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RESEARCH ARTICLE

# Clinical features and genotype–phenotype correlations in epilepsy patients with de novo *DYNC1H1* variants

Claudia Cuccurullo[1,2] | Emanuele Cerulli Irelli[3] | Lorenzo Ugga[4] | Antonella Riva[5,6] | Alessandra D'Amico[7] | Sara Cabet[8] | Gaetan Lesca[9,10] | Leonilda Bilo[1] | Federico Zara[5,6] | Catrinel Iliescu[11] | Diana Barca[12] | France Fung[13] | Katherine Helbig[13] | Xilma Ortiz-Gonzalez[13] | Helenius J. Schelhaas[14] | Marjolein H. Willemsen[15] | Inge van der Linden[14] | Laura Canafoglia[16] | Carolina Courage[17,18] | Samuele Gommaraschi[18] | Pedro Gonzalez-Alegre[19] | Tanya Bardakjian[19] | Steffen Syrbe[20] | Elisabeth Schuler[20] | Johannes R. Lemke[21] | Stella Vari[22] | Gitte Roende[23] | Mads Bak[24] | Mahbulul Huq[25] | Zoe Powis[26] | Katrine M. Johannesen[27] | Trine Bjørg Hammer[27] | Rikke S. Møller[27] | Rachel Rabin[28] | John Pappas[28] | Mary L. Zupanc[29] | Neda Zadeh[30] | Julie Cohen[31] | Sakkubai Naidu[32] | Ilona Krey[33] | Russell Saneto[34,35] | Jenny Thies[36] | Laura Licchetta[37] | Paolo Tinuper[37] | Francesca Bisulli[37] | Raffaella Minardi[38] | Allan Bayat[27,39] | Nathalie Villeneuve[40] | Florence Molinari[41] | Hormos Salimi Dafsari[42,43,44,45,46,47] | Birk Moller[42,43] | Marie Le Roux[48] | Clara Houdayer[49] | Marilena Vecchi[50] | Isabella Mammi[51] | Elena Fiorini[52,53] | Jacopo Proietti[52,53] | Sofia Ferri[52] | Gaetano Cantalupo[52,53,54] | Domenica Immacolata Battaglia[55,56] | Maria Luigia Gambardella[55] | Ilaria Contaldo[55] | Claudia Brogna[55,57] | Marina Trivisano[58] | Angela De Dominicis[59] | Stefania Maria Bova[59] | Elena Gardella[27] | Pasquale Striano[5,22] | Antonietta Coppola[1]

**Correspondence**
Claudia Cuccurullo and Antonietta Coppola, Department of Neuroscience, Reproductive and Odontostomatological Sciences, Federico II University, via Pansini 5, Naples 80131, Italy.
Email: c.cuccurullo93@gmail.com and antonietta.coppola1@gmail.com

**Abstract**

**Objective:** *DYNC1H1* variants are involved on a disease spectrum from neuromuscular disorders to neurodevelopmental disorders. *DYNC1H1*-related epilepsy has been reported in small cohorts. We dissect the electroclinical features of 34 patients harboring de novo *DYNC1H1* pathogenic variants, identify subphenotypes on the *DYNC1H1*-related epilepsy spectrum, and compare the genotype–phenotype correlations observed in our cohort with the literature.

For affiliations refer to page 20.

**Methods:** Patients harboring de novo *DYNC1H1* pathogenic variants were recruited through international collaborations. Clinical data were retrospectively collected. Latent class analysis was performed to identify subphenotypes. Multivariable binary logistic regression analysis was applied to investigate the association with DYNC1H1 protein domains.

**Results:** *DYNC1H1*-related epilepsy presented with infantile epileptic spasms syndrome (IESS) in 17 subjects (50%), and in 25% of these individuals the epileptic phenotype evolved into Lennox–Gastaut syndrome (LGS). In 12 patients (35%), focal onset epilepsy was defined. In two patients, the epileptic phenotype consisted of generalized myoclonic epilepsy, with a progressive phenotype in one individual harboring a frameshift variant. In approximately 60% of our cohort, seizures were drug-resistant. Malformations of cortical development were noticed in 79% of our patients, mostly on the lissencephaly–pachygyria spectrum, particularly with posterior predominance in a half of them. Midline and infratentorial abnormalities were additionally reported in 45% and 27% of subjects. We have identified three main classes of subphenotypes on the *DYNC1H1*-related epilepsy spectrum.

**Significance:** We propose a classification in which pathogenic de novo *DYNC1H1* variants feature drug-resistant IESS in half of cases with potential evolution to LGS (Class 1), developmental and epileptic encephalopathy other than IESS and LGS (Class 2), or less severe focal or genetic generalized epilepsy including a progressive phenotype (Class 3). We observed an association between stalk domain variants and Class 1 phenotypes. The variants p.Arg309His and p.Arg1962His were common and associated with Class 1 subphenotype in our cohort. These findings may aid genetic counseling of patients with *DYNC1H1*-related epilepsy.

**KEYWORDS**

*DYNC1H1*-related epilepsy, dynein, infantile epileptic spasms syndrome, lissencephaly/pachygyria, MCDs

## 1 | INTRODUCTION

The cytoplasmic dynein 1 heavy chain 1 (*DYNC1H1*) gene (Mendelian Inheritance in Man [MIM] 600112) located on chromosome 14q32.31 encodes for a subunit of the cytoplasmic dynein complex. DYNC1H is an adenosine triphosphatase (ATPase)-dependent motor protein, involved in retrograde axonal transport, neuronal mitosis and migration, and signaling affecting gene expression.[1–4] The structure of DYNC1H1 may be divided into a C-terminal motor domain and a N-terminal stem or tail domain, linked by the neck domain. The motor region includes the stalk or microtubule-binding domain, and six ATPase subdomains, which form a ring. The tail domain includes regions for homodimerization and binding with intermediate and light-intermediate chains of the dynein complex.[5–7]

Heterozygous pathogenic *DYNC1H1* variants have been associated with a broad neurological phenotypic spectrum, including intellectual disability (ID) with cortical neuronal migration defects (MRD13; MIM 614563),[6,8,9] an autosomal dominant axonal form of Charcot–Marie–Tooth disease (CMT2O; MIM 614228),[10] spinal muscular atrophy lower extremity-predominant 1 (SMALED1; MIM 158600),[11–13] or a combined phenotype consisting of both peripheral neuropathy and central nervous system (CNS) involvement.[14]

The genotype–phenotype correlation underlying the *DYNC1H1*-related spectrum has been initially attributed to specific domain mutations. *DYNC1H1* variants falling within the tail domain have been implicated in predominant neuromuscular phenotypes, whereas those associated with primarily CNS involvement have clustered in

**Key points**

- *DYNC1H1*-related epilepsy manifests itself in half of cases within the first year with IESS, which can evolve into LGS.
- Epilepsy can be grouped into Class 1, including IESS and/or LGS; Class 2, including other DEEs; and Class 3, including focal epilepsy or genetic generalized epilepsy.
- MCDs are seen in approximately 80% of patients. Lissencephaly–pachygyria with anteroposterior gradient may be a specific pattern. Midline and infratentorial anomalies may be common.
- In *DYNC1H1*-related epilepsy, pathogenic variants may reside either in motor or in tail domains of DYNC1H1 protein.
- The variants p.Arg309His in the tail domain and p.Arg1962His in the motor domain are common *DYNC1H1* variants and are usually associated with Class 1 phenotype.

the motor domains.[15,16] In recent years, pathogenic *DYNC1H1* variants have been reported with epileptic seizures and encephalopathy in small cohorts, and few genotype–phenotype correlations have been provided. Particularly, in patients with *DYNC1H1*-related epilepsy, variants have been supposed to be more likely located in the motor domain.[6,15–19]

We report on the electroclinical features of 34 patients harboring rare pathogenic de novo *DYNC1H1* variants and identify subphenotypes on the *DYNC1H1*-related epilepsy spectrum. Furthermore, we review the electroclinical features of 58 individuals with *DYNC1H1*-related epilepsy reported in literature, which allows us to provide a deeper clinical characterization and to explore genotype–phenotype correlations.

## 2 | MATERIALS AND METHODS

### 2.1 | Patient selection

Patients with de novo *DYNC1H1* pathogenic variants were recruited from 29 epilepsy centers through international collaborations. *DYNC1H1* variants were identified through massively parallel sequencing technologies (either panel for malformations of cortical development or exome sequencing) and interpreted according to the American College of Medical Genetics and Genomics guidelines.[20] In all cases, pathogenic variants were validated using Sanger sequencing and segregation analysis was performed on available family members. *DYNC1H1* variants were distinguished according to their effect and the localization within the protein functional domains.[5–7] Data on clinical phenotype, neuroimaging, and electroencephalography (EEG) were retrospectively collected through a structured spreadsheet. Motor and intellectual development were assessed through developmental milestones, neurological examination, and when available intellectual quotient. Seizures were classified according to the International League Against Epilepsy criteria[21] and were assigned, when possible, to defined epileptic syndromes.[22–25] Patients' brain magnetic resonance imaging (MRI) studies were independently assessed by two neuroradiologists. A third neuroradiologist was consulted in the case of disagreement. Malformations of cortical development (MCDs) were assessed according to the recent classification.[26] Brain abnormalities were categorized into three groups: infratentorial, median line, and supratentorial malformations.

Written informed consent was obtained for genetic analysis and all clinical and neurophysiological investigations. Clinical data gathered during routine diagnostic and clinical courses were provided to the principal investigator by each referring clinician in a deidentified form. The study complies with anonymized retrospective study regulations and was reviewed by the local ethics committee.

### 2.2 | Previously published cases

A systematic search on MEDLINE PubMed was performed with the terms “DYNC1H1” and “EPILEPSY” (last search January 31, 2023). All cases with sufficient electroclinical information were reviewed. Multiple descriptions of the same individual in different publications have been identified by mutation (cDNA/protein change), sex, age at study, and age at seizure onset.

### 2.3 | Statistical analysis

Continuous variables were summarized as mean ± SD or median (interquartile range), as appropriate. Categorical variables were summarized as frequencies and percentages within the cohort of patients.

