (F)	*De novo*	Pathogenic	chr15:g.26812818	NM_000814.4:c.745C>A	p.Gln249Lys
	EG1542 (M)	*De novo*	Pathogenic	chr15:g.26812796	NM_000814.4:c.767T>A	p.Leu256Gl
	T23950 (F)	Unknown	Likely pathogenic	chr15:g.26806281	NM_000814.4:c.878T>A	p.Leu293His
	T25708 (M)	*De novo*	Pathogenic	chr15:g.26806246	NM_000814.4:c.913G>A	p.Ala305Thr
CACNA1A	EG1371 (M)	*De novo*	Pathogenic	chr19:g.13566019	NM_023035.2:c.301G>C	p.Glu101Gln
	T21924 (F)	Unknown, father unavailable	VOUS [c]	chr19:g.13476262	NM_023035.2:c.653C>T	p.Ser218Leu
	T23039 (F)	*De novo*	Pathogenic [a]	chr19:g.13414398	NM_023035.2:c.2137G>A	p.Ala713Thr
	T24139 (M)	Mosaic mother	Pathogenic [a]	chr19:g.13414398	NM_023035.2:c.2137G>A	p.Ala713Thr
	EG1519 (F)	*De novo*	Pathogenic	chr19:g.13368235	NM_023035.2:c.4531G>T	p. Ala1511S
DNM1	T24107 (F)	Mosaic parent	Pathogenic [a]	chr9:g.130982480	NM_004408.2:c.709C>T	p.Arg237Trp
GNAO1	T25023 (M)	*De novo*	Pathogenic [a]	chr16:g.56385408	NM_020988.2:c.836T>A	p.Ile279Asn
IQSEC2	T17563 (F)	*De novo*	Pathogenic	chrX:g.53279555	NM_001111125.1:c.2203C>T	p.Gln735*
ALG13	T22647 (F)	Unknown, father unavailable	Pathogenic [a]	chrX:g.110928268	NM_001099922.2:c.320A>G	p.Asn107Ser

DS, Dravet syndrome; EE, epileptic encephalopathy; EIEE, early infantile epileptic encephalopathy; EIMFS, epilepsy of infancy with migr
myoclonic encephalopathy; EOEE, early onset epileptic encephalopathy; GEFS+, Generalized epilepsy with febrile seizures plus; ID, intel
spasms; LGS, Lennox-Gastaut syndrome; MAE, epilepsy with myoclonic-atonic seizures; SGE, symptomatic generalised epilepsy; VOUS, v
[a] Recurrent mutation that has previously been reported as causing EE. [b] Previously reported [29]. [c] Clinical Variant (rs121908225) associa
migraine type 1 with progressive cerebellar ataxia.

Table 3. Clinical features of individuals with *SLC1A2* mutations

Identifier	Age Gender	Epilepsy syndrome	Development prior to seizure onset	Age of seizure onset	Seizure type at onset	Development after seizure onset	Other seizure types	Age of seizure offset	EEG	Neuroimaging	
Individuals identified in this cohort											
T23159	17y F	EME	Never normal	2d	Myoclonic (virtually continuous multifocal twitching, particularly in sleep)	Profound ID	Tonic (generalised and focal) Myoclonic SE Possible NCSE	Ongoing	Multifocal Slow background	3m: normal 1y: almost complete absence of myelination of cerebrum with normal myelination of cerebellum and brain stem, thin CC, T2 hyperintensity in lentiform nuclei 3y: cerebral atrophy, complete absence of myelination of subcortical and periventricular white matter, normal myelination of cerebellum, brain stem, thalami and basal ganglia, thin CC, thinning of cortical grey matter, no cerebellar atrophy 13y: extreme supratentorial atrophy involving cortex, deep grey matter and white matter. Sparing of posterior fossa with brainstem and cerebellum are of normal size and signal intensity	
EG1291	6y F	EOEE	Never normal	5d	Epileptic spasms	Profound ID	Myoclonic Myoclonic SE Tonic Tonic-clonic Focal	Ongoing	Multifocal (no hypsarrhythmia)	6m: Delayed myelination 3y: Progress in myelination, frontal atrophy, thin CC	
Individuals identified in Epi, Epilepsy Phenome/Genome et al. 2013											
n	8y F	IS	Unknown	1mth	Focal tonic Myoclonic	Some regression Severe ID		Unknown	Hypsarrhythmia Disorganised background Bifrontal central frequent discharges	Normal	

