BRIEF COMMUNICATION

Unexplained early onset epileptic encephalopathy: Exome screening and phenotype expansion

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Summary

Early onset epileptic encephalopathies (EOEEs) represent a significant diagnostic challenge. Newer genomic approaches have begun to elucidate an increasing number of responsible single genes as well as emerging diagnostic strategies. In this single-center study, we aimed to investigate a cohort of children with unexplained EOEE. We performed whole-exome sequencing (WES), targeting a list of 137 epilepsy-associated genes on 50 children with unexplained EOEE. We characterized all phenotypes in detail and classified children according to known electroclinical syndromes where possible. Infants with previous genetic diagnoses, causative brain malformations, or inborn errors of metabolism were excluded. We identified disease-causing variants in 11 children (22%) in the following genes: *STXBP1* (n = 3), *KCNB1* (n = 2), *KCNT1*, *SCN1A, SCN2A, GRIN2A, DNM1*, and *KCNA2*. We also identified two further variants (in *GRIA3* and *CPA6*) in two children requiring further investigation. Eleven variants were de novo, and in one paternal testing was not possible. Phenotypes were broadened for some variants identified. This study demonstrates that WES is a clinically useful screening tool for previously investigated unexplained EOEE and allows for reanalysis of data as new genes are being discovered. Detailed phenotyping allows for expansion of specific gene disorders leading to epileptic encephalopathy and emerging sub-phenotypes.

KEY WORDS: **infantile spasms, epilepsy, encephalopathy.**

Early onset epileptic encephalopathy (EOEE) represents a challenging group of age-dependent electroclinical syndromes, which may be the result of a specific congenital or acquired structural brain lesion, metabolic disorder, trisomy, copy number variation (CNV), or single-gene defect. Although single-gene disorders are increasingly being identified, there is considerable genetic heterogeneity within individual EOEEs as well as phenotypic heterogeneity for many presumed monogenic causes. However, a large proportion of cases remain unexplained. As molecular diagnostics evolve, there is a need to describe the various clinical and research approaches. Developing a deeper understanding of the broader clinical spectrum and interpretation of genotype correlations requires accurate phenotyping. In this study we describe a cohort of previously investigated children with unexplained sporadic EOEE and report the use of whole-exome sequencing (WES), followed by analysis of selected epilepsy genes in affected probands.

Patients and Methods

Patients with EOEE (95% infants <1 year, remainder <2 years onset) without specific etiology identified between 1997 and 2012 were included. All clinical, imaging, and neurophysiology data were reviewed in detail to clearly define phenotypes. Phenotypes were classified into known electroclinical syndromes according to International League Against Epilepsy (ILAE) classification, where possible, or the electroclinical evolution defined as "nonspecific" with generalized or focal (multifocal) epileptiform discharges. Patients were investigated previously for inborn errors of metabolism, structural brain malformation (magnetic resonance [MR] imaging), single-gene disorders, and chromosomal microarray, and only patients with negative results were included (Table S1). Approval from the hospital ethics committee and informed parental consent to collect clinical data and perform WES were obtained.

Exome sequencing and validation of variants (Appendix S1)

Proband exome sequencing was performed at Atlas Biolabs (http://www.atlas-biolabs.de). Parents did not undergo exome sequencing, but did have Sanger sequencing undertaken to test for the presence/absence of relevant variants. Alignment of sequence data, data cleaning, and variant identification was performed using standard bioinformatics procedures including Mendel, MD and NextCode Health (Appendix S1). All heterozygous variants with a frequency >0.05% in the National Heart, Lung and Blood Institute (NHLBI) Exome Variant Server and our own Irish exome database were excluded. A minor allele frequency of 0.25% was selected for homozygous variants or variants with at least two heterozygous variants per gene. Variants were filtered for presence in 137 epilepsy-associated genes including X-linked genes (Table S2). Variants were prioritized for validation and inheritance testing based on the presence of heterozygous/homozygous variants in dominant/recessive genes, genotype–phenotype correlation, absence of variants in control populations, and predicted variant effects.