Latent class analysis (LCA) was performed to investigate the existence of underlying subphenotypes on the *DYNC1H1* spectrum. LCA is a statistical technique used to identify underlying groups, termed “latent classes,” within a population based on patterns of responses to categorical variables. LCA has been considered particularly useful compared to conventional clustering techniques when dealing with categorical data. The

following indicators were included in LCA: infantile onset of epilepsy (i.e., <2 years); epilepsy syndromes, namely, infantile epileptic spasms syndrome (IESS), Lennox–Gastaut syndrome (LGS), genetic generalized epilepsy (GGE), focal epilepsy, developmental and epileptic encephalopathy (DEE) with prominent focal seizures, and DEE with prominent generalized seizures; brain MRI abnormalities consistent with lissencephaly–pachygyria spectrum; and presence of moderate/severe ID. Missing data were handled during class assignment with a maximum likelihood estimator that calculates the likelihood of each individual given their available information, provided that at least one indicator was available and assumed missing at random. Log-likelihood test, Akaike information criterion (AIC), Bayesian information criterion (BIC), and entropy were used for model statistics and to assess the optimal number of classes. Comparisons between observed classes were conducted using unpaired *t*-tests and Mann–Whitney *U*-tests for continuous variables, depending on their normality, whereas chi-squared tests or Fisher exact tests were employed for categorical variables. Both unadjusted *p*-values and *p*-values adjusted for the false discovery rate (FDR) method are reported.

Furthermore, to assess the robustness of our LCA solution, a two-step cluster analysis was performed using complete data analysis with the same indicators.

Next, multivariable binary logistic regression analysis was performed to assess the association between DYNC1H1 domains and specific epilepsy subphenotypes identified through LCA, adjusted for age at onset, sex, variant effect, and ethnic background. Finally, another multivariable binary logistic regression model was developed to assess the variables independently predicting severe ID, by including both DYNC1H1 functional domains and clinical variables showing a value of $p < .2$ at univariable analysis in the model.

Statistical analyses were performed using SPSS Statistics software (v27, IBM) and implemented with R Studio (v4.2.3).

# 3 | RESULTS

## 3.1 | Clinical features

We studied 30 novel probands and updated the available electroclinical information for another four patients already partially described.[16,27,28] The total *DYNC1H1* cohort included 34 cases.

The probands were 20 males and 14 females aged between 8 months and 40 years (mean ± SD = 154.5 ± 131.8 months, median = 117 months) at our last assessment. Nearly all patients presented with ID, and brain MRI showed abnormal findings in 30 of 34 (88%) of them (MRI was not available for one patient). Clinical and genetic data are detailed below and reported in Table 1.

## 3.2 | Deep phenotyping in patients with *DYNC1H1*-related epilepsy

### 3.2.1 | Epilepsy

The mean age at seizure onset was 54 ± 61 months (range = 2 months–20 years, median = 12 months). Infantile spasms (IS) were the main seizure type at onset (13/34, 38%) and appeared at a mean age of 6.5 ± 6 months (range = 2–24 months, median = 5 months). The remaining 21 patients (mean age at onset = 67 ± 68 months, median = 42 months) presented with a variety of seizure types (Table 1), the most frequent of which were focal onset either motor or nonmotor seizures (9/34, 26%).

Seventeen subjects experienced IS at some point in their lives and were diagnosed with IESS (50%), with four of these individuals subsequently progressing to LGS (12%). In 12 patients, focal onset epilepsy with structural and genetic etiologies was defined, albeit in four of them the onset was with bilateral tonic–clonic seizures as well as febrile seizures in one subject. Finally, two patients presented with myoclonic seizures. Among them, in one subject epilepsy began in adolescence with myoclonic seizures, associated with segmental myoclonus, cerebellar signs, and photosensitivity, suggesting the diagnosis of progressive myoclonus epilepsy (PME). This individual also presented with infrequent absences with eyelid myoclonia. However, the criteria of either PME or epilepsy with eyelid myoclonia were not met.[25]

EEG recordings at onset of seizures showed a hypsarrythmic pattern in eight patients affected with IESS, whereas focal or multifocal epileptiform discharges were observed in the other five patients. At our last assessment, the hypsarrythmic pattern was replaced by multifocal epileptiform discharges within a slow background activity in four patients with IESS. Conversely, in one patient the EEG initially showed multifocal epileptiform abnormalities within a moderately well-organized background pattern during wakefulness and sleep, whereas a hypsarrythmic pattern appeared approximately 1 year later. The evolution of the EEG could not be evaluated in three patients. Focal or multifocal epileptiform discharges, with or without bilateral diffusion, were described in nine patients with focal onset epilepsy (Figure 1). In the patient presenting with a PME-like phenotype, the EEG at onset was characterized by spike–wave complexes at 3–3.5 Hz synchronous

with the myoclonic jerks; photosensitivity was reported 7 years after epilepsy onset, and bilateral spike–wave complexes at a minor frequency of 2.5–3 Hz persisted after 20 years, within a normal-frequency background activity (Figure 1A,B).

At our last assessment, approximately 60% of patients (20/34) did not achieve seizure control, whereas 40% (14/34) were seizure-free, including seven patients with focal onset seizures and five patients with IESS (5/17, 30%). Patients were treated with a mean number of two antiseizure medications (ASMs; range = 0–6), whereas the mean number of all the ASMs tried was four (range = 1–12). The most used current ASMs were valproic acid (13/34, 38% of patients), lamotrigine (9/34, 26%), clobazam (8/34, 23%), carbamazepine/oxcarbazepine (7/34, 20%), and vigabatrin (5/34, 15%). Ketogenic diet and adrenocorticotropic hormone/steroids were tried in five of 34 (15%) and 13 of 34 (38%), respectively, separately or in some cases combined. A vagus nerve stimulator was implanted in one patient.

#### 3.2.2 | Neurological assessment and additional clinical features

In our cohort, no patient had a phenotype suggestive of spinal muscular atrophy (SMA) or Charcot–Marie–Tooth disease. Five patients presented with signs of peripheral neuropathy on electroneurography and electromyography. However, in the majority, nerve conduction studies and electromyography were not assessed, as it was not deemed necessary on clinical grounds.

All patients but two (32/34, 94%) presented with developmental concerns and ID, with severe delay in 21 patients (62%). Eight patients were observed with developmental regression after epilepsy onset, six of whom were diagnosed with IESS. Nine patients were not able to walk unsupported at our last assessment. Mild and nonspecific facial dysmorphisms were described in 10 patients, including retrognathia, prognathism, hypo- or hypertelorism, epicanthus. In six patients, skeletal anomalies were noticed, including arthrogryposis multiplex congenita, clubfeet, hip dysplasia, and scoliosis. Eye concerns were reported in eight patients (i.e., bilateral cataract, strabismus, keratoconus).

#### 3.2.3 | Neuroimaging

Brain MRI was performed in all patients except for one. MRI images were available in 14 patients. Infratentorial anomalies were described in nine subjects (9/33, 27%), mainly including brainstem and cerebellar vermian hypoplasia. Median line anomalies were noticed in 15 subjects (15/33, 45%), mostly consisting of corpus callosum dysgenesis and white matter volume reduction. In the supratentorial malformations group, we included MCDs, which were present in 26 subjects (26/33, 79%). The most frequently depicted MCDs, reported in 17 patients (17/26, 65%), were included on the lissencephaly–pachygyria spectrum. Particularly, eight patients with lissencephaly or subcortical band heterotopia showed a distinctive anteroposterior increasing gradient. Among the other MCDs, polymicrogyria was depicted in six subjects, periventricular nodular heterotopias were noticed in another six, and focal cortical dysplasia in other two (Figure 2). Five patients had co-occurring MCDs (Table S1).[18] Fluorodeoxyglucose positron emission tomography was performed in two patients and showed cerebellar hypometabolism in the patient with a myoclonic epileptic phenotype and mild hypometabolism of left cerebral cortex in the other individual.

### 3.3 | Genetic landscape

The probands harbored 31 pathogenic *DYNC1H1* variants, including missense variants in the majority (30/34 probands, 88%), one frameshift variant, two in-frame deletions, and one in-frame duplication.

Overall, 12 of 31 variants (39%) were located in the tail domains, 15 of 31 (48%) variants were positioned in the motor domains, and among these, five resided in the stalk region, and four of 31 (13%) variants were in the linker (Figure 3A).

Two mutations recurred in our cohort, and genotype–phenotype correlations are shown in Table S2.

Among subjects harboring nonmissense variants, the individual with the frameshift variant p.Arg2610GlyfsTer23 showed a distinctive PME-like phenotype.[28]

### 3.4 | Literature review

We identified 58 subjects with *DYNC1H1*-related epilepsy reported in literature, harboring 46 different variants (Table S3).[6,9,15–19,29–38] Eighteen variants were located in the tail domain, 26 variants resided in the motor domain, of which 14 were within the stalk region, and two variants were in the neck. The variants p.Arg3344Gln/Trp, p.Arg309His, and p.Arg1962Cys recurred in unrelated subjects.

The seizure types were not available in 20 of 58 children. Fifteen patients presented with spasms, the EEGs showing hypsarrhythmia in eight of them, and focal or multifocal epileptic abnormalities in five.[17–19,32,33,37]

**TABLE 1** Electroclinical and genetic findings in our cohort.

| | | | Epilepsy | | | | |
|---|---|---|---|---|---|---|---|
| | | | Onset | | Follow-up | | |
| Pt | Gender/last evaluation | Mutation cDNA, protein/domain site | Age | Sz type at onset (frequency) | Epilepsy syndrome/Sz type | Sz freedom (age) | Previous ASMs |
| 1 | M/36 years[27] | c.1798G > A; p.Ala600Thr/ tail domain, inside the homodimerization region | 1 year | Spasms, bilateral TC Sz (daily) | IESS and LGS/TC, tonic, myoclonic, myoclonic–atonic, gelastic sz, spasms, atypical abs | No | ACTH, PB, FLB, LTG, VGB, KD |
| 2 | F/8 years 8 months[27] | c.926G > A; p.Arg309His/ tail domain, inside the homodimerization region | 3 years | Atonic sz (daily) | IESS and LGS/ atonic Sz, atypical abs, spasms | No | FLB |
| 3 | F/23 months | c.5828G > A; p.Arg1943Gln/ motor domain (AAA1 subdomain) | 5 months | Spasms (unk) | IESS/none | Yes (5 months) | ACTH, VGB |
| 4 | M/19 months | c.5885G > A, p.Arg1962His/motor domain (AAA1 subdomain) | 5 months | Spams (daily) | IESS/spasms, in clusters | No | Steroids |
| 5 | M/24 years | c.4670 T > C; p.Ile1557Thr/neck domain | 1 year | FS (unk) | Focal to bilateral TC Sz | No | LCM |
| 6 | F/28 years | c.874C > T; p.Arg292Trp/ tail domain, outside the homodimerization region | 18 years | Bilateral TC Sz during sleep (weekly) | Focal onset nonmotor Sz | No | VPA |
| 7 | M/32 years[28] | c.7828delC; p.Arg2610Gly fsTer23/motor domain | 12 years | Myoclonic Sz (weekly) | Myoclonic Sz, myoclonic abs, bilateral TC Sz | No | LEV, CLB |
| 8 | M/28 years | c.10051A > T; p.Ile3351Phe/stalk domain including MTBD | 20 years | Focal onset motor Sz with impaired awareness (daily) | Focal onset motor Sz | No | NA |
| 9 | M/12 years 4 months | c.10175 T > C; p.Met3392Thr/stalk domain including MTBD | 5 years | Myoclonic Sz (daily, upon awakening) | Myoclonic Sz | No | OXC, VPA, LEV, TPM, PB, CBZ, FLB, PHT, VGB |