ACTH, adrenocorticotropic hormone; ADHD, attention deficit hyperactivity disorder; AZD, acetazolamide; CBZ, carbamazepine; CC, corpus callosum; CLB, clobazam; CLZ, clora
cerebral spinal fluid; CT, computed tomography; CSE, convulsive status epilepticus; CZP, clonazepam; d, days; DZP, diazepam; EE, epileptic encephalopathy; EIEE, early infanti
migrating focal seizures; EME, early myoclonic encephalopathy; EOEE, early onset epileptic encephalopathy; ETX, ethosuxamide; F, female; FBM, felbamate; FIAS, focal impair
GORD, gastro-oesophageal reflux disease; GPFA, generalised paroxysmal fast activity; GSW, generalised spike wave; h, hours; ID, intellectual disability; IS, infantile spasms; KD
levetiracetam; LTG, lamotrigine; LGS, Lennox-Gastaut Syndrome; M, male; MAD, modified Atkins diet; MDZ, midazolam; mth, months; NCSE, non-convulsive status epilepticus
nitrazepam; OCBZ, oxcarbazepine; PB, phenobarbitone; PFA, paroxysmal fast activity; PHT, phenytoin; PRM, primidone; PSW, polyspike wave; PEG, percutaneous endoscopic
status epilepticus; SGE, symptomatic generalised epilepsy; SPECT, single-photon emission computed tomography; STP, stiripentol; SSW, slow spike wave; TPM, topiramate; V
w, weeks; y, years; ZNS, zonisamide

[a] medications current at last follow-up underlined

Table 4. Clinical features of individuals with *GABRB3* mutations

Identifier	Age Gender	Epilepsy syndrome	Development prior to seizure onset	Age of seizure onset	Seizure type at onset	Development after seizure onset	Other seizure types	Age of seizure offset	EEG	Neuroimaging
Individuals identified in this cohort										
T25111	19y F	EE	Delayed at 6m	12y	Tonic-clonic	Severe ID	Absence Atonic Tonic-clonic CSE Focal impaired awareness evolving to bilateral convulsion Tonic	Ongoing	Generalised Atypical fast background Right and bisynchronous epileptiform activity PFA Multifocal	Normal
T22598	SUDEP 2y 11mth F	EE	Delayed	6mth	Facial grimace and horizontal head shaking with retained awareness	Delayed	Tonic-clonic Focal with retained awareness Focal SE Atonic Atypical absence Tonic	Ongoing at death	Multifocal Slow background	Incomplete myelination Plagiocephaly
T25708	14y M	LGS	Never normal Feeding via NG tube from 5m	5mth	Altered facial expression, choking, stiffening of arms, eye rolling	Severe ID	Tonic-clonic Atypical absence Tonic Myoclonic Episodes of kicking or thrashing of limbs and body Bouts of laughter lasting <3 minutes	Ongoing	Almost continuous GSW Slow background Multifocal SSW GPFA	8m: mild prominence of ventricles and subarachnoid spaces 2y: atrophy and hypomyelination of central white matter and ventricles 6y: delayed but progressing myelination empty sella with flatten pituitary gland 11y: normal
T23950	7y M	EOEE	Never normal	1mth	Myoclonic	Profound ID	Tonic Focal motor Clonic	Unknown (lost to follow up)	Multifocal sharp waves	Cortical atrophy Thin CC Increased T2 in globus pallidus
EG0258	9y M	Dravet syndrome-like	Normal	8mth	Febrile Tonic-clonic	Mild ID Delayed by 2.5y	Tonic-clonic Absence Myoclonic Atonic	Ongoing	Frontal, temporal and central sharp waves Frontal and temporal R sharp waves	Normal
EG1542	3y M	EOEE	Unknown	1d	Tonic-clonic	Delayed by 3mth Severe ID	Spasms Focal motor Tonic	Ongoing	Hypsarrhythmia Polymorphous delta intermixed with polyspikes Diffuse background slowing	Hypomyelination
EG0254	12y M	MAE	Normal	1y	Febrile Tonic-clonic	Mild ID Delayed by 2.5y	Myoclonic-atonic Atonic Myoclonic Absence	1.5y	2.5-3Hz GSW Diffuse slowing	Normal
Individuals identified in Epi, Epilepsy Phenome/Genome et al. 2013										
dr	13y F	IS	Normal	5mth	Infantile Spasms	Mild delay	Myoclonic Focal impaired awareness	Unknown	Hypsarrhythmia evolved from multifocal discharges Background slowing	Normal
gs	18y F	LGS	Delayed	10mth	Focal impaired awareness	Severe ID	Tonic-clonic CSE	Unknown	Generalised slowing GSW Bilateral synchronous L>R sharp waves or spikes L temporal or generalised onset to seizures L temporal paroxysmal features Bilateral independent	Normal

									hemispheric seizures Bilateral independent sharp waves or spike in temporal and occipital regions	
jw	20y M	LGS	Mild delay	10mth	IS	Mild ID	Tonic-clonic Atypical absence Myoclonic Atonic	Unknown	2Hz sharp-spike wave Bilateral occipital slow wave Asymmetric photic driving Poorly organised background Multifocal	Normal
jr	11y M	LGS	Delayed	10mth	IS (some letters say FS, shortly after birth Mum noticed jerking movements)	Moderate ID	FS Tonic-clonic Tonic Atypical absence Myoclonic Atonic	Unknown	Generalised 2Hz discharges Irregular spikes with myoclonic jerks and accounting for 40-50% of sleep recording Slow background	Small acute infarct in splenium of CC

