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Genetics and genotype-phenotype correlations in early onset epileptic encephalopathy with burst suppression

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Abstract

Objective—We sought to identify genetic causes of early onset epileptic encephalopathies with burst suppression (Ohtahara syndrome and early myoclonic encephalopathy) and evaluate genotype-phenotype correlations.

Methods—We enrolled 33 patients with a referral diagnosis of Ohtahara syndrome or early myoclonic encephalopathy without malformations of cortical development. We performed detailed phenotypic assessment including seizure presentation, EEG, and MRI. We confirmed burst suppression in 28 out of 33 patients. Research-based exome sequencing was performed for patients without a previously identified molecular diagnosis from clinical evaluation or research-based epilepsy gene panel.

Results—In 17/28 (61%) patients with confirmed early burst suppression, we identified variants predicted to be pathogenic in *KCNQ2* (n=10), *STXBP1* (n=2), *SCN2A* (n=2), *PNPO* (n=1), *PIGA* (n=1), and *SEPSECS* (n=1). In 3/5 (60%) patients without confirmed early burst suppression, we identified variants predicted to be pathogenic in *STXBP1* (n=2) and *SCN2A* (n=1). The patient with the homozygous *PNPO* variant had a low CSF pyridoxal-5-phosphate level. Otherwise, no early laboratory or clinical features distinguished the cases associated with pathogenic variants in specific genes from each other or from those with no prior genetic cause identified.

Interpretation—We characterize the genetic landscape of epileptic encephalopathy with burst suppression, without brain malformations, and demonstrate feasibility of genetic diagnosis with clinically available testing in over 60% of our cohort with *KCNQ2* implicated in one third. This electroclinical syndrome is associated with pathogenic variation in *SEPSECS*.

Introduction

Ohtahara syndrome (OS), or early infantile epileptic encephalopathy (EIEE), and early myoclonic encephalopathy (EME) comprise a group of early onset epileptic encephalopathies with burst suppression (EOEE-BS). Onset is within the first three months of life, most often within one month. In OS, spasms are the predominant seizure type. In EME, myoclonic seizures predominate, non-epileptic myoclonus is common, and burst suppression may be more prominent in sleep. While historically depicted as separate entities, OS and EME have more recently been considered part of a spectrum, with overlap in clinical presentation and etiology; we will use the broader term EOEE-BS to encompass both syndromes.[1-4] Known genetic causes of EOEE-BS include brain malformations (e.g., polymicrogyria and lissencephaly), inborn errors of metabolism (e.g., pyridoxine- and other vitamin-dependent epilepsies, mitochondrial disorders, and amino acidopathies), and other genetic etiologies (e.g., pathogenic variants in *ARX, KCNQ2, SCN2A, SIK1, SLC25A22, STXBP1*).[2, 5-13] Based on current literature, single gene variants explain at least 20-30% of epileptic encephalopathies.[14-16] We sought to determine the contribution of genetic etiologies to EOEE-BS and to delineate genotype-phenotype correlations in a cohort of patients with EOEE-BS without malformations of cortical development. We highlight the importance of identifying genetic causes for these young patients with refractory epilepsy who could potentially benefit from gene-based treatments in this era of emerging precision medicine.[17]

Patients and methods

Cohort ascertainment and phenotypic analysis

Patients were recruited locally at Boston Children's Hospital, through collaborating researchers and physicians, and Aaron's Ohtahara Foundation (www.ohtahara.org), a family support organization. This study was approved by the Boston Children's Hospital Institutional Review Board. All patients had been diagnosed with OS or EME by treating pediatric neurologists. We reviewed history obtained from medical records and from families via phone and/or written surveys, including birth history, seizure types and frequency, physical examination features, developmental history, family history, and clinical genetic/metabolic evaluations. We reviewed primary EEG and MRI original data and reports as available. MRIs were reviewed with a neuroradiologist (EY) in 26 patients (1-11 MRIs/patient); original images were not available in the remainder, for whom reports were reviewed. We visually assessed overall volume, ventricle size, sulcal widening, callosal and white matter volume, gray matter volume, cortical morphology, cerebellar volume or malformation, myelination, focal signal abnormality, prior injury, diffusion abnormality, mass effect, basal ganglia, spectroscopy, visual pathways, and the pituitary gland. EEGs before 3 months of age or first available EEG were reviewed by pediatric epileptologists (AMB, AP, HEO) in 17 patients. Original tracings were not available in the remainder, for whom reports were reviewed. We evaluated background features and epileptiform activity of neonatal EEGs according to American Clinical Neurophysiology Society guidelines.[18] Burst suppression was defined as an invariant pattern with periods of suppression <5 uV alternating with periods of high amplitude bursts of activity, devoid of normal waking and sleep features, and lacking reactivity.[18, 19]

Based on available data and the above criteria, we classified cases as EOEE-BS vs. other severe EOEE without a specific electroclinical syndrome. We include in this report patients without malformations of cortical development. Our analysis focuses on 28 patients with confirmed EOEE-BS. We report in parallel the genetic analysis of five patients with similar clinical presentation without confirmed burst suppression.