| Current ASMs | EEGs (age) | MRIs (age) | Developmental stages | Neurological examination | EKG/EMG findings |
|---|---|---|---|---|---|
| VPA, RUF, CBZ, CLZ | Slow background activity; diffuse, high-voltage, SW complexes (36 years) | Bilateral lissencephaly (P-A gradient), basal ganglia dysgenesis (29 years) | Severe ID; single words at 2 years, sitting at 1 year, walking at 2 years 6 months assisted (lost) | Tetraparesis (>lower limbs), not able to walk, OCF at 3rd pc, strabismus | Unremarkable/ unremarkable |
| LTG, VPA, CLB | Bilateral frontal–central–temporal EDs, subcontinuous during sleep (8 years 8 months) | Bilateral lissencephaly (P-A gradient), transmantle NH, CC thickening, basal ganglia dysgenesis, brainstem and vermian hypoplasia (2 years) | Severe ID; single words at 2 years, sitting at 10 months, walking at 3 years assisted | Diffuse hypotrophy and hypotonia, OCF at 3rd pc | Unremarkable/ NA |
| None | Hypsarrythmic pattern (5 months) | Insular pachygyria, posterior CC thinning, mild ventriculomegaly (15 months) | Mild ID; single words at 13 months, first sentence at 1 year 11 months, walking 15 at months | Unremarkable except for hyperkinetic behavior | NA/NA |
| CLB, TPM, VGB, KD | Hypsarrythmic pattern (5 months); frequent multifocal EDs, slow background activity, absence of well-formed sleep figures (13 months) | Lissencephaly–pachygyria spectrum (A-P gradient), WM volume reduction, PNH (2 days) | Severe ID; never able to sit, walk, or speak | Severe diffuse hypotonia, no eye contact, no head control, clubfeet, arthrogryposis | Sinus bradycardia with sinus arrhythmia/NA |
| CBZ, LTG, VPA, CLB | Bilateral sharp wave–slow wave activity (1 year); no EDs (24 years) | Mild generalized atrophy (24 years) | Severe ID; never able to speak sentences (24 years) | Spastic tetraparesis, hip dysplasia, scoliosis | Unremarkable/ NA |
| LEV, LTG | Left parietal–occipital sharp wave–slow wave activity, increasing during sleep (28 years) | Unremarkable (28 years) | Unremarkable, except for mild ID | Unremarkable | Unremarkable/ NA |
| VPA, PB | 3–3.5-Hz SWs with myoclonic jerks (12 years); photosensitivity (19 years); 3–3.5-Hz SWs with eyelid myoclonia (28 years); normal background activity with diffuse 2.5–3-Hz SWs (32 years) | CC mild thickening, superior vermian hypoplasia (32 years) | Unremarkable, except for deficit of verbal fluency and long-term visual memory | Dysarthria, segmental myoclonus | Unremarkable/ unremarkable |
| LTG, CLB | Unremarkable (20 years and 28 years) | Mild generalized atrophy (21 years) | Severe ID; never able to speak sentences; delayed autonomous walking | Spastic hypertonia, scoliosis, keratoconus | NA/NA |
| Steroids | Multifocal EDs (5 years); left temporal slowing and bilateral (L > R) sharp waves, multifocal EDs at drowsiness and during sleep (12 years) | WM volume reduction, bilateral hippocampi malrotation, posterior CC thinning (12 years);[18] FDG-PET: cerebellar hypometabolism (12 years) | Mild ID; single words at 2 years, first sentence at 3 years 6 months, sitting at 9 months, walking at 18 months | OCF <3rd pc, hyperactive behavior | Unremarkable/ unremarkable |

(Continues)

**TABLE 1** (Continued)

| Pt | Gender/last evaluation | Mutation cDNA, protein/domain site | Epilepsy | | | | Previous ASMs |
|---|---|---|---|---|---|---|---|
| | | | Onset | | Follow-up | | |
| | | | Age | Sz type at onset (frequency) | Epilepsy syndrome/Sz type | Sz freedom (age) | |
| 10 | M/2 years | c.10247_10279dup; p.Leu3416_Asn3426dup/ stalk domain including MTBD | 2 months | Spasms (daily, clusters) | IESS/spasms | No | ACTH, VGB, TPM, CBZ, DZP |
| 11 | M/9 months | c.385A > G; p.Lys129Glu/ tail domain, outside the homodimerization region | 4 months | Focal onset motor Sz, myoclonic Sz (daily) | IESS/focal onset motor Sz, myoclonic Sz, spasms, bilateral TC Sz | Yes (9 months) | LEV, VGB, CLB, steroids, VPA |
| 12 | F/10 years | c.3278 T > C; p.Phe1093Ser/tail domain, inside the homodimerization region | 7 months | Spasms (unk) | IESS/none | Yes (7 months) | ACTH |
| 13 | F/6 years 4 months | c.1861_1863 delGAC; p.Asp621del/tail domain, inside the homodimerization region | 3 years 6 months | Focal onset nonmotor Sz (unk) | Focal onset nonmotor Sz, focal to bilateral TC Sz | No | NA |
| 14 | F/14 years | c.6989G > A; p.Gly2330Glu/motor domain (AAA1 subdomain) | 5 months | Spasms (unk) | IESS and LGS/ atonic Sz, tonic Sz | No | ACTH |
| 15 | F/8 months (deceased) | c.5885G > A; p.Arg1962His/motor domain | 3 months | Spasms (unk) | IESS/spasms | No | VGB, ACTH |
| 16 | F/9 years 6 months | c.4700G > A; p.Arg1567Gln/neck domain | 9 years | Focal to bilateral TC Sz (unk) | Focal to bilateral TC Sz | Yes (9 years) | CLZ |
| 17 | M/9 years 1 month | c.8945G > A; p.Arg2982His/motor domain | 10 months | Bilateral TC Sz (daily) | FS (1 episode), bilateral TC Sz | Yes (3 years) | VPA |
| 18 | F/3 years | c.10420C > T; p.Arg3474Trp, mosaic/ stalk domain including MTBD | 10 months | Myoclonic Sz (unk) | IESS and LGS/ myoclonic Sz, spasms, atypical abs, tonic, atonic, TC Sz | No | PER, KD, RUF, TPM, VPA, ZNS, LEV, CBD |

| Current ASMs | EEGs (age) | MRIs (age) | Developmental stages | Neurological examination | EKG/EMG findings |
|---|---|---|---|---|---|
| CBZ, TPM, VPA | Left temporal EDs (2 months); central–temporal EDs, with worsening during sleep (1 year 6 months) | WM volume reduction, posterior pachygyria, PMG, hippocampi malrotation, CC and vermian dysgenesis (2 years) | Severe ID; never able to walk or speak sentences | OCF <3rd pc, rare eye contact, severe axial/limb hypotonia, severe dysphagia | NA/peripheral demyelinating neuropathy, mainly affecting the motor conduction (10 months) |
| LEV, VGB, VPA | Hypsarrythmic-like pattern (4 months); slow background activity, multifocal sharp waves in the left parietal–central regions with bilateral diffusion (7 months) | Lissencephaly–pachygyria spectrum (P-A gradient; 4 months) | Severe ID; no single words at 9 months, not able to sit alone at 9 months | Axial/limb hypotonia, no eye contact, strabismus | Unremarkable/ NA |
| None | Sharp waves in the temporal regions (R > L; 9 months); bilateral spike and polyspike activity (posterior dominant; 1 year) | NHs (10 years),[18] FDG-PET: mild hypometabolism of left cerebral cortex (10 years) | Moderate ID; single words at 2 years, walking at 2 years | ASD, limb hypotonia | NA/ unremarkable |
| LTG | Unremarkable (5 years 4 months) | CC thickening, pons and vermian hypoplasia, pachygyria (P-A gradient; 4 years 7 months) | ID; crawling at 16 months, walking at 20 months | Strabismus | NA/NA |
| LTG, CLB, CBD, VNS (14 years) | Bilateral independent temporal sharp waves (L > R) during sleep, GPFA (14 years) | Right Heschl gyrus cortical dysplasia (14 years) | Severe ID; never able to speak, walking at 4 years | Strabismus, bilateral cataract | Unremarkable/ unremarkable |
| VGB | Hypsarrythmic pattern, burst suppression; paucity of sleep figures (5 months) | Dilated cerebral ventricles (36 Gestational Week Ultrasound); bilateral frontal–parietal PMG, PNH, lateral ventricle dilation, WM volume reduction (8 months) | Severe ID; never acquired head control, no social smile, no eye contact; feeding concerns | Severe axial hypotonia | Unremarkable/ NA |
| LEV, OXC | Bilateral independent central–temporal sharp waves (9 years 6 months) | Pachygyria (A-P gradient), hippocampal thickening, cortical dysplasia (9 years 4 months) | Severe ID; sitting at 24 months, crawling at 29 months | OCF <3rd pc self-injurious behavior, Gowers' sign, delayed response to sensation | NA/chronic denervation with unremarkable sensory/ motor nerve conduction velocities |
| None | Generalized background slowing (9 years 1 month) | Mild sylvian enlargement (5 years 8 months) | Severe ID; never able to speak, sit, or walk | OCF at 3rd pc, stereotypies, axial/ limb hypotonia | NA/NA |
| LCM, CLB, CBD | Hypsarrhythmialike pattern (15 months); slow background activity, bilateral SWs (2–2.5 Hz), GPFA in sleep (2 years) | Unremarkable (12 months) | Severe ID; never able to speak, sitting at 7 months, walking at 15 months | OCF at 3rd pc, ASD | Unremarkable/ NA |

(Continues)

