The Genetic Landscape of Epilepsy of Infancy with Migrating Focal Seizures

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Abstract

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Objective: Epilepsy of infancy with migrating focal seizures (EIMFS) is one of the most severe developmental and epileptic encephalopathies. We delineate the genetic causes and genotype-phenotype correlations of a large EIMFS cohort.

Methods: Phenotypic and molecular data were analyzed on patients recruited through an international collaborative study.

Results: We ascertained 135 patients from 128 unrelated families. 93/135 (69%) had causative variants (42/55 previously reported) across 23 genes, including 9 novel EIMFS genes: *de novo* dominant *GABRA1, GABRB1, ATP1A3*; X-linked *CDKL5, PIGA*; and recessive, *ITPA, AIMP1, KARS, WWOX*. The most frequently implicated genes were *KCNT1* (36/135, 27%) and *SCN2A* (10/135, 7%). Mosaicism occurred in two probands (*SCN2A, GABRB3*) and three unaffected mothers (*KCNT1*). Median age of seizure onset was 4 weeks, with earlier onset in the *SCN2A, KCNQ2*, and *BRAT1* groups. Epileptic spasms occurred in 22% patients. 127 patients had severe to profound developmental impairment. All but 7 patients had ongoing seizures. Additional features included microcephaly, movement disorders, spasticity, and scoliosis. Mortality occurred in 33% at median age 2 years 7 months.

Interpretation: We identified a genetic cause in 69% of patients with EIMFS. We highlight the genetic heterogeneity of EIMFS with 9 newly implicated genes, bringing the total number to 33. Mosaicism was observed in probands and parents, carrying critical implications for recurrence risk. EIMFS pathophysiology involves diverse molecular processes from gene and protein regulation to ion channel function and solute trafficking.

Introduction

Epilepsy of infancy with migrating focal seizures (EIMFS) is a rare, devastating developmental and epileptic encephalopathy (DEE) characterized by seizure migration between cerebral hemispheres and profound developmental impairment, often with regression.[1–3] Seizure onset occurs in the first six months of life, with seizures that often increase in frequency over the first few months and are refractory to anti-epileptic drugs. Described in 1995, initially as migrating partial seizures of infancy,[1] EIMFS was for many years considered a severe infantile-onset epilepsy syndrome of unknown etiology. Between 2011 and 2019, EIMFS has been associated with pathogenic variants in 24 genes. Most cases have heterozygous dominant *de novo* variants, although homozygous and compound heterozygous variants in recessive disorders have also been described. To date, the gene most frequently associated with EIMFS is *KCNT1*, discovered in 6 of 12 cases by Barcia et al in 2012.[4] Sodium channel genes have also been implicated, including *SCN1A, SCN2A*, and *SCN8A*.[5–8]

Here, we report the largest EIMFS series to date comprising 135 patients, and define the genetic architecture of this devastating syndrome. We identify the most frequently implicated genes and report novel genes for this syndrome. We describe the phenotypic

spectrum and analyze genotype-phenotype correlations and inheritance patterns that inform prognosis and reproductive risk counseling.

Methods

Participants and phenotyping

Our EIMFS cohort comprised 135 patients who were referred to the Epilepsy Genetics Research Program at the University of Melbourne by collaborators in the United Kingdom (34), Australia (30), China (22), Italy (20), USA (18), Canada (4), New Zealand (3), United Arab Emirates (2), and Sweden (2). 55 cases have been previously reported, of whom 42 had been solved genetically.[3,5,7,9–20] The Human Research Ethics Committees of Austin Health and the Institutional Review Boards of collaborating groups approved the project. Informed written consent was obtained from the parents or legal guardians of all patients.

The syndrome of EIMFS was defined by (1) focal seizures with observed clinical and/or electrical migration between hemispheres and (2) seizure onset before age 6 months. Evidence of developmental slowing or regression was usually present.

We obtained histories and medical records from families, referring clinicians, and hospitals about each patient's epilepsy, development, comorbidities, family history, and death where applicable. Where possible, a validated seizure questionnaire was completed with the family.[21] Investigations, including EEG, neuroimaging, and clinical genetic testing reports were also analysed.

Molecular Analysis

Molecular analysis was carried out using whole exome sequencing (WES) of the proband or proband-parent trio, or targeted sequencing using molecular inversion probes (MIPs)[22] to identify variants in known and candidate EIMFS genes, as determined by the collaborative center. Variants were filtered using conventional methods[16,22] and verified using polymerase chain reaction (PCR) or standard methods in clinical laboratories. Clinical testing with chromosomal microarray analysis and/or epilepsy gene panels were performed in some patients. When available, parental DNA was sequenced by PCR, MIPs, or WES, for segregation and for phasing of recessive variants. For patients or parents, when evidence of mosaicism was observed from WES or MIP data, or suspected based on PCR, additional methods were used for confirmation, as published.[7,9] Droplet digital PCR was used to confirm that the variant in the mother of Patients 11 and 12 was definitively heterozygous and not mosaic (Supplementary table 1).