Genetic analysis

We reviewed all clinical genetic testing performed for each case. When a clinical genetic diagnosis was not available, we performed research-based genetic assessment. One patient was diagnosed by a research gene panel, and the remainder (N=23) underwent exome sequencing to investigate known or novel genetic causes. DNA extracted from blood or saliva underwent whole exome sequencing capture using either the Agilent SureSelect XTHuman All Exon v4 or Illumina Rapid Capture Exome enrichment kit. Sequencing of 100bp paired-end reads was obtained using Illumina HiSeq (Illumina, SanDiego, CA). Coverage was >90% or >80% meeting 20x coverage with the two methods respectively. The pipeline for data analysis in both cases included alignment and quality score recalibration with Burrows-Wheeler Aligner (BWA, bio-bwa.sourceforge.net/) and variant calling with GATK (www.broadinstitute.org/gatk/). Variant annotation, filtering and interpretation was completed with the assistance of XBrowse (https://atgu.mgh.harvard.edu/xbrowse). Clinical chromosomal microarray results were available in 23/33 patients.

We described variants using human genome 19 (hg19) coordinates. We used inheritance pattern, *in silico* predictions, control databases [including the Exome Aggregation Consortium (ExAC), Exome Variant Server, 1000 Genomes database, and dbSNP], clinical laboratory reports, and findings in the literature to assess pathogenicity. For candidate genes we prioritized *de novo* heterozygous, compound heterozygous, or homozygous variants in genes with a plausible role in epilepsy. After assessing known epilepsy genes, we considered other candidate genes. Variants considered pathogenic or likely pathogenic were confirmed by PCR.

Statistical analysis

Descriptive statistics were applied as indicated. Fisher's exact test was used to compare proportions of patients with resolution of burst suppression before 2 months. The Mann-Whitney U test was used to compare timing of seizure onset in the group with likely pathogenic *KCNQ2* variants versus the remainder of the cohort, with a two-tailed p-value of less than 0.05 considered significant in both cases.

Results

Cohort

We studied 33 patients with a referral diagnosis of OS or EME without malformations of cortical development. After reviewing EEG data and/or reports, we confirmed early burst suppression in 28 patients, whom we classified as EOEE-BS. The remaining five patients (Patients 12, 13, 17, 30, and 33) had abnormal early EEGs but did not have confirmed burst suppression. Of these five patients, two had excess discontinuity with multifocal sharp waves, two had multifocal sharp waves without discontinuity, and one had a normal background initially but evolved to transient burst suppression at 5 months then hypsarrhythmia. They were classified as other severe EOEE without a specific electroclinical syndrome. We performed genetic analysis of these cases based on the referral diagnosis of EOEE given by their treating pediatric neurologists. Of the 28 patients with EOEE-BS, 5 were born pre-term (range 30 – 36.5 weeks) and the remainder at term. Median age of seizure onset was 1 day (range 1 day – 3 months). Seizure types at onset included spasms, myoclonic, tonic, or focal motor seizures (Table 1). All had active seizures at last follow-up except one; all had persistently abnormal EEGs. Patients were followed for a median of 15.6 months (range 1 month to 18 years) to last follow-up or death. In 23/28 (82%) patients with EOEE-BS, burst suppression resolved with evolution to another epileptic encephalopathy pattern by median age 3.5 months (range 1-16 weeks) (Table 1). In 4/28 (14%) patients with EOEE-BS, burst suppression persisted at last EEG (range 1.3-6 months). One patient had no follow-up EEGs. Three of 28 progressed to West syndrome.

All patients with EOEE-BS had severe global developmental delay or intellectual disability at last follow-up. Physical examinations reported normal occipito-frontal circumference in 23, microcephaly in 4 (3 congenital, 1 acquired), and head size unknown in one patient; central pattern of hypotonia or spastic quadriparesis in all; visual impairment (likely cortical) in all when adequate information was available; and abnormal movements in 8 (myoclonus, chorea, or tremors). Eleven (39%) died between 33 days and 7.6 years. Clinical

details are summarized in Table 1 and Supplemental Table 1. None of the 5 patients without burst suppression had died at last follow-up, and none had abnormal movements, but they were otherwise similar in clinical presentation to the group with burst suppression (Supplemental Table 1, Patients 12, 13, 17, 30, and 33).