**TABLE 1** (Continued)

| Pt | Gender/last evaluation | Mutation cDNA, protein/domain site | Epilepsy | | | | Previous ASMs |
|---|---|---|---|---|---|---|---|
| | | | Onset | | Follow-up | | |
| | | | Age | Sz type at onset (frequency) | Epilepsy syndrome/Sz type | Sz freedom (age) | |
| 19 | F/25 years | c.1424A > G; p.Gln475Arg/tail domain, inside the homodimerization region | 12 years | Bilateral TC Sz (rare) | Bilateral TC Sz, focal onset Sz with impaired awareness | Yes (25 years) | None |
| 20 | M/40 years | c.926G > A; p.Arg309His/ tail domain, inside the homodimerization region | 18 months | FS, atypical abs (daily) | LGS-like/atypical abs, bilateral TC Sz, spasms, tonic Sz, SE (triggered by fever) | No | ACTH, VPA, VGB, PB, TGB, LEV |
| 21 | M/15 years | c.10973G > A; p.Gly3658Glu/motor domain (AAA5 subdomain) | 3 years | Focal onset motor Sz with impaired awareness (daily) | Focal onset Sz with impaired awareness, focal to bilateral TC Sz | No | LEV, LTG, CLB, TPM |
| 22 | M/2 years | c.4366G > A; p.Glu1456Lys/neck domain | 5 months | Spams (daily) | IESS/spasms | No | Steroids, TPM, VPA |
| 23 | M/14 years (deceased) | c.4104_4112del; p.Asn1368_Leu1370del/ tail domain, outside the homodimerization region | 1 month | Spasms (unk) | IESS/spasms (1 year), myoclonic Sz (8 months), bilateral tonic Sz (3 years) | No | NA |
| 24 | M/9 years[16] | c.9041A > G; p.Asn3014Ser/motor domain | 6 years | Atonic Sz (sporadic) | LGS-like/atonic Sz | Yes | LEV |
| 25 | F/17 months | c.8135G > A; p.Cys2712Tyr/motor domain | 6 months | Spasms (daily) | IESS/spasms | No | VGB, steroids |
| 26 | M/15 years | c.2342T > C p.Leu781Pro/ tail domain, inside the homodimerization region | 7 years, 6 months | Focal onset motor Sz (weekly) | Focal onset Sz, sometimes with bilateral TC diffusion | Yes | CBZ |
| 27 | M/11 years | c.5884C > T; p.Arg1962Cys/motor domain (AAA1 subdomain) | 4 years | Focal motor Sz with impaired awareness (unk) | Focal motor Sz with impaired awareness | Yes | VPA |

| Current ASMs | EEGs (age) | MRIs (age) | Developmental stages | Neurological examination | EKG/EMG findings |
|---|---|---|---|---|---|
| OXC, LCM | Multifocal EDs with bilateral diffusion, slow background activity (25 years) | NA | Moderate ID; walking at 24 months, first sentence at 8 years | Unremarkable | Unremarkable/ NA |
| LTG, VPA, PER, CLZ | Right posterior EDs (32 months); slow background activity, continuous multifocal EDs, enhancing in sleep (36 years) | Pachygyria (P-A gradient; 15 years) | Severe ID; single words at 32 months, never able to speak sentences, walking at 32 months | Scoliosis, tetraparesis | NA/NA |
| PER, VPA | Bilateral (L > R) frontal–central–temporal EDs upon awakening and during sleep (2 years 9 months); bilateral frontal EDs during sleep, poor representation of sleep figures (10 years) | Pachygyria (A-P gradient), vermian hypoplasia (15 years) | Severe ID; first sentences at 6 years, sitting at 6 months, walking at 36 months | OCF <3rd pc, ASD, axial hypotonia, poor motor coordination | NA/NA |
| VGB, KD | Hypsarrhythmia (5 months); multifocal EDs, accentuated during sleep; slow background activity, sleep stages cannot be distinguished (20 months) | Bilateral perisylvian PMG, bilateral small hippocampi (17 months) | Severe ID; unable to sit or stand unaided, nonverbal; regression in minor milestones at time of spasms onset | Axial/limb hypotonia, arthrogryposis multiplex congenita, right clubfoot, poor deep tendon reflexes (>lower limbs) | Unremarkable (8 months)/NA |
| NA | Multifocal EDs, accentuated during sleep, moderately well-organized background pattern (6 months); multifocal EDs, myoclonus associated with diffuse SW complexes (12 months); hypsarrhythmialike pattern (15–30 months) | WM volume reduction, CC dysgenesis, pons, brainstem, and vermian hypoplasia, PNH (30 months) | Severe ID; sitting position never acquired, nonverbal | Touch-induced nonepileptic myoclonus, startlelike | NA/NA |
| LEV | Slow background activity, diffuse SWs at 1–2.5 Hz, with maximum in bilateral occipital regions (6 years) | CC dysgenesis, arachnoid cysts, periventricular hyperintensities and cortical atrophy (2 years) | ID; delayed age at sitting, walking at 23 months, speaks few single words, ADHD | Bilateral congenital cataract, cutis laxa, bluish sclerae, Gowers' sign, axial/ limb hypotonia | Unremarkable/ signs of left axonal loss of sensory and motor neurons |
| VGB | Hypsarrhythmia with right frontal focus (6 months) | Bifrontal PMG (7 months) | ID; sitting at 16 months, words not acquired at 16 months | Bilateral congenital cataract, hypotonia | NA/NA |
| VPA, LTG | Ictal EEG (8 years): recorded seizure originating from right parietal–central–temporal site, lasting approximately 2 min; sleep EEG (14 years): bilateral independent spikes in frontal regions | Simplified gyral pattern, especially in frontal regions, dysmorphic brainstem and cerebellar vermis (11 years) | Mild ID; sitting at 6 months, walking at 13 months, first words at 12 months, first sentences at 4 years; WISC-IV (13 years) total IQ = 79 | Walking on wide base, pes cavus, hypoelicitable patellar reflexes, clumsy gross and fine motor skills | Unremarkable/ normal sensorimotor conduction velocity (13 years) |
| None | Focal spikes and sharp waves at right frontal site; slow background activity (4 years) | Simplified gyral pattern (frontal regions) and dilated lateral ventricles (2 years) | Severe ID; sitting at 16 months, walking at 48 months, no acquired language | Microcephaly, hyperactivity, ASD, strabismus | Unremarkable/ NA |

(Continues)

**TABLE 1** (Continued)

| Pt | Gender/last evaluation | Mutation cDNA, protein/domain site | Epilepsy | | | | Previous ASMs |
|---|---|---|---|---|---|---|---|
| | | | Onset | | Follow-up | | |
| | | | Age | Sz type at onset (frequency) | Epilepsy syndrome/Sz type | Sz freedom (age) | |
| 28 | F/14 years | c.10232C > T; p.Pro3411Leu/stalk domain including MTBD | 2 years | Focal motor Sz during sleep (unk) | IESS/asymmetric spasms upon awakening, tonic and TC Sz during sleep (weekly) | No | VGB |
| 29 | M/22 months | c.925C > T; p.Arg309Cys/ tail domain, inside the homodimerization region | 2 months | Focal motor Sz (weekly) | Focal motor Sz | Yes (17 months) | CBZ |
| 30 | M/26 years | c.1705C > T; p.Arg569Trp/ tail domain, inside the homodimerization region | 11 months | Bilateral TC Sz (unk) | Focal to bilateral TC Sz (monthly), tonic Sz | Yes | LEV, OXC, CBZ, VGB, TPM, LTG, CLB |
| 31 | M/15 years | c.10958 T > A; p.Val3653Glu/motor domain (AAA5 subdomain) | 6 years 7 months | Tonic Sz (sporadic) | Focal tonic Sz, FS | Yes (9 years) | NA |
| 32 | F/9 years | c.3169 T > C; p.Tyr1057His/tail domain, inside the homodimerization region | 2 years | Spasms (daily) | IESS/spasms, tonic, focal to bilateral motor Sz | Yes (5 years) | CBZ, OXC, TPM, CLZ |
| 33 | F/16 years | c.4868G > A; p.Arg1623Gln/neck domain | 5 months | Spasms (unk) | IESS | Yes (14 years) | ACTH, CLZ |
| 34 | M/9 years | c.926G > A; p.Arg309His/ tail domain, inside the homodimerization region | 2 months | Asymmetric spasms (daily) | IESS/asymmetric spasms and tonic Sz in clusters, Sz with eyelid myoclonia, bilateral TC Sz | No | VGB, VPA, CLB, steroids, TPM, KD, FLB |

Abbreviations: $^{18}$FDG-PET, $^{18}$F-fluorodeoxyglucose positron emission tomography; abs, absences; ACTH, adrenocorticotropic hormone; ADHD, attention-deficit/hyperactivity disorder; A-P, anteroposterior; ASD, autism spectrum disorder; ASM, antiseizure medication; CBD, cannabidiol; CBZ, carbamazepine; CC, corpus callosum; CLB, clobazam; CLZ, clonazepam; DZP, diazepam; ED, epileptiform discharge; EEG, electroencephalogram; EKG, electrocardiogram; EMG, electromyography; F, female; FLB, felbamate; FS, febrile seizures; GPFA, generalized paroxysmal fast activity; ID, intellectual disability; IESS, infantile epileptic spasms syndrome; IQ, intelligence quotient; KD, ketogenic diet; L, left; LCM, lacosamide; LEV, levetiracetam; LGS, Lennox–Gastaut syndrome; LTG, lamotrigine; M, male; MCD, malformation of cortical development; MRI, magnetic resonance imaging; MTBD, microtubules binding domain; NA, not available; NH, nodular heterotopia; OFC, occipital–frontal circumference; OXC, oxcarbazepine; P-A, posteroanterior; PB, phenobarbital; pc, percentile; PER, perampanel; PHT, phenytoin; PMG, polymicrogyria; PNH, periventricular nodular heterotopia; Pt, patient; R, right; RUF, rufinamide; SE, status epilepticus; SW, spike–wave; Sz, seizures; TC, tonic–clonic; TGB, tiagabine; TPM, topiramate; unk, unknown; VGB, vigabatrin; VNS, vagus nerve stimulator; VPA, valproate; WISC-IV, Wechsler Intelligence Scale for Children, Fourth Edition; WM, white matter; ZNS, zonisamide.