Results

Of the 50 probands, eleven (22%) were considered to have disease-causing variants in known epileptic encephalopathy and epilepsy-associated genes, 10 of which were de novo: *STXBP1* (n = 3), *KCNB1* (n = 2), *KCNT1* (n = 1), *KCNA2* (n = 1), *DNM1* (n = 1), *SCN2A* (n = 1), and *SCN1A (*n = 1). For the *GRIN2A* variant, paternal testing was not possible. Variants of interest were found in two further patients in *GRIA3* (de novo) and *CPA6* (maternally inherited). Phenotypes, inheritance, and molecular characteristics for these patients are described in Tables 1 and 2 and also discussed below. Other variants and clinical data for remaining patients are described in Tables S3 and S1, respectively.

Discussion

Because of wide genotype and phenotype heterogeneity, it is difficult to predict with certainty the responsible gene for many EOEEs. This study demonstrates the clinical utility of proband WES screening of 137 epilepsy-associated genes in previously investigated children with unexplained EOEE. Detailed electroclinical phenotyping allowed us to expand some of the phenotypes described.

Sodium channelopathies were identified in two patients with variants in *SCN1A* and *SCN2A,* respectively. The *SCN1A* variant (patient 5, Tyr426Asn), a known pathogenic variant, was undetected by prior commercial testing (polymerase chain reaction [PCR] and multiplex ligation-dependent probe amplification [MLPA]), demonstrating the ability of WES in detecting previously "missed" cases of

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Table 1. Clinical features of patients with disease causing (n = 11) and candidate gene (n = 2) variants

Patient (gender)	Gene, CDS change	Phenotype	Seizures (onset, type, frequency, evolution, outcome)	EEG (onset and evolution)	Other clinical features	Neuroimaging
1 (M)	*KCNT1*, c.1193G>A	MPSI	10 weeks, clonic and tonic hourly; then dyscognitive, improved, but refractory epilepsy	MF, migrating; 7 years (MF/ESES)	Profound ID, 9 years, contractures, hypertonia, poor vision, PEG, RTIs	Myelination delay, atrophy
2 (M)	*STXPB1*, c.296A>G	Nonspecific (MF)	Day 2, clonic and subtle (eye flicker), hourly; developed clonic, dyscognitive, tonic; improved, 1 year Sz-free	MF, mainly; 6 years (infrequent discharges, slow)	Profound ID, bottom shuffle, 7 years, mild PM, stereotypies, autism, orally fed, hypotonia	Normal × 2
3 (M)	*STXBP1*, c.1216C>T	Ohtahara syndrome	Day 2, flexor and extensor-tonic, 20 per day/clusters, jerks, dyscognitive, months Sz-free, now rare brief Szs	BS (awake and sleep); 6 years MF and slow	Profound ID, 13 years, ocular wobble, limb spasticity, PM, PEG	Progressive atrophy
4 (F)	*STXBP1*, c.236C>T	Ohtahara syndrome	Day 5, focal and trunk jerks, 4–20/day, evolved to extensor spasms; tonic and dyscognitive, ongoing	BS (awake and sleep); 18 months (MF, slow)	Profound ID, died 33 months, PM, hypertonia, PEG	Thin corpus callosum
5 (M)	*SCN1A*, c.1276T>A	Dravet syndrome	4.5 months, multifocal myoclonic, stimulus-evoked, several per day/clusters; developed clonic, GTCS, FC, SE; Sz-free for 1 year	GSW with myoclonus; 4 years (slow)	Ambulant, severe ID, 10 years, mild PM, autism, orally fed	Normal
6 (F)	*GRIN2A*, c.1845C>A	Nonspecific (MF)	9 months, Jerks, then GTCS, several/day; developed tonic trunk, startles, clonic, intermittent dyscognitive	MF and G; 11 years (slow)	Profound ID, self-injury, 15 years died, PM, PEG, self-injury, athetosis, cataract, duplex kidney	Atrophy
7 (M)	*KCNB1*, c.1141G>A	Nonspecific (MF)	3 months, startle episodes evolved to spasm-like, 40/day, stimulus induced tonic seizures, dyscognitive, focal motor seizures	MF; 7 years (MF, photosensitive, slow)	Profound ID, 8 years, PM, orally fed, limb hypertonia, wheelchair bound	Normal
8 (F)	*KCNB1*, c.1248C>G	Nonspecific (MF)	14 months, tonic–clonic (prolonged), days or weeks apart/clusters developed; tonic–clonic, DAs, provoked by multiple stimuli (emotion, fever, tripping)	MF; 14 years (MF and G, slow)	Severe-profound ID, 15y, hand-wringing, scoliosis, ataxia, drools, in-toeing	Normal
9 (F)	*GRIA3*, c.743C>T	Infantile spasms	6 weeks, clonic and tonic, clusters 5/day, developed spasms at 4 months, ongoing focal seizures	MF, discontinuous; 7 years (MF/some G)	Severe ID, speaks, potty trained, ambulant, 10 years, orally fed	Normal
10 (F)	*CPA6*, c.619C>G + c.799G>A	Nonspecific (G)	5 months, dyscognitive, tonic, arm jerks, 3/day clusters, became Sz-free for 2 years; developed LGS	G, poly-spike, slow; 15 years (near ESES/MF)	Severe ID, some language, ambulant, 16 y, orally fed, mild tremor, drooling	Normal
11 (M)	*KCNA2*, c.869T>G	Novel phenotype (G)	7 weeks, nonspecific events at 3 months, absences 9 months, ongoing frequent absences (typical and atypical), occasional GTCS	Focal and GSW (3 Hz); evolved to pure GSW	Moderate ID, cerebellar ataxia, tremulousness	Normal
12 (M)	*DNM1*, c.865A>T	Infantile spasms	Fetal hiccoughs, nods at 11 weeks, subtle eye spasms, post vaccine encephalopathy, infantile spasms at 7 months, tonic seizures at 20 months, refractory seizures	MF discharges, then hypsarrhythmia at 8 months; slowing and MF	Profound ID, dysmorphism, inverted nipples, overgrowth, nystagmus, dyskinesia, died 3y	Delayed myelination