ACTH, adrenocorticotropic hormone; ADHD, attention deficit hyperactivity disorder; AZD, acetazolamide; CBZ, carbamazepine; CC, corpus callosum; CLB, clobazam; CLZ, clora
cerebral spinal fluid; CT, computed tomography; CSE, convulsive status epilepticus; CZP, clonazepam; d, days; DZP, diazepam; EE, epileptic encephalopathy; EIEE, early infant
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w, weeks; y, years; ZNS, zonisamide

[a] medications current at last follow-up underlined

Table 5. Clinical features of individuals with *CACNA1A* mutations

Case	Age Gender	Epilepsy syndrome	Development prior to seizure onset	Age of seizure onset	Seizure type at onset	Development after seizure onset	Other seizure types	Age of seizure offset	EEG	Neuroimagin
Individuals identified in this cohort										
T24139 (brother of T24629)	5y M	EOEE Initially diagnosed with hyperekplexia	C-PAP ventilation for 4h after birth	At birth (abnormal movements in utero)	Jittery movements at birth, myoclonic seizures in NICU	Regression at 2y Severe ID	Tonic-clonic Focal tonic Focal impaired awareness CSE Ongoing myoclonic seizures (spontaneous and reflex to noise and touch)	Ongoing	Multifocal Diffuse slowing R tempoparietal spikes	Normal
T24629 (sister of T24139)	15y F	EOEE Initially diagnosed with hyperekplexia	Delayed	5h (abnormal movements in utero)	Focal myoclonic	Severe ID	Tonic-clonic Focal CSE Tonic	Ongoing	Multifocal Beta activity R>L sharp and slow waves	2d: suggestive of hyp damage in periventri area 4y: normal
T23039	7y F	EOEE	Normal until 4mth	1-2h	Tonic-clonic	Severe ID	Myoclonic Focal CSE Focal impaired awareness	Ongoing	GSW PSW Mild slowing bi-mid central frontal area	4d: normal 18mth: bimesial temp lobe increased T2 sig
EG1371	4y M	EIMFS	Delayed	4w	Tonic with focal features	Severe ID	Asymmetric tonic Focal motor Focal NCSE	Ongoing	Migration of rhythmic left predominant 2-4 Hz bi-occipital activity to right 5-6 Hz theta activity L>R posterior quadrant slowing	2mth: normal
EG1519	12y F	EOEE	Delayed	1d	CSE	Moderate ID	2 additional CSE at 14mth and 24mth without intervening seizures Tonic with R hemifacial clonic and L eye deviation	Ongoing	Bi-frontal, L fronto-central and temporal slowing	Normal
Individuals identified in Epi, Epilepsy Phenome/Genome et al. 2013										
EPGP011141	19y F	EOEE → LGS	Unremarkable	1h	Myoclonic	Severe ID Regression	Tachypnoeic episode Tonic-clonic Focal impaired awareness CSE Drop attacks Myoclonic (reflex to loud noise) Tonic	Ongoing	GSW PSW Generalised slowing	CT newborn period: 5w: normal SPECT 7y: areas of m decreased radiopharmaceutical in L frontal, parietal a temporal lobes

ACTH, adrenocorticotropic hormone; ADHD, attention deficit hyperactivity disorder; AZD, acetazolamide; CBZ, carbamazepine; CC, corpus callosum; CLB, clobazam; CLZ, clora cerebral spinal fluid; CT, computed tomography; CSE, convulsive status epilepticus; CZP, clonazepam; d, days; DZP, diazepam; EE, epileptic encephalopathy; EIEE, early infanti migrating focal seizures; EME, early myoclonic encephalopathy; EOEE, early onset epileptic encephalopathy; ETX, ethosuxamide; F, female; FBM, felbamate; FIAS, focal impair GORD, gastro-oesophageal reflux disease; GPFA, generalised paroxysmal fast activity; GSW, generalised spike wave; h, hours; ID, intellectual disability; IS, infantile spasms; KD levetiracetam; LTG, lamotrigine; LGS, Lennox-Gastaut Syndrome; M, male; MAD, modified Atkins diet; MDZ, midazolam; mth, months; NCSE, non-convulsive status epilepticus nitrazepam; OCBZ, oxcarbazepine; PB, phenobarbitone; PFA, paroxysmal fast activity; PHT, phenytoin; PRM, primidone; PSW, polyspike wave; PEG, percutaneous endoscopic status epilepticus; SGE, symptomatic generalised epilepsy; SPECT, single-photon emission computed tomography; STP, stiripentol; SSW, slow spike wave; TPM, topiramate; V w, weeks; y, years; ZNS, zonisamide

[a] medications current at last follow-up underlined

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