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RESEARCH ARTICLE

# Dissecting genetics of spectrum of epilepsies with eyelid myoclonia by exome sequencing

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## Abstract

**Objective:** Epilepsy with eyelid myoclonia (EEM) spectrum is a generalized form of epilepsy characterized by eyelid myoclonia with or without absences, eye closure-induced seizures with electroencephalographic paroxysms, and photosensitivity. Based on the specific clinical features, age at onset, and familial occurrence, a genetic cause has been postulated. Pathogenic variants in *CHD2*, *SYNGAP1*, *NEXMIF*, *RORB*, and *GABRA1* have been reported in individuals with photosensitivity and eyelid myoclonia, but whether other genes are also involved, or a single gene is uniquely linked with EEM, or its subtypes, is not yet known. We aimed to dissect the genetic etiology of EEM.

**Methods:** We studied a cohort of 105 individuals by using whole exome sequencing. Individuals were divided into two groups: EEM− (isolated EEM) and EEM+ (EEM accompanied by intellectual disability [ID] or any other neurodevelopmental/psychiatric disorder).

**Results:** We identified nine variants classified as pathogenic/likely pathogenic in the entire cohort (8.57%); among these, eight (five in *CHD2*, one in *NEXMIF*, one in *SYNGAP1*, and one in *TRIM8*) were found in the EEM+ subcohort (28.57%). Only

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one variant (*IFIH1*) was found in the EEM− subcohort (1.29%); however, because the phenotype of the proband did not fit with published data, additional evidence is needed before considering *IFIH1* variants and EEM− an established association. Burden analysis did not identify any single burdened gene or gene set.

**Significance:** Our results suggest that for EEM, as for many other epilepsies, the identification of a genetic cause is more likely with comorbid ID and/or other neurodevelopmental disorders. Pathogenic variants were mostly found in *CHD2*, and the association of *CHD2* with EEM+ can now be considered a reasonable gene–disease association. We provide further evidence to strengthen the association of EEM+ with *NEXMIF* and *SYNGAP1*. Possible new associations between EEM+ and *TRIM8*, and EEM− and *IFIH1*, are also reported. Although we provide robust evidence for gene variants associated with EEM+, the core genetic etiology of EEM− remains to be elucidated.

**KEYWORDS**
*CHD2*, generalized epilepsy, *IFIH1*, *NEXMIF*, *SYNGAP1*, *TRIM8*

## 1 | INTRODUCTION

Epilepsy with eyelid myoclonia (EEM) is a generalized form of epilepsy first described by Jeavons in 1977.[1,2] It is clinically characterized by the triad of eyelid myoclonia (EM) with or without absences, eye closure-induced seizures with electroencephalographic (EEG) paroxysms, and photosensitivity.[3,4] Onset is typically in childhood, with a peak at approximately 8 years of age (typical range = 2–14) and a preponderance among girls.[4,5] EM is the clinical hallmark, consisting of jerking of the eyelids often associated with jerky upward deviation of the eyeballs and retropulsion of the head. However, the presence of only EM does not meet the criteria for the syndromic diagnosis, as EM can be observed as an isolated trait in a range of conditions, including otherwise healthy individuals. Furthermore, some features of EEM, including eye-closure sensitivity, EM, and photosensitivity, are not specific to EEM and may be present in other epilepsy syndromes.[6]

Although this is a well-characterized electroclinical condition, only recently has EEM been included among the genetic generalized epilepsies (GGEs) by the International League Against Epilepsy (ILAE) classification.[2] Genetic susceptibility to EEM has been suggested, based on the specific clinical features, age at onset, and especially, familial and twin occurrence.[5,7–9] So far, a unique gene for this condition has not been identified. Although the main feature of this syndrome is EM, photoparoxysmal response (PPR) is also very common in EEM. PPR is a pathological EEG activity induced by intermittent photic stimulation.[10] PPR probably has significant genetic heterogeneity and an overall complex genetic architecture, with linkage reported to several loci.[11] Photosensitivity is an abnormal clinical sensitivity to flickering light, often associated with PPR on EEG.[10] Photosensitivity occurs in some epileptic encephalopathies, such as Dravet syndrome due to *SCN1A* variants and encephalopathy associated with *CHD2* variants.[12–14] *CHD2* has also been implicated in epilepsy with photosensitivity due to 15q26.1 deletion.[15] In a study of 238 individuals with GGEs with photosensitivity, de novo *CHD2* variants were found in three of 36 individuals with EEM.[16] Although this was the first gene to be associated with EEM, *CHD2* variants had also been reported in association with other epileptic conditions with varying degrees of severity,[12–14] all of which share photosensitivity. Thus, *CHD2* is currently considered a gene in which

**Key points**

- Pathogenic/likely pathogenic variants affecting *CHD2*, *NEXMIF*, *SYNGAP1*, and *TRIM8* are found in nearly 30% of EEM+ individuals
- Pathogenic variants affecting *CHD2* and EEM+ can be considered a reasonable gene–disease association
- The core genetic etiology of both EEM+ and EEM− remains to be elucidated
- This genetic study and recent literature suggest that EEM is a spectrum of conditions, where EEM with comorbidities may be a different entity from isolated EEM

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some variants are associated with photosensitive epilepsy rather than with EEM in particular. Besides *CHD2*, variants in other genes have been reported in association with an EEM phenotype, including *SYNGAP1*, *NEXMIF*, and *RORB*. Loss of function variants in *SYNGAP1* variants have been reported in people presenting with EEM associated with moderate to severe intellectual disability (ID) alone and in association with reflex eating seizures.[17,18] In one third of these individuals with *SYNGAP1* variant, the EEM evolved into myoclonic–atonic seizures or atonic seizures.[18] *NEXMIF* loss of function variants have been reported in females with delayed motor milestones, impaired language development, moderate to profound ID, behavioral abnormalities including autism-spectrum disorder, and an early onset seizure phenotype consisting of EEM overlapping with myoclonic–atonic epilepsy.[19] Variants in *RORB* have been found in people with EEM associated with bilateral tonic–clonic seizures.[20] In addition, variants in *GABRA1* have been described in photosensitive GGE.[21]

These data suggest that the genetic etiology of EEM is heterogeneous; however, the full spectrum is yet to be discovered, and most studies have taken a candidate gene approach. Here, we aimed to unravel the genetic etiology of EEM using a genome-wide approach in a large cohort of well-characterized individuals.

## 2 | MATERIALS AND METHODS

This research was approved by local review boards or ethics committees (number 11/LO/2016). For all individuals, written informed consent for research use of clinical and genetic data was obtained from patients, their parents, or legal guardians in the case of minors or those with ID.

Burden analysis was conducted by comparing the data with control groups from the Epi25 Collaborative study. Subjects were ascertained by the Epi25 Collaborative and provided signed informed consent at the participating centers according to local or national ethical requirements and their standards at the time of collection. Data reuse and analysis were approved by the Epi25 Collaborative (cases) and dbGaP (controls).

### 2.1 | Selection of patients

Patients with an electroclinical diagnosis of EEM were included in this study according to the ILAE criteria (frequent EM, with or without absences, induced by eye closure and photic stimulation).[2] Exclusion criteria were any of severe or profound ID (according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition), major dysmorphism/organ defects suggestive of chromosomal abnormalities, and a known genetic condition. Only patients selected by a neurologist with expertise in epilepsy were enrolled. Clinical and neurophysiological features of epilepsy, family history, general and neurological examination, neuroradiological findings, and drug history were collected.

The cohort was subdivided into two groups based on recent electroclinical studies[4] suggesting that EEM associated with other clinical features, including a higher rate of ID and behavioral disorders, is a different entity from "EEM-only," with a more benign profile based on seizure remission and a more favorable neuropsychiatric outcome. Thus, the recruited individuals were divided into EEM+ (EEM accompanied by ID or any other neurodevelopmental/psychiatric disorder) and EEM− (no ID or other neurodevelopmental/psychiatric disorder associated).

Genomic DNA was obtained from peripheral blood from patients and, where possible, their parents.

### 2.2 | Cohort description

The cohort comprises 151 individuals: 105 people with EEM− or EEM+, and 46 parents. Whole exome sequencing (WES) was undertaken at deCODE genetics (Reykjavik, Iceland; 101 cases), the Telethon Institute of Genetics and Medicine, (Naples, Italy; 18 cases), or University College London (nine cases, including five Australian cases), or through the Epi25 initiative (23 cases).

WES and quality control (QC) methods are described in Data S1.

### 2.3 | Analysis of WES data

#### 2.3.1 | De novo variant analysis

Putative de novo variants (DNVs) in trios were identified using DeNovoGear[22] and selected to have a nonreference genotype in the child, and reference genotypes in both parents. DNV calls were annotated using ANNOVAR[23] and selected based on the following filters: (1) posterior probability for observing a de novo event (pp_dnm) ≥ .9; (2) scaled Combined Annotation Dependent Depletion (CADD) score ≥20; and (3) gene harboring the variant is among the 191 monoallelic or X-linked epilepsy-related genes in the Genomics England UK 100 000 Genomes Project (GEL) Genetic Epilepsy Syndromes (version 2.489) panel, and designated by GEL with a "green" rating (i.e., those for which there is a high level of evidence for gene–disease [epilepsy] association).[24,25] Variants

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that remained following these filters were visually inspected using the Integrative Genomics Viewer (IGV) browser,[26] and further validated in proband and parents using Sanger sequencing technology, where polymerase chain reaction primers were designed to amplify the target regions harboring the variant and the products were Sanger sequenced and analyzed on a 3130XL instrument. Variants with only pp_dnm ≥ .9 and CADD score ≥20 were categorized as "technical candidates" and inspected in IGV if considered clinically relevant by comparing the neuropsychiatric/epilepsy phenotype reported in the literature (PubMed) in association with variants in that specific gene and the phenotype of the individual carrying the specific variant. Clinical relevance was considered when the phenotype overlapped (e.g., reported EM with/without absences, with/without neuropsychiatric conditions). The pathogenicity of the DNVs was evaluated according to the American College of Medical Genetics and Genomics Association (ACMG) guidelines.[27] This de novo analysis thus identified individuals with EEM carrying a DNV in a gene already linked with the condition.

#### 2.3.2 | Screening of candidate genes associated with photosensitivity in epilepsy

The WES data of the entire cohort were screened for variants in five genes (*CHD2*, *NEXMIF*, *GABRA1*, *SYNGAP1*, and *RORB*) known to be associated with photosensitivity and EM as described.[9,16,18,21,28] ANNOVAR[23] was used for variant annotation. The variants were not filtered for frequency or CADD scores.[29] These variants were visually inspected using the IGV browser,[26] and the variants confirmed in IGV were further validated using Sanger sequencing. ACMG guidelines were used to evaluate the pathogenicity of these variants.

To establish the quality and reliability of the variants, the whole exome coverage of each individual of the cohort and the coverage of the capture kit intervals containing these five candidate genes were estimated using Genome Analysis Toolkit v3.5.[30] One consistently inadequately covered interval (<90% coverage at 10× in all exomes) in the *CHD2* gene (15:93521465-93 521 613) was separately Sanger sequenced in 58 cases with available DNA.

### 2.4 | Screening for candidate genes associated with epilepsy

The entire cohort was screened for rare variants across the canonical coding sequences of 191 monoallelic or X-linked epilepsy-related genes from the GEL Genetic Epilepsy Syndromes (version 2.489) panel.[24,25] Only genes designated by GEL with a "green" rating (i.e., those with high level of evidence for gene–disease association) were included. ANNOVAR[23] was used for variant annotation, and the Genome Aggregation Database (gnomAD) dataset was used for variant filtering based on allele frequency and allele count. The filtering criteria for the variants were (1) exonic function: nonsynonymous or splicing; (2) gnomAD allele frequency ≤ .0005 and gnomAD allele count ≤8; and (3) classified as deleterious using at least one prediction tool (Sorting Intolerant From Tolerant [SIFT] score ≤ .0574, PolyPhen score ≥ .1575). The clinical relevance of the filtered variants was then evaluated by comparing the neuropsychiatric and epilepsy phenotype reported in the literature (PubMed) in association with variants in that specific gene and the phenotype of the individual carrying the specific variant. The variant was considered to be clinically relevant if the phenotype was overlapping (reported EM with/without absences, with/without neuropsychiatric conditions). The pathogenicity of the variants was evaluated using the ACMG criteria.

### 2.5 | Autosomal recessive model of analysis

A detailed description is available in Data S1.

### 2.6 | Burden analysis

The study cohort was generated by combining data from different studies after the QC and restricting to the samples of European ancestry (total number = 794). It comprises cases with EEM ($n = 100$), Italian controls from the Epi25 Collaborative ($n = 319$), controls from the MIGen (Myocardial Infarction Genetics) Consortium ($n = 72$), and controls from an Alzheimer study ($n = 303$). The data generation process for the Epi25 Consortium has been described.[31]

A detailed explanation of the variant calling, QC, and statistical analysis is provided in Data S1.

## 3 | RESULTS

### 3.1 | Clinical features of the population

Genomic DNA from 105 individuals (75 females) was collected from three countries (Italy, the UK, and Australia). DNA was available from both parents for 24 individuals (trios) of whom two were siblings (thus, we sequenced 46 parents). The remaining 81 were singletons; among these,

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we also had four siblings from Family EEM_27, two siblings from Family EEM_28, and two from Family EEM_29. A total of 151 individuals were sequenced. Seventy-seven subjects were classified as EEM− (20 singletons) and 28 as EEM+ (19 singletons). Clinical details are summarized in Table 1, with further details in Table S1.

## 3.2 | Genetic results

### 3.2.1 | De novo analysis of trios

The DNVs discovered in our cohort are summarized in Table 2. We identified four DNVs from four trios, which can be deemed pathogenic according to ACMG guidelines and will be discussed here. Twenty-three "technical candidates" were also identified in 11 individuals, but the function of the genes and/or available data on related phenotype are not strong enough to support any conclusion on these variants, which thus remain variants of unknown significance (VUSs; summarized in Table S2).

The proband of trio EEM_16 had a missense DNV in *CHD2* (NM_001271: c.4598T>G, p.Leu1533Arg) that was not present in gnomAD and hence novel. This variant was predicted to be damaging/deleterious by PolyPhen and SIFT and showed a CADD score of 32. This individual had an EEM+ phenotype, with ID and psychiatric disturbances. She had only EM with absences, which were often self-induced or precipitated by stress or intermittent photic stimulation. A second DNV in *CHD2* was found in the proband of trio EEM_19 (NM_001271.4: c.3455+2T>G). This splicing variant had a CADD score of 25.9 and was absent in gnomAD. This individual also had an EEM+ phenotype consisting of ID, behavioral problems, and self-induced, stress- or intermittent photic stimulation-precipitated EM with absences. No other seizure types were reported. For this case, we also obtained RNA from fresh blood and performed a Northern blot analysis showing that the variant leads to the deletion of exon 27 (skipping of the exon) compared to the wild type. The two *CHD2* variants reported in this study are novel.

The proband of Family EEM_05 had a frameshift deletion in *NEXMIF* (NM_001008537: c.2171delG, p.Ser724fs) that was absent in gnomAD. As described,[32] she had drug-resistant epilepsy starting at the age of 9 years with recurrent episodes of prolonged nonconvulsive status epilepticus characterized by mydriasis, EM, and reduced responsiveness to environmental stimuli. She also had mild ID and was thus classified as having EEM+.

The proband of Family EEM_03 had a novel de novo frameshift insertion in *TRIM8* (NM_030912: c.1154dupA, p.Glu385fs), with a CADD score of 32. This variant was absent in gnomAD. *TRIM8* has been little studied; however, one report on this gene suggests its association with epileptic encephalopathy and absences and a possible association with nephrotic syndrome.[33] EM was specifically reported in one individual: Patient 2 from Allen et al.[12] Our proband had an EEM+ phenotype consisting of behavioral disturbances, language disorder, drug-resistant EM associated with bilateral tonic–clonic seizures, and other comorbidities (multiple breast fibroadenoma, pustular acne, keloids). Nephrotic syndrome was excluded in our patient. Notably, the gene product is expressed in the brain, breast, and skin, and is downregulated in breast cancer.[34]

**TABLE 1** Demographic characteristics of the population.

| Characteristic | *n* | Details/range |
|---|---|---|
| Population | 105 affected individuals (30 M, 75 F) | 81 singletons<br>24 from trios |
| Clinical classification | | 77 EEM−<br>28 EEM+ |
| Age at present study, years | 30 (mean) | Range = 11–83 |
| Family history | 71 yes<br>31 no<br>3 NA | |
| ID | 31 yes<br>4 borderline ID<br>68 no<br>2 NA | |
| Onset of epilepsy | 7.68 years old | Range = .75–20 years old |

Abbreviations: EEM, epilepsy with eyelid myoclonia; F, female; ID, intellectual disability; M, male; NA, not available.

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**TABLE 2** List of four pathogenic/likely pathogenic de novo variants identified in four affected probands.

| Family | Gene name | Position (NCBI37) | Consequence | cDNA change | Protein | Computational analysis score, PolyPhen-2 and SIFT | CADD scores (Phred scaled) | Clinical classification | ACMG classification |
|---|---|---|---|---|---|---|---|---|---|
| EEM_03 | *TRIM8* | 10:104416609 | Frameshift insertion | c.1154dupA | p.Glu385fs | Unknown | 32 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| EEM_05 | *NEXMIF* | X:73962221 | Frameshift deletion | c.2171delG | p.Ser724fs | Damaging (1); deleterious (.01) | 34 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| EEM_16 | *CHD2* | 15:93555580 | Missense | c.T4598G | p.Leu1533Arg | Damaging (1); deleterious (0) | 32 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| EEM_19 | *CHD2* | 15:93534749 | Splice site | c.3455+2T>G | NA | No change in donor site | 25.9 | EEM+ | Pathogenic (*PS2*, *PM2*) |

Abbreviations: CADD, Combined Annotation Dependent Depletion; EEM, epilepsy with eyelid myoclonia; NA, not available; SIFT, Sorting Intolerant From Tolerant.

### 3.2.2 | Screening of the five EEM candidate genes

The variants identified through screening of *CHD2*, *NEXMIF*, *GABRA1*, *SYNGAP1*, and *RORB* genes in our cohort are summarized in Table 3.

Ten variants were identified in these five candidate genes. Two novel DNVs in *CHD2* (in probands of Families EEM_16 and EEM_19) and one in *NEXMIF* (proband of Family EEM_05) have already been reported above (see Tables 2 and 3 for comparison).

Four more *CHD2* variants (NM_001271: c.4173dupA, p.Gln1392Tfs*17; NM_001271: c.3454C>T, p.Arg-1152Trp; NM_001271.3: c.2577+7T>C; and NM_001271.3: c.3734dupA, p.Tyr1246IlefsTer13) were identified in four different singletons. The NM_001271.3: c.3734dupA, p.Tyr1246IlefsTer13 variant causing a frameshift insertion was observed in EEM_AUS_24, an individual with EEM+ (EEM, mild ID, and severe depression associated with mania). This variant could not be Sanger confirmed, because further DNA was not available for testing. Computational evidence and family information were not available. However, we could classify the variant as pathogenic according to the ACMG, as it is frameshift within a gene where loss of function is a known mechanism of disease. Furthermore, this variant was previously reported as a de novo pathogenic variant in an individual with EEM,[16] and in another unrelated individual with Lennox–Gastaut syndrome.[35]

The missense *CHD2* variant (NM_001271: c.C3454T, p. Arg1152Trp) was identified in EEM-AUS-21, an individual with EEM+ (EEM, mild ID, language delay, autism spectrum disorder). This variant had a CADD score of 34 and was predicted to be damaging and deleterious by PolyPhen and SIFT, thereby supporting pathogenicity according to ACMG guidelines.[27] The same variant was reported in ClinVar as a likely pathogenic variant for developmental and epileptic encephalopathy.[36] Sanger sequencing confirmed the variant; the variant was not found in the parents; thus, it was considered de novo and classified as pathogenic according to ACMG guidelines.

A splice site variant in *CHD2* (NM_001271.3: c.2577+7T>C) was observed in EEM-BR-05. This individual had only EEM. The same variant was confirmed by Sanger sequencing and also found in the unaffected mother. There is a matrilineal history of epilepsy (not otherwise specified). The higher gnomAD minor allele frequency (.003) and the nonsegregation with the condition support a benign classification for this variant, according to the ACMG guidelines. Our observation supports an earlier ClinVar report that also classified this variant as a benign variant in the case of developmental and epileptic encephalopathy.

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We identified a novel frameshift variant in *CHD2* (NM_001271.3: c.3734dupA), in EEM-CT-11, an EEM+ individual with mild ID. This variant was confirmed through Sanger sequencing. Although we lack family data, we could classify the variant as pathogenic according to the ACMG, as it is frameshift within a gene where loss of function is a known mechanism of disease.[27]

A novel variant in *NEXMIF* (NM_001008537: c.G370A, p.Ala124Thr) was observed in one singleton (EEM-CT-10). This individual is a female who had EEM. Multiple lines of computational evidence (PolyPhen score and prediction = 0, benign; SIFT score and prediction = .318, tolerated; CADD score = .866) support a benign impact.[27]

Two further rare novel variants were identified in *SYNGAP1* (NM_006772: c.1531+1G>T) and *GABRA1* (NM_000806: c.C1051A, p.Pro351Thr). The former was a splice site variant found in EEM-AUS-22, an individual with EEM+ (EEM with developmental delay, speech and language disorder, and mild to moderate ID, drug resistance, and ataxia). Sanger sequencing confirmed the variant in the proband and its absence in parents; thus, it was considered de novo. According to ACMG guidelines, the absence in gnomAD and the de novo nature of the variant support pathogenicity.[27] The *GABRA1* (NM_000806: c.C1051A, p.Pro351Thr) variant was missense, found in EEM-UK-02, an individual with EEM−, with a CADD score of 24.2 and predicted to be probably damaging (PolyPhen score = .497) but tolerated (SIFT score = .201). This variant could not be Sanger confirmed. Due to the lack of segregation data and computational evidence, the variant was classified as a VUS. We did not find any variants of interest in *RORB*.

### 3.2.3 | Screening of 191 monoallelic or X-linked epilepsy-related genes in the GEL Genetic Epilepsy Syndromes (version 2.489) panel

The 56 variants from this analysis are tabulated in Table S3. Seven variants occurred in more than one individual: six in two individuals and one in three individuals (Table 4).

Three variants (*CACNA1E* [NM_001205294: c.G6161A, p.Arg2054His], *MBD5* [NM_018328: c.C1535T, p.Ser512Phe], *NBEA* [NM_015678: c.A4631G, p.N1544S]) were present in a proband and an unaffected parent. These variants were likely benign due to their lack of segregation.

A variant in *IFIH1* (NM_022168: c.G1853A, p.Arg618Gln) was found in a proband (EEM-MO-04) affected by EEM and her unaffected mother. There was a matrilineal history of epilepsy (two cousins and one aunt with epilepsy). Multiple lines of computational evidence support a deleterious effect on the gene and support pathogenicity according to ACMG guidelines; it was classified as likely pathogenic.

A variant in *DNM1L* (NM_001278463: c.C305T, p.Thr102Met) was found in three individuals: two unrelated affected individuals and the unaffected mother of one of them (with an unspecified matrilineal family history of epilepsy). This variant has a gnomAD frequency of .000223471 and a CADD score of 26.7, and was predicted to be damaging (PolyPhen score = .951) but tolerated (SIFT score = .173). Further information on the cosegregation of this variant with the disease in multiple affected family members is required to confirm the pathogenicity of this variant according to ACMG guidelines.[27]

Two variants were found in two unrelated individuals; the variant *CACNA1G* (NM_001256332: c.A3372C, p.Glu1124Asp) was found in an individual whose parents were not available for testing and one unaffected father whose affected child did not carry the variant. This variant is predicted to be damaging (PolyPhen score = .938) but tolerated (SIFT score = .239), with a CADD score of 23.9. The variant was reported in gnomAD with a frequency of .0000637105 and was classified as a VUS. The variant in *SETBP1* (NM_001130110: c.G46A, p.Glu16Lys) was found in two unrelated, affected individuals (EEM-BR-02, whose parents were not available for testing; and EEM-SS-13, whose three affected siblings that did not carry the variant). This variant was predicted to be probably damaging (PolyPhen score = .538) and deleterious (SIFT score = .006), with a CADD score of 24.7. The variant was reported in gnomAD with a frequency of .000063743 and was classified as a VUS.

The remaining 41 variants were unique in the proband cohort (present in only one individual); five variants occurred in unaffected parents as well. At present, there is not sufficient evidence to state that the remaining 36 variants could be associated with the EEM phenotype observed in these cases. Thus, these remaining variants are not discussed further.

### 3.2.4 | Autosomal recessive model of analysis

The cohort did not show any homozygous candidate variants for EEM. However, possible compound heterozygosity was found in 10 individuals (six singletons, one unaffected mother, one proband and the unaffected mother of the proband, one unaffected father). IGV confirmed two heterozygous variants; results are summarized in Table S4. We could not determine whether these heterozygous variants are compound heterozygous, and these variants are not discussed further (Table 4).

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**TABLE 3** Variants identified through screening of *CHD2*, *NEXMIF*, *GABRA1*, *SYNGAP1*, and *RORB* genes.

| Sample | Gene name | Position (NCBI.37) | Consequence | cDNA change |
|---|---|---|---|---|
| EEM-AUS-24 | *CHD2* | 15:93545442 | Frameshift insertion | c.4173dupA |
| EEM-AUS-21 | *CHD2* | 15:93534746 | Missense | c.C3454T |
| EEM-BO-09 (EEM_19 proband) | *CHD2* | 15:93534749 | Splice site | c.3455+2T>G |
| EEM-BO-06 (EEM_16 proband) | *CHD2* | 15:93555580 | Missense | c.T4598G |
| EEM-BR-05 | *CHD2* | 15:93518187 | Splice site | c.2577+7T>C |
| EEM-CT-11 | *CHD2* | 15:93540315 | Frameshift variant | c.3734dupA |
| EEM-MO-02 (EEM_05 proband) | *NEXMIF* | X:73962221 | Frameshift deletion | c.2171delG |
| EEM-CT-10 | *NEXMIF* | X:73964022 | Missense | c.G370A |
| EEM-AUS-22 | *SYNGAP1* | 6:33406341 | Splice site | c.1531+1G>T |
| EEM-UK-02 | *GABRA1* | 5:161322866 | Missense | c.C1051A |

Abbreviations: ACMG, American College of Medical Genetics and Genomics Association; CADD, Combined Annotation Dependent Depletion; EEM, epilepsy with eyelid myoclonia; NA, not available; SIFT, Sorting Int olerant From Tolerant.

**TABLE 4** List of shared variants, identified in the cohort, in 191 epilepsy-related genes of the GEL Genetic Epilepsy Syndromes (version 2.489) panel.

| Sample | Gene name | Position (NCBI.37) | Consequence | cDNA change | Protein |
|---|---|---|---|---|---|
| EEM_22 mother | *CACNA1E* | 1:181765942 | Missense | c.G6161A | p.Arg2054His |
| EEM-SS-05 (EEM_22 proband) | *CACNA1E* | 1:181765942 | Missense | c.G6161A | p.Arg2054His |
| EEM_16 father | *CACNA1G* | 17:48676971 | Missense | c.A3372C | p.Glu1124Asp |
| EEM-TO-01 | *CACNA1G* | 17:48676971 | Missense | c.A3372C | p.Glu1124Asp |
| EEM-NA-05 | *DNM1L* | 12:32861094 | Missense | c.C305T | p.Thr102Met |
| EEM-NA-03 (EEM_03 proband) | *DNM1L* | 12:32861094 | Missense | c.C305T | p.Thr102Met |
| EEM_03 mother | *DNM1L* | 12:32861094 | Missense | c.C305T | p.Thr102Met |
| EEM-MO-04 (EMA_06 proband) | *IFIH1* | 2:163134116 | Missense | c.G1853A | p.Arg618Gln |
| EEM_06 mother | *IFIH1* | 2:163134116 | Missense | c.G1853A | p.Arg618Gln |
| EEM-MO-09 (EEM_10 proband) | *MBD5* | 2:149227047 | Missense | c.C1535T | p.Ser512Phe |
| EEM_10 mother | *MBD5* | 2:149227047 | Missense | c.C1535T | p.Ser512Phe |
| EEM-BO-09 (EEM_19 proband) | *NBEA* | 13:35751209 | Missense | c.A4631G | p.Asn1544Ser |
| EEM_19 father | *NBEA* | 13:35751209 | Missense | c.A4631G | p.Asn1544Ser |
| EEM-BR-02 | *SETBP1* | 18:42281357 | Missense | c.G46A | p.Glu16Lys |
| EEM-SS-13 (EEM_27 proband) | *SETBP1* | 18:42281357 | Missense | c.G46A | p.Glu16Lys |

Abbreviations: ACMG, American College of Medical Genetics and Genomics Association; CADD, Combined Annotation Dependent Depletion; EEM, epilepsy with eyelid myoclonia; SIFT, Sorting Intolerant From Tolerant; TCS, tonic–clonic seizure.

### 3.2.5 | Burden analysis

After performing a stringent sample QC and excluding outliers (see Table S5, Figures S1–S6), we included 694 controls and 100 cases in the downstream analysis. No significant inflation was identified (QQ-plots, Figures S8–S10; values given in Table S6).

We did not identify any single burdened gene or gene set (Figures S11–S13).

## 4 | DISCUSSION

We conducted a genetic study using WES on a cohort of 105 individuals affected by EEM. At a genome-wide level, we did not identify any single burdened gene-carrying variants causing EEM. Our sample size may still be too small to identify any such gene. Our results do demonstrate, on the other hand, that the phenotypic umbrella of EEM is genetically heterogeneous.

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| Protein | Computational analysis score, PolyPhen-2 and SIFT | CADD scores (Phred scaled) | Clinical classification | ACMG classification |
|---|---|---|---|---|
| p.Gln1392Thrfs*17 | Unknown | 23.3 | EEM+ | Pathogenic (*PVS1*) |
| p.Arg1152Trp | Damaging (1); deleterious (0) | 34 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| NA | No change in donor site | 25.9 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| p.Leu1533Arg | Damaging (1); deleterious (0) | 32 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| NA | No change in donor site | 11.51 | EEM− | Benign (*BP4*, *BS1*) |
| p.Tyr1246IlefsTer13 | Unknown; deleterious (.01) | 33 | EEM+ | Pathogenic (*PVS1*, *PM2*) |
| p.Ser724fs | Damaging (1); deleterious (.01) | 34 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| p.Ala124Thr | Benign (0); tolerated (.318) | .866 | EEM− | Benign (*BP4*) |
| NA | No change in donor site | 24.2 | EEM+ | Pathogenic (*PS2*, *PM2*) |
| p.Pro351Thr | Probably damaging (.497); tolerated (.201) | 24.2 | EEM | Unknown significance |

| Computational analysis score, PolyPhen-2 and SIFT | CADD scores (Phred scaled) | Clinical classification | ACMG classification |
|---|---|---|---|
| Damaging (.938); tolerated (.239) | 23.9 | Unaffected | Likely benign (*BS4*) |
| Damaging (.938); tolerated (.239) | 23.9 | EEM− | |
| Damaging (.983); tolerated (.307) | 10.35 | Unaffected | Unknown significance |
| Damaging (.983); tolerated (.307) | 10.35 | EEM− | |
| Damaging (.951); tolerated (.173) | 26.7 | EEM− | Unknown significance |
| Damaging (.951); tolerated (.173) | 26.7 | EEM+ | |
| Damaging (.951); tolerated (.173) | 26.7 | Unaffected (maternal grandmother is affected by bilateral TCS) | |
| Damaging (.999); deleterious (0) | 35 | EEM− | Likely pathogenic (*PP3*) |
| Damaging (.999); deleterious (0) | 35 | Two cousins and one aunt from mother's side with epilepsy | |
| Benign (.147); deleterious (.007) | 20.7 | EEM− | Likely benign (*BS4*) |
| Benign (.147); deleterious (.007) | 20.7 | Unaffected | |
| Benign (.213); deleterious (.007) | 22.5 | EEM+ | Likely benign (*BS4*) |
| Benign (.213); deleterious (.007) | 22.5 | Unaffected | |
| Probably damaging (.538); deleterious (.006) | 24.7 | EEM− | Unknown significance |
| Probably damaging (.538); deleterious (.006) | 24.7 | EEM+ | |

Overall, we found nine variants that we could classify as pathogenic/likely pathogenic in the entire cohort (9/105, 8.6%); among these, eight were found in the EEM+ subcohort (8/28, 28.6%), with only one found in the EEM− subcohort (1/77, 1.3%). Overall, the yield of our testing is low; however, considering that the majority of the pathogenic variants were found in the EEM+ group, our yield is closer to what is commonly reported in WES studies in people affected by epilepsy and neurodevelopmental disorders (up to 40%).[37,38] Our results are also in line with a recent electroclinical study[4] that defined two clinical EEM clusters: cluster 1 or "EEM-plus," where EEM is associated with a higher rate of ID, behavioral disorders, earlier age at epilepsy onset, EM status epilepticus, generalized paroxysmal fast activity, self-induced seizures, febrile seizures, and poor drug response; and cluster 2 or "EEM-only," with a more benign profile based on seizure remission and a

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more favorable neuropsychiatric outcome.[4] EEM-plus does not completely overlap with our EEM+ classification. However, ID and behavioral disorders are shared features of EEM-plus and EEM+ and are also features shared with the group of patients with epilepsy with the highest chance of an underlying genetic cause. On the other hand, EEM-only completely overlaps with our definition of EEM− and sits more comfortably with the ILAE-defined broad category of GGE. Taken together, the published clinical study and our data confirm that EEM is a spectrum of conditions; EEM with comorbidities may be a different entity from isolated EEM.

Focusing on the single affected genes, we identified a pathogenic variant in *CHD2* in five individuals (5/105, 4.76% of our cohort; among these, three are novel). All of these were found in individuals whose clinical history was classified as EEM+. Variants affecting *CHD2* have already been reported in a wide range of epileptic conditions. The common denominator across these conditions appears to be photosensitivity, which may occur alone, or in the context of a GGE, a myoclonic–atonic epilepsy, or EEM.[16] *CHD2* variants have also been reported in a more severe phenotype, with ID and behavioral problems.[39] *CHD2* variants have not yet been associated with EEM−. Thus, we suggest that CHD2-EEM+ can be considered a reasonable gene–disease association.

One rare pathogenic variant affecting *NEXMIF* was found in an individual with EEM+. *NEXMIF* pathogenic variants have been reported in individuals with ID, autism spectrum disorder, and epilepsy. *NEXMIF* lies on the X chromosome, and males show a more severe phenotype than females, including severe ID, West syndrome, and Lennox–Gastaut syndrome.[19] Females typically show a milder phenotype, with a clinical presentation overlapping between EEM and myoclonic–atonic epilepsy. Interestingly Stamberger et al. reported that 24% of their studied cohorts fit the clinical diagnosis of EEM.[19] These individuals were all females with ID and/or behavioral problems,[19] further supporting the association of EEM+ with *NEXMIF*.

Another two pathogenic variants were found in two other individuals with EEM+, one in *SYNGAP1* and one in *TRIM8*. Variants in *SYNGAP1* cause a developmental epileptic encephalopathy with a distinctive syndrome combining EEM and myoclonic–atonic seizures, as well as a predilection to seizures triggered by eating.[18] Similar to individuals with *NEXMIF* variants, EEM has been reported only in individuals with *SYNGAP1* variants associated with ID/behavioral problems, providing further evidence to strengthen the association of EEM+ with *SYNGAP1*. Variants affecting *TRIM8* are associated with a complex phenotype featuring epilepsy, neurodevelopmental delay, and nephrotic syndrome.[40] The epilepsy phenotype has been reported in 16 individuals with de novo truncating variants, including 11 individuals with early onset, drug-resistant focal or generalized epilepsy, comorbid with ID. In these individuals, the epilepsy phenotype has not been described in detail; one individual had hippocampal sclerosis on magnetic resonance imaging[40]; three individuals had early onset, drug-resistant developmental and epileptic encephalopathy, of whom one had electrical status epilepticus during sleep[33]; one individual had infantile spasms[41]; and one had early onset EEM with developmental delay and ID, in keeping with our classification of EEM+.[12] Of interest, *TRIM8* is expressed in brain, breast, and skin.[34] Expression of the murine homologue, *Trim8*, is broad across the brain of 2-week-old wild-type mice and enriched in the dendritic regions of hippocampal neurons and Purkinje cells.[41] Although the evidence for the association between *TRIM8* and EEM+ is not as robust as for *CHD2*, *NEXMIF*, and *SYNGAP1*, it may represent a new association.

In individuals with EEM−, we identified only one likely pathogenic variant, affecting the gene *IFIH1*, in which heterozygous variants have been so far described in an Arabic population with Aicardi–Goutières syndrome, a condition that can feature epileptic encephalopathy associated with various degrees of neurodevelopmental delay and basal ganglia calcification.[42] This variant was classified as pathogenic; however, the phenotype of our proband did not fit Aicardi–Goutières syndrome. Although this association can be considered plausible based on the genetic criteria, additional cases are needed before considering *IFIH1* variants and EEM− an established association.

We also found 23 DNVs whose predicted computational scores support pathogenicity. However, these genes have not yet been associated with an epilepsy phenotype, and the available data on their function were not strong enough to underpin any conclusion. More data are needed for these genes and may emerge from ongoing genetic studies in epilepsies.

In conclusion, the core genetic etiology of both EEM+ and EEM− remains to be elucidated; most cases in our cohort did not have an identified genetic cause; if one exists, it may emerge from studies of common variation and lie in oligo- or polygenic frameworks.

## AUTHOR CONTRIBUTIONS

**Antonietta Coppola and S. Krithika:** Conception and design of the work; patient recruitment and collection of the data; analysis and interpretation of the data; drafting and revision of the manuscript. **Michele Iacomino, Costin Leu, Laura Hernandez-Hernandez, Nick Lench, Helena Martins, Vincenzo Nigro, Michele Pinelli, Tommaso Pippucci, and Ravishankara Bellampalli:** Genetic analysis and drafting. **Dheeraj Bobbili and Patrick May:** Genetic and statistical analysis; drafting of the manuscript; critical revision for important intellectual

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content. **Irene Bagnasco, Leonilda Bilo, Daniela Buti, Susanna Casellato, Claudia Cuccurullo, Edoardo Ferlazzo, Lucio Giordano, Giuseppe Gobbi, Stefano Meletti, Tullio Messana, Barbara Salis, Vito Sofia, Salvatore Striano, Laura Tassi, Aglaia Vignoli, Anna Elisabetta Vaudano, and Maurizio Viri:** Patient recruitment and acquisition of the data. **Simona Balestrini, Pasquale Striano, Ingrid E. Scheffer, Federico Zara, and Sanjay M. Sisodiya:** Conception and design of the work; patient recruitment; critical revision for important intellectual content.

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## CONFLICT OF INTEREST STATEMENT

A.C. has received speaker's honoraria from Eisai and Jazz Pharmaceuticals, and has served as a scientific consultant for BIAL and UCB. A.E.V. has received personal compensation as a scientific advisory board member for Angelini Pharma. P.S. has received speaker's honoraria from UCB, Jazz Pharmaceuticals, Proveca, BioMarin, and Neuraxpharm, and has served as a scientific consultant for Angelini Pharma and UCB. S.M.S. has received honoraria for educational events from Eisai and Zogenix, and institutional contributions for advisory boards, educational events, or consultancy work from Eisai, Jazz Pharmaceuticals, Stoke Therapeutics, UCB, and Zogenix. The other authors have nothing to disclose.

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## ETHICS STATEMENT

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. As specified in the Materials and Methods section, this research was approved by local review boards or ethics committees (number 11/LO/2016).

## PATIENT CONSENT STATEMENT

As specified in the Materials and Methods section, written informed consent for research use of clinical and genetic data was obtained from patients, their parents, or legal guardians in the case of minors or those with intellectual disability.

## PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

No figures/tables/excerpts were reproduced in this text, and all data are appropriately cited.

## CLINICAL TRIAL REGISTRATION

No clinical trial registration was needed for this research.

## DATA AVAILABILITY STATEMENT

Deidentified data are available on reasonable request.

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## REFERENCES

1. Jeavons PM. Nosological problems of myoclonic epilepsies in childhood and adolescents. Dev Med Child Neurol. 1977;19:3–8.
2. Specchio N, Wirrell EC, Scheffer IE, Nabbout R, Riney K, Samia P, et al. International league against epilepsy classification and definition of epilepsy syndromes with onset in childhood: position paper by the ILAE task force on nosology and definitions. Epilepsia. 2022;63:1398–442.
3. Appleton RE, Panayiotopoulos CP, Acomb BA, Beirne M. Eyelid myoclonia with typical absences: an epilepsy syndrome. J Neurol Neurosurg Psychiatry. 1993;56:1312–6.
4. Cerulli Irelli E, Cocchi E, Ramantani G, Caraballo RH, Giuliano L, Yilmaz T, et al. Electroclinical features and long-term seizure outcome in patients with eyelid Myoclonia with absences. Neurology. 2022;98:e1865–76.
5. Giuliano L, Fatuzzo D, Mainieri G, Maira G, Elia M, Ferlazzo E, et al. Eyelid myoclonia with absences: electroclinical features and prognostic factors. Epilepsia. 2019;60:1104–13.
6. Smith KM, Wirrell EC, Andrade DM, Choi H, Trenité DKN, Jones H, et al. Clinical presentation and evaluation of epilepsy with eyelid myoclonia: results of an international expert consensus panel. Epilepsia. 2023;64:2330–41.
7. Adachi M, Inoue T, Tsuneishi S, Takada S, Nakamura H. Eyelid myoclonia with absences in monozygotic twins. Pediatr Int. 2005;47:343–7.
8. Capovilla G, Striano P, Gambardella A, Beccaria F, Hirsch E, Casellato S, et al. Eyelid fluttering, typical EEG pattern, and impaired intellectual function: a homogeneous epileptic condition among the patients presenting with eyelid myoclonia. Epilepsia. 2009;50:1536–41.
9. Sadleir LG, Vears D, Regan B, Redshaw N, Bleasel A, Scheffer IE. Family studies of individuals with eyelid myoclonia with absences. Epilepsia. 2012;53:2141–8.
10. Kasteleijn-Nolst Trenite DG. Photosensitivity in epilepsy. Electrophysiological and clinical correlates. Acta Neurol Scand Suppl. 1989;125:3–149.
11. Tauer U, Lorenz S, Lenzen KP, Heils A, Muhle H, Gresch M, et al. Genetic dissection of photosensitivity and its relation to idiopathic generalized epilepsy. Ann Neurol. 2005;57:866–73.
12. pi4K Consortium, Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, et al. De novo mutations in epileptic encephalopathies. Nature. 2013;501:217–21.
13. Suls A, Jaehn JA, Kecskes A, Weber Y, Weckhuysen S, Craiu DC, et al. De novo loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. Am J Hum Genet. 2013;93:967–75.
14. Thomas RH, Zhang LM, Carvill GL, Archer JS, Heavin SB, Mandelstam SA, et al. CHD2 myoclonic encephalopathy is frequently associated with self-induced seizures. Neurology. 2015;84:951–8.
15. Courage C, Houge G, Gallati S, Schjelderup J, Rieubland C. 15q26.1 microdeletion encompassing only CHD2 and RGMA in two adults with moderate intellectual disability, epilepsy and truncal obesity. Eur J Med Genet. 2014;57:520–3.
16. Galizia EC, Myers CT, Leu C, de Kovel CGF, Afrikanova T, Cordero-Maldonado ML, et al. CHD2 variants are a risk factor for photosensitivity in epilepsy. Brain. 2015;138:1198–207.
17. Mignot C, von Stulpnagel C, Nava C, Ville D, Sanlaville D, Lesca G, et al. Genetic and neurodevelopmental spectrum of SYNGAP1-associated intellectual disability and epilepsy. J Med Genet. 2016;53:511–22.
18. Vlaskamp DRM, Shaw BJ, Burgess R, Mei D, Montomoli M, Xie H, et al. SYNGAP1 encephalopathy: a distinctive generalized

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developmental and epileptic encephalopathy. Neurology. 2019;92:e96–e107.
19. Stamberger H, Hammer TB, Gardella E, Vlaskamp DRM, Bertelsen B, Mandelstam S, et al. NEXMIF encephalopathy: an X-linked disorder with male and female phenotypic patterns. Genet Med. 2021;23:363–73.
20. Rudolf G, Lesca G, Mehrjouy MM, Labalme A, Salmi M, Bache I, et al. Loss of function of the retinoid-related nuclear receptor (RORB) gene and epilepsy. Eur J Hum Genet. 2016;24:1761–70.
21. Johannesen K, Marini C, Pfeffer S, Møller RS, Dorn T, Niturad CE, et al. Phenotypic spectrum of GABRA1: from generalized epilepsies to severe epileptic encephalopathies. Neurology. 2016;87:1140–51.
22. Ramu A, Noordam MJ, Schwartz RS, Wuster A, Hurles ME, Cartwright RA, et al. DeNovoGear: de novo indel and point mutation discovery and phasing. Nat Methods. 2013;10:985–7.
23. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164.
24. Investigators GPP, Smedley D, Smith KR, et al. 100,000 genomes pilot on rare-disease diagnosis in health care—preliminary report. N Engl J Med. 2021;385:1868–80.
25. Martin AR, Williams E, Foulger RE, Leigh S, Daugherty LC, Niblock O, et al. PanelApp crowdsources expert knowledge to establish consensus diagnostic gene panels. Nat Genet. 2019;51:1560–5.
26. Robinson JT, Thorvaldsdottir H, Wenger AM, Zehir A, Mesirov JP. Variant review with the integrative genomics viewer. Cancer Res. 2017;77:e31–4.
27. Richards S, Aziz N, Bale S, Bick D, das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
28. Samanta D, Willis E. KIAA2022-related disorders can cause Jeavons (eyelid myoclonia with absence) syndrome. Acta Neurol Belg. 2020;120:205–7.
29. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46:310–5.
30. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303.
31. Koko M, Motelow JE, Stanley KE, Bobbili DR, Dhindsa RS, May P, et al. Association of ultra-rare coding variants with genetic generalized epilepsy: a case-control whole exome sequencing study. Epilepsia. 2022;63:723–35.
32. Cioclu MC, Coppola A, Tondelli M, Vaudano AE, Giovannini G, Krithika S, et al. Cortical and subcortical network dysfunction in a female patient with NEXMIF encephalopathy. Front Neurol. 2021;12:722664.
33. Assoum M, Lines MA, Elpeleg O, Darmency V, Whiting S, Edvardson S, et al. Further delineation of the clinical spectrum of de novo TRIM8 truncating mutations. Am J Med Genet A. 2018;176:2470–8.
34. Tian Z, Tang J, Liao X, et al. TRIM8 inhibits breast cancer proliferation by regulating estrogen signaling. Am J Cancer Res. 2020;10:3440–57.
35. Lund C, Brodtkorb E, Oye AM, Rosby O, Selmer KK. CHD2 mutations in Lennox-Gastaut syndrome. Epilepsy Behav. 2014;33:18–21.
36. Benson KA, White M, Allen NM, Byrne S, Carton R, Comerford E, et al. A comparison of genomic diagnostics in adults and children with epilepsy and comorbid intellectual disability. Eur J Hum Genet. 2020;28:1066–77.
37. Scala M, Bianchi A, Bisulli F, Coppola A, Elia M, Trivisano M, et al. Advances in genetic testing and optimization of clinical management in children and adults with epilepsy. Expert Rev Neurother. 2020;20:251–69.
38. Chen WL, Mefford HC. Diagnostic considerations in the epilepsies-testing strategies, test type advantages, and limitations. Neurotherapeutics. 2021;18:1468–77.
39. Bernardo P, Galletta D, Iasevoli F, D'Ambrosio L, Troisi S, Gennaro E, et al. CHD2 mutations: only epilepsy? Description of cognitive and behavioral profile in a case with a new mutation. Seizure. 2017;51:186–9.
40. Weng PL, Majmundar AJ, Khan K, Lim TY, Shril S, Jin G, et al. De novo TRIM8 variants impair its protein localization to nuclear bodies and cause developmental delay, epilepsy, and focal segmental glomerulosclerosis. Am J Hum Genet. 2021;108:357–67.
41. Sakai Y, Fukai R, Matsushita Y, Miyake N, Saitsu H, Akamine S, et al. De novo truncating mutation of TRIM8 causes early-onset epileptic encephalopathy. Ann Hum Genet. 2016;80:235–40.
42. Al Mutairi F, Alfadhel M, Nashabat M, et al. Phenotypic and molecular Spectrum of Aicardi-Goutieres syndrome: a study of 24 patients. Pediatr Neurol. 2018;78:35–40.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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