Continued

Table 1. Continued.

Patient (gender)	Gene, CDS change	Phenotype	Seizures (onset, type, frequency, evolution, outcome)	EEG (onset and evolution)	Other clinical features	Neuroimaging
13 (F)	*SCN2A*, c.2995G>A	Ohtahara syndrome	Encephalopathic at birth, tonic seizures awake and sleep, persist some with desaturation, became brief	BS (awake and sleep); 4 months (MF, discontinuous, slow)	Profound ID, axial hypotonia, peripheral hypertonia	Normal

EEG, electroencephalogram; ESES, electrical status epilepticus during slow wave sleep; F, female; FC, febrile convulsions; GTCS, generalized tonic–clonic/bilateral convulsive seizures; G, generalized epileptiform discharges on EEG; ID, intellectual disability (cognitive impairment); LGS, Lennox-Gastaut syndrome; M, male; MPSI, malignant migrating partial seizures of infancy; MRI, magnetic resonance imaging; MF, multifocal epileptiform discharges; PEG, percutaneous endoscopic gastrostomy; PM, progressive microcephaly; RTIs, respiratory tract infections; Sz, seizure; SE, status epilepticus.

Table 2. Molecular and inheritance characteristics

Patient	Gene, inheritance	Rare or novel	Previous report	SIFT/Polyphen 2 prediction	Variant location and DNA change	Protein change	Location of variant (protein)
1	*KCNT1*, de novo	Novel	Heron (2012)*	Deleterious/probably damaging	chr9:138657034 (G/A)	Arg398Gln	Cytoplasmic domain
2	*STXPB1*, de novo	Novel	No	Deleterious/probably damaging	chr9:130422358 (A/G)	Tyr99Cys	Syntaxin-binding protein 1 chain
3	*STXBP1*, de novo	Novel	Same codon*	Deleterious/probably damaging	chr9:130438188 (C/T)	Arg406Cys	Syntaxin-binding protein 1 chain
4	*STXBP1*, de novo	Novel	No	Deleterious/probably damaging	chr9:130420720 (C/T)	Pro79Leu	Syntaxin-binding protein 1 chain
5	*SCN1A*, de novo	Rare	Nabbout (2003)*	Deleterious/probably damaging	chr2:166903381 (A/T)	Tyr426Asn	S6 helical–cytoplasmic domain
6	*GRIN2A*, mother negative, father NT	Rare	Endele (2010)*	Deleterious/probably damaging	chr16:9923442 (G/T)	Asn615Lys	Cytoplasmic domain
7	*KCNB1*, de novo	Novel	No	Deleterious/probably damaging	chr20:47990956 (C/T)	Gly381Arg	Selectivity motif within pore-forming segment
8	*KCNB1*, de novo	Novel	No	Deleterious/probably damaging	chr20:47990849 (G/C)	Phe416Leu	Cytoplasmic domain
9	*GRIA3*, de novo	Novel	No	Benign/tolerated	chrX:122488807 (C/T)	Ala248Val	Extracellular domain
10	*CPA6*, maternal	Rare	Sapio (2012)*	Both deleterious/probably damaging	chr8:68419039 (G/C) + chr8:68396042 (C/T)	Gln207Glu+ Gly267Arg	Carboxypeptidase A6 chain (both)
11	*KCNA2*, de novo	Novel	No	Deleterious/probably damaging	chr1:111146536 (A/C)	Leu290Arg	Voltage sensor domain
12	*DNM1*, de novo	Novel	No	Deleterious/probably damaging	chr9:13098449 (A/T)	Ile289Phe	Dynamin-type G domain
13	*SCN2A*, de novo	Rare	Nakamura (2013)*	Deleterious/probably damaging	chr2:166210777 (G/A)	Glu999Lys	Cytoplasmic domain