Neuroimaging

A summary of MRI features is presented in Table 1, and more detailed review of patients for whom we had electronic imaging data is presented (Supplemental Table 3). Of the 28 patients with EOEE-BS, 16 had normal initial MRIs in the neonatal or early infantile period, with slightly open opercula in some cases. One patient had subtle dysgenesis of the corpus callosum (Patient 20), and one had pontine hypoplasia (Patient 19). Thirteen patients had diffusely low cerebral volume either on initial MRI or development of volume loss over time. Nine had delayed or abnormal myelination. Four had findings of uncertain significance in relationship to the epileptic encephalopathy (transverse sinus thrombosis, intraventricular hemorrhage, arachnoid cyst, germinolytic and connatal cysts). No findings were specifically associated with a given gene. Notably, two patients (Patients 8 and 31) had evidence of hypoxic-ischemic injury on neuroimaging, but this did not exclude genetic etiology. In one of these two individuals (Patient 8), we observed a pathogenic variant in *KCNQ2*. There was also a patient in the group without burst suppression who had neuroimaging findings suggestive of central hypoxic-ischemic injury as well as a likely pathogenic variant in *SCN2A* (Patient 17). The clinical course of these 3 patients deviated from that expected for the degree of hypoxic-ischemic injury.

Metabolic testing

While several patients had metabolic abnormalities of unclear relationship to their EOEE-BS, only one patient (Patient 18) had a metabolic derangement clearly linked to the underlying diagnosis. This patient had low CSF pyridoxal-5-phosphate (P5P) level in the setting of a subsequently identified homozygous *PNPO* pathogenic variant. One patient had high phenylalanine in association with diagnosis of phenylketonuria (Patient 26), which was treated early and did not explain her OS phenotype.

Amongst the 5 patients without burst suppression, Patient 13 with a deletion including *STXBP1*, had low CSF folate (not while taking valproic acid). She initially responded to folinic acid, as has one case in the literature,[20] with improvement in seizures and development. The effect waned over years.

Identification of variants in known disease-associated genes

Based on clinical and research genetic testing including exome sequencing when clinical testing was negative, we identified the following known or suspected genetic causes of EOEE-BS in 28 patients: 10 patients (35.7%) each with a likely pathogenic or pathogenic variant in *KCNQ2* that was disease-associated and/or that we established as *de novo*; 2 patients (7.1%) each with established or presumed *de novo STXBP1* pathogenic variant, including one partial gene deletion; 2 patients (7.1%) each with a *de novo SCN2A* likely pathogenic variant; one patient (3.6%) with a homozygous *PNPO* pathogenic variant, one patient (3.6%) with a hemizygous *PIGA* likely pathogenic variant, and one patient (3.6%)

with a homozygous likely pathogenic *SEPSECS* variant (Tables 1 and 2). In the 5 patients without confirmed burst suppression, we identified the following known or suspected genetic causes of epileptic encephalopathy: 2 patients (Patients 12 and 13) with pathogenic variants in *STXBP1* and one patient with a suspected pathogenic variant in *SCN2A*. The overall yield of 61% with known or suspected genetic causes in the group with confirmed burst suppression is comparable to the yield of 60% in the group without confirmed suppression, and the genetic etiologies overlap. A summary of the genetic findings, including references for those variants previously reported in the literature, *in silico* prediction scores, and parental testing when available, are indicated in Table 2, divided by gene. All identified genetic etiologies except *SEPSECS* have been established in association with EOEE-BS previously.[5, 7-9, 21-26] All of these variants were absent from control databases, except one *PNPO* variant that has 2 alleles in the ExAC database but none in the homozygous state. There is a single allele in the ExAC database for a different missense variant (p.Ala1333Val) affecting the same amino acid of the gene *SCN2A* as the variant identified in Patient 16 in our series. There were no clear patterns to medication response by genetic subgroup.