| Current ASMs | EEGs (age) | MRIs (age) | Developmental stages | Neurological examination | EKG/EMG findings |
|---|---|---|---|---|---|
| PB | Spikes in the bilateral central regions (2 years); SWs in frontal–temporal regions, accentuated during sleep (14 years) | Dilated lateral and third ventricles, MCDs of the frontal lobes (17 months; 8 years) | ID; sitting at 10 months, walking at 24 months, first single words at 11 years, sentence production not acquired | Diffusehypotrophism, dystonic posture of upper limbs, simple and complex motor tics | NA/NA |
| CBZ | Bilateral independent spikes in posterior bilateral regions and vertex (3 months) | Bilateral posterior subcortical band heterotopia (3 months) | Severe ID; sitting 9 at months, walking at 22 months, single words at 10 months, sentence production not acquired | Broad-based gait, possible only with support; poor fine motor coordination | NA/NA |
| VPA, CBZ, OXC, TPM, CLB, LTG | Frontal sharp waves (11 months), multifocal EDs (26 years) | Unremarkable (7 years, 15 years) | Severe ID, sitting at 8 months, walking at 18 months, first words at 32 months, able to produce simple sentences | Fine and gross motor impairment, behavior disorder | Normal/NA |
| None | Focal EDs, unremarkable background activity (6 years 7 months) | Right PNH (8 years) | Moderate ID | Fine and gross motor impairment | Normal/NA |
| VPA | Focal sharp waves, slow background activity (9 years) | Bilateral perisylvian PMG (2 years) | Severe ID; sitting at 18 months, walking at 24 months, not able to speak sentences; developmental regression | Fine and gross motor impairment | Normal/ axonal motor neuropathy |
| VPA | Bilateral SW and polyspike-wave complexes (3 months); isolated left central spikes (8 years) | Pachygyria (A-P gradient) and PMG, myelination delay, hypotrophic hippocampus, CC dysgenesis (4 years 8 months) | Severe ID; not able to sit, language not acquired | Spastic dystonic tetraparesis, stereotypies, scoliosis, bilateral cataract | Normal/ axonal motor neuropathy |
| FLB, VPA, CLB | Hypsarrhythmialike pattern (2 months); slow background activity, high-voltage spikes, sharp waves and fast rhythms in the anterior regions (9 years) | Pachygyria–lissencephaly, subcortical bilateral heterotopia, more evident in the occipital–parietal regions, dysmorphic basal ganglia, hypoplastic pons (2 months) | Severe ID; sitting at 36 months, able to produce only vocalizations | Diffuse hypotrophy, limb hypotonia, drooling, cardiac tamponade at 2 years of unidentified etiology | Unremarkable/ unremarkable |

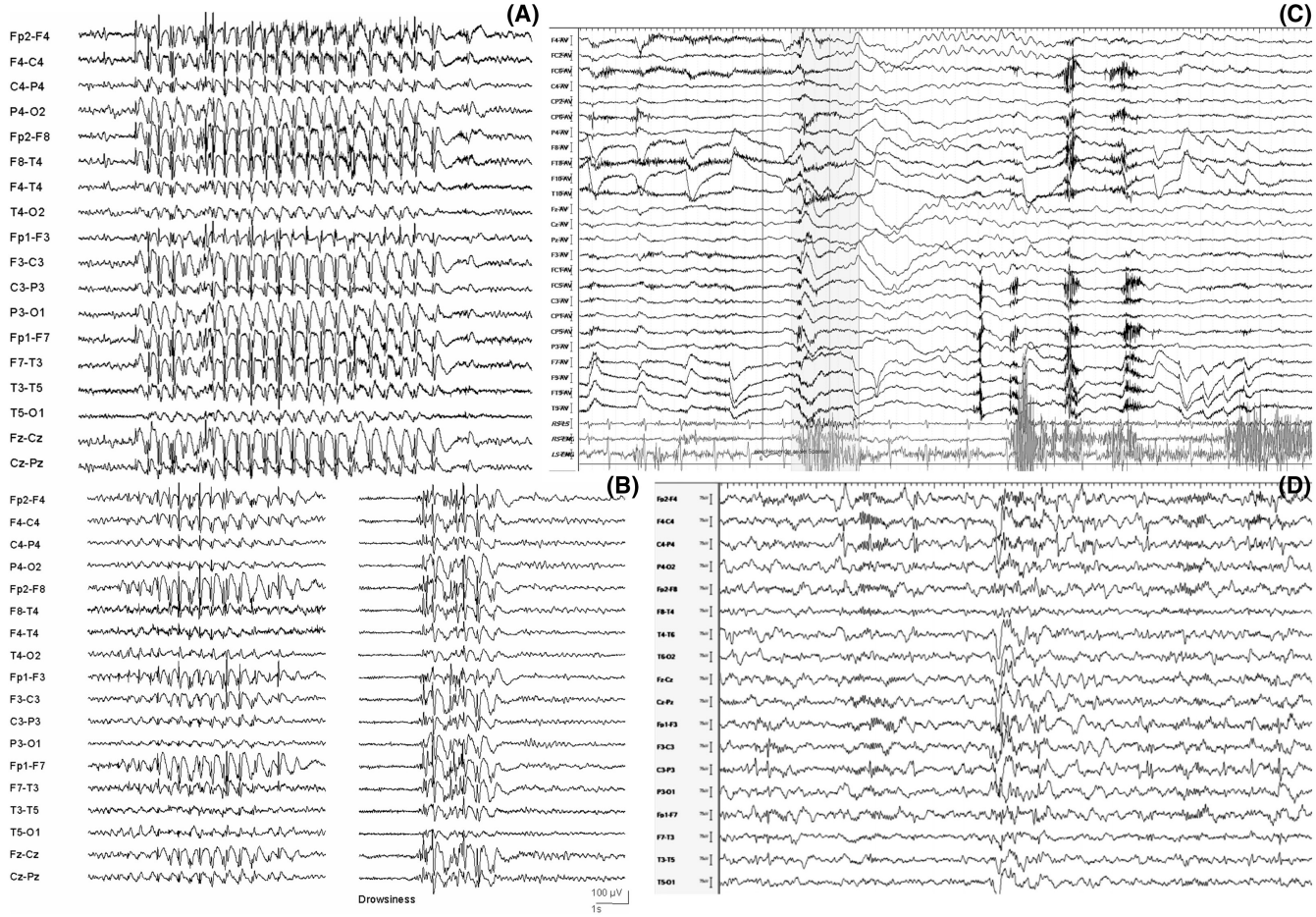


**FIGURE 1** Electroencephalographic (EEG) findings. (A, B) EEG recording of Patient 7 when he was 32 years old, showing generalized spike–wave complexes at 2.5–3 Hz (A) and asymmetric polyspike–wave complexes (emphasized over the right frontal areas), accentuated during drowsiness (B). (C) EEG recording of Patient 9 when he was 10 years old, showing ictal recording at awakening characterized at the onset by a high-amplitude slow wave followed by an electrodecremental response, corresponding to a bilateral muscular activation lasting approximately 1 s; then brief sinusoidal rhythmic theta activity over the frontal regions follows. (D) Sleep EEG recording of Patient 26 at the age of 14 years, showing normal sleep figures (k complex and sleep spindles) and focal sharp waves and spike–wave complexes over the frontocentral regions, bilaterally independent, more frequent on the left side.

In 14 patients, epilepsy onset was with focal seizures.[6,16,18,31,38] The EEG was available in four of these subjects and showed multifocal epileptiform discharges, with striking activation during sleep up to a continuous spike-and-wave pattern in one child.[18] Three patients were described to have a phenotype LGS-like, albeit EEGs were not provided.[6,35,36] Among patients with spasms or definite IESS, pathogenic variants were in the motor domain in seven subjects[17–19,32] and within the stalk region in four subjects.[17–19]

Brain MRI disclosed MCDs in 48 of 58 patients, included on the lissencephaly–pachygyria spectrum in 42 of 58 patients (72%)[6,9,16,18,19,29,32,34–38] and with anteroposterior increasing gradient in 26 of them.[6,18,29,34,36–38] Midline anomalies were noticed in 21 patients, more frequently as corpus callosum dysgenesis or agenesis and white matter volume reduction.[6,9,15,16,18,19,29,31,32,35,37] Infratentorial abnormalities such as pons or cerebellar vermian hypoplasia were described in two patients.[6,16]

Overall, considering MRI findings of both our cohort and the 58 patients reported in literature, MCDs were found in 84.1% of patients with *DYNC1H1*-related epilepsy. Particularly, lissencephaly–pachygyria may be observed in 59.3% of cases, and it shows a characteristic anteroposterior increasing gradient in the 64.4% of these (Figure 3B).

Almost all patients, except for two with focal seizures,[6] presented with ID and developmental delay.

Bilateral cataract was described as an additional clinical feature in six subjects.[15,16,18,31,32,34]

Four patients harbored the p.Arg309His variant, in the tail domain.[18,34] The epileptic phenotype was not described in three of these individuals,[34] who all showed prominent posterior pachygyria on brain MRI and severe