NT, not tested (the father of patient 6 was not available); NA, not applicable: Rare variants are variants with a minor allele frequency <0.05% (heterozygous alleles) or 0.25% (homozygous or variants in genes with ≥2 variants to identify compound heterozygous genes). Patient 3: a variant in the same codon leading to an Arg406His amino acid change has been reported in EOEE (Saitsu et al., 2008)*.

*References: see Table S3.

SCN1A-negative Dravet syndrome. The variant in *SCN2A* (patient 13, Glu999Lys), confirms *SCN2A* as a major gene implicated in Ohtahara syndrome.[1]

Potassium channelopathies are emerging as a major cause of epileptic encephalopathy. Approximately 50% of cases of migrating partial seizures of infancy (MPSI) occur due to de novo *KCNT1* variants. Variants in *KCNT1* are most commonly reported in autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE). The variant in patient 1 (Arg398Gln) leads to a strong gain-of-function effect, reported previously to cause ADNFLE with psychiatric disturbance, and most recently in a family with both MPSI and ADNFLE.[2] De novo *KCNB1* variants (both novel) were identified in two patients with epileptic encephalopathy, adding to three previously described patients to date.[3] The *KCNB1*-related phenotype is expanded here to include prominent stimulus and photosensitive epilepsy. We describe a patient with the recently discovered potassium channelopathy due to a novel variant in *KCNA2* (p.Leu290Arg) predicted to disrupt the critical voltage sensor domain of Kv1.2. Three patients have recently been reported with *KCNA2* variants also affecting the critical voltage-sensor domain, producing a gain-in-function effect.[4,5] Remarkably, when compared to our patient, all exhibit similar phenotypes characterized by early onset generalized seizures (predominantly 3 Hz absence seizures) and a pronounced cerebellar ataxia. These findings suggest a potential genotype–phenotype correlation for variants affecting the voltage-sensor domain of Kv1.2.

STXBP1 (encoding syntaxin binding protein 1) plays a role in exocytosis of synaptic vesicles and is emerging as one of the most important non–ion channel proteins responsible for a variety of neurodevelopmental disorders including EOEE. Two patients described had Ohtahara syndrome and one had a nonspecific less-severe epilepsy, marked stereotypies, and autistic-type behavior (patient 2). Dynamin-1 is a GTPase localized to presynaptic terminals that plays a role in synaptic vesicle endocytosis/membrane recycling.[6] This is the second report to confirm infantile spasms (patient 12) as the sole presentation of *DNM1* mutation, associated with particularly poor outcome. *GRIN2A* encodes the alpha subunit of the glutamate-activated NMDA receptor recently implicated in the epilepsy aphasia spectrum of disorders including Landau-Kleffner syndrome (LKS). The *GRIN2A* variant (Asn615Lys) in patient 6 confirms its role in EOEE as one previous case was described, and its functional relevance has been reported.[7] Although both of these patients had EOEE, they are not so similar as to determine any genotype–phenotype correlation, and in patient 6, a later epilepsy aphasia disorder (e.g. LKS/continuous spike-wave during sleep [CSWS]) did not develop.[7]

GRIA3 (encoding the ionotropic glutamate receptor, GluR3) is associated with seizures and intellectual disability.[8] Mutations in *GRIA3* are known to be X-linked, with affected males and healthy female carriers. Although the *GRIA3* variant in patient 9 is de novo, the proband is female, and further investigation for potential disease mechanisms is warranted, including X-inactivation studies, functional studies, and investigation of *GRIA3* variants in other EOEE cohorts, to determine pathogenicity of the variant identified.

Although many genetically proven EOEEs occur de novo, patient 10 has two maternally inherited in cis variants in *CPA6* (Gln207Glu+Gly267Arg). *CPA6* has an established role in epilepsy but has not been reported in epileptic encephalopathy. Functional studies have reported effects for both variants seen in our patient.[9,10] Gly267Arg has been reported in the heterozygous state in temporal lobe epilepsy.[9,10] Gln207Glu has been reported in an individual with presumed compound heterozygous mutations, but no parental testing was performed.[9] Because both parents of patient 10 have unexplained epilepsy but not epileptic encephalopathy, it is unlikely that the *CPA6* variants identified are the cause of the EOEE phenotype. However, these variants of unknown significance may have a modifying effect on disease predisposition based on complex inheritance of multiple variants.

It is not possible to directly compare our diagnostic rate with those of previous studies which use next-generation sequencing (NGS) or gene-panel approaches in children with epilepsy because of significant variation in methodologies of studies, with reported "hit-rates" ranging from 10% to 72%.[11] This variation is reflected in (1) the sequencing method (whole genome/exome/targeted/other panel-based method), (2) the pipeline applied (trio/family/proband alone analysis and the number and type of genes included for proband alone analysis), (3) the likelihood of the gene variant reported being disease-causing, (4) the type of epileptic disorder and clinicodemographic characteristics of the cohort included, and (v) the extent of preceding investigations of the cohort being investigated. Cost comparisons of the different approaches, including research resources where utilized, are also difficult to determine.

Our study represents a previously investigated EOEE cohort that remained unexplained following neuroimaging (all had brain MRI), metabolic (all), and genetic investigation (array comparative genomic hybridization [CGH] in all, karyotype in 72%, and an average of two single-gene tests per patient; range of 0–8 single gene tests) prior to exome sequencing. For each proband, to validate relevant variants, exome data filtering of 137 selected genes was performed as well as Sanger sequencing. This approach may not offer the same degree of read depth when compared to other targeted sequencing approaches, for example, the approach originally reported by Lemke et al. (2012),[12] or in some commercial NGS gene panels. However, targeted sequencing/panel-based approaches may be limited by the number of genes targeted, and in the current era of relatively frequent gene discovery regular updates to panels may be necessary to incorporate new genes. In a recent study of infants with epilepsy and developmental delay,[11] a smaller

targeted gene panel of 38 genes resulted in a 12.7% diagnostic "hit rate" for the unexplained subgroup of patients, whereas a 327-targeted panel resulted in many variants of unknown significance and was therefore abandoned. Our study of proband-only exome analysis, although not a targeted sequencing approach, allowed for reassessment of exome data for newly discovered genes, which detected variants in *DNM1, KCNA2,* and *KCNB1* shortly after their discovery without further patient testing.

Trio sequencing is a powerful method for determining causal variants in unexplained sporadic epileptic encephalopathy, although it is usually applied on a research basis.[13,14] In two recent studies specifically investigating unexplained infantile spasms, 35- and 63-gene panels did not identify any causal variants when compared to trio sequencing, which identified causal variants in 28% and 36%, respectively.[13,14] Further candidate genes are often identified using trio sequencing.[6,13,14] However, NGS technology and its applications are rapidly changing. The increased ability of whole genome sequencing to reduce sequencing artifacts compared to exome sequencing has been reported recently,[15] and as computer programs used to analyze exome/genome sequence data improve, CNV analysis will also become possible.

It is an exciting prospect that patient-specific therapies related to the molecular pathology identified for newly described epileptic encephalopathies may emerge, although much work is needed in this area. A beneficial effect of acetazolamide and zonisamide (two related drugs) was observed in our patient with the *KCNA2* variant, an interesting observation given that patients with the related gene disorder involving *KCNA1* may experience acetazolamide-responsive episodic ataxia. However, larger patient numbers and responses are warranted to investigate these findings as this finding may reflect natural evolution of *KCNA2* encephalopathy.[4,5]

The current era is one in which genomic technology and approaches to its applications are changing rapidly, and further studies exploring the utility of these approaches and replication of candidate gene findings are warranted to forge diagnostic and treatment pathways, as well as to characterize the phenotypes of children with unexplained EOEE.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline clinical characteristics/phenotype and previous investigations

Table S2. Epilepsy-associated gene list

Table S3. Heterozygous and compound heterozygous variants present in patients with early onset epileptic encephalopathy

Appendix S1. Supplemental Methods.