Results

Cohort Description and Phenotype

Our cohort comprised 135 children (78 girls, 56 boys) with EIMFS. All patients had evidence of interhemispheric seizure migration, either clinically and/or by EEG, in accordance with our inclusion criteria. We present the phenotypic summary, the genetic findings, and phenotype-genotype correlations of the cohort (Table 1, Table 2

Supplementary table 1). Age at study ranged from 1 month to 16 years (median 3 years 10 months).

117 patients were singletons; 14 had siblings or half-siblings with EIMFS, and 4 had siblings with other DEEs. Twelve patients were from 9 consanguineous families (3 sibships, 6 singletons). 36/124 patients had a first- or second-degree relative with epilepsy or febrile seizures, including the 18 siblings and half-siblings above.

The median age of seizure onset was 4 weeks (range day 1 to 6 months). Three patients (Patients 1, 52, 71) had suspected *in utero* seizures with postnatal seizures described between day 1 and 2 weeks. Onset in the first week of life was seen in patients with the follow genes: *SCN2A* (10 patients, median 3.5 days), *KCNQ2* (4 patients, median 3 days), and *BRAT1* (5 patients, median 1 day).

Seizure type at onset included focal seizures (121), tonic seizures (5), and epileptic spasms (1), with additional seizure types (Supplementary Table 1). Epileptic spasms occurred in 30/135 (22%) patients. All patients had refractory epilepsy in the course of their disease; however, 3 with *SCN2A* variants and 2 with *KCNQ2* variants were seizure-free for 1 year or more.

Seizure migration was observed clinically in 111 patients, and on at least one EEG in 112 patients, with 88 patients displaying both clinical and electrical migration. In addition to focal seizures and ictal interhemispheric migration, interictal EEG findings for the 134 patients for whom data were available included focal/multifocal spikes in all patients. Hypsarrhythmia (classic or modified) was seen in 13 patients, an electrodecremental pattern without hypsarrhythmia in 11, and a burst-suppression pattern in 19, with some patients displaying different patterns at different times.

We obtained data regarding development for 134 patients; 127/134 (95%) patients had severe to profound intellectual disability (ID). While all patients showed developmental slowing at the time of refractory migrating focal seizures, 2 patients went on to have normal development, 3 had mild and 2 moderate ID. All 7 children could walk. 6/7 patients with milder phenotypes had variants in *SCN2A, KCNQ2*, and *SLC12A5*; the other child with moderate ID remains genetically unsolved. Spasticity or spastic quadriparesis was reported in 40/135 (30%) patients. Hypotonia occurred in 82/135 (61%) patients, including some with a mixed picture of axial hypotonia and appendicular hypertonia. 16/135 (12%) had scoliosis. Movement disorders were present in 36/135 (27%) patients; descriptions included choreoathetosis and dyskinesia. Head circumference data were available for 124 individuals; 61/124 (49%) had microcephaly. Patients 39, 104, and 105 had thermal dysregulation with recurrent episodes of hypo- or hyperthermia.

MRI data were available for 133 patients, 55/133 (41%) were normal, 54/133 (41%) showed atrophy, 17/133 (13%) had white matter or T2 hyperintensities and 14/133 (11%) had delayed myelination (Supplementary table 1). Of the 50 MRI scans performed within a month of seizure onset, 27/50 (54%) were normal, and 6/50 showed atrophy (12%). Thirteen patients had a normal first MRI with progressive atrophy on subsequent imaging.

Our cohort had a high mortality rate; 45/135 (33%) patients died at a median age of 2 years 7 months (range 34 days to 12 years). Causes of death were available in 25/45 patients and included complications of ongoing seizures and status epilepticus (8), infections including pneumonia (6), cardiac and/or respiratory failure (8). None were reported as Sudden Unexpected Death in Epilepsy. In 25/45 patients who died, pathogenic variants were identified in the following genes: *KCNT1* (9), *SCN2A* (1), *SCN1A* (2), *GABRB3* (1), *HCN1* (1), *SCN8A* (1), *SMC1A* (1), *BRAT1* (4), *SLC12A5* (1), *SLC25A22* (2), *ITPA* (1), and *KARS* (1), with 20 remaining unsolved. Of the 90 patients that are alive, 35/90 (39%) are still under 3 years.

Genetic etiologies of EIMFS—Causative genetic variants were identified in 93/135 (69%) of our cohort (51 new patients, 42 reported previously): 67/93 (72%) had autosomal dominant, 3/93 (3%) X-linked, and 23/93 (25%) patients had homozygous or compound heterozygous autosomal recessive diseases (Figure 1). For some probands who underwent clinical testing, parental sequencing was not performed if the variant was a recurrent pathogenic variant. Of the 42 unsolved cases, 27 have had WES and 8 have had WGS.

67/70 autosomal dominant or X-linked variants were heterozygous single nucleotide variants, 1/70 was a 3-base pair deletion and 2/70 patients had heterozygous deletions that encompass epilepsy-related genes. 55/70 (79%) were confirmed to be *de novo*, 8/70 had inherited variants, 7 with *KCNT1* pathogenic variants and 1 with an X-linked recessive *PIGA* variant from his mother.

We describe 9 novel genes for EIMFS as well as variants in 14 of the previously reported 24 EIMFS genes (Figure 1, Supplementary table 2). Variants in novel EIMFS genes and analysis of pathogenicity are presented in Table 2 (Supplementary table 1).

Novel dominant EIMFS genes—Three patients had heterozygous *de novo* variants in autosomal dominant genes: *GABRA1, GABRB1*, and *ATP1A3*. We identified 2 individuals with pathogenic variants in GABA receptor subunit-encoding genes not previously associated with EIMFS, but reported in association with other developmental and epileptic encephalopathies: one case each for *GABRA1*[23] and *GABRB1*[24]. Patient 64 with a pathogenic *ATP1A3* variant was 3 years old and had a choreoathetoid and hyperkinetic movement disorder, consistent with this gene being implicated in both epilepsy and movement disorders.[25,26]

Novel X-linked EIMFS genes—We identified one boy (Patient 68) with a *de novo* variant in *CDKL5*, classically an X-linked dominant gene affecting females. His in-frame 3 base pair deletion occurred at a site previously reported in a girl with *CDKL5*.[27] We observed two boys with variants in the X-linked recessive gene *PIGA*, one inherited from an unaffected mother and one *de novo*.

Novel recessive EIMFS genes—Four patients had inherited homozygous or compound heterozygous recessive variants in novel EIMFS genes: *ITPA, AIMP1, KARS*, and *WWOX* (Table 2, Supplementary table 1).

Patient 88, the son of non-consanguineous Nepalese parents, had a recurrent homozygous variant in *ITPA* (p.W151*), previously reported in a Pakistani family, suggesting a founder effect for this variant.[28] Patient 88 had microcephaly, profound impairment, choreoathetosis, and MRI signal abnormalities in the corticospinal tracts similar to the reported case. He also had a non-dilated cardiomyopathy with depressed systolic function, whereas published cases have dilated cardiomyopathy.[28] He had a similarly affected deceased brother, for whom DNA was not available (Supplementary table 1).

Patient 89 had compound heterozygous variants in *AIMP1*. Patient 90 had compound heterozygous variants in *KARS*, which encodes a protein in the same multi-tRNA synthetase complex (MSC) as *AIMP1*. Onset of seizures was at 2 months, and he died at 6 months of age after status epilepticus. His brother had the same pathogenic variants and DEE, but not an EIMFS phenotype based on the limited available phenotypic data.

We identified compound heterozygous variants in *WWOX* in Patient 93, a recurrent missense change (p.E17K)[29] and a novel intronic deletion predicted to alter splicing. She had profound ID, spasticity, scoliosis and acquired microcephaly with a head circumference of 89th percentile at 6 months and <1st percentile at 6 years 9 months.

Established EIMFS Genes—The most commonly involved gene was *KCNT1* (36/135, 27% cohort, including 20 reported cases[3,9,12,14]). We identify *KCNT1* variants in 16 new patients: 5 with novel *KCNT1* variants (2 with p.R356W, one each with p.M267T, p.F909L and p.F932L), and 11 previously described (Supplementary table 2). Twenty-four *KCNT1* variants were *de novo* and 5 were of unknown inheritance (recurrent pathogenic variants). Notably, one individual (Patient 2) with a *de novo* variant in *KCNT1* had consanguineous parents; no recessive variants were identified in epilepsy-associated genes for this patient.

We observed autosomal dominant inheritance of *KCNT1* variants in 6 families affecting 7 patients. Two half-siblings came from a previously reported remarkable family in which the mother had Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFLE) and the heterozygous *KCNT1* variant, confirmed not to be mosaic by ddPCR.[9] With one partner, she had a child with EIMFS (Patient 11) and one with ADNFLE, and with a second partner, she had a child with EIMFS (Patient 12) and one with focal epilepsy. Three individuals with *KCNT1* variants (Patients 22, 23, and 29) had unaffected mothers who were mosaic for their respective variants. Mosaicism was initially suspected by PCR of maternal DNA and confirmed by allele-specific restriction digest and gel electrophoresis for Patient 29, as reported.[9] Patients 6 and 36 inherited *KCNT1* variants from their unaffected fathers, whose variants were identified by PCR. Both variants were recurrent with c.2849G>A p.R950Q reported in EIMFS and NFLE, and c.862G>A p.G288S occurring in both NFLE and a child with developmental delay and seizures and NFLE (Supplementary table 2). Mosaicism was not suspected from the PCR results.

SCN2A was the second most commonly implicated gene in our cohort in 10/135 (7%) patients; 8 individuals have been previously reported.[7,10] The two new individuals have recurrent pathogenic variants seen in patients with DEEs (p.E999K and p.V251I) but not in

EIMFS (Supplementary table 2). Patient 42 harbored a mosaic variant in *SCN2A* in 34% of leukocytes on WES, confirmed with cloning using Invitrogen's topoisomerase.[7]

Additional known EIMFS genes with heterozygous *de novo* variants included *SCN1A* (7), *KCNQ2* (4 patients: 3 missense variants, 1 deletion including exon 1 of *KCNQ2*, described below), *GABRB3* (3, including Patient 61, who had a mosaic variant), *HCN1, SCN8A*, and *SMC1A* (1 each) (Supplementary table 2).

We identified two patients with deletions encompassing more than one epilepsy-related gene. Patient 54 had a heterozygous *de novo* 6.8 Mb deletion that included two known EIMFS genes, *SCN1A* and *SCN2A*. Patient 58 had a heterozygous *de novo* 229-kb deletion that included exon 1 of *KCNQ2*, which we considered likely to be causative; the deleted region also included *EEF1A2*, implicated in epilepsy and ID[30]. Given the established gene-disease association between *KCNQ2* and EIMFS, we attributed pathogenicity to *KCNQ2*, but it is possible that *EEF1A2* played a role as well, particularly as *KCNQ2* truncations and deletions are typically associated with self-limited neonatal seizures while missense and inframe deletions are associated with severe phenotypes.

Eight patients with EIMFS from 6 consanguineous families harbored homozygous recessive variants, involving *BRAT1* and *SLC12A5* in 2 families each, and *PLCB1* and *SLC25A22* each in one family[13,15,16]. We found compound heterozygous variants in *BRAT1* (3 patients from 2 families), *QARS* (1 singleton), *SLC12A5* (2 siblings from 1 family),[13] and *TBC1D24* (4 singletons).

EIMFS phenotype-genotype correlation

Phenotypic data are summarized according to each gene in Table 1. We highlight the phenotypes of the two most commonly identified genes for EIMFS here.

KCNT1 EIMFS patients (n=36)—Thirty-six patients (20 female) with *KCNT1* variants were studied at age 2 months to 16 years (median 3 years 9 months). Median seizure onset was 3.5 weeks (range day 1 to 5 months). All had severe to profound impairment, and none was seizure-free. Ten had microcephaly. Ten had spasticity, 2 scoliosis, and 6 movement disorders. EEG data was available for 35/36 patients and showed burst suppression in 8/35, hypsarrhythmia or modified hypsarrhythmia in 8/35, and electrodecremental patterns in 4/35 (Supplementary table 1). MRI brain studies in 19 individuals showed a combination of abnormal or delayed myelination, thin corpus callosum, white matter hyperintensities, and progressive cerebral atrophy. Brain MRI was normal in 17 patients. Nine had died, at a median age of 4 years.

SCN2A EIMFS (n=10)—There were 10 individuals (7 female) with *SCN2A* EIMFS[7] studied at age 12 months to 15 years (median 6 years) with median seizure onset at 3.5 days (range day 1 to 8 weeks). All EEGs showed multifocal spikes, 2 patients had burst-suppression, 1 hypsarrhythmia and 1 electrodecremental pattern. All patients had MRIs performed, 8 more than 1 month after seizure onset. 5/8 were abnormal with findings ranging from cerebral atrophy, white matter hyperintensities and, in one case, prominent cerebellar foliae and bilateral hippocampal sclerosis. Six patients had profound and 2 severe

developmental impairment. Three patients had one or more year of seizure freedom, and one had normal developmental outcome. Eight patients had movement disorders and three were microcephalic. Patient 39 died at 22 months with increasing seizures and aspiration pneumonia.

Discussion

We describe the phenotypic spectrum and genetic landscape of 135 patients with EIMFS. EIMFS is an age-dependent DEE syndrome characterised by interhemispheric migration of focal seizures with onset in the first 6 months of life. We highlight the extensive genetic heterogeneity of EIMFS (Figure 1), which is similar to that in other epilepsy syndromes such as infantile spasms (West syndrome) and Lennox-Gastaut syndrome, but with a considerably higher yield on current testing. We identify the genetic etiology in 69% of our cohort, including 9 novel EIMFS genes and 14 of 24 previously reported EIMFS genes. The most commonly involved genes were *KCNT1* (27%) and *SCN2A* (7%), together explaining 34% of EIMFS. This pattern differs from that reported in patients with neonatal-onset DEEs, [31] including Ohtahara Syndrome,[32] for which *KCNQ2* and *SCN2A* are most common. Our yield is higher than in other series of heterogeneous DEEs that find a pathogenic variant in about 50% of cases.[24,31–35] These studies highlight the importance of classifying a patient's epilepsy syndrome, which influences genetic testing and interpretation.[36]

Phenotypic features of our EIMFS cohort

All patients had onset of seizures in the first 6 months of life characterized by electrical and/or clinical interhemispheric seizure migration, associated with significant impact on development. We identify several key phenotypic features not well recognised as part of the EIMFS spectrum: 22% patients had epileptic spasms, 2 patients had normal outcome, and 45/135 children died, with an overall mortality of 33%.

Epileptic spasms have only been rarely reported in EIMFS.[3] In this series, 30/135 patients (22%) had epileptic spasms, including those with *KCNT1, SCN2A, SCN1A, GABRB3, CKDL5, PIGA, BRAT1, TBC1D24, AIMP1, QARS* and *WWOX* pathogenic variants. Epileptic spasms are a hallmark of *CDKL5* encephalopathy, but EIMFS had not been previously described in this disease. Whether other genes predispose to epileptic spasms in patients with EIMFS will require larger cohorts to enable phenotype-genotype correlation.

The median age of seizure onset was 4 weeks, driven in part by 36 patients with *KCNT1* EIMFS with median onset age 3.5 weeks. Interestingly, genes previously associated with seizure onset in the first week of life, *SCN2A, KCNQ2,* and *BRAT1*, had similar onset ages in EIMFS patients.

While seizure outcome and developmental prognosis are generally poor in EIMFS, there are rare reports of mildly affected patients.[37] While all patients experienced refractory epilepsy early in their course, 7 became seizure-free at a median age of 24 months, including 3 *SCN2A* patients with median offset at 3.5 months[7]. We observed severe to profound intellectual disability in 95% of patients, However, Patient 58 (*KCNQ2*), and Patient 40 (*SCN2A)* had normal developmental outcome. Mild to moderate impairment occurred in

Patient 44 (*SCN2A*), Patients 55 and 56 (*KCNQ2*), Patient 78 (*SLC12A5*), and Patient 124 (unsolved).

Other features included microcephaly (49%), spasticity (30%), movement disorder (27%), and premature death (33%). Median age of death was 2 years 7 months (45 patients). This rate is higher than in Dravet syndrome (17% by 20 years) and comparable to Ohtahara Syndrome (30% by 8 months).[32,38] Of the genetically solved patients, 9/36 (25%) of patients with *KCNT1* variants and 4/5 with *BRAT1* variants died. The mortality in the unsolved patients was 48% (20/42).

Novel genetic causes of EIMFS

We identified 9 novel EIMFS genes in our cohort—dominant genes *GABRA1, GABRB1, ATP1A3*; X-linked genes *CDKL5, PIGA;* and recessive genes *ITPA, AIMP1, KARS*, and *WWOX*—encoding a wide range of proteins. These genes have not been described in patients with EIMFS, but they have been associated with other epilepsy syndromes, including infantile spasms and Dravet Syndrome (Supplementary table 2).

Considering recessive EIMFS genes first, here we implicated three genes that form part of the multi-tRNA synthetase complex (MSC); two new to EIMFS (*KARS* and *AIMP1*) and one previously reported in a sibling pair with EIMFS (*QARS*[39]). KARS and QARS combine with other aminoacyl-tRNA synthetases (ARS) and three scaffolding proteins (AIMP1, AIMP2 and AIMP3) to form the multi-tRNA synthetase complex. There were several unifying features among our patients with variants in these multi-tRNA synthetase complex genes. The patients with *AIMP1* and *QARS* variants had microcephaly, epileptic spasms, and spasticity; the patient with the *KARS* variant died by age 6 months. These findings suggest that other multi-tRNA synthetase complex genes could be associated with EIMFS.

We establish a role for *ITPA* in EIMFS. *ITPA* encodes inosine triphosphate pyrophosphatase, which removes non-canonical purines from the cell and is important in DNA and cell cycle integrity. Our patient shares features with published cases, including hypotonia, microcephaly and movement disorder.[28] Reported patients have an early infantile encephalopathy with epilepsy; however, the epileptology has not been delineated. The MRI for our patient showed abnormal signal in the corticospinal tracts, particularly the posterior limbs of internal capsules, as previously reported;[28] interestingly, our patient's MRI additionally showed increased T2-weighted signal in the bilateral basal ganglia.

We present the first case of EIMFS (Patient 93) associated with biallelic compound heterozygous variants of *WWOX* in the context of profound ID, microcephaly, and scoliosis. Reported *WWOX* encephalopathy cases[29] have had DEEs with infantile spasms and Lennox-Gastaut syndrome; our patient also developed epileptic spasms.

Ion channel genes featured as novel dominant causes of EIMFS. We emphasize the role of GABA-related mechanisms by implicating two new GABA receptor subunit genes, *GABRA1* and *GABRB1*, along with our finding that three patients had variants in the known EIMFS gene *GABRB3*.[40] *GABRG2* has also recently been associated with EIMFS. [41] We thus expand the phenotypic spectrum of GABA subunit genes to include mild Genetic

Generalized Epilepsies and a range of DEEs, including EIMFS, infantile spasms, Dravet syndrome, and early infantile DEE (Supplementary table 2). *ATP1A3*, encoding a sodium-potassium ATPase, has been previously associated with alternating hemiplegia of childhood, rapid-onset dystonia-parkinsonism and early onset DEEs.[25,26] Our 3 year old patient had EIMFS and a hyperkinetic movement disorder and hemiplegic episodes.

X-linked inheritance has not been reported before in EIMFS. *PIGA*, encoding phosphatidylinositol glycan anchor biosynthesis class A, is involved in the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor protein and is associated with a range of phenotypes from paroxysmal nocturnal hemoglobinuria to Ohtahara syndrome.[32,42,43] Pathogenic variants in *CDKL5* cause a well-recognized infantile DEE, predominantly in females although rare males are reported.[44,45] Our male patient has an in-frame 3-base pair deletion at a site previously mutated in a girl with *CDKL5* encephalopathy with well controlled epilepsy.[27] We hypothesize that this variant results in a hypomorphic effect on the CDKL5 protein and thus a milder phenotype in the reported girl[27] and permits survival in a non-mosaic male, albeit with the severe presentation of EIMFS.

Mosaicism

Mosaicism is increasingly recognized in human genetic disease, with critical implications for causation and recurrence risk counseling.[46] We identified two probands with mosaic variants in *SCN2A* and *GABRB3*, causing EIMFS; they were as severely affected as patients with germline variants. For some diseases, the percentage mosaicism of a pathogenic variant correlates with disease severity,[47] however, our patients had profound impairment. Potential variability in percentage mosaicism between tissues, such as brain and blood, has been hypothesised to explain these differences.

Mosaicism in a proband means there is minimal recurrence risk for the proband's parents. In contrast, we found three parents who were mosaic for their child's variant, placing them at risk of having additional affected children. A dedicated evaluation for parental mosaicism in all patients that initially appear to be *de novo* may have important implications for counseling, particularly if one parent has had epilepsy, febrile seizures, or neurological disorder.[46]

Inheritance and genetic counseling

We observed several genetic patterns across our cohort, with most patients harboring *de novo* heterozygous pathogenic variants (Figure 2). One child from a consanguineous family had a *de novo* heterozygous *KCNT1* variant, highlighting that patients from consanguineous pedigrees are also subject to the stochastic nature of *de novo* mutagenesis.

We observed autosomal dominant variants inherited from unaffected parents, including three who were mosaic for the variants, and from an affected parent with milder epilepsy. We also observed autosomal recessive inheritance in 17% of our cohort: homozygous variants in children of parents who were consanguineous or from the same geographic region, and compound heterozygous variants in non-consanguineous pedigrees. While recessive inheritance has been considered rare in DEEs,[48] it should be considered in both

consanguineous and non-consanguineous families. Finally, we observed X-linked recessive inheritance with a *PIGA* variant in an affected boy inherited from his unaffected mother.

In summary, we present 135 patients of EIMFS, for whom 69% have identified genetic etiologies, including 9 novel and 14 known EIMFS genes in our cohort, bringing the total number of EIMFS genes to 33. This heterogeneous landscape points to diverse mechanisms leading to EIMFS. We highlight the complex genetic architecture of EIMFS as variants arise *de novo*, and may be mosaic, or are inherited, from affected or unaffected parents. As pathogenic variants are identified in larger numbers of EIMFS patients, stronger genotype-phenotype correlations will emerge and inform prognosis and comorbidities. Early precise genetic diagnosis will allow timely direction of patients to appropriate therapies, accurate reproductive counseling, and future innovative precision medicine trials for this devastating syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

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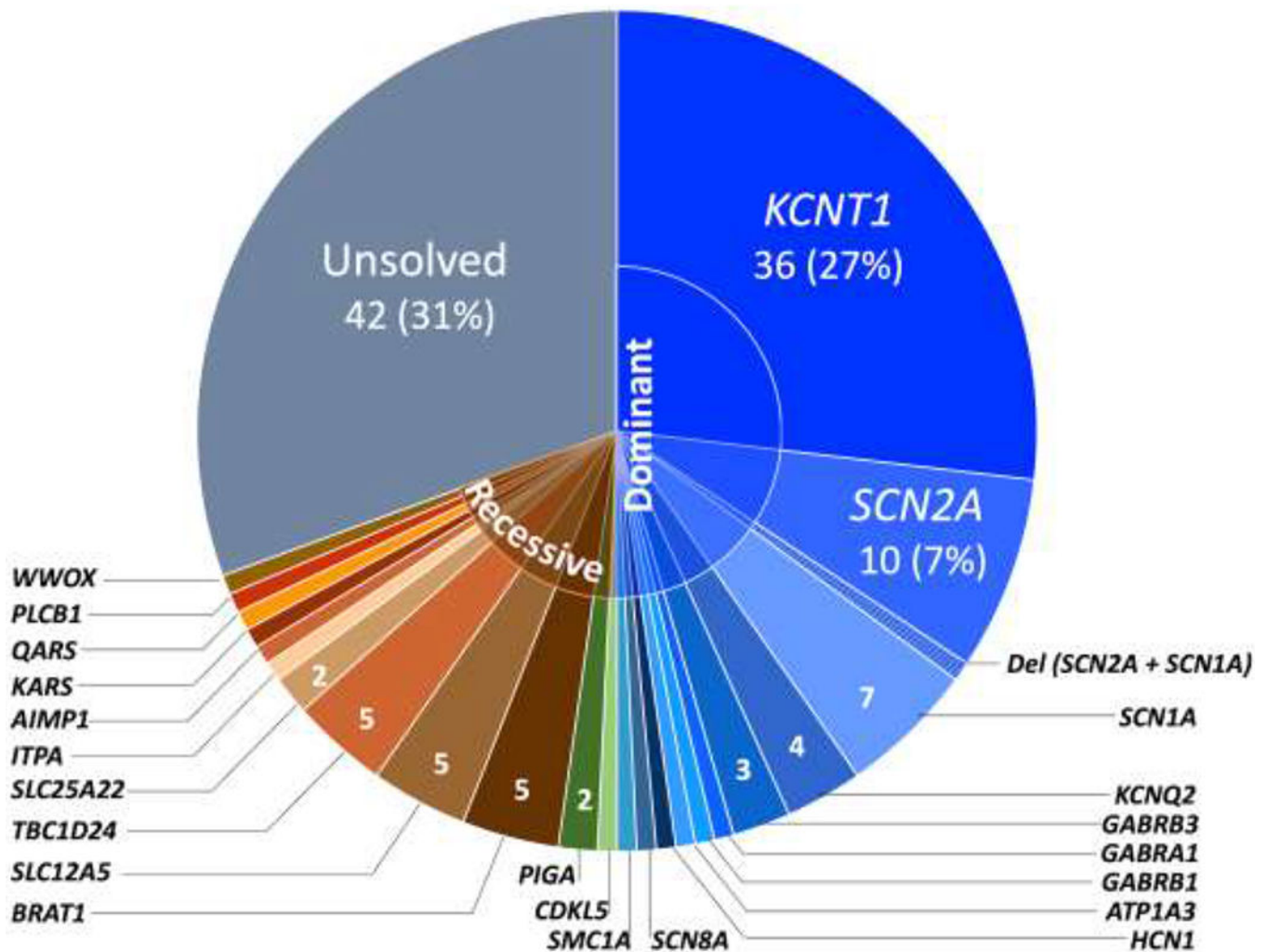
Figure 1.
The molecular genetic landscape of EIMFS. 69% of patients have an identified molecular cause: 50% patients have variants in genes that follow dominant inheritance (blue, most are *de novo*), rare patients have X-linked genes (green) and rare patients have variants following homozygous or compound heterozygous (brown) patterns. Bold gene names are genes newly associated with EIMFS. Numbers indicate the number of patients with a pathogenic variant in each gene in our cohort.

Figure 2.
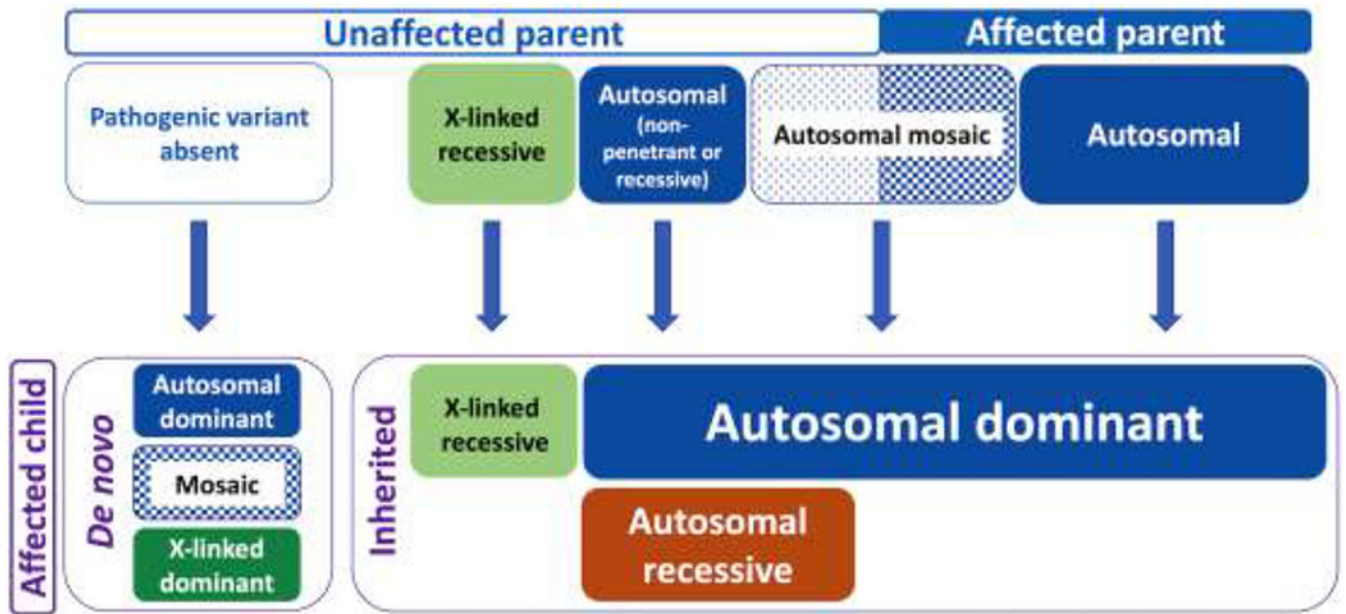Inheritance patterns of pathogenic variants associated with EIMFS. Autosomal dominant, recessive, and X-linked inheritance may occur. Mosaicism should be considered in affected children as well as unaffected and affected parents. Lower levels of mosaicism are more likely to be present in unaffected individuals, while higher levels of mosaicism can occur with mildly or severely affected individuals.[46]

Table 1

Phenotype-genotype data of 135 patients with EIMFS.

Gene (n)	Age (#) D(n); age(#)	Age sz onset (#)	Sz types at onset: F;T;C;O (n)	Sp (n)	Sz-free (n)	Micro-cephaly (n)	Profound-severe Impairment (n)	Motor Disorders: S;H;M;Sc (n)
Cohort (135)	3.4y D45; 2.6y	4w	F121;T5;C3;O6	30	7	61/124 ^	127/134 ^	S40; H82; M36; Sc16
Dominant Genes (67/135, 50%, 22 male)								
KCNT1 (36)	3.2y D9; 4y	3.5w	F31;T2;C1;O2	7	-	10/32	36	S10; H22; M6; Sc2
SCN2A (10)	6y D1; 22m	3.5d	F9;C1	4	3	3	8	S3; H6; M8; Sc;2
SCN2A/1A (1)	18m	6w	F	-	-	-	1	-
SCN1A (7)	2.3y D2; 2.3y,12y	2w *	F7	1	-	1	7	S1; H1; M1; Sc2
KCNQ2 (4)	4.8y	3d	F4	-	3	1	1	S1; H2; M1; Sc1
GABRB3 (3)	4.4y D1; 4.5y	8w	F2;C1	1	-	2	3	S2; H3; M3
GABRA1 (1)	4.3y	2m	F	-	-	-	1	H
GABRB1 (1)	2y	4m	F	-	1	-	1	H
ATP1A3 (1)	3y	4w	F	-	-	-	1	H; M
HCN1 (1)	14m - D	2d	T	-	-	1	1	S; H; M
SCN8A (1)	6m - D	1d	F	-	-	-	1	-
SMC1A (1)	9m - D	7w	T	-	-	1	1	H
X-linked Genes (3/135, 2%, 3 male)								
CDKL5 (1)	3m	4w	F	1	-	-	1	H; M
PIGA (2)	2y, 3.8y	2m,5m	F2	2	-	-	2	S2; H2; M2
Recessive Genes (23/135, 17%, 11 male)								
BRAT1 (5)	10m D4; 6m	1d *	F4;O1	1	-	5	5	S5; M1
SLC12A5 (5)	5y D1; 2.5y	3m	F5	-	-	4	4	H3
TBC1D24 (5)	2.1y	2d	F5	1	-	2	5	-
SLC25A22 (2)	14m - D 3.9y - D	1w,2w	F2	-	-	2	2	H2
ITPA (1)	18m - D	1d	F	-	-	1	1	H; M
AIMP1 (1)	4y	6w	F	1	-	1	1	S; M; Sc
KARS (1)	6m - D	2m	F	-	-	-	1	-
QARS (1)	3y	4w	F	1	-	1	1	S
PLCB1 (1)	7m	6m	F	-	-	-	1	H
WWOX (1)	6y	2.5m	F	1	-	1	1	S; H; Sc
Unsolved (42/135, 31%, 20 male)								
Unsolved (42)	4y D20; 3.2y	5w	F38;T1;O3	9	-	25/35	40/41	S12; H32; M9; Sc7

Abbreviations: C=clonic; d=day; D=deaths; F=focal; H=hypotonia; m=months; M=movement; n=number; O=other (includes reflex, myoclonic, tonic-clonic, spasms); S=spasticity; Sc=scoliosis; Sp=epileptic spasms; Sz=seizure; T=tonic; w=week; y=year; #=median;

^
denotes data not available on all patients so denominator stated where applicable;

*
in utero seizures suspected

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Table 2

Variants in novel EIMFS genes and *in silico* analysis of pathogenicity

Patient	Gene	Inheritance	gDNA (GRCh37/hg19)	Amino acid	rs ID	ExAc and gnomAD#	Poly-phen	SIFT	Ref	How identified	ACMG Classification[49]
62	GABRA1	De Novo	Chr5:16131 8039C>T	p.P280L	NA	0	PD	D	NA	WGS	LP
63	GABRB1	De Novo	Chr4:47405633T>C	p.I247T	NA	0	B	D	NA	WES	LP
64	ATP1A3	De Novo	Chr19:42472985A>G	p.L924P	NA	0	PD	D	NA	WES	LP
68	CDKL5	De Novo	ChrX:18606120_18606122del	p.L201del	NA	0	NA	NA	NA	NGS panel	LP
69	PIGA	Maternal^	ChrX:15349697C>T	p.R119Q	NA	0	B	D	NA	WES	LP
70	PIGA	De Novo	ChrX:15349649G>A	p.A135V	NA	0	PD	D	14	NGS panel	LP
88	ITPA	Both parents	Chr20:3202527G>A	p.W151*	rs200086262	5/120822 15/282558	NA	NA	28	WES	P
89	AIMP1	Maternal	Chr4:107248643C>T	p.R73*	rs373122636	4/120776 6/251184	NA	NA	NA	WES	LP
		Paternal	Chr4:107248689_107248690del	p.Q88Rfs*25	rs766457850	0 5/250996	NA	NA	NA	WES	LP
90	KARS	Maternal	Chr16:75669578C>G	p.E293D	NA	0	PD	D	NA	NGS panel	VUS
		Paternal	Chr16:75678291dup	p.S13Vfs*34	NA	0	NA	NA	NA	NGS panel	LP
93	WWOX	Maternal	Chr16:78133724G>A	p.E17K	rs780345312	0 2/196758	PD	D	29	WES	VUS
		Paternal	Intronic Deletions: Chr16:78,180,536-78,185,186 x1 and Chr16:78,200,089-78,218,016 x1	NA	NA	0	NA	NA	NA	CMA	VUS

Abbreviations: B = Benign; CMA = clinical microarray; D = Deleterious; ExAC = Exome Aggregation Consortium; gnomAD = Genome Aggregation Database; LP = likely pathogenic; NA = Not applicable; NGS = Next Generation Sequencing; P = pathogenic; PD = Possibly Damaging; SIFT = Sorting Intolerant from Tolerant; VUS = variant of unknown significance; WES = Whole Exome Sequencing; WGS = Whole Genome Sequencing; # Where applicable no variants were seen as homozygotes or hemizygotes variants in gnomAD;

^
asymptomatic mother