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Original article

# Diagnostic value of genetic testing, with focus on *CACNA1A*, in children with episodic neurologic disorders: a single-centre retrospective study

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ABSTRACT

*Background:* Episodic neurologic disorders, such as paroxysmal torticollis, paroxysmal tonic upward gaze deviation, migraine, and episodic ataxia, represent a diagnostic challenge in paediatric patients. Variants in the *CACNA1A* and other genes such *SCN8A* and *DEPDC5*, have been implicated in episodic ataxia, hemiplegic migraine, and related conditions. However, the diagnostic yield of *CACNA1A* testing in paediatric populations with these symptoms remains uncertain.

*Methods:* We conducted a retrospective study at Hospital Sant Joan de Déu, Barcelona, analysing 32 paediatric patients with episodic neurologic disorders. Clinical evaluation, neuroimaging, video EEG, and genetic testing were performed. Clinical and genetic data were correlated to identify predictors of pathogenic variants.

*Results:* The cohort included 32 patients (21 females), with a mean age at symptom onset of 1.3 years. Paroxysmal torticollis (12/32) and paroxysmal tonic upgaze deviation (9/32) were the most frequent initial symptoms. Pathogenic variants were identified in 6/32 patients, of whom 2 carried *CACNA1A* variants. Positive genetic findings were significantly associated with developmental delay ($p = 0.0056$) and paroxysmal tonic upgaze deviation ($p = 0.0185$). Additional variants were identified in genes not classically linked to episodic disorders, including *KAT6A*, *NFIX*, and *DEPDC5*. Neuroimaging abnormalities were observed in 7/22 patients, and EEG abnormalities in 3/16.

*Conclusions:* Genetic testing provides important insights in the evaluation of paediatric patients with episodic neurologic disorders, particularly in those with developmental delay, paroxysmal tonic upgaze, or episodic ataxia. Although the overall diagnostic yield remained low, consistent with other paroxysmal movement disorders, these findings support the integration of genetic testing into the diagnostic algorithm and underscore the need to consider broader genetic aetiologies. Larger studies are warranted to confirm these observations.

## 1. Introduction

Episodic neurologic disorders in childhood including paroxysmal torticollis [1], paroxysmal tonic upward gaze deviation [2], hemiplegic migraine [3], benign paroxysmal vertigo [4], and episodic ataxia [5], represent a diagnostic challenge for paediatric neurologists [6–8]. These manifestations are often transient, heterogeneous, and age-dependent, and their clinical presentation can overlap with a wide spectrum of acquired and genetic conditions [9]. The episodic nature of the symptoms may delay recognition and complicate the differentiation from epileptic seizures, migraine, or paroxysmal movement disorders, leading to uncertainty in the diagnostic process.

Among genetic causes, variants in the *CACNA1A* gene have emerged as one of the most relevant contributors. Pathogenic variants in this gene

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are associated with disorders such as episodic ataxia type 2, familial hemiplegic migraine, spinocerebellar ataxia type 6, and other paroxysmal neurological presentations [10–13].

Clinical descriptions have also linked *CACNA1A* to early-onset phenotypes including developmental delay, epileptic encephalopathy, and paroxysmal ocular motor disorders, suggesting a broader spectrum than initially recognised. For the clinician, however, it remains difficult to determine in which specific scenarios *CACNA1A* testing is most informative, especially in children without a family history of migraine or ataxia [14–17].

The increasing availability of next-generation sequencing has facilitated the detection of *CACNA1A* variants in paediatric cohorts. Nevertheless, the diagnostic yield of genetic testing in children presenting with episodic neurologic symptoms remains uncertain. Most reports to date are limited to isolated cases or small series, often focused on selected phenotypes such as episodic ataxia or hemiplegic migraine. This scarcity of systematic data leaves paediatric neurologists with limited guidance on how to integrate genetic testing into their diagnostic algorithm for children presenting with paroxysmal torticollis, ocular motor disturbances, or episodic ataxia. In addition, the identification of variants in other genes not traditionally linked to episodic disorders further complicates the interpretation and raises the possibility of broader genetic contributions [4,18–21].

These episodic presentations overlap with paroxysmal movement disorders and stroke-like episodes, and some conditions such as alternating hemiplegia of childhood (AHC), episodic ataxia (EA) due to *CACNA1A*, and *GLUT1* deficiency can present with stroke-like imaging changes.

To address these gaps, we conducted a retrospective study in a cohort of children presenting with episodic neurologic disorders at a tertiary paediatric neurology centre. The objectives were to evaluate the diagnostic utility of genetic testing, with particular attention to *CACNA1A*, and to explore whether specific clinical features—such as developmental profile, or type of episodic manifestation—may help predict a positive genetic result. By doing so, this study aims to provide practical evidence to guide paediatric neurologists in the evaluation of children with these complex and often overlapping episodic presentations.