KCNQ2 subgroup

Likely pathogenic and pathogenic variants in *KCNQ2* identified in our series were all heterozygous missense variants in exons 4-7 and 15 (Table 2). The domains affected included the transmembrane domains, cytoplasmic domains, and the pore-forming domain. Six of the 10 identified variants were previously reported, including 2 with different missense variants affecting the same amino acid position also reported; one of these was identified in two unrelated patients in our series (Patients 5 and 6).[9, 24, 27-29]

Tonic seizures and/or spasms were present at last follow-up in all patients except one who was seizure-free at last follow-up. There was a trend towards earlier seizure onset in the *KCNQ2* cohort compared to the rest of the cohort, which did not meet statistical significance (Mann-Whitney U test $p = 0.08$), specifically within the first week for all 10 patients in the *KCNQ2* cohort compared to 10/18 patients in the rest of the EOEE-BS cohort. All 10 had burst suppression identified by 1.5 months, resolving by 2 months in 8/10 (1 week to 5 months, median 1.25 months). This is a higher proportion of patients with resolution of burst suppression by 2 months compared to the other patients in the cohort (Fisher exact test, $p<0.001$). Earlier resolution of burst suppression did not appear to correlate with better clinical outcome with regard to epilepsy, development, or survival (3/10 died). There was no clear pattern of medication response. Only two patients tried ezogabine, a potassium channel opener. One patient had an early positive response but developed a rash which necessitated treatment cessation. In the second patient, treatment had to be stopped due to urinary retention. Neuroimaging showed low volume in 6/10 (acquired/progressive in two, mild and stable or only one time point available in four), delayed or dysmyelination in 5/10, and evidence of central hypoxic-ischemic injury in one. Motor examination demonstrated spasticity in all but two patients (2 months and 1.3 years at last follow-up, respectively). Two patients had non-epileptic myoclonus (Patients 1 and 3).

The *KCNQ2* subgroup was similar in clinical presentation to the series of 8 patients with *KCNQ2* variants reported by Weckhuysen and colleagues and to other reports of *KCNQ2*-related epileptic encephalopathy.[9, 24, 27-29] One difference is that 9/10 patients in our series had seizures at last follow-up (range 1 month to 4 years, mean 1.9 years), whereas 7/8 patients in their series had offset of seizures at age range 9 months to 4 years.[9] With regard to neuroimaging, deep gray matter signal abnormalities have been reported to occur transiently.[9] While technical differences across MRIs from different sites are a potential confounder, our *KCNQ2* cohort did not demonstrate patterns of basal ganglia signal abnormality that were clearly distinct from normal signal, and the thalamic signal increase was not found in our cohort. The decreased white matter volume and abnormal myelination reported in a subset of patients is similar to that seen commonly in our cohort but not specific to *KCNQ2*.[9]

STXBP1 subgroup

Pathogenic variants in *STXBP1*, similar to reported variants, were found in two patients with confirmed burst suppression (one deletion, one missense) and two patients without confirmed burst suppression (one splice site, one frameshift) (Table 2).[7, 8, 30-35] Two of the 4 patients had burst suppression identified at approximately 2 months, resolving by 4 months and 13 months, respectively. Two patients did not have burst suppression after careful review of EEGs. All 4 patients had prominent spasms in the first 3 months of life. They had tonic seizures, spasms, or head drops at last follow-up, ranging from 13 months to 16 years. Neuroimaging was normal except for mild hypoplasia of the corpus callosum in one patient. Motor examination showed spasticity in 3 and diffuse hypotonia in 1 (Patient 12). Despite literature reports of movement disorders, the only abnormal movements noted were hand stereotypies in one patient without burst suppression (Patient 13).[36]

SCN2A subgroup

Likely pathogenic missense variants were identified in the gene *SCN2A* in three patients: two with confirmed burst suppression identified in the first week of life, evolving within 2 months including a period of modified hypsarrhythmia, and one with high amplitude multifocal sharp waves at 2 weeks but no identified burst suppression. None were previously reported in the literature but all were *de novo* and absent from control databases. One patient (Patient 15) had a different missense variant at the same amino acid position as a variant identified in a family with benign familial neonatal-infantile seizures.[37] Tonic seizures and spasms were prominent early in all three patients. Neuroimaging was normal in one patient, showed diffuse atrophy in another, and was suggestive of central hypoxic-ischemic injury in the third. Motor examination demonstrated spasticity in the two patients with follow-up beyond the first year and diffuse hypotonia in one patient with follow-up up to 6 weeks of life. None had abnormal movements.

Other genes, known or novel association with burst suppression

The patient with a *PNPO* homozygous variant (Patient 18) was born prematurely at 33 weeks gestation, presented with spasms and focal clonic seizures on day of life 1, and had burst suppression on the first documented EEG at 2.5 months. The EEG evolved to an abnormal but continuous background with multifocal sharp waves by 3.5 months.

Neuroimaging showed diffuse low volume and non-occlusive transverse sinus thrombosis. The patient had normal head growth, pigmentary retinopathy, diffuse hypotonia, and hyperkinetic movements.

