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Published in:
European Journal of Paediatric Neurology

DOI:
10.1016/j.ejpn.2020.10.004

Publication date:
2021

European Journal of Paediatric Neurology 30 (2021) 144–154

Contents lists available at ScienceDirect

European Journal of Paediatric Neurology

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ARTICLE INFO

Article history:
Received 1 May 2020
Received in revised form
11 October 2020
Accepted 14 October 2020

Keywords:
Paroxysmal disorders
Congenital cerebellar ataxia
Episodic ataxia
Cognitive difficulties
Paroxysmal tonic upward gaze
Epilepsy

ABSTRACT

Background: *CACNA1A*-related disorders present with persistent progressive and non-progressive cerebellar ataxia and paroxysmal events: epileptic seizures and non-epileptic attacks. These phenotypes overlap and co-exist in the majority of patients.
Objective: To describe phenotypes in infantile onset *CACNA1A*-related disorder and to explore intra-familial variations and genotype-phenotype correlations.
Material and methods: This study was a multicenter international collaboration. A retrospective chart review of *CACNA1A* patients was performed. Clinical, radiological, and genetic data were collected and analyzed in 47 patients with infantile-onset disorder.
Results: Paroxysmal non-epileptic events (PNEE) were observed in 68% of infants, with paroxysmal tonic upward gaze (PTU) noticed in 47% of infants. Congenital cerebellar ataxia (CCA) was diagnosed in 51% of patients including four patients with developmental delay and only one neurological sign. PNEEs were found in 63% of patients at follow-up, with episodic ataxia (EA) in 40% of the sample. Cerebellar ataxia was found in 58% of the patients at follow-up. Four patients had epilepsy in infancy and nine in childhood. Seven infants had febrile convulsions, three of which developed epilepsy later; all three patients had CCA. Cognitive difficulties were demonstrated in 70% of the children. Cerebellar atrophy was found in only one infant but was depicted in 64% of MRIs after age two.

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https://doi.org/10.1016/j.ejpn.2020.10.004

Conclusions: Nearly all of the infants had CCA, PNEE or both. Cognitive difficulties were frequent and appeared to be associated with CCA. Epilepsy was more frequent after age two. Febrile convulsions in association with CCA may indicate risk of epilepsy in later childhood. Brain MRI was normal in infancy. There were no genotype-phenotype correlations found.

1. Introduction

CACNA1A pathogenic variants are have been reported in association with chronic progressive and non-progressive cerebellar syndromes and also with paroxysmal phenotypes: paroxysmal dyskinesia, migraine, and epilepsy. These syndromes co-exist in the majority of patients. Other reported comorbidities include psychomotor delay, cognitive impairment, attention deficit hyperactivity disorder and autism [1–4]. The age of disease onset varies from infancy to adulthood [5].

The CACNA1A gene encodes a pore-forming alpha-1A subunit of calcium channels (see Fig. 2 in Lacinová, 2005) [6]. Calcium channels are responsible for the initiation of multiple calcium dependent physiological events: gene transcription, cell division, exocytosis, muscle contraction through mediation the entry of calcium ions into excitable cells [7].

Calcium channels are subdivided according to their sensitivity to peptide toxins. N-type calcium channels sensitive to ω-Aga IVA toxin were named P/Q-type calcium channels (P for Purkinje cells) [6]. P/Q-type calcium channels are expressed in the cerebellar Purkinje and granule cells, thalamus, cortex and hippocampus [8]. Calcium channels are multi-subunit complexes. The P/Q type channels consist of the principal subunit alpha 1 and auxiliary subunits: beta, alpha-2/delta, and gamma subunits [6]. There are at least 6 classes of alpha-1 subunits: alpha-1A, B, C, D, E, and S, which are encoded by 6 different genes [6]. Each alpha-1A subunit comprises four domains, NH and COOH tails, and intracellular loops [8]. Each domain contains six putative alpha-helical membrane spanning segments (S1–S6) including one pore-forming segment. The positively charged transmembrane segments (S4) constitute the calcium channel's voltage sensor. The pore-forming alpha-1A subunit is encoded by the CACNA1A gene.

The *CACNA1A* gene contains 47 exons. It is alternatively spliced [9]. In general, several types of *CACNA1A* pathogenic changes are responsible for clinical presentations, such as: point mutations (missense, in frame insertion/deletion, premature stop codon), deletions, or large gene rearrangements, small expansion of a CAG repeat predicted to encode for polyglutamine repeats in the COOH tail in exon 47 of the coding region of the *CACNA1A* [10], 19p13.13 del contiguous gene syndrome [1] and bi-allelic *CACNA1A* phenotype [11]. Different heterozygous variants act through variable pathogenic mechanisms and cause different but highly overlapping clinical phenotypes.

Based on functional studies, a loss of function (LOF) mechanism has been associated with Episodic Ataxia type 2 (EA2) and epilepsy [12–14]. Gain of function (GOF) missense mutations cause familial hemiplegic migraine type 1 (FHM1; MIM 141500). Spinocerebellar ataxia type 6 (SCA6; MIM 183086) is attributable to CAG expansion and characterized by late onset slowly progressive permanent cerebellar ataxia, which has been associated with toxic effects of polyglutamine accumulation [10].

We retrospectively studied clinical, genetic, and radiological data of 47 patients with infantile onset disease due to pathogenic variants in the CACNA1A gene. Our objectives were to describe the various phenotypes and their frequency as well as to determine genotype/phenotype correlations. We analyzed intrafamilial variations between siblings and generations.