## 2. Methods

### 2.1. Study design

We conducted a retrospective study at the Movement Disorders Unit, Hospital Sant Joan de Déu, Barcelona, Spain, covering the period between 2018 and 2024. The study was approved by the institutional Ethics Committee (PIC-129-24), and all procedures were performed in accordance with ethical standards for human research.

### 2.2. Study subjects

Eligible participants were children presenting with paroxysmal neurologic symptoms, including tonic upgaze deviation, paroxysmal nystagmus, paroxysmal torticollis, episodic ataxia, alternating hemiplegia of childhood, or hemiplegic migraine. Patients with syncope or stroke-like episodes were excluded. Neurological symptoms were documented using standardised clinical assessments. Family history was restricted to first-degree relatives (parents and siblings) with epilepsy, migraine, or paroxysmal disorders; reports not meeting formal criteria for migraine were excluded. For the neurological examination, only findings from the initial assessment were considered.

### 2.3. Genetic testing

Genetic analysis was performed using next-generation sequencing (NGS). Clinical exome sequencing (CES) was performed up to 2022; from 2023 onwards, whole-exome sequencing (WES) was used. After 2022, CES was preferentially requested in younger children or in those with more striking clinical features, as results could be obtained more rapidly. Gene selection for phenotype-driven analyses was guided by patient-specific Human Phenotype Ontology (HPOs) terms. *CACNA1A* was interrogated in all patients: sequence-level variants were assessed by NGS, and—according to protocol and availability—copy-number analysis (e.g., MLPA for *CACNA1A*) and triplet-repeat expansion testing were also performed; earlier cases were retrospectively tested when feasible. In selected cases, additional analyses such as Chromosome Microarray Analysis (CMA) or whole-genome sequencing (WGS) were performed. All analyses were carried out at the Hospital Sant Joan de Déu genetics laboratory. Variants were classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines [22,23], and candidate variants were confirmed by Sanger sequencing, with segregation studies performed when parental samples were available. Individual *in silico* predictors such as AlphaMissense, MutationTaster, PROVEAN, SIFT and CADD were not used to apply ACMG criteria. At the protein level, REVEL was the only predictor considered to inform final classification when appropriate [24]. For transparency, we report the values of the aforementioned individual predictors, but they did not influence ACMG-based classification. Only variants classified as pathogenic or likely pathogenic were considered as a definite diagnosis.

### 2.4. Statistics

Descriptive statistics were performed for all clinical and genetic data. Continuous variables were compared between genetically positive and negative groups using the Mann–Whitney *U* test. Categorical variables were analysed using Fisher's exact test, with odds ratios (OR) calculated when appropriate. Statistical significance was set at $p < 0.05$. Analyses were performed using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

A total of 32 children were included, of whom 21 were female. The mean current age at the time of review was 7.1 ± 4.1 years (± SD (standard deviation), range: 5 months–19 years), and the mean age at symptom onset was 1.3 ± 0.04 years (± SE (standard error), range: 1 month–5.7 years). The mean age at last follow-up was 5.1 ± 4.1 years (±SD, range: 6 months–17,9 years). Clinical characteristics are summarised in Table 1.

### 3.1. Clinical presentation

Paroxysmal torticollis was the most frequent presenting symptom (12/32), followed by paroxysmal tonic upgaze deviation (9/32). Less frequent initial symptoms were benign paroxysmal vertigo (4/32), episodic nystagmus (2/32), alternating hemiplegia of childhood (2/32), episodic ataxia (2/32), and hemiplegic migraine (1/32).

The age at onset differed across phenotypes. Paroxysmal torticollis typically appeared during the first year of life (mean ± SD, 0.5 ± 0.4 years, range 1 month–1.3 years), whereas paroxysmal tonic upgaze deviation showed a slightly later onset (mean ± SD, 1.1 ± 0.7 years, range 1 month–2 years). In contrast, episodic ataxia and hemiplegic migraine were generally observed after the age of two years.

17/32 had a positive family history of paroxysmal disorders, migraine, or epilepsy in first-degree relatives. Neurodevelopmental problems were documented in a subset of children, with developmental delay in 6/32 patients, intellectual disability in 2/32, and autism spectrum disorder in 2/32.

### 3.2. Diagnostic investigations

Lumbar puncture was performed in 6/32 children. Abnormal results

**Table 1**
Clinical characteristics of children with episodic neurological symptoms.

| Patient | Sex | Currentage(years) | Initialsymptom | Ageofonset(years) | Ageatlastfollow – up(years) | Familyhistory | Abnormalocularmovement | Torticollis | Episodicataxia | Vertigo | Migraine | Alternatinghemiplegia | Developmentaldelay | Intellectualdisability | Initialphysicalexamination | Lumbarpuncture | Neuroimage | VideoEEG | Genetictesting | Finaldiagnosis | Positivegenetictest |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | F | 9.8 | Nys | 0.2 | 9,8 | Y | Y | N | Y | N | N | N | Y | N | Abnormal | N | Normal | Normal | CES, WGS, MLPA, SCNA | *SCN8A* (LP) | *Y* |
| P2 | M | 19.1 | Mig | 5.0 | 17.9 | N | N | N | N | N | Y | N | N | N | Abnormal | N | Normal | Normal | CES, arrayCGH | N P/LP | N |
| P3 | F | 8.8 | Ton | 1.8 | 2.0 | N | Y | N | N | N | N | N | N | – | Normal | N | N | Normal | CES | N P/LP | N |
| P4 | F | 14.9 | EpA | 1.5 | 10.1 | Y | Y | N | Y | N | N | N | Y | Y | Abnormal | Normal | Abnormal | N | CES | N P/LP | N |
| P5 | F | 9.8 | AHC | 2.8 | 9.8 | Y | N | N | N | N | N | Y | N | ASD | Abnormal | Normal | Abnormal | Normal | CES | MOGAD | N |
| P6 | M | 12.8 | Nys | 1 | 12.8 | N | Y | N | N | Y | N | N | N | N | Normal | N | Normal | Abnormal | CES | *DEPDC5* (LP) | Y |
| P7 | F | 7.6 | Ton | 1.8 | 1.7 | N | Y | N | N | N | N | N | N | – | Normal | N | N | Normal | CES | N P/LP | N |
| P8 | F | 8.1 | Ton | 1.6 | 3.6 | Y | Y | N | N | N | N | Y | N | – | Normal | N | Normal | Normal | CES | N P/LP | N |
| P9 | M | 10.2 | Tor | 0.2 | 6.3 | Y | N | Y | Y | N | N | N | Y | N | Normal | N | Normal | N | CES | N P/LP | Y |
| P10 | F | 7.2 | Tor | 1.0 | 4 | N | N | Y | Y | N | N | N | N | – | Normal | N | N | N | CES | N P/LP | N |
| P11 | F | 5.3 | Tor | 0.3 | 4.8 | N | N | Y | N | N | N | N | N | – | Abnormal | N | Abnormal | N | CES | N P/LP | Y |
| P12 | F | 13.8 | Tor | 0.3 | 12.7 | N | N | Y | N | Y | Y | N | N | N | Normal | N | Normal | N | CES | N P/LP | N |
| P13 | M | 6.3 | Tor | 1.3 | 6.2 | Y | N | Y | N | N | N | N | N | ASD | Normal | N | N | N | CES, arrayCGH | N P/LP | N |
| P14 | F | 7.4 | Tor | 0.4 | 1.3 | N | N | Y | Y | Y | N | N | N | . | Normal | N | Abnormal | Normal | CES | N P/LP | N |
| P15 | M | 5.6 | Tor | 0.4 | 2.1 | N | N | Y | N | N | N | N | N | . | Normal | N | N | N | CES | GER | N |
| P16 | F | 5.4 | Tor | 0.5 | 2.2 | N | N | Y | N | N | N | N | Y | . | Abnormal | N | Normal | Abnormal | CES | *NFIX* (LP) | Y |
| P17 | M | 3.4 | Ton | 1.2 | 2.9 | N | Y | N | N | N | N | N | N | – | Normal | N | N | Normal | CES | N P/LP | N |
| P18 | F | 3.8 | Ton | 2.0 | 2.8 | N | Y | N | N | N | N | N | N | – | Normal | Abnormal | Normal | Normal | CES | N P/LP | N |
| P19 | M | 4.3 | Ver | 2.0 | 3.3 | Y | N | N | Y | Y | N | N | N | . | Abnormal | N | N | Normal | CES | IDA | N |
| P20 | F | 3.6 | Ton | 0.8 | 3.6 | Y | Y | N | Y | N | N | N | N | – | Abnormal | N | N | Normal | CES | *CACNA1A* (P) | Y |
| P21 | F | 3.4 | Tor | 0.3 | 3.3 | N | N | Y | N | N | N | N | N | – | Abnormal | N | N | N | CES, MLPA, SCA | N P/LP | N |
| P22 | M | 2.8 | Tor | 1.2 | 2.8 | N | N | Y | Y | Y | N | Y | N | – | Normal | N | Normal | N | WES, MLPA, SCA | N P/LP | N |
| P23 | F | 2.6 | Tor | 0,1 | 2.6 | Y | N | Y | N | N | N | N | N | – | Normal | N | Normal | N | CES | N P/LP | Y |
| P24 | F | 4.3 | Ver | 1,2 | 2.7 | N | N | N | N | Y | N | N | N | – | Abnormal | N | N | N | CES | N P/LP | N |
| P25 | F | 3.9 | Ver | 1.1 | 2.9 | N | N | Y | Y | N | N | N | N | – | Normal | N | Normal | N | WES | N P/LP | N |
| P26 | F | 4.5 | Tor | 0,3 | 4.5 | N | N | Y | N | N | N | N | N | – | Normal | N | Normal | N | CES, MLPA, SCA | N P/LP | N |
| P27 | F | 10.8 | EpA | 5.7 | 10,8 | N | N | N | Y | Y | N | N | N | N | Abnormal | N | Normal | N | CES | N P/LP | N |
| P28 | M | 10.9 | Ton | 0.1 | 10.9 | N | Y | N | N | N | N | N | Y | Y | Abnormal | Abnormal | Abnormal | Abnormal | CES | *CACNA1A* (P) | Y |
| P29 | M | 3.8 | Ton | 0.8 | 3.8 | Y | Y | N | N | N | N | N | Y | – | Abnormal | Abnormal | Abnormal | Normal | CES | *KAT6A* (P) | Y |
| P30 | F | 6.9 | Ver | 4.3 | 6.2 | N | N | N | N | Y | N | N | N | N | Normal | N | Normal | N | CES | N P/LP | N |
| P31 | F | 4.3 | AHC | 0.2 | 4.3 | N | N | N | N | N | Y | Y | N | – | Normal | Normal | Normal | Normal | WES, arrayCGH | N P/LP | N |
| P32 | M | 0.5 | Ton | 0.1 | 0.5 | N | Y | N | N | N | N | N | N | – | Normal | N | Abnormal | N | CES, arrayCGH | N P/LP | N |