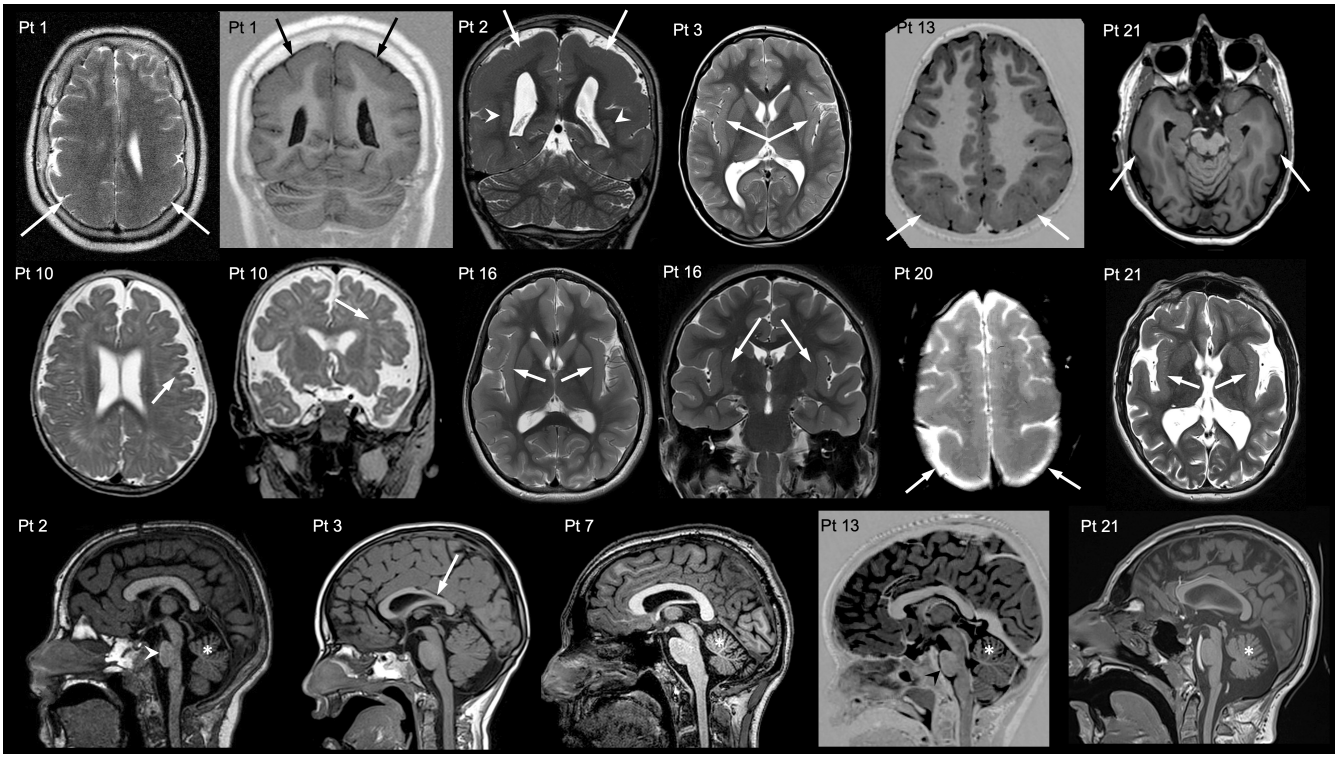


**FIGURE 2** Neuroradiological findings. Magnetic resonance imaging depicts bilateral posterior lissencephaly–pachygyria spectrum in Patients 1, 2, 13, and 20, whereas a posteroanterior increasing gradient is evident in Patient 16 (arrows). Insular pachygyria is present in Patients 3 and 16. Bilateral temporoinsular pachygyria is depicted in Patient 21 on axial T1- and T2-weighted sequences (arrows), as well as ventriculomegaly due to white matter volume loss. Polymicrogyria is present in Patient 10 (arrows). In Patient 2, nodular periventricular heterotopias are evident (arrowheads). Midsagittal images (lower row) show corpus and isthmic corpus callosum thinning in Patient 3 (arrows), thickening of the corpus callosum in Patients 2, 7, and 13, cerebellar vermis hypoplasia in Patients 2, 7, 13, and 21 (asterisks), and brainstem hypoplasia in Patients 2 and 13 (arrowheads).

ID. The other p.Arg309His subject[18] presented with drug-resistant spasms and severe ID. Among the three patients with p.Arg1962Cys, in the motor domain,[6,18,19] two were affected with drug-resistant IESS[18,19] and one was described as having focal seizures; pachygyria was noticed in two of them,[6,18,19] with posterior predominance in one subject.[6]

## 3.5 | Latent class analysis

LCA favored a three-class solution (log-likelihood = −246, AIC = 549, BIC = 621, entropy = .81) against a two-class solution (log-likelihood = −268, AIC = 573, BIC = 621, entropy = .69). The three-class solution was also favored against a four-class solution (log-likelihood = −235, AIC = 549, BIC = 646, entropy = .82) due to lower BIC and model parsimony. To assess the robustness of our results, a two-step cluster analysis using complete data analysis was performed, confirming the three-cluster solution, yielding reproducible results (Table S4). The probability of each indicator to be assigned to each class is represented in Figure S1.

The first class included 57 (62%) patients, the second class included 22 (23.9%) patients, and the third class included 13 (14.1%) patients. The three classes did not significantly differ in terms of distribution of DYNC1H1 functional domains, whereas Class 1 was characterized by the lowest age at seizure onset ($p < .001$), higher representation of severe ID ($p < .001$), epileptic spasms ($p < .001$), tonic seizures ($p = .03$), lissencephaly–pachygyria spectrum on brain MRI ($p = .04$), hypsarrythmic pattern on EEG ($p = .03$), and major motor abnormalities on neurological examinations ($p = .03$), and patient phenotypes fulfilled criteria for IESS and/or LGS. Class 2 was characterized by later epilepsy onset and lower rates of tonic seizures/spasms compared with Class 1, and intermediate rates of ID and major motor abnormalities; the epilepsy syndromes included in this class were DEE/epileptic encephalopathy (EE; i.e., other than LGS and IESS; Figure 4). Class 3 showed the lowest rate of cognitive abnormalities, with patients diagnosed as either focal epilepsy or GGE.

After FDR adjustment, the majority of statistically significant differences between the described classes were confirmed, except for tonic seizures (FDR-adjusted $p = .07$), the

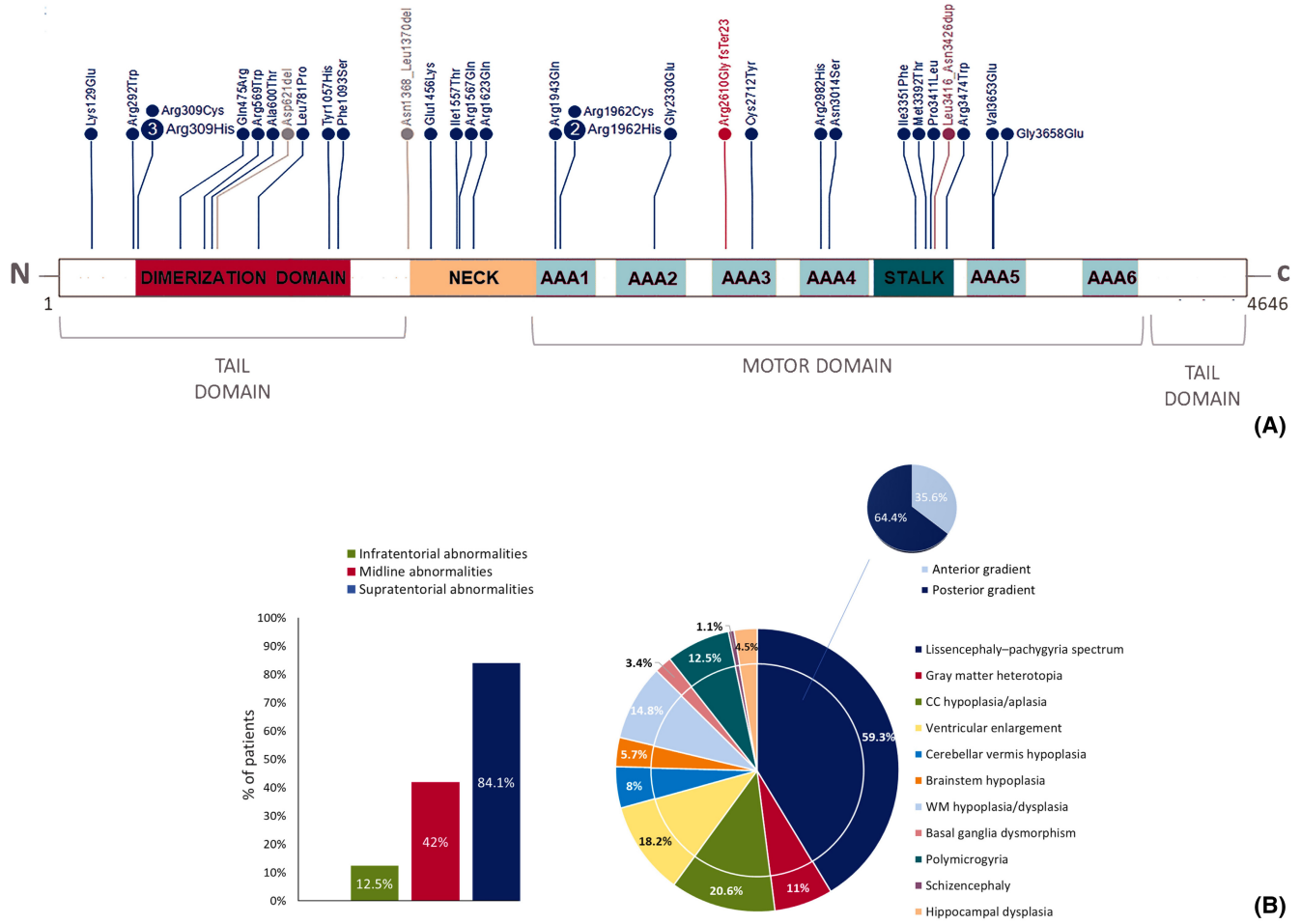


**FIGURE 3** (A) Distribution of DYNC1H1 pathogenic variants in our cohort along the protein domains. (B) Brain magnetic resonance imaging (MRI) findings categorized by malformation types. The left part of the figure presents brain MRI findings categorized into three groups: infratentorial malformations, median line malformations, and supratentorial malformations. The right part of the figure represents brain MRI findings according to these malformation types. Percentages shown in the figure are based on a combination of data from our cohort and previous literature. CC, corpus callosum; WM, white matter.

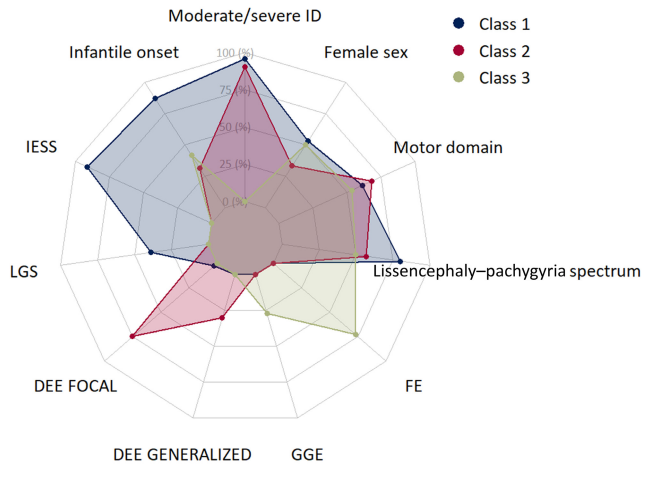


**FIGURE 4** Classes of *DYNC1H1*-related epilepsy spectrum identified through latent class analysis. DEE, developmental and epileptic encephalopathy; FE, focal epilepsy; GGE, genetic generalized epilepsy; ID, intellectual disability; IESS, infantile epileptic spasms syndrome; LGS, Lennox–Gastaut syndrome.

lissencephaly–pachygyria spectrum observed on brain MRI (FDR-adjusted $p = .08$), hypsarrhythmic patterns on EEG (FDR-adjusted $p = .07$), and major motor abnormalities observed during neurological examinations (FDR-adjusted $p = .055$). A detailed description of the characteristics of the three classes, along with both unadjusted and FDR-adjusted $p$-values, is summarized in Table 2.