2. Materials and methods

This study was a multicenter retrospective analysis of 47 patients from seven medical centers. Clinicians collected data by reviewing patients' charts and completing a data collection form for children who had an infantile disease presentation. The data collection form was developed by the authors. The variables chosen were based on a consensus opinion of experts with established clinical experience treating *CACNA1A* patients. Data collection occurred from July 2019 through December 2019. Data collection was based on a cohort of patients who were diagnosed from 2008 to 2019. Genetically confirmed diagnosis of *CACNA1A*-related

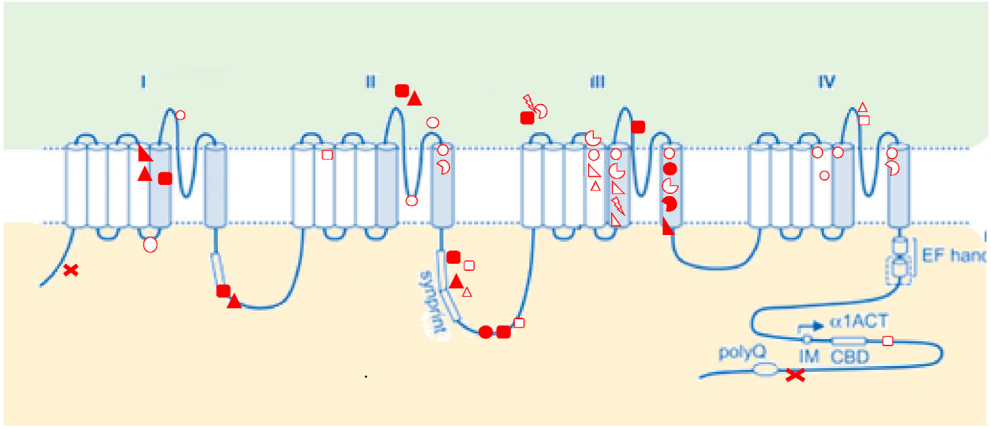

Fig. 1. *CACNA1A* -related phenotypes and the position of their associated pathogenic variants in the protein. The figure demonstrates the lack of correlation between variant positions and associated phenotype.

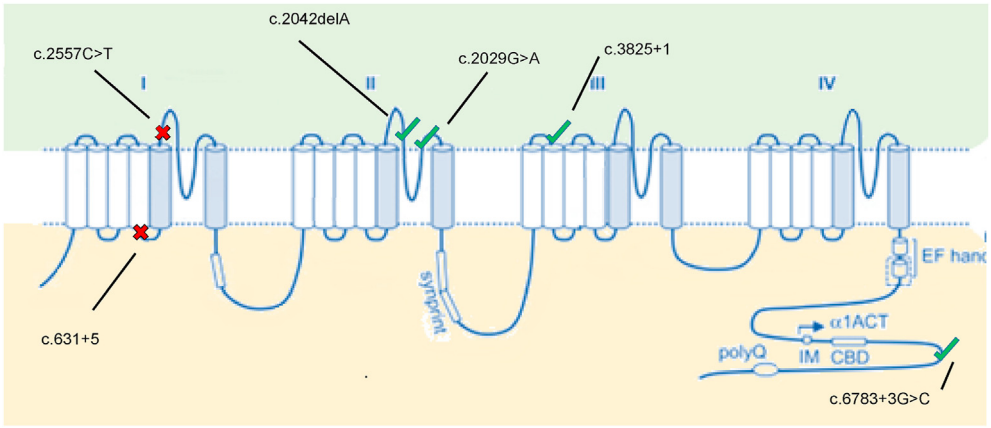

Fig. 2. Variant positions in the protein for families with and without anticipation The figure demonstrates a lack of consistency between variant positions and anticipation.

disorder was the primary inclusion criteria. Disease onset between birth to 24 months was the secondary inclusion criteria. Patients who were younger than two years of age on the last date of examination were excluded from the follow-up analysis.

2.1. Data collection form

The data form included the following variables that were recorded as being present or absent in the patients' chart: developmental milestones, neurological symptoms, epilepsy and non-epileptic events, cognitive function, and neuroimaging findings. These variables were recorded at two time points: in the infantile period and on the date of last examination (Appendix Tables 1A and 1B).

Family history of patients with autosomal dominant (AD) pathogenic variants were collected and categorized in order to compare clinical phenotypes between siblings and between generations. Forty different variants of the CACNA1A gene were reported by the clinicians. The variants were evaluated by their protein position and the effect on the protein: LOF, GOF or untested.

2.2. Clinical parameters for data collection

Congenital cerebellar ataxia (CCA) was defined as a combination of developmental delay (DD) and two or more additional neurological signs such as oculomotor abnormalities, hypotonia, or cerebellar signs: head or body titubation, dysarthria, nystagmus, tremor, or ataxic gait. We defined suspected CCA (sCCA) as a combination of DD and at least one neurological sign such as oculomotor abnormality, hypotonia, or cerebellar sign. We also defined a subgroup of patients who demonstrated CCA without non-epileptic paroxysmal events as predominantly CCA.

Paroxysmal non-epileptic events (PNEEs) are recurrent episodes that resemble epileptic seizures but do not result from abnormal electrical cortical activity. PNEEs include several types of involuntary movements, such as recurrent attacks of tremor, dystonia, chorea, athetosis, and ataxia [15]. We also defined a subgroup of patients who demonstrated PNEEs without CCA or DD as predominantly PNEEs.

Cognitive difficulties included intellectual disability and borderline intellectual function. Intellectual disability (ID) was defined according to the DSM-5 criteria. We also used the definition of Borderline intellectual functioning (BIF) that delimits "normal" intellectual functioning from intellectual disability (IQ 71–85) [16].

We compared intra-familial variations by describing AD patients who had a parent or a sibling that was also diagnosed with *CACNA1A*. We devised a scaling system where points were assigned to each patient/parent or patient/sibling dyad. Every symptom was assigned a point if it was present in the individual and then each individual further received a cumulative score. Additional points were added to the cumulative score based on the time of symptom onset i.e., whether it initially appeared in adulthood, childhood, or infancy according to the following scale: 0 = asymptomatic, 1 = adult onset, 2 = childhood onset, 3 = infantile onset. Cognitive difficulties were scored on a scale of 0–2 with: 0 = no cognitive difficulties, 1 = borderline intellectual function, 2 = intellectual disability.