The patient with the hemizygous *PIGA* variant (Patient 20) was born prematurely at 30 weeks gestation and had onset of focal motor and likely myoclonic seizures at less than 10 days. He had burst suppression on first available EEG documentation at 4.5 months, which evolved at 5 months to hypsarrhythmia. Neuroimaging showed dysgenesis of the corpus callosum. The patient had a cleft palate, diffuse hypotonia evolving to spasticity, chorea, and tremors. While facial dysmorphisms were described in the four previously reported patients with *PIGA* pathogenic variant associated epileptic encephalopathy, cleft palate and movement disorder were not described.[38]

The patient with the homozygous *SEPSECS* variant (Patient 19) was born at term and had onset of spasms at 3 weeks. He had burst suppression on first available EEG at 5 months, evolving at 12 months to hypsarrhythmia then later to generalized slowing with multifocal sharp waves. Neuroimaging was notable for cerebral more than cerebellar volume loss, consistent with the known association of *SEPSECS* variants with autosomal recessive progressive cerebello-cerebral atrophy (PCCA).[21] This patient also presented with microcephaly and diffuse hypotonia.

Patients without identified genetic etiology

For 11 patients with EOEE-BS and 2 without early burst suppression, a genetic etiology was not definitively identified. In 7 of 13 patients, genetic variants of uncertain significance were identified (Supplemental Table 2). At this point, there is insufficient evidence to classify the variants as being likely or known pathogenic in the context of the patients' histories. *INPP4A* is a strong candidate gene for Patient 26. There is a single previously reported patient with a homozygous *INPP4A* variant with microcephaly, severe developmental delay, cortical visual impairment, infantile onset myoclonic epilepsy, and cerebellar hypoplasia who had a similarly affected deceased brother.[39] Patient 26 did not have cerebellar hypoplasia but shared the other features. Eleven of 13 patients had chromosomal microarray testing, and two patients had duplications in known genomic hotspots for copy number variation in association with epilepsy, one in 16p11.2 (Patient 22) and one in 15q11.2 between breakpoints 1 and 2 (Patient 28); however, these were not felt to fully explain the severe epileptic encephalopathy phenotype.[40, 41]

Of these 13 patients, 11 had burst suppression identified between the first week of life and 3 months. One (Patient 30) had an initial normal EEG evolving after 3 months through transient periods of burst suppression and hypsarrhythmia. Another patient (Patient 33) had initial excess discontinuity then generalized slowing with nearly continuous bi-posterior sharp waves. EEG evolution in the 11 patients with EOEE-BS included persistent burst suppression in 4 patients, a period of hypsarrhythmia or modified hypsarrhythmia in 6 patients, and no follow-up in 1 patient. Myoclonic seizures were predominant at onset in six patients (three with spasms as well), whereas myoclonic seizures were prominent at onset in only two patients with identified genetic (*KCNQ2*) variants. Neuroimaging in the 11 patients with EOEE-BS was normal for age initially in 8 patients, showed nonspecific diffuse

atrophy in 3, demonstrated evidence of hypoxic ischemic injury in 1, and demonstrated abnormal T2 signal in the deep gray matter of 3 (two of these cases may be explained by vigabatrin exposure). The patients without burst suppression both had volume loss, and one (Patient 33) had thick perisylvian cortex without polymicrogyria (Supplemental Tables 1 and 3). To summarize all 13 patients with and without burst suppression together, two had congenital microcephaly and eleven had normal head growth. Motor examination demonstrated either diffuse hypotonia or spastic quadriparesis. Three had congenital malformations (Patients 21, 31 and 33). One patient had non-epileptic myoclonus (Patient 27) and one had tremors (Patient 26).

Discussion

This study provides insight into the genetic landscape of EOEE-BS, including OS and EME, as well as detailed phenotypic evaluation by genetic subtype. Pathogenic variants in known genes explain over 60% of cases of EOEE-BS, encompassing the syndromes OS and EME, in patients without a cortical malformation. We newly associate the gene *SEPSECS* with EOEE-BS in a single case and identify a strong candidate gene *INPP4A*. Additional cases would confirm these associations. The early seizure types varied such that strict classification into OS or EME is challenging and does not differentiate genetic etiology. Prominent myoclonic seizures at onset (as in EME) were over-represented in patients without an identified genetic etiology.