## 3.6 | Genotype–phenotype correlations

Multivariable binary logistic regression revealed a borderline significant association ($p = .066$) between stalk domain and Class 1 (namely the LGS-IESS subgroup), after adjusting for sex, age at onset, ethnic background, and variant effect (Table S5). We did not check genotype–phenotype correlations for other identified classes due to the low sample size. In addition, a second multivariable binary logistic regression model showed that spasms

**TABLE 2** Clinical characteristics according to clinical subgroups identified by latent class analysis.

| | Class 1, *n* = 57 | Class 2, *n* = 22 | Class 3, *n* = 13 | Unadjusted *p* | FDR-adjusted *p* |
|---|---|---|---|---|---|
| Sex, female, *n* (%) | 25 (53.2) | 6 (33.3) | 5 (50) | .35 | .460 |
| Gene domain, *n* (%) | | | | | |
| Tail domain | 23 (40.4) | 7 (31.8) | 6 (50) | .57 | .625 |
| Motor domain | 15 (26.3) | 8 (36.4) | 2 (16.7) | .4 | .5 |
| Stalk domain | 16 (28.1) | 3 (13.6) | 4 (33.3) | .3 | .416 |
| Neck domain | 3 (5.3) | 4 (18.2) | 0 | .09 | .160 |
| Age at last observation, years, median (IQR) | 4.1 (2–10) | 12 (6.4–24.2) | 11 (2.9–30) | .003* | .009* |
| Age at epilepsy onset, years, median (IQR) | .6 (.3–1) | 3 (.9–6.5) | 3.7 (1–11.5) | <.001* | <.001* |
| Intellectual disability, *n* (%) | | | | | |
| None | 0 | 0 | 3 (25) | <.001* | <.001* |
| Mild intellectual disability | 3 (6.7) | 0 | 9 (75) | <.001* | <.001* |
| Moderate intellectual disability | 3 (6.7) | 7 (35) | 0 | .003* | .009* |
| Severe intellectual disability | 39 (86.7) | 13 (65) | 0 | <.001* | <.001* |
| Seizure types, *n* (%) | | | | | |
| Spasms | 32 (82.4) | 1 (5.6) | 0 | <.001* | <.001* |
| Tonic seizures | 12 (32.4) | 2 (11.1) | 0 | .03* | .068 |
| Atonic seizures | 5 (13.5) | 1 (5.9) | 0 | .3 | .4 |
| GTCS | 11 (29.7) | 8 (44.4) | 4 (36.4) | .6 | .6 |
| Focal seizures | 7 (18.9) | 12 (66.7) | 8 (72.7) | <.001* | .001* |
| Myoclonic seizures | 7 (18.9) | 3 (16.7) | 3 (27.3) | .7 | .7 |
| Absences | 5 (13.5) | 1 (5.6) | 1 (9.1) | .6 | .6 |
| MRI features, *n* (%) | | | | | |
| Lissencephaly–pachygyria | 44 (80) | 12 (57.1) | 6 (50) | .04* | .08 |
| Polymicrogyria | 10 (18.2) | 0 | 1 (8.3) | .09 | .16 |
| Gray matter heterotopia | 8 (14.5) | 1 (4.8) | 1 (8.3) | .45 | .53 |
| EEG features, *n* (%) | | | | | |
| Hypsarrhythmia | 12 (35.3) | 1 (6.7) | 0 | .03* | .068 |
| Clinical examination, *n* (%) | | | | | |
| Major motor abnormalities | 26 (74.3) | 10 (55.6) | 3 (27.3) | .02* | .055 |
| Cataract | 7 (19.4) | 1 (5.3) | 1 (9.1) | .3 | .4 |
| Microcephaly | 11 (30.6) | 4 (21.1) | 1 (9.1) | .3 | .4 |

Abbreviations: EEG, electroencephalographic; FDR, false discovery rate; GTCS, generalized tonic–clonic seizures; IQR, interquartile range; MRI, magnetic resonance imaging.

*Indicates statistically significant variables ($p < .05$).

(odds ratio [OR] = 11.51, 95% confidence interval [CI] = 1.19–111.33, $p = .03$) and stalk domain (OR = 20.81, 95% CI = 1.15–374.75, $p = .04$) were independently associated with severe ID (Table S6).

## 4 | DISCUSSION

DYNC1H1 is one of two known cytoplasmic heavy chain dyneins and has essential functions in all cells, including orientation of the mitotic spindle, nuclear migration during mitosis, organelle motility, and autophagosomal and endosomal trafficking. In the CNS, DYNC1H1 retrogradely transports cargoes in the axon and dendritic shafts, including synaptic vesicle precursors, neurotransmitters, neurotrophic factor receptors, and mRNA.[2,3] It further contributes to neuronal development by promoting myelination of both CNS and peripheral nerves.[4]

In recent years, pathogenic variants in *DYNC1H1* have been classified with *DYNC1H1*-related neuromuscular disorders with variants in the tail domain and concomitant *DYNC1H1*-related neurodevelopmental disorders

with variants in the residual protein domains.[39] The latter disease entity includes brain malformations and epileptic encephalopathies, along with structural epilepsies.[6,17]

In our *DYNC1H1*-related epilepsy cohort, age at onset of epilepsy was during infancy in the majority of patients and in more than half of individuals was within the first year of life. The most frequent seizure type at onset was IS, occurring in 38% of our cohort at a median age of 5 months. IESS was defined overall in half of our cohort, and in approximately half of these subjects the EEG showed a hypsarrythmic pattern. IS were drug-resistant in approximately 60% of our IESS patients. Moreover, in approximately one quarter of these individuals, the epileptic phenotype had evolved into LGS. Focal onset epilepsy was the second most common epileptic condition (35% of our cohort). A distinctive PME-like phenotype was reported in one subject, who harbored the frameshift variant p.Arg2610GlyfsTer23.[28]

In the 58 reported *DYNC1H1*-related epileptic individuals, epilepsy onset usually occurred in infancy, and the most frequent epileptic phenotypes described were IESS and focal epilepsy. Among patients reported with IESS, IS were drug-resistant in approximately 70% of cases.[18,19,32,37] When comparing these cases with our cohort, we conclude that the epileptic phenotype related to *DYNC1H1* variants was usually manifested during childhood and in approximately half of cases within the first year of life. IESS is the most common epileptic syndrome related to *DYNC1H1*-related epilepsy, and in 60%–70% of cases IS may be resistant to several lines of ASMs and/or other treatment, including vigabatrin, steroids, and ketogenic diet, used separately or in combination. Concerning the previously reported cases affected with IESS,[18,19,32,37] in our cohort the evolution from this syndrome to LGS was reported in four individuals.

The observation that de novo variants in *DYNC1H1* are found in patients with EE, autism spectrum disorder, and ID has been already discussed by Lin et al.[17] Among both our cohort and the reviewed 58 patients, >90% of subjects presented with ID and developmental concerns. Moreover, in eight subjects of our cohort, developmental regression after epilepsy onset was observed, six of whom were diagnosed with IESS, and among the previously reported subjects, regression was reported in four.[19,38] These findings suggest that *DYNC1H1* may be considered as a disease gene in the evaluation of DEEs.[23]

MCDs were disclosed on brain MRI in 79% of our cohort and 83% of the *DYNC1H1*-related epilepsy cases herein reviewed. The most frequently observed MCDs, disclosed overall in 60% of our cohort and cases reported in literature, were included on the lissencephaly–pachygyria spectrum.[6,9,16,18,19,29,32,34–38] Specifically, lissencephaly–pachygyria with anteroposterior increasing gradient may be confirmed as an evocative pattern on the spectrum of *DYNC1H1*-related disorders, as it was observed in approximately 65% of subjects with MCDs in our cohort and reported in literature.[6,18,29,34,36–38] Besides MCDs, midline anomalies have been noticed in 27% of our cohort and 36% of patients reported in literature.[6,9,15,16,18,19,29,31,32,35,37] Corpus callosum dysgenesis and white matter volume reduction with consequent lateral ventricle enlargement were reported as the most common midline abnormalities. Finally, infratentorial anomalies mainly consisting of brainstem and/or cerebellar vermian hypoplasia have been disclosed in a relevant percentage of patients (45%) in our cohort, although these have been noticed in only two cases herein reviewed.[6,16] To conclude, the neuroimaging features in *DYNC1H1*-related epilepsy affirm that MCDs included on the lissencephaly–pachygyria spectrum are the most frequent anomalies in these individuals and show that the posterior predominance of pachygyria is an evocative pattern. Moreover, midline and infratentorial anomalies may also be common in these patients and should be investigated. Among further clinical features, eye concerns, mainly bilateral cataracts[15,16,18,31,32,34] and nonspecific facial dysmorphisms,[15,16,34] are described in a minority of the individuals in both literature and our cohort.

In our cohort, we identified three main classes of *DYNC1H1*-related epilepsy spectrum. Class 1 included the patients diagnosed with IESS and/or LGS; it was characterized by the lowest age at seizure onset, frequent presentation with epileptic spasms and hypsarrhythmia on EEG, higher representation of severe ID, lissencephaly–pachygyria spectrum on brain MRI, and major motor abnormalities on neurological examinations. Class 2 was characterized by a later epilepsy onset and lower rates of tonic seizures/spasms compared with Class 1 and intermediate rates of ID and major motor abnormalities; the epilepsy syndromes included in this class were DEE/EE (i.e., other than LGS and IESS). Class 3 included patients with the lowest rate of cognitive abnormalities, diagnosed as either focal epilepsy or GGE. Our probands harbored 31 different *DYNC1H1* variants, mainly missense, distributed either in the tail or motor domains (39% and 48%). A borderline significant association was only shown between stalk domain and Class 1.

Two variants recurred in our cohort. Three subjects harbored the p.Arg309His variant within the tail domain. They presented with spasms and a phenotype consistent with IESS, LGS, or both. Moreover, brain MRI showed lissencephaly–pachygyria, prominent posteriorly in two subjects, who also presented further MCDs (i.e. nodular heterotopia) and midline or infratentorial abnormalities. In the literature, p.Arg309His was reported to be a frequent *DYNC1H1* variant and the individuals in whom

it was identified usually presented with posterior lissencephaly–pachygyria and drug-resistant spasms.[18,34] Additionally, in our cohort one individual harbored the p.Arg309Cys variant; his phenotype was characterized by focal onset motor seizures controlled with carbamazepine monotherapy and posterior subcortical band heterotopia on brain MRI. Finally, the second recurrent variant in our cohort was p.Arg1962His/Cys, which involves the motor domain of DYNC1H1 protein. The p.Arg1962His was identified in two individuals, whose phenotype was consistent with Class 1, as was the phenotype of the p.Arg309His subjects, showing drug-resistant spasms and a hypsarrythmic EEG pattern. The subject harboring p.Arg1962Cys showed instead a less severe epileptic phenotype, consisting of focal seizures, controlled after ASM discontinuation. Conversely, the p.Arg1962Cys subjects reported in the literature were described as having an epileptic phenotype similar to our p.Arg1962His patients and Class 1 *DYNC1H1*-related epilepsy spectrum.[6,18,19] We may therefore conclude that p.Arg309His variant in the tail domain is usually associated with the Class 1 phenotype of *DYNC1H1*-related epilepsy, whereas p.Arg309Cys seems to be less specific for lissencephaly–pachygyria spectrum and related to a less severe epileptic phenotype. Similarly, the p.Arg1962His variant in the motor domain was associated with Class 1 *DYNC1H1*-realted epilepsy in our cohort, whereas p.Arg1962Cys was associated with both Class 1 and a less severe Class 2 epileptic phenotype.