2.3. Statistical analysis

Descriptive statistics were presented by mean with standard deviation, median, range, frequency, and relative frequency where appropriate. Conditional probabilities were based on relative frequencies. Patients' characteristics were presented at disease onset and last follow-up. Intra-familial variability was described by mean score with standard deviation for patient dyads at disease onset and last follow-up. Patient dyads were matched, and comparisons were performed via Matched-Pairs t-Test with statistical threshold set at 5% for bilateral significance. Researchers performed the data analysis in IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). The study was performed with institutional ethics committee approval [0075-17WOMC].

3. Results

A total of 47 patients with infantile onset *CACNA1A* were included in the study. All but two infants were born at term and the perinatal period was unremarkable. Median age at infantile onset was 9 months (range 0–22 months). Median age at last follow-up was 8.75 years (total range 2–18.5 years, interquartile range 4.75–13 years) (N = 43). Four patients under the age of 2 years at the time of last examination were excluded from the last follow-up analysis (Table 1, Table 2, Appendix Tables 1a and 1b).

3.1. Clinical characteristics of infantile onset

Nearly all infants 96% (45/47) had either PNEEs or CCA with approximately one quarter of them that had a combination of both 23% (11/47). The most common presenting phenotype was a PNEE which was found in 68% (32/47) infants. The most common type of PNEE was paroxysmal tonic upward gaze (PTU) which was identified in 69% (22/32) infants with PNEEs and represents 47% (22/47) of the sample. PTU was associated with CCA (5 patients), other types of PNEE (4), and epilepsy (2). Four patients had isolated PTU

Table 1
Clinical and radiological features of patients with infantile onset *CACNA1A*-related disorder.

Onset Age 0–22 months				Outcome Age 2–18 years			
Sign/Symptom	Number	Category %	f/N%	Sign/Symptom	Number	Category %	f/N %
Total	47		100%	**Total**	43		100%
Asymptomatic	0		0	Asymptomatic	2		5%
DD	29	100%	62%				
Isolated DD	1	3%	2%				
PNEE	32	100%	68%	**PNEE**	27	100%	63%
PNEE-PTU	22	69%	47%	PNEE-PTU	6	22%	14%
PNEE-EA	5	16%	11%	PNEE-EA	17	63%	40%
PNEE-PT	9	28%	20%	PNEE-PT	5	19%	12%
PNEE-PV	3	9%	7%	PNEE-PV	2	7%	5%
Multiple PNEEs	6	19%	13%	Multiple PNEEs	13	48%	30%
				PNEE-MGR	10	37%	23%
				PNEE-HM	4	15%	9%
Predominantly PNEE	17	53%	36%	Predominantly PNEE	16	59%	37%
CCA + sCCA	24	100%	51%	**CA**	25	100%	58%
CCA	20	83%	42%				
sCCA	4	17%	9%				
Predominantly CCA	11	46%	23%	Predominantly CA	14	56%	33%
Predominantly sCCA	2	8%	4%				
Epilepsy	4		9%	Epilepsy	9		21%
Febrile convulsion	7		15%	Febrile convulsion	1		2%
Neurological signs							
Cerebellar signs	20		43%				
Hypotonia	16		34%				
Oculomotor signs	12		26%				
Persistent dystonia	3		6%				
Pyramidal signs	3		6%				
Oculomotor apraxia	1		2%	Oculomotor apraxia	1		2%
				Coma-like	5		12%
Cognitive difficulties							
				Evaluated	40	100%	93%
				Cognitive difficulties	28	70%	65%
				Intellectual Disability	20		
				Borderline Intelligence	8		
				Learning Disability	8		19%
				ADHD	4		9%
				ASD	2		5%
				Death	1		2%
Brain MRI				**Brain MRI**			
Done	32	100%	68%	Done	22	100%	51%
Abnormal	5	16%	11%	Abnormal	14	64%	33%
Cerebellar atrophy	1	3%	2%	Pancerebellar atrophy	8		
				Vermian atrophy	6		
Abnormal findings	1-Thin ocular nerves, wide cisterna magna 2-Arachnoid cyst 3-Thin corpus callosum 4-Hyperintense signal of posterior white matter			Including abnormal findings	1-Caudate and Putamen atrophy 2-Dentate nuclei hyperintensities 3-Cerebral white matter hyper signal 4-Hippocampal hypoplasia and Pons hypotrophy 5-Hyperintense signal of posterior white matter		

Abbreviations:DD- Developmental delay; CCA-Congenital cerebellar ataxia; sCCA- Suspected Congenital cerebellar ataxia; PNEE-Paroxysmal non-epileptic event; PTU-Paroxysmal tonic upward gaze; PT-Paroxysmal torticollis; PV- Paroxysmal vertigo; EA-Episodic ataxia; MGR-Migraine; HM-Hemiplegic migraine; ADHD-Attention deficit hyperactivity disorder; ASD-Autism spectrum disorder.

during infancy. Three out of four had a strong family history of paroxysmal events: two patients had a parent with migraine and a brother with PTU, and one patient had a parent with childhood onset epilepsy. The only patient who had isolated infantile PTU developed EA and cerebellar ataxia later, but a precise age of the onset was not reported. Other types of PNEEs included: paroxysmal torticollis (PT) in 28% (9/32), EA in 16% (5/32), vertigo in 9% (3/32), and multiple types PNEEs 19% (6/32). Approximately one third of the infants 36% (17/47) had predominantly PNEEs. Four of the PNEE patients had a combination of PNEE and DD (Table 1).

Developmental delay was the second most common phenotype overall. It was found in 62% (29/47) of patients. In 69% (20/29) of DD patients, the DD was combined with *two* or more additional neurological signs resulting in a diagnosis of CCA. Four patients were diagnosed with sCCA from a combination of DD and only *one* neurological sign. A total of 51% (24/47) of patients were considered to have CCA based on these criteria. Approximately one third of the infants 28% (13/47) had predominantly CCA. One patient had isolated DD.