AHC = Alternating Hemiplegia of Childhood, ASD = Autism Spectrum Disorder, CES = Clinical Exome Sequencing, EpA = Episodic ataxia, GER = Gastroesophageal reflux disease, IDA = Iron deficiency anemia, LB = likely benign, LP = likely pathogenic, Mig = hemiplegic migraine, MOGAD = MOG Antibody-Associated Disease, MLPA = Multiplex ligation-dependent probe amplification, N = No, N P/LP = No P/LP variant identified, Nys = paroxysmal nystagmus, P = pathogenic, SCA = Trinucleotide repeat expansion analysis, Ton = Tonic upgaze deviation, Tor = Paroxysmal torticollis, VUS = variant of unknown significance, WES= Whole Exome Sequencing, WGS =Whole Genome Sequencing, Y = yes.

were found in 3/6 (P18, P28, P29), mostly related to altered neurotransmitter metabolites, which guided further investigations. For example, P29 had low levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), while P28 showed abnormal GABA and biopterin levels. In these cases, lumbar puncture was performed to exclude *GLUT1* deficiency, neurotransmitter disorders, and opsoclonus–myoclonus syndrome, or to confirm MOG-antibody associated disease (MOGAD) (Table 2).

Brain MRI was carried out in 22/32 patients and showed abnormalities in 7/22. The findings were heterogeneous, ranging from incidental anomalies such as arachnoid cysts (P11) or elongated perivascular spaces (P14), to progressive structural changes. Notably, 2/22 children developed cerebellar atrophy over time (P4 and P28). P4 had a normal MRI at 1.1 years but showed marked cerebellar atrophy at 7.8 years, while P28 presented with early vermian atrophy at 0.7 years, which progressed significantly by 3 years of age. 2/22 patients (P29, P32) had a thin corpus callosum, consistent with an underlying genetic disorder. 1/22 patient (P5) developed multifocal T2/FLAIR lesions involving basal ganglia, subcortical white matter, and cerebellar peduncles at 3.7 years, compatible with MOGAD (Table 2).

Video EEG was performed in 16/32 patients, with abnormal results in 3/16. P6 showed isolated focal paroxysms during sleep, P16 had right rolandic epileptiform discharges, and P28 exhibited irregular spike–wave activity with electroclinical events including myoclonic seizures (Table 2).

### 3.3. Genetic testing

All children underwent next-generation sequencing: clinical exome sequencing (CES) in 30/32, whole-exome sequencing (WES) in 3/32, and whole-genome sequencing (WGS) in 1/32; the WGS case had a prior negative CES. Additional targeted analyses included MLPA for *CACNA1A* (4/32), CMA (4/32), and trinucleotide repeat expansion analysis for spinocerebellar ataxia (3/32). In total, 6/32 patients had a positive molecular result (Table 3).

2/32 patients carried pathogenic variants in *CACNA1A*. One was a nonsense variant and the other an in-frame INDEL, both confirmed as *de novo* (P20, P28) and associated with early-onset episodic symptoms and developmental delay. The years in which genetic studies were performed are detailed in Table 4.

Single pathogenic or likely pathogenic variants were also identified in other genes. P1 carried a maternally inherited *SCN8A* missense variant and presented with nystagmus and paroxysmal vertigo, later developing seizures. P6 had a truncating variant in *DEPDC5*, inherited from his father, and showed episodic nystagmus with epileptiform activity on EEG. P16 had a splicing variant in *NFIX*, inherited from his father, and presented with torticollis and focal rolandic epileptiform activity. P29 carried a *de novo* truncating variant in *KAT6A*, associated with tonic upgaze deviation, developmental delay, and a thin corpus callosum.

Supplementary Table 1 summarises other genetic findings in the cohort, including variants of uncertain significance and likely benign variants. These were not included in the main analysis but are reported for completeness and potential future reference.

### 3.4. Clinical–genetic correlations

Comparative analyses were performed to identify predictors of a positive genetic test. Although patients with a positive result tended to have an earlier symptom onset (median 0.65 vs 1.20 years), this difference did not reach statistical significance ($p = 0.055$). By contrast, developmental delay was strongly associated with a positive test (odds ratio [OR] = 24.0, $p = 0.0056$). Paroxysmal tonic upgaze deviation was also significantly associated with a positive genetic test (OR = 13.6, $p = 0.0185$) (Fig. 1). Other variables, including sex or family history were not statistically significant predictors. Multivariate analysis was not

**Table 2**
CSF, neuroimaging, and electroencephalographic findings in children with episodic neurologic disorders.

| Patient | CSF | Brain MRI | VideoEEG |
|---|---|---|---|
| P1 | N | Normal | Normal |
| P2 | N | Normal | Normal |
| P3 | N | N | Normal |
| P4 | Normal | Initial brain MRI (1.1 y): normal, follow-up MRI (7.8 y): cerebellar atrophy | N |
| P5 | Normal | Initial MRI (3.2 y): normal, follow-up MRI (3.7 y): multifocal T2/FLAIR lesions involving basal ganglia, subcortical white matter, and cerebellar peduncles | Normal |
| P6 | N | Normal | PSG (8.1 y): Isolated focal paroxysms in the frontal regions of both hemispheres during sleep. |
| P7 | N | N | Normal |
| P8 | N | Normal | Normal |
| P9 | N | Normal | N |
| P11 | N | MRI (1.3 y): Slight increase in extra-axial spaces, small arachnoid cysts in the anterior temporal poles, and a small cyst in the septum pellucidum. | N |
| P12 | N | Normal | N |
| P14 | N | MRI (2.4 y): Elongated perivascular spaces | Normal |
| P16 | N | Normal | VideoEEG (2.3 y): Right rolandic epileptiform anomalies. |
| P17 | N | N | Normal |
| P18 | 5-HIAA 84 nmol/L (RV: 122–257), ratio HVA/5-HIAA 4,18 (RV: 0,92–2,63), biopterin 8 nmol/L (RV:11–39) | Normal | Normal |
| P19 | N | N | Normal |
| P20 | N | N | Normal |
| P22 | N | Normal | N |
| P23 | N | Normal | N |
| P25 | N | Normal | N |
| P26 | N | Normal | N |
| P27 | N | Normal | N |
| P28 | GABA 28 nmol/L (RV:37–92), Biopterin 68 nmol/L (RV: 11–39), Vitamin B6 3 nmol/L (RV:24–87) | Initial MRI (0.7 y): Cerebellar atrophy, predominantly vermian. Follow-up MRI (3.0 y): Significant progression of cerebellar atrophy. | VideoEEG (4.8 y): Irregular spikes or spike-wave activity in frontocentral regions, with low persistence. One epileptic seizure and five electroclinical events during sleep, characterized by eye opening, limb movements, and myoclonic seizures |
| P29 | 5-HIAA 108 nmol/L (RV: 170–490), HVA 245 nmol/L (RV:344–906), ratio HVA/MHPG 4 (RV: 5–30) | MRI (1.5 y): thin and short corpus callosum | Normal |

(*continued on next page*)

**Table 2** (*continued*)

| Patient | CSF | Brain MRI | VideoEEG |
|---|---|---|---|
| P30 | N | Normal | N |
| P31 | Normal | Normal | Normal |
| P32 | N | MRI (0.5 y): thin corpus callosum | N |

5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG 3-methoxy-4-hydroxyphenylglycol; RV = reference value.

feasible due to the limited sample size.

### 3.5. Follow-up diagnoses

During longitudinal follow-up, three children received additional or alternative diagnoses: P5 was diagnosed with MOGAD, P15 with gastro-oesophageal reflux disease, and P19 with iron-deficiency anaemia. The clinical course and follow-up of all patients is presented in Table 4.

## 4. Discussion

This study evaluated the diagnostic contribution of genetic testing, with particular focus on *CACNA1A*, in a cohort of children presenting with episodic neurologic disorders. The results show that the presence of paroxysmal upgaze deviation and developmental delay are strong predictors of a positive genetic finding. In addition, several children harboured variants in genes not traditionally associated with episodic disorders, illustrating the genetic heterogeneity of these conditions.

Two children in our cohort carried pathogenic variants in *CACNA1A*, corresponding to a diagnostic yield of 6.3 %. This figure is comparable to previously reported yields in small paediatric series, where rates between 5 % and 15 % have been described [25–28]. The identification of *CACNA1A* variants in patients without a family history supports the inclusion of *CACNA1A* testing in the diagnostic work-up of children with episodic ataxia, hemiplegic migraine, or paroxysmal ocular motor disturbances, even in apparently sporadic cases. Importantly, neuroimaging findings of progressive cerebellar atrophy were observed in two patients with *CACNA1A* variants, reinforcing the value of combining genetic testing with longitudinal MRI assessment [10,29].

The presence of developmental delay and abnormal ocular movements emerged as the strongest predictors of a pathogenic genetic test. Children with developmental delay were 24 times more likely to have a positive result, and paroxysmal tonic upgaze deviation was present in more than 80 % of genetically positive cases compared to less than one third of genetically negative cases. By contrast, although patients with a positive genetic test tended to have an earlier onset of symptoms, this difference did not reach statistical significance. These findings provide practical markers to guide clinicians in prioritising genetic testing in children with episodic neurologic symptoms.

Although *CACNA1A* remains the most established gene in the context of paediatric episodic disorders, our results underscore the heterogeneity of genetic findings. Variants were identified in *SCN8A* [30], *DEPDC5* [31–35], *NFIX* [36], and *KAT6A* [37], expanding the spectrum of genes that can underlie episodic phenotypes. In most cases, the phenotypes were not classical for these genes, suggesting that paroxysmal neurologic symptoms may represent part of a broader neurodevelopmental disorder rather than a gene-specific hallmark.

The case of *DEPDC5* is notable, as this gene is classically associated with focal epilepsies but here presented with episodic ocular motor disturbances [7,20]. Similarly, *KAT6A* variants are typically linked to developmental delay/intellectual disability, yet our patient manifested with tonic upgaze deviation and corpus callosum abnormalities. A recent report described a patient with a truncating *KAT6A* mutation who, in addition to intellectual disability and autistic features, also presented with infantile seizures and paroxysmal startle episodes, underscoring the phenotypic heterogeneity of *KAT6A*-related disorders [37]. These examples emphasize the need for broad genomic approaches (e.g., exome sequencing with HPO-guided analysis) rather than restricting testing to the *CACNA1A* gene.

Our findings also demonstrate the value of combining genetic testing with multimodal investigations. MRI abnormalities were present in one third of tested patients, and while not specific, they provided important diagnostic clues in cases with progressive cerebellar atrophy or structural anomalies [10,29]. EEG contributed additional information in a minority of cases, particularly when paroxysmal symptoms overlapped with epileptic activity, as in the patient with *DEPDC5*-related disease [7,20].

Three patients received alternative diagnoses during follow-up: MOGAD [38], gastro-oesophageal reflux [39], and iron-deficiency anaemia [40]. These conditions can mimic genetic episodic disorders and emphasise the importance of maintaining a broad differential diagnosis. The co-existence of acquired and genetic conditions also illustrates the challenges faced by clinicians in real-world practice.

Most previous studies of paediatric episodic disorders have focused on selected phenotypes, such as hemiplegic migraine [41], paroxysmal torticollis [18], eye movement disorders [12], or episodic ataxia [29,42,43], often in familial cases. Our study differs by evaluating a heterogeneous cohort of sporadic patients, reflecting the diagnostic uncertainty encountered in paediatric neurology clinics. The observed prevalence of *CACNA1A* variants and the association with early-onset developmental phenotypes are in line with established pathophysiological mechanisms, whereby altered calcium channel function disrupts cerebellar circuits and cortical excitability.

The identification of non-*CACNA1A* variants also parallels reports of increasingly diverse genetic backgrounds in paroxysmal disorders. For instance, *SCN8A* have been linked to epilepsy and developmental encephalopathies, but emerging evidence suggests they may also contribute to intermittent movement or ocular motor phenotypes [30,44,45]. Similarly, *NFIX* mutations have been associated with structural brain anomalies, which may predispose to episodic symptoms. These findings align with a growing body of literature recognising episodic symptoms as a shared manifestation of multiple neurodevelopmental disorders.

This study has several limitations. The retrospective design may have introduced bias in data collection. The small sample size limits the statistical power and precludes subgroup analyses by phenotype. Approximately three quarters of the cohort remained without a definitive genetic diagnosis, highlighting the current limitations of exome-based approaches. Some variants may have been missed due to technical limitations, while others may reside in non-coding regions not covered by current methods. Functional studies will also be essential to clarify the significance of variants of uncertain significance. Although all patients underwent NGS, MLPA and triplet-repeat expansion testing for *CACNA1A* were not uniformly implemented across the entire cohort and were introduced at different time points; thus, copy-number changes or repeat-expansion mechanisms could have been under detected. Moreover, HPO-driven gene lists evolved during the study period, potentially introducing ascertainment heterogeneity in gene-level interrogation.

From a clinical perspective, genetic testing should be prioritised in children presenting with episodic neurologic symptoms when developmental delay or paroxysmal tonic upgaze deviation are present. Although early age of onset was not statistically significant in this study, the trend towards earlier onset in genetically positive cases suggests it may still contribute to clinical suspicion. *CACNA1A* testing remains particularly relevant when cerebellar involvement is documented, but broader exome-based approaches are recommended given the genetic heterogeneity observed. Although the diagnostic yield was relatively low, in line with other studies, the identification of both *CACNA1A* and non-*CACNA1A* variants underscores the relevance of genomic testing in this heterogeneous group.

**Table 3**
Genetic variants and *in silico* predictions in children with episodic neurologic disorders.

| Patient | Genomic DNA Variant (GRCh37) | Gene (transcript) | cDNA Change HGVS | Protein change | Variant Type | ACMG criteria | Inheritance | Alpha missense | Mutation Taster | Provean | SIFT | CADD | REVEL | Previous published (PMID) | gnomAD Exomes (v4.1.0 alleles) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | chr12-52156456-G-A | *SCN8A* (NM_014191.4) | c.2540G > A | p.(Arg847Gln) | Missense | LP (PS4-mod-PM2-sup, PP3-str,) | Maternally inherited | Deleterious (str) | Uncertain | Uncertain | P sup | 33 | Deleterious | 37440794 38251463 | 0 |
| P6 | chr22-32210991-C-T | *DEPDC5* (NM_001242896.3) | c.1459C > T | p.Arg487* | Nonsense | LP (PS4-mod, PVS1-vstr, PM2-sup) | Paternally inherited | NA | Uncertain | NA | NA | 38 | NA | 24814846 23542697 23542701 32086284 29761115 28102150 30093711 34587683 37471090 | 0.0004 % |
| P16 | chr19-13183924-G-T | *NFIX* (NM_001271043.2) | c.646+1G > T | p.? | Splicing | LP (PVS1-mod, PM2-sup, PP1-sup) | Paternally inherited | NA | Uncertain | NA | NA | 35 | NA | N | 0 |
| P20 | chr19-13409692-C-A | *CACNA1A* (NM_023035.3) | c.2767G > T | p.Glu923* | Nonsense | P (PVS1-vstr, PM6-mod, PM2-sup) | *De novo* | Benign (mod) | Uncertain | NA | NA | 36 | NA | 19486177 10371528 25735478 27250579 | 0 |
| P28 | chr19-13368252-AAG- | *CACNA1A* (NM_001127222.2) | c.4512_4514del | p.Phe1505del | In-frame INDEL | P (PM1-str, PS3 mod, PM2 sup) | *De novo* | NA | NA | NA | NA | NA | NA | 24836863 26716990 | 0 |
| P29 | chr8-41801382-A-C | *KAT6A* (NM_006766.5) | c.2112T > G | p.Tyr704* | Nonsense | P (PVS1-vstr, PM2-sup) | *De novo* | NA | Uncertain | NA | NA | 36 | NA | N | 0 |

LB= Likely benign, LP = Likely pathogenic, mod = moderate, N = No, NA = not available, P= Pathogenic, str = strong, sup = supporting, VUS = variant of unknown significance, vstr = very strong.

**Table 4**
Year of genetic testing and clinical follow-up in patients with episodic neurologic disorders.

| Patient | CES | WES | WGS | CMA | Expansions | MLPA | Clinical course/Follow-up |
|---|---|---|---|---|---|---|---|
| P1 | 2018 | | 2020 | | 2019 | 2018 | Episodic ataxia, diazepam, still FU |
| P2 | 2018 (2021) | | | 2021 | | | Discharged – transition to adult |
| P3 | 2018 | | | | | | Lost to FU |
| P4 | 2018 | | | | | | Lost to FU |
| P5 | 2019 | | | | | | Still FU |
| P6 | 2019 | | | | | | Occasional episodes, no treatment, still FU |
| P7 | 2021 | | | | | | Discharged – resolution |
| P8 | 2020 | | | | | | Discharged – resolution |
| P9 | 2021 | | | | | | Last episode 4y ago, headache, still FU |
| P10 | 2020 | | | | | | Discharged – resolution |
| P11 | 2020 | | | | | | Improved, FU for learning difficulties |
| P12 | 2021 | | | | | | Occasional vertigo, discharged |
| P13 | 2021(2023) | | | 2021 | | | Improved torticollis, ASD dx |
| P14 | 2022 | | | | | | Discharged – resolution |
| P15 | 2022 | | | | | | Discharged – resolution |
| P16 | 2022 | | | | | | Still FU |
| P17 | 2023 | | | | | | Discharged – resolution, language disorder |
| P18 | 2023 (2024) | | | | | | Discharged – resolution |
| P19 | 2023 | | | | | | Discharged – resolution |
| P20 | 2023 | | | | | | Bicarbonate + acetazolamide (partial improvement), still FU |
| P21 | 2023 | | | | 2024 | 2024 | Discharged – resolution |
| P22 | 2023 | 2024 | | | 2024 | 2024 | Discharged – resolution |
| P23 | 2023 | | | | | | Improved torticollis, irritability (ataxia?), still FU |
| P24 | 2024 | | | | | | Discharged – resolution |
| P25 | | 2023 | | | 2024 | | Discharged – resolution |
| P26 | 2024 | | | | | 2024 | Discharged – resolution |
| P27 | 2022 | | | | | | Functional disorder? (suspected), still FU |
| P28 | 2018 | | | | | | Ataxia + nystagmus, acetazolamide + bicarbonate, still FU |
| P29 | 2022 | | | | | | Psychomotor delay, still FU |
| P30 | 2022 | | | | | | Discharged – resolution |
| P31 | | 2024 | | 2024 | | | Persistent episodes, partial improvement with verapamil, still FU |
| P32 | 2024 | | | 2024 | | | Improved, still FU |

Dx = diagnosis; CES = clinical exome sequencing; WES = whole-exome sequencing; WGS = whole-genome sequencing; CMA = chromosomal microarray analysis; MLPA = multiplex ligation-dependent probe amplification; Expansions = trinucleotide repeat expansion analysis for spinocerebellar ataxia; FU = follow-up. Years in parentheses indicate reanalysis dates.

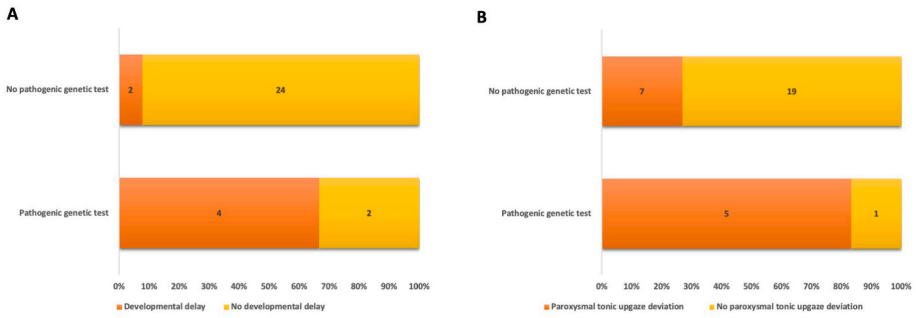


**Fig. 1.** Association of developmental delay (A) and paroxysmal tonic upgaze deviation (B) with pathogenic genetic results in children with episodic neurologic disorders.

## Author information

JDOE conceived and design the study; MC, LMS, DY, CX, JO, AASM, CHD, LM, JA, AS, JDOE collected and analysed the data; MC drafted the manuscript; MC, LMS, DY, CX, JO, AASM, CHD, LM, JA, AS, JDOE revised the manuscript. All authors reviewed, edited, and approved the manuscript.

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## Conflict of interests

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2025.11.005.

## Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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