## 5 | CONCLUSIONS

We report the electroclinical features of subjects affected by *DYNC1H1*-related epilepsy. Epileptic phenotypes are manifested within the first year of life in approximately half of cases, and IESS is the most common epileptic syndrome. IS may be resistant to multiple ASMs, and the evolution to LGS may occur. However, *DYNC1H1* may be responsible for other epileptic phenotypes. We have identified three main classes on *DYNC1H1*-related epilepsy spectrum that represent clusters with different degrees of severity. *DYNC1H1* variants were distributed either in tail or motor domains, and a borderline significant association was only identified between stalk domain and Class 1, including patients affected by IESS and LGS.

The most commonly reported variants were p.Arg309His/Cys, in the tail domain, and p.Arg1962His/Cys, in the motor domain. The variants p.Arg309His and p.Arg1962His were both associated with a Class 1 phenotype, whereas p.Arg309Cys and p.Arg1962Cys were found to be related to a less severe Class 2 phenotype.

*DYNC1H1*-related epilepsy is a rare condition. Although our cohort is the largest described to date, we have not found significant associations between *DYNC1H1* variant distribution in functional domains and the occurrence of epilepsy.

This work will aid in genetic counseling of patients with *DYNC1H1*-related epilepsy and their families and guide the care of these patients.

## 6 | COLLABORATORS

Elena Fontana, Judith Cohen, Silvia Masnada, Pierangelo Veggiotti, Christina Lam, and Anna-Elina Lehesjoki collaborated in this study.

### AUTHOR CONTRIBUTIONS

**Claudia Cuccurullo:** Writing of the manuscript; editing and revision. **Antonietta Coppola:** 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. **Emanuele Cerulli Irelli:** Genetic and statistical analysis; drafting of the manuscript; critical revision for important intellectual content. **Lorenzo Ugga, Alessandra D'Amico, Sara Cabet:** Reviewing and classification of neuroradiological data. **Gaetan Lesca, Leonilda Bilo, Federico Zara, Catrinel Iliescu, Diana Barca, France Fung, Katherine Helbig, Xilma Ortiz-Gonzalez, Helenius J. Schelhaas, Marjolein H. Willemsen, Inge van der Linden, Laura Canafoglia, Carolina Courage, Samuele Gommaraschi, Pedro Gonzalez-Alegre, Tanya Bardakjian, Steffen Syrbe, Elisabeth Schuler, Johannes R. Lemke, Stella Vari, Gitte Roende, Mads Bak, Mahbulul Huq, Zoe Powis, Katrine M. Johannesen, Trine Bjørg Hammer, Rikke S. Møller, Rachel Rabin, John Pappas, Mary L. Zupanc, Neda Zadeh, Julie Cohen, Sakkubai Naidu, Ilona Krey, Russell Saneto, Jenny Thies, Laura Licchetta, Paolo Tinuper, Francesca Bisulli, Raffaella Minardi, Allan Bayat, Nathalie Villeneuve, Florence Molinari, Hormos Salimi Dafsari, Birk Moller, Marie Le Roux, Clara Houdayer, Marilena Vecchi, Isabella Mammi, Elena Fiorini, Jacopo Proietti, Sofia Ferri, Gaetano Cantalupo, Domenica Immacolata Battaglia, Maria Luigia Gambardella, Ilaria Contaldo, Claudia Brogna, Marina Trivisano, Angela De Dominicis, Stefania Maria Bova:** Patient recruitment and acquisition of the clinical and genetic data. **Pasquale Striano, Elena Gardella, Antonella Riva:** Conception and design of the work; patient recruitment; critical revision for important intellectual content.

## AFFILIATIONS

[1]Epilepsy Center, Department of Neuroscience, Reproductive and Odontostomatological Sciences, Federico II University of Naples, Naples, Italy
[2]Neurology and Stroke Unit, Ospedale del Mare Hospital, Naples, Italy
[3]Department of Human Neurosciences, Sapienza University of Rome, Rome, Italy
[4]Department of Advanced Biomedical Sciences, University Federico II, Naples, Italy
[5]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy
[6]Medical Genetic Unit, Istituti di Ricovero e Cura a Carattere Scientifico Istituto Giannina Gaslini, Genoa, Italy
[7]Department of Radiology, "Tortorella" private hospital, Salerno, Italy
[8]Pediatric and Fetal Imaging, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, Lyon, France
[9]Service de Génétique, Hospices Civils de Lyon, Bron, France
[10]Institut NeuroMyoGene, CNRS UMR5310, INSERM U1217, Université Claude Bernard Lyon 1, Lyon, France
[11]Department of Clinical Neurosciences, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[12]Department of Pediatric Neurology, Expertise Center for Rare Diseases in Pediatric Neurology, member of the EpiCARE European Reference Network, "Prof. Dr. Alex. Obregia" Clinical Hospital, Bucharest, Romania
[13]Department of Pediatrics and Neurology, Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA
[14]Department of Neurology, Epilepsy Center Kempenhaeghe, Heeze, the Netherlands
[15]Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands
[16]Integrated Diagnostics for Epilepsy, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[17]Folkhälsan Research Center, Helsinki, Finland
[18]Department of Biomedical and Clinical Science, University of Milan, Milan, Italy
[19]Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Pennsylvania, Philadelphia, USA
[20]Division of Paediatric Epileptology, Center for Pediatrics and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany
[21]Center for Rare Diseases, University of Leipzig Medical Center, Leipzig, Germany
[22]Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, "G. Gaslini" Institute, University of Genoa, Genoa, Italy
[23]Department of Pediatrics and Adolescent Medicine, University Hospital Rigshopitalet, Copenhagen, Denmark
[24]Department of Clinical Genetics, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark
[25]Department of Pediatrics, Wayne State University, Detroit, Michigan, USA
[26]Ambry Genetics, Department of Emerging Genetic Medicine, CGC 15 Argonaut, Aliso Viejo, California, USA
[27]Department of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Center, Dianalund, Denmark
[28]Clinical Genetic Services, Department of Pediatrics, NYU Grossman School of Medicine, New York, New York, USA
[29]Children's Health of Orange County, Orange, California, USA
[30]Genetics Center and Division of Medical Genetics, Children's Hospital of Orange County, Orange, California, USA
[31]Department of Neurology, Kennedy Krieger Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[32]Department of Neurogenetics, Kennedy Krieger Institute, Baltimore, Maryland, USA
[33]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany
[34]Department of Neurology, Seattle Children's Hospital, University of Washington, Seattle, Washington, USA
[35]Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, Washington, USA
[36]Seattle Children's Research Institute, University of Washington, Seattle, Washington, USA
[37]IRCCS, Istituto Delle Scienze Neurologiche di Bologna, full member of the EpiCARE European Reference Network, Bologna, Italy
[38]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
[39]Department of Regional Health Research, University of Southern Denmark, Odense, Denmark
[40]Pediatric Neurology Department, Timone Children Hospital, Marseille, France
[41]Biolab, PolitoBIOMedLab, Department of Electronics and Telecommunications, Politecnico di Torino, Turin, Italy
[42]Department of Pediatrics, Faculty of Medicine, University of Cologne and University Hospital Cologne, Cologne, Germany
[43]Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany
[44]Max Planck Institute for Biology of Ageing, Cologne, Germany
[45]Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases, University of Cologne, Cologne, Germany
[46]Department of Paediatric Neurology, Evelina's Children Hospital, Guy's & St. Thomas' Hospital NHS Foundation Trust, London, UK
[47]Randall Division of Cell and Molecular Biophysics, Muscle Signaling Section, King's College London, London, UK
[48]Department of Pediatric Neurology and Neurosurgery, CHU, Angers, France
[49]Service de Génétique Médicale, Université d'Angers, CHU d'Angers, Inserm, CNRS, MITOVASC, SFR ICAT, Angers, France
[50]University of Padua, Padua, Italy
[51]Medical Genetics Unit, Mirano Hospital, Venice, Italy
[52]Child Neuropsychiatry Unit, University Hospital of Verona, full member of the EpiCARE European Reference Network, Verona, Italy
[53]Center for Research on Epilepsy in Pediatric Age, University Hospital of Verona, Verona, Italy
[54]Innovation Biomedicine Section, Department of Engineering for Innovation Medicine, University of Verona, Verona, Italy
[55]Pediatric Neurology Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[56]Università Cattolica del Sacro Cuore, Rome, Italy
[57]Neuropsychiatric Unit, ASL Avellino, Avellino, Italy
[58]Neurology, Epilepsy, and Movement Disorders, Bambino Gesù Children's Hospital, IRCCS, full member of the EpiCARE European Reference Network, Rome, Italy
[59]Child Neurology Unit, Buzzi Children's Hospital, Milan, Italy



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## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest with regard to the present study. 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.

## DATA AVAILABILITY STATEMENT

The identified data are available on reasonable request.

## PATIENT CONSENT STATEMENT

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.

## ORCID

*Claudia Cuccurullo* https://orcid.org/0000-0001-5118-5103
*Emanuele Cerulli Irelli* https://orcid.org/0000-0003-3906-555X
*Antonella Riva* https://orcid.org/0000-0001-9152-5571
*Gaetan Lesca* https://orcid.org/0000-0001-7691-9492
*France Fung* https://orcid.org/0000-0001-8448-7423
*Katherine Helbig* https://orcid.org/0000-0001-8249-0549
*Laura Canafoglia* https://orcid.org/0000-0002-5385-761X
*Katrine M. Johannesen* https://orcid.org/0000-0002-7356-3109
*Rikke S. Møller* https://orcid.org/0000-0002-9664-1448
*Ilona Krey* https://orcid.org/0000-0002-9168-7615
*Laura Licchetta* https://orcid.org/0000-0001-7979-9895
*Francesca Bisulli* https://orcid.org/0000-0001-6827-0099
*Allan Bayat* https://orcid.org/0000-0003-4986-8006
*Florence Molinari* https://orcid.org/0000-0001-5111-7215
*Marie Le Roux* https://orcid.org/0000-0002-5231-658X
*Jacopo Proietti* https://orcid.org/0000-0002-5584-4724
*Domenica Immacolata Battaglia* https://orcid.org/0000-0003-0491-4021
*Stefania Maria Bova* https://orcid.org/0000-0002-4092-7412
*Elena Gardella* https://orcid.org/0000-0002-7138-6022
*Pasquale Striano* https://orcid.org/0000-0002-6065-1476
*Antonietta Coppola* https://orcid.org/0000-0002-4845-4293

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## SUPPORTING INFORMATION

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

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