At 61%, the frequency of pathogenic variants in this study is higher than previous reports of a 20-30% yield from exome sequencing in early life epileptic encephalopathy.[14-16] These prior studies did not focus on EOEE-BS; each evaluated a broader group of epileptic encephalopathies including those with older age of onset than our cohort.[14-16] In patients with early life epilepsy (onset under 3 years) enrolled through the Pediatric Epilepsy Research Consortium, the yield of diagnosis with clinical genetic testing (predominantly including epilepsy gene panels and chromosomal microarray testing) was 27-33%.[42] There have been several reports of yield for specific genes including *KCNQ2*, *STXBP1,* and *SCN2A* within EOEE-BS and severe overlapping epileptic encephalopathy.[5, 7-9] This is, however, the first comprehensive report of genetic etiologies in a cohort of patients with clinical diagnosis of OS or EME without brain malformations. Our subjects were referred either locally at Boston Children's Hospital, through the family support group Aaron's Ohtahara Foundation, or through collaborating physicians. While our cohort may have a referral bias, it was likely biased towards enrollment of patients without a clinical explanation; thus, we do not think that we have overestimated the role of genetic influence on EOEE.

The largest genetic subgroup we identified was the group of patients with *KCNQ2* variants, comprising 35.7% of this series of 28 patients with EOEE-BS and 30% of the total 33 patients enrolled with severe EOEE without malformations. This is higher than the 10% yield of *KCNQ2* pathogenic variants in a similar previously reported series of patients with unexplained neonatal or early-infantile seizures and psychomotor retardation.[9] Frequency of variants in *STXBP1* and *SCN2A* in this cohort is within expected based on prior reports.[5, 7, 8] Overall it is clear that variants in these three genes, *KCNQ2*, *STXBP1* and

SCN2A, are among the most frequent causes of EOEE-BS. However, it is evident that EOEE-BS is highly heterogeneous in terms of genetic etiology.

For the *KCNQ2* subgroup, clinical presentation is similar to the prior report, except for absence of deep gray signal abnormalities on neuroimaging.[9] We speculate some of these differences could reflect differences in age at time of MRI and in MRI acquisition. Our data suggest that prominent early and persistent tonic seizures and spasms, and early resolution of burst suppression, may be features suggestive of a *KCNQ2* pathogenic variant.

Most of the metabolic abnormalities were non-specific with one exception (low CSF P5P in a patient with a pathogenic *PNPO* homozygous variant), emphasizing the need for evaluating metabolic causes and seeking a specific molecular diagnosis. Otherwise, we did not observe clear phenotypic distinctions between the genetic subgroups, especially early in the clinical course. Phenotyping relied on clinical reports rather than primary data in some cases, but this is not expected to skew results in a particular direction.

Imaging evidence of hypoxia-ischemia did not exclude a genetic diagnosis in our series. This finding suggests that genetic evaluation should be considered for patients in whom hypoxic-ischemic encephalopathy (HIE) is expected to be the cause of early seizures if the clinical course varies from that expected for the degree and pattern of HIE.

We provide further support for the role of the gene *PIGA* and new evidence for the role of the gene *SEPSECS* in the pathogenesis of EOEE-BS. Four *PIGA* variants in six patients with EOEE have been reported, including one variant (p.R412X) associated with neonatal onset with burst suppression in three patients.[38, 43] Thus, our patient's variant is only the second unique variant in *PIGA* identified in association with EOEE-BS, and represents a different mechanism from the other genetic causes of EOEE-BS. This finding supports further investigation for pathogenic variants in genes with overlapping roles in glycosyl-phophatidylinositol biosynthesis as potential additional causes of EOEE-BS in other patients.

Pathogenic variants in *SEPSECS* are an identified cause of progressive cerebello-cerebral atrophy and are associated with early life epilepsy, but this is the first report of association with EOEE-BS.[21-23] Previously reported patients had epilepsy onset ranging from 11 months through the second year of life with seizure types including infantile spasms and myoclonic seizures.[21-23] Disruption of selenoprotein biosynthesis, as caused by pathogenic variants in this gene, is another likely molecular cause of EOEE-BS.[21]

In this era of evolving rational therapy and precision medicine in epilepsy, having an established genetic diagnosis often influences treatment. EOEE-BS is typically refractory to medications, and thus even modest improvements in quality of life from directed therapy would be valuable. In the case of vitamin-dependent epilepsies, treatment with pyridoxine, folinic acid, or P5P is critical.[44] For more recently established treatments, efficacy remains to be determined and in some cases requires knowledge of which variants are loss or gain of function. For example, ezogabine is an anti-epileptic drug acting on potassium channels and may be beneficial in patients with loss of function *KCNQ2* pathogenic variants, though formal studies have yet to be performed.[45, 46] While many variants are considered dominant

negative, some may in contrast enhance potassium channel activity.[47, 48] Care must be taken to adequately assess the type and degree of dysfunction, which can be done through emerging international collaborative efforts centered around *KCNQ2* such as the Rational Intervention for KCNQ2/3 Epileptic Encephalopathy Project (www.RIKEE.org). Sodium channel acting medications, such as phenytoin in particular, appear to be most beneficial in patients with gain of function *SCN2A* pathogenic variants.[49]

We conclude that the majority of children with EOEE without structural malformations have identifiable genetic etiologies. Genetic testing should now be pursued on a clinical basis to arrive at a definitive diagnosis, put an end to additional needless laboratory investigations, and potentially drive treatment decisions. In addition, a genetic diagnosis can empower families with the knowledge that they need to care and advocate for their children as well as to make decisions regarding family planning. Appropriate and timely clinical diagnosis is already impacting treatment. Finally, in this era of precision medicine, it is important that infants with epilepsy be appropriately diagnosed in order to be eligible for gene-specific clinical trials as they emerge. In conclusion, our findings together suggest that early and efficient genetic testing would be of high yield and clinical benefit for patients with EOEE-BS and other cases of severe EOEE resembling OS and EME.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EIEE	early infantile epileptic encephalopathy
EME	early myoclonic encephalopathy
EOEE-BS	early onset epileptic encephalopathy with burst suppression
OS	Ohtahara syndrome

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Table 1

Summary of clinical characteristics of patients with confirmed burst suppression, by genetic sub-group.

	***KCNQ2* het variants (N = 10)**	***STXBP1* het variants (N = 2)**	***SCN2A* het variants (N = 2)**	***PNPO* hom variant (N =1)**	***SEPSECS* hom variant (N =1)**	***PIGA* X-linked variant (N =1)**	**Unexplained (N=11)**
Gender N male (%)	6 (60%)	2 (100%)	1 (50%)	0 (0%)	1 (100%)	1 (100%)	5 (50%)
Age at seizure onset (median and range)	1 day (1 – 7 days)	1 month (both)	1 day (both)	1 day	3 weeks	>10 days	6 days (1 day – 3 months)
Primary seizure types at onset N (%)	Spasms – 5 (50%) Tonic – 6 (60%) Focal motor or apneic– 4 (40%) Myoclonic - 1 (10%)	Spasms (both)	Tonic – 2 (100%) Tonic-clonic → spasms – 1 (50%)	Focal clonic, spasms	Spasms	Focal motor, myoclonic	Spasms – 8 (73%) Tonic – 4 (36%) Myoclonic – 6 (55%)
Age BS identified (median and range)	3.5 days (1 day to 7 weeks)	2 months (both)	<7 days (both)	2.5 months	5 months (first available)	4.5 months (first available)	13 days (5 days to 3 months)
Age BS resolved (median and range)	1.25 months (1 week to 5 months)	8.5 months (4 months, 13 months)	1.3 months (20 days, 2 months)	3.5 months	12 months	5 months	4.5 months (3.5 to 16 months); BS persisted in 4 (5 weeks – 6 months)
EEG evolution, patterns over time	Discontinuous evolving to BS within 2 months – 2 (20%) Excess MF sharp waves – 10 (100%) Hyps (full or modified) – 4 (40%)	MF sharp waves with discontinuity → slow spike wave – 1 (50%) MF sharp waves - 1 (50%)	Modified hyps – 2 (100%) Multifocal sharp waves with generalized slowing – 1 (50%)	Multifocal sharp waves with generalized slowing	BS in sleep/MF with slowing in waking → hyps → MF with generalized slow	Hysp	BS persists - 4 (36%) BS alternates with hyps – 2 (18%) Hyps or modified hyps +/− evolves to MF with slowing – 4 (36%) No follow-up – 1 (9%)
MRI findings	Low volume – 6 (60%) Delayed/ dysmyelination – 5 (50%) Central hypoxic ischemic injury – 1 (10%)	Normal, slight under-opercularization – 2 (100%)	Normal – 1 (50%) Diffuse atrophy – 1 (50%)	Diffuse low volume, thin brainstem, delayed myelination, thrombus R transverse sinus	Cerebral more than cerebellar volume loss, delayed + dysmyelination, pontine hypoplasia	Dysgenesis of corpus callosum	Normal initially – 8 (73%) Diffuse atrophy – 3 (27%) Hypoxic ischemic injury – 1 (9%) T2 signal deep gray – 2 (18%)
Abnormal movements N (%)	Non-epileptic myoclonus - 2 (20%)	0 (0%)	0 (0%)	Hyperkinetic	0 (0%)	Chorea, tremors	Non-epileptic myoclonus - 1 (9%) Tremors – 1 (9%)

BS = burst suppression, het = heterozygous, hom = homozygous hyps = hypsarrhythmia

Table 2

Summary of suspected pathogenic variants in established epilepsy genes for a series of 34 patients with referral diagnosis of Ohtahara syndrome or early myoclonic encephalopathy. All except Patients 12, 13, 17, 31, and 34 had confirmed early burst suppression.

ID	Epilepsy gene	Method(s) of identification	Suspected or known pathogenic mutation	Zygosity	Inheritance pattern	Phenotype	In Silico Predictions
1	*KCNQ2*	Research exome	c.601C>T (p.Arg201Cys)[29]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.998 SIFT: 0
2	*KCNQ2*	Clinical gene panel + research exome	c.667T>C (p.Ser223Pro)	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.993 SIFT: 0
3	*KCNQ2*	Research panel	c.793G>A (p.Ala265Thr) [9, 27, 29]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.588 SIFT: 0.01
4	*KCNQ2*	Clinical gene panel	c.821 C>T (p.Thr274Met) [9, 27]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.990 SIFT: 0
5	*KCNQ2*	Research exome	c.841G>A (p.Gly281Arg) [28, 29]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.994 SIFT: 0
6	*KCNQ2*	Research exome	c.841G>A (p.Gly281Arg) [28, 29]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.994 SIFT: 0
7	*KCNQ2*	Clinical gene panel	c.846C>G (p.Asp282Glu)	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.906 SIFT: 0
8	*KCNQ2*	Clinical gene panel and research exome	c.881 C>T (p.Ala294Val) [24, 27, 28]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.821 SIFT: 0
9	*KCNQ2*	Research exome	c.937G>A (p.Gly313Arg)	Heterozygous	*De novo*	EOEE-BS	PP-2: 1.00 SIFT: 0
10	*KCNQ2*	Clinical gene panel	c.1742G>A (p.Arg581Gln)	Heterozygous	Presumed *de novo*	EOEE-BS	PP-2: 0.999 SIFT: 0
11	*STXBP1*	Clinical single gene sequencing	c.416C>T (p.Pro139Leu)[32, 34]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.996 SIFT: 0.001
12	*STXBP1*	Research exome	c.794+1G>T (splice variant intron 9) [35]	Heterozygous	*De novo*	EOEE	N/A
13	*STXBP1*	Clinical single gene sequencing	c.901delC (p.Gln301Argfs*7)[8, 34]	Heterozygous	Presumed *de novo*	EOEE	N/A
14	*STXBP1*	Clinical chromosomal microarray	9q34.11 deletion including exons 1-4 of *STXBP1* [7, 30, 31, 33]	Heterozygous	*De novo*	EOEE-BS	N/A
15	*SCN2A*	Clinical gene panel	c.1289A>G (p.Glu430Gly)[37]	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.995 SIFT: 0
16	*SCN2A*	Research exome	c.3997G>A (p.Ala1333Thr)	Heterozygous	*De novo*	EOEE-BS	PP-2: 0.999 SIFT: 0
17	*SCN2A*	Research exome	c.2505A>T (p.Leu835Phe)	Heterozygous	*De novo*	EOEE	PP-2: 0.261 SIFT: 0

ID	Epilepsy gene	Method(s) of identification	Suspected or known pathogenic mutation	Zygosity	Inheritance pattern	Phenotype	In Silico Predictions
18	*PNPO*	Clinical gene panel	c.686G>A (p.Arg229Gln)	Homozygous	AR	EOEE-BS	PP-2: 1.00 SIFT: 0
19	*SEPSECS*	Clinical exome	c.176C>T (p.Ala59Val)	Homozygous	AR	EOEE-BS	PP-2: 0.446 SIFT: 0.02
20	*PIGA*	Research exome	c.424G>A (p.Ala142Thr)	Hemizygous	X-linked	EOEE-BS	PP-2: 1.00 SIFT: 0.03

AD = autosomal dominant; AR = autosomal recessive; EOEE = early onset epileptic encephalopathy; EOEE-BS = early onset epileptic encephalopathy with burst suppression, PP-2 = PolyPhen2. If known disease-associated variants, different amino acid change at the same location as a known disease associated variant, or similar truncations are in the literature these are referenced. Transcript IDs: All *KCNQ2* variants NM_172107.2, *STXBP1* variants NM_003165.3, *SCN2A* variants #15 (clinical) has transcript NM_021007.2, #16 and #17 (research) have transcript NM_001040142.1. *PNPO* variant reported above is transcript NM_01829.3. *SEPSECS* variant reported above is transcript NM_016955.3. All of these variants were confirmed to be absent in control databases, except the *PNPO* variant has 2 alleles in the ExAC database but no documented homozygotes. For patient 16, there is a single allele with a different missense variant affecting the same amino acid in the ExAC database, p.Ala1333Val.