Febrile convulsions (FC) were reported in 15% (7/47) of patients.

Table 2
Evolution of the presenting clinical phenotype of patients with infantile onset *CACNA1A*-related disorder (n = 47).

Diagnosis at infantile onset	Predominantly PNEE n = 17		Predominantly CCA/ sCCA n = 13		Combined PNEE and CCA/ sCCA n = 11		DD and PNEE n = 4		Isolated DD n = 1	Isolated FC n = 1
Diagnosis at last follow-up	PTU	2 (12%)	CA	13 (100%)	PTU	1 (9%)	EA	1 (25%)	EA + ID 1	EA + MGR + ID 1
	EA	8 (67%)	PTU + HM	1 (8%)	PTU + MGR	1 (9%)	EA + MGR	1 (25%)		
	Multiple PNEE	6 (35%)	HM	2 (8%)	EA	1 (9%)	PV + HM	1 (25%)		
	PNEE Combined with:	1 (6%)	MGR	1 (8%)	EA + MRG	1 (9%)	CA	0		
	CA	1 (6%)	EA + MGR	1 (8%)	Multiple PNEE	1 (9%)	ID	0		
	ID	3 (18%)	ID	12 (92%)	CA	11 (100%)	BIF	1 (25%)		
	BIF	0	BIF	1 (8%)	ID	5 (45%)	LD	2 (50%)		
	Epilepsy	0	Epilepsy	5 (38%)	BIF	3 (27%)	Epilepsy	0		
	Coma-like	1 (6%)	Coma-like	2 (15%)	Epilepsy	4 (36%)	Coma-like	1 (25%)		
	MRI CA	2 (12%)	MRI CA	8 (62%)	Coma-like	2 (18%)	MRI CA	1 (25%)		
	ASD	2 (12%)	Death	1 (8%)	MRI CA	3 (27%)	Under age 2 years	1 (25%)		
	Asymptomatic	3 (18%)								
	Under age 2 years									

Abbreviations:DD- Developmental delay; CCA-Congenital cerebellar ataxia; sCCA-suspected Congenital cerebellar ataxia; CA-Cerebellar ataxia, PNEE-Paroxysmal non-epileptic event; PTU-Paroxysmal tonic upward gaze; EA-Episodic ataxia; HM-Hemiplegic migraine, MGR-Migraine; PV-Paroxysmal vertigo; ID-Intellectual disability; BIF-Borderline intellectual function; LD-Learning disability, Epi-Epilepsy; MRI CA-MRI cerebellar atrophy; ASD-Autism spectrum disorder; FC-Febrile convulsion.

One patient had isolated FC. Epilepsy was diagnosed in 9% (4/47) of the patients. These patients presented with multiple seizure types including: focal seizures, primary generalized seizures, secondary generalized seizures, and recurrent status epilepticus.

3.2. Clinical characteristics at last follow-up

PNEEs remained the most common phenotype found at the last follow-up meeting for 63% (27/43) of patients. Some of the cases of PNEEs were sustained since infancy while others were childhood-onset. The proportion of sustained PNEEs for children who had it in infancy was 74% (20/27). The types of PNEEs varied over time with EA becoming the most frequent type of paroxysm (63%, 17/27) and nearly half of patients with PNEEs (48%, 13/27) demonstrating multiple types of PNEEs. Only 22% (6/27) of those with PNEEs had PTU at the time of last follow-up.

Cerebellar ataxia was diagnosed in more than half (58%, 25/43) of follow-up patients. All of the patients with CCA at infantile onset were found to have cerebellar ataxia at last follow-up. The four infants with sCCA were all diagnosed with CA by the time of last follow-up. There were 40 patients at follow-up who were tested for cognitive difficulties (the seven missing cases were removed pairwise for comparisons). Seventy percent (28/40) of the follow-up patients who were screened for their cognitive function were found to have cognitive difficulties: 20 were diagnosed with intellectual disability and eight had borderline intelligence function. The large majority of CCA patients (95%, 21/22) had cognitive difficulties and when CCA was predominant, 100% (13/13) of the patients were found to have cognitive difficulties at last follow-up. This was in contrast to the predominantly PNEEs cases of which only 33% (4/12) of them developed cognitive difficulties.

Dystonia was found in 57% (27/47) of patients. Types of dystonia included: persistent jaw opening, persistent hand dystonia, and paroxysmal dystonias: paroxysmal tonic upward gaze, paroxysmal torticollis, and paroxysmal hand and trunk dystonia. There was no single definitive trigger in these cases. The most common reported trigger of PTU was fatigue followed by febrile diseases. Stress was the other dystonia-inducing event, reported in a few patients with PTU.

The following oculomotor abnormalities were demonstrated in 26% (12/47) of patients: abnormal ocular alignment, including strabismus and alternating intermittent or persistent exotropia and esotropia. Five patients had nystagmus, which was considered to be a cerebellar sign.

Epilepsy was diagnosed in 21% (9/43) of patients at last follow-up. All four of the patients with infantile onset epilepsy continued to have epilepsy at last follow-up. The proportion of infants with FC who developed epilepsy was high, at 43% (3/7). All three patients had CCA. The other four patients with infantile FC who did not develop epilepsy had PNEE or isolated FC. All of the patients with epilepsy also had concurrent cerebellar ataxia. Migraines were diagnosed in 33% (14/43) of patients and four of them had hemiplegic attacks.

Coma-like attacks were reported in five patients at the time of last follow-up. The most common triggers were minimal head trauma and febrile disease. One of the patients has been previously described in a detailed case report of clinical and radiological presentation as well as possible pathogenic mechanisms [3].

3.3. Brain magnetic resonance imaging

Magnetic resonance imaging (MRI) of the brain was performed on 32 patients during infancy. Only one patient with CCA demonstrated cerebellar atrophy at the age of eight months. Four other infants showed incidental findings of inconsequential abnormalities (Table 1). By the time of last follow-up, 64% (14/22) of the patients with MRI had developed vermian or pan-cerebellar atrophy (Table 1, Table 2, Appendix Tables 1A,1B).

3.4. Intra-familial variability

There were ten AD patient/parent dyads and six AD patient/sibling dyads with *CACNA1A* who had complete data for analysis. The severity of the clinical phenotypes at infantile onset and at last follow-up were combined within each dyad. Cumulative severity scores of patients were matched with parent or sibling and the matched-pairs were compared. Children had, on average, higher severity scores than their parents (mean = 7.4, SD = 1.6 vs. mean = 3.3, SD = 2.7, p = 0.001). Patients had, on average, similar severity scores compared to their siblings, (mean = 7.2, SD = 1.2 vs. mean = 7.7, SD = 1.6, p = 0.456).

3.5. Genetic data

Over half, 53%, (25/47) of infants presented with de novo heterozygous *CACNA1A* pathogenic variants.

Forty CACNA1A gene variants *CACNA1A* were reported in this study. To our knowledge, 28 of them were novel variants that have not been previously described. There were two reported in CLINVAR and nine that were already previously published in the

literature (Appendix Table 1A). The variants are unique in each family and spread out in different domains throughout the protein (Figs. 1 and 2).

One variant, published by Bahamonde et al. as a GOF [17] variant, showed that a single amino acid deletion (ΔF1502) in the S6 Segment of CaV2.1 Domain III associated with congenital ataxia increases channel activity and promotes Ca2+ influx. Eleven variants were defined as LOF variants and one variant was hypothesized by Tonelli to be a LOF variant [18] The other 10 were nonsense variants (stop codons, splicing and frameshift). In our cohort, all nine patients with epilepsy had untested variants; LOF variants were found in five out of seventeen patients with EA, four out of fourteen patients with migraines, and two patients who had PNEEs and became free of symptoms after the age of two years.

4. Discussion

We studied 47 patients with infantile onset *CACNA1A* related disorder. Nearly all of the patients demonstrated either PNEEs or CCA at onset and a quarter of them had a combination of phenotypes manifesting the coexistence of both PNEEs and CCA. We described clinical features of both of these syndromes separately and analyzed the clinical course of patients with a combination of PNEEs, CCA, and predominant phenotypes.

4.1. Paroxysmal non-epileptic events and paroxysmal tonic upward gaze

The most common clinical presentation in our cohort were PNEEs with PTU being the most frequent type of PNEE. PTU is a poor localizing sign and it has a variety of etiologies and pathophysiological mechanisms. It was described as a motor component of epileptic seizures, in semi-voluntary movements of motor stereotypies and tics, and in dystonia. PTU in *CACNA1A* patients has been described previously [2,4,19–21].

The PTU attacks in our patients were never isolated. In the majority of patients, PTU appeared in association with other non-epileptic paroxysmal events, epilepsy, or CCA (Table 1). Seven patients who demonstrated PTU without other features had a strong family history of PTU in other siblings, parental EA, and migraines. Our data suggest that co-existence of PTU with other neurological signs such as other PNEE, developmental delay, or family history of paroxysmal events may be indicative of *CACNA1A* related disorder. This finding is supported by other studies [2,4,20,21]. Whether isolated PTU is associated with *CACNA1A* pathogenic variants is not clear and needs further research.

The clinical course of PTU episodes varies in the majority of patients. These episodes either improve, decrease in frequency, or resolve completely as has been reported by other authors [4,20,22]. Humbertcloud et al. suggested that children with PTU and other PNEEs are at high risk of impaired cognitive abilities (Humbertclaude et al., 2019). In our study only one third (33%) of infants with predominantly PNEE developed cognitive difficulties.

4.2. Developmental delay and congenital cerebellar ataxia

The second most common phenotype in patients with infantile onset *CACNA1A* was DD. About 69% of these patients were diagnosed with CCA when DD was combined with *two* or more additional neurological signs and 83% were diagnosed with CCA based on *one* or more neurological signs. CCA has previously been reported in association with pathogenic variants in *CACNA1A* [3,24–27].

CCA is characterized by severe hypotonia, hypo-activity and oculomotor abnormalities in the neonatal period and it is followed by DD associated with more clear features of cerebellar disturbance, such as an impairment of smooth goal directed movements and also of fine-tuning of speed, force and direction [28–30]. Twenty of 29 patients with DD met all criteria for CCA, whereas four patients had DD with only one neurological sign and were diagnosed with sCCA. All patients with sCCA went on to develop cerebellar ataxia after the age of two years. These data suggest that CCA should be a diagnostic consideration in infants harboring *CACNA1A* pathogenic variants when they present with a combination of DD and even a single neurological sign such as: hypotonia, oculomotor abnormality, or cerebellar signs.

Although the motor component of *CACNA1A*-related cerebellar ataxia may be slow or non-progressive (Travaglini et al., 2017), *CACNA1A* patients are at a high risk of cognitive problems [4]. We found that all our patients with cerebellar ataxia had cognitive difficulties (Tables 1 and 2).

The pathogenesis of cerebellar ataxia in *CACNA1A*-related disorder is not well understood. Travaglini et al. [27] suggested that in cases of GOF mutations, Purkinjie cell damage and neuronal loss in the cerebellum are a result of an imbalance between the hyperexcitability of Purkinjie cells and hyperpolarizing small-conductance Ca2+-activated inhibitor K+ channels of Purkinjie cells [27,31]. The hyperexcitability of Purkinjie cells is a result of a decreased voltage threshold for activation in somatic and dendritic CaV2.1 channels.

4.3. Cognitive difficulties

Cognitive problems in association with *CACNA1A* have been previously reported [1,23,32,33]. Cognitive difficulties in our sample included borderline intellectual functioning and intellectual disability. Due to overlap between the two major phenotypes, CCA and PNEEs, we compared the frequency of cognitive difficulties in patients with predominantly CCA and predominantly PNEE phenotypes. We found that all patients with predominantly CCA developed cognitive difficulties compared to a third of the patients with predominantly PNEE phenotypes. This suggests that the most common association with cognitive difficulties in infantile onset *CACNA1A* disorder is CCA. The pathophysiologic basis of cognitive dysfunction in *CACNA1A* disorder is unclear, Damaj et al. [1] suggested that dysregulation of the cerebellar connections within cortical and limbic structures may possibly impair cortical and limbic processes, including motor memory consolidation.

4.4. Epilepsy

Four patients manifested epilepsy in infancy. The number of patients with epilepsy increased after the age of two. All patients continued taking antiepileptic therapy. Cerebellar ataxia was an additional co-morbid phenotype evident in all nine patients.

Epileptic patients from our sample demonstrated different seizure types, including prolonged non-convulsive status epilepticus. Otherwise, seizure semiology consisted of: generalized myoclonus (defined by authors as hyperekplexia), recurrent status epilepticus, intractable seizures, and epileptic encephalopathy which has been reported in association with *CACNA1A* mutations [1,22,34–38]. The pathogenesis of *CACNA1A* related epileptogenicity is complicated [10,22,37,39,40]. Due to the complexity of epilepsy pathogenesis in *CACNA1A* related disorder, patients may need medications with different mechanisms of action.

Three patients out of the seven who presented with FC in infancy developed epilepsy for an incidence of 43%. All three patients had congenital cerebellar ataxia. The incidence of epilepsy in the general population following FC is approximately 2–15.8% [41]. Our results indicate a potentially higher risk of epilepsy in *CACNA1A*

patients with CCA following FC compared to the risk of epilepsy following FC in the regular population. This would require further investigation for broader generalization.

4.5. Neurological signs

We found abnormal ocular alignment, including strabismus and alternating intermittent or persistent exotropia and esotropia, in one quarter of the patients. Tantsis et al. [21] reviewed oculomotor findings in their own patients with *CACNA1A* syndrome. Due to the high frequency of oculomotor abnormalities in these patients, the authors proposed that early onset of abnormal eye movements, associated with DD or cerebellar atrophy, is an indication of *CACNA1A* disorder [21]. Abnormal eye movements have been reported in other genetic syndromes of cerebellar disorders. Abnormal ocular alignment in patients with spinocerebellar ataxia type 3 has been reported by Ghasia et al. [42]. The authors suggested that the brainstem, the deep cerebellar nuclei, and the superior cerebellar peduncle involvement were the physiological basis for this disorder [42].

Dystonia was an additional neurological sign in our patients. Dystonia was noted in up to 25% of individuals with SCA6 [43] and was similarly reported in SCA1, SCA2, and SCA3 [44,45]. Dystonia in *CACNA1A*-related phenotype can appear as a paroxysmal dystonia, specifically as PTU or PT. It can be triggered by fatigue, febrile disease, stress, without any apparent trigger [2,45,46], or as persistent disorder [11].

4.6. Brain magnetic resonance imaging

Cerebellar atrophy was depicted in only one infant. These data correlate with the Tantsis et al. [21] study which showed only one of nine patients who demonstrated cerebellar atrophy before the age of two. By the time of last follow-up, 14 of 22 patients were found to have cerebellar atrophy on MRI. Four patients with cerebellar ataxia had normal brain MRI results, with the oldest patient being 9 years old. These data suggest that cerebellar atrophy was not a mandatory feature of the infantile onset *CACNA1A* disorder before the age of two years, and it did not strongly correlate with the clinical phenotype. Humbertclaude et al. [23] suggested a correlation between cerebellar atrophy and cognitive problems but we didn't establish a clear relationship in our analysis.

4.7. Course of the disease

CACNA1A patients demonstrated a progressive and degenerative clinical course (Table 2). Only two patients with predominantly PNEEs became symptom-free after age two. Most of the patients with predominantly CCA developed new comorbidities: epilepsy, coma-like attacks, and PNEEs including paroxysmal dyskinesia and migraine. All these patients showed cognitive difficulties. Patients with predominantly PNEEs developed new types of paroxysmal events, although cerebellar ataxia and cognitive problems were less frequent comorbidities. Half of the patients with FC developed epilepsy. Two patients with monosymptomatic presentation: isolated DD and isolated FC developed complex phenotype: paroxysmal dyskinesia, migraine, and cognitive difficulties. About half of the patients developed cerebellar atrophy.

4.8. Intra-familial variations

Intra-familial variability, from asymptomatic carriers to seriously affected family members, has been discussed by several authors [1,8,47]. Our data indicate that the severity of clinical features in most of the families tends to be greater in children compared to their parents but similar in siblings. The mechanism of anticipation in these affected families is not clear. Angelini et al. hypothesized that widespread distribution of Ca channels in the brain, various modifier genes, and other factors, may explain the incomplete penetrance in *CACNA1A* related disorder. In order to figure out possible mechanisms of anticipation we compared pathogenic variant positions in the protein between families with and without anticipation (Fig. 2). We did not find any consistency. Anticipation related to the CACNA1A gene has been reported in Spinocerebellar ataxia type 6 (SCA6) due to a CAG repeat expansion [48]. Whether an increase in (CA)n-repeat (D19S1150) and (CAG)n-repeat in the 3-prime-UTR could possibly result in worsening of clinical phenotypes between generations is unclear. Parental mosaicism is an additional explanation for a milder phenotype in the parent compared to the child. These possibilities were not studied in patients from our cohort and further investigation is needed.

4.9. Genotype-phenotype correlation

To find genotype-phenotype correlations we matched clinical phenotypes to pathogenic variant positions in the protein. We did not find any associations (Fig. 1). LOF mutations in *CACNA1A* have been associated traditionally with EA type 2 [14] and GOF mutations have been associated with FHM1 (OMIM). Our findings suggest that LOF variants may be associated with different phenotypes. These data correlate with the Xiao Jiang results [37]. Two patients with AD variants inherited from healthy parents and became symptom free, were found to carry LOF variants in the cytoplasmic region: one patient in the 3′ prime position and the other patient in the 5′ prime position of the protein. The first patient carried the variant: c.6783+3G > C which is located close to the end of the protein and hence, may not affect the protein function. The second patient carried a LOF variant: c.146dupA; p.Q50AfsX26, located at the beginning of the protein and was suspected to significantly affect the protein function. Both dominant negative effect and haploinsufficiency, proven by nonsense mediated decay mechanism [12], have been suggested to act as molecular mechanisms in CACNA1A genes. Therefore, we failed to understand the mechanism by which this damaged protein resulted in a transient PNEE phenotype in this patient and his healthy parent.

4.10. Limitations

This retrospective study was limited by several factors. The relative rarity of infantile onset *CACNA1A* made the recruitment of a large sample very challenging. As a result, the statistical associations that could be explored were limited. There was no comparison group and therefore causation could not be established. Rather, this study reinforced many existing hypotheses and shed new light regarding the clinical presentation and natural history of *CACNA1A* syndrome. A detailed analysis of the epileptic manifestations of *CACNA1A* –related disorder as well as the efficacy of various medications in *CACNA1A*-related epileptic seizures and paroxysmal non-epileptic events was beyond the scope of the current investigation. Further research is needed, such as a prospective cohort study, to continue exploring the evolution and clinical outcomes of *CACNA1A* disorders and to assist in the development of a consensus therapy.

5. Conclusions

CACNA1A patients presented with either PNEEs, CCA, or both. The most common clinical phenotype were PNEEs with the most frequent type being PTU in infancy and EA in childhood. *CACNA1A* disorder is a neurodegenerative disease. All infants with DD and

one or more neurological signs developed cerebellar ataxia. This combination appears to be a strong indicator for developing cerebellar ataxia. Cognitive difficulties were frequent and CCA was strongly associated with it. Half of the patients with FC developed epilepsy, which was more than expected. The overall number of patients with epilepsy increased over time. Cerebellar atrophy was rarely present on brain MRI in infants but appeared in many patients after the age of two. Abnormal ocular alignment was present in one quarter of the patients. The severity of clinical features tended to be greater in children compared to their parents but not significantly different in siblings. There were no genotype-phenotype correlations found in this study.

Appendix

Table 1A
Clinical, radiological and genetic characteristics of 47 participants during infancy

Patient	Patient's Ref.	Age (mos)	Gender	De novo	AD	DD	CCA	sCCA	PNEE					Epi	FC	LD	PS	PD	OA	MRI-CA
									PTU	PT	PV	EA	HD							
1	Blumkin et al., 2010, 2015	0	M	c.4043G > A; p.Arg1348Gln		+	+		+											
2	Blumkin et al., 2015	0.2	F	c.815G > A; p.Cys272Tyr		+	+		+											
3		1	M	c.5223G > A; p.Arg1663Gln		+	+													
4		10	F	c.3799G > A; p.Glu1267Lys		+	+		+					+	+					
5		11	F	c. 5419C > T; p.Ala1807Thr		+	+		+					+						
6	Blumkin et al., 2015	0.2	F	c.4048G > T; p.Val1350Leu		+	+		+											
7	Travaglini et al., 2017	12	M	c.4025C > T; p.Ile1342Thr		+	+										+			
8	Travaglini et al., 2017	22	F	c.653C > T; p.Ser218Leu		+		+												
9	Travaglini et al., 2017	7	M	c.4186G > A; p.Val1396Met		+	+								+					+
10	Travaglini et al., 2017	3	M	c.4055G > A; p.Arg1352Gln		+	+													
11		12	M	c.4532C > A; p.Ala1511Asp		+	+										+			
12		12	F	c.5006G > A; p.Arg1669Gln		+	+												+	
13		9	F	c.4186G > A; p.Val1396Met		+	+								+					
14	Carreno et al., 2011	12	F	c.4160A > G; p.Tyr1387Cys		+	+													
15	Vila-Pueyo et al., 2014	9	M		p.Glu533Lys					+		+	+					+		
16	Vila-Pueyo et al., 2014	13	F		p.Glu533Lys				+	+		+								
17	Cuenca-Leónet al.2008.	12	M	c.3734A > G; p.Tyr1245Cys						+										
18	Bahamonde et al., 2015	12	M	c.4503-4505del; p.Phe1502del		+	+			+				+						
19		9	F		c.631+5G > C rs786200963										+					
20		12	M		c.631+5G > C rs786200963	+			+		+	+								
21		7	F	c.1922del; p.Asn641Ilefs*18					+						+					
22		9	F		c.2557C > T; p.Gln863Ter				+											
23		15	F		c.2557C > T; p.Gln863Ter				+											
24	Roubertie et al., 2008	10	M		c.2206C > T; p.Gln736Ter				+						+	+				
25	Roubertie et al., 2008	10	F		c.2206C > T; p.Gln736Ter				+											
26	Roubertie et al., 2008; Humbertclaude et al., 2018	16	F		c.2206C > T; p.Gln736Ter					+						+				
27	Humbertclaude et al., 2018	2	M	c.5115_5126del: p.Y1706_11709del					+											
28	Humbertclaude et al., 2018	4	M		c.6190C > T				+	+										
29	Humbertclaude et al., 2018	11	F		c.2042delA:p.Q681RfsX17	+														
30	Humbertclaude et al., 2018	8	F		c.2042delA:p.Q681RfsX17	+	+		+	+										
31	Humbertclaude et al., 2018	9	M		c.3414delC; p.Lys1139Argfs*48		+			+						+				
32	Humbertclaude et al., 2018	2	F		c.6783+3G > C		+				+									
33	Humbertclaude et al., 2018	6	F		c.146dupA; p.Gln50Alafs*26		+				+									
34	Humbertclaude et al., 2019	9	F		c.3825+1G > A	+	+					+								
35	Humbertclaude et al., 2019	4	F				+							+		+				

(continued on next page)

Table 1A (*continued*)

Patient	Patient's Ref.	Age (mos)	Gender	De novo	AD	DD	CCA	sCCA	PNEE					Epi	FC	LD	PS	PD	OA	MRI-CA
									PTU	PT	PV	EA	HD							
					c.4373C > T; p.Thr1458Met															
36	Humbertclaude et al., 2019	10	M		c.4251T > G; p.Cys1417Trp	+	+							+						
37	Humbertclaude et al., 2019	12	M		c.2029G > A; p.GLY677Arg	+			+											
38	Humbertclaude et al., 2019	7	F		c.2029G > A; p.GLY677Arg	+	+	+		+										
39		1	F	c.2003A > C; p.Asp668Ala		+		+												
40	Allen et al., 2013	1	F	c.2134G > A; p.Ala712Thr		+		+							+					
41		11	F	c.4988G > A p.Ser1663Asn		+	+		+	+										
42		6	F		c.5356G > C		+			+	+									
43		9	M	c.1227dupT; p.Asp410Ter			+					+								
44	Burk et al., 2014	0.5	M		c.904G > A; P.Asp302Asn	+	+			+	+							+		
45		3	F	c.3566del; p.Asn1189Thrfs*53		+	+	+		+										
46		2	F	c.5267G > A; p.Arg1756Gln		+	+	+		+										
47		0	F	c.4084C > T; p. Arg1362Trp		+		+												

Abbreviations + Positive Empty cell Negative; AD-Autosomal dominant; DD- Developmental delay; CCA-Congenital cerebellar ataxia; sCCA-suspected Congenital cerebellar ataxia; PNEE-Paroxysmal non-epileptic events; PTU-Paroxysmal tonic upward gaze; PT-Paroxysmal torticollis; PV- Paroxysmal vertigo; EA-Episodic ataxia; HD-Hemi dystonia; Epi-Epilepsy; FC-Febrile convulsion; LD-Language delay; PS-Pyramidal signs; PD-Persistent dystonia; OA-Oculomotor apraxia.

Table 1B
Clinical, radiological characteristics of 43 participants at follow-up

Patients	Age (month)	A	CA	PNEE						Epi	Coma-like	ID/BIF	LP	LD	ADHD	ASD	BP	PD	OA	PS	Death	MRI-CA
				PTU	EA	PT	PV	MGR	HM													
1	217		+	+	+	+		+		+	+	+						+				+
2	117		+									+					+					
3	74		+									+	+					+				
4	54		+		+					+			+		+							
5	48		+							+		+						+				
6	84		+									+										
7	156		+	+					+	+		+										+
8	176		+						+			+										+
9	78		+							+	+	+									+	+
10	61		+					+				+										+
11	108		+							+		+								+		+
12	45		+									+										
13	31		+							+		+										
14	144		+						+		+	+										+
15	36			+	+	+								+		+		+				
16	120				+	+								+	+	+						
17	132					+	+		+													
18	120		+							+	+	+								+		+
19	168				+			+				+										
20	108				+			+			+			+	+							
21	<24																					
22	84			+				+														
23	35				+																	
24	180				+									+								
25	<24																					
26	193					+		+				+										
27	93		+		+							+										
28	141				+							+										
29	190				+							+										+
30	105		+		+			+				+										
31	36				+									+								
32	76	+																				
33	128	+																				
34	169						+							+								+
35	176				+							+										+
36	86				+							+										
37	109		+		+			+				+										+

Table 1B (*continued*)

Patients	Age (month)	A	CA	PNEE						Epi	Coma-like	ID/BIF	LP	LD	ADHD	ASD	BP	PD	OA	PS	Death	MRI-CA
				PTU	EA	PT	PV	MGR	HM													
38	222		+									+								+		+
39	24		+									+										
40	44		+							+		+										
41	48		+									+										
42	<24																					
43	60				+			+														
44	<24																					
45	57		+	+															+			
46	86		+	+				+							+							
47	156		+									+										+

Abbreviations:+.
Positive.
Empty cell Negative.
A- Asymptomatic; CA-Cerebellar ataxia; PNEE-Paroxysmal non-epileptic events; PTU-Paroxysmal tonic upward gaze; PT-Paroxysmal torticollis; PV- Paroxysmal vertigo; EA-Episodic ataxia; MGR-Migraine; HM-Hemiplegic migraine; Epi-Epilepsy; ID/BIF-Intellectual disability/Borderline intellectual function; LP-Language problems; LD-Learning disabilities(dyslexia, dysgraphia, dyscalculia); ADHD-Attention deficit hyperactivity disorder; ASD-Autism spectrum disorder; BP- Behavioral problems; PD-Persistent dystonia; OA-Oculomotor apraxia; PS-Pyramidal signs.

Phenotype	CCA and CA	Predominantly PNEE without epilepsy	Epilepsy at onset	Epilepsy after age 2 years	Epilepsy with EA	Migraine	Coma- like	Asymptomatic
SNP								
Potentially LoF								

References

[1] L. Damaj, A. Lupien-Meilleur, A. Lortie, É. Riou, L.H. Ospina, L. Gagnon, et al., CACNA1A haploinsufficiency causes cognitive impairment, autism and epileptic encephalopathy with mild cerebellar symptoms, Eur. J. Hum. Genet. 23 (11) (2015) 1505–1512, https://doi.org/10.1038/ejhg.2015.21.

[2] L. Blumkin, E. Leshinsky-Silver, M. Michelson, A. Zerem, S. Kivity, D. Lev, et al., Paroxysmal tonic upward gaze as a presentation of de-novo mutations in CACNA1A, Eur. J. Paediatr. Neurol. 19 (3) (2015) 292–297, https://doi.org/10.1016/j.ejpn.2014.12.018.

[3] L. Blumkin, M. Michelson, E. Leshinsky-Silver, S. Kivity, D. Lev, T. Lerman-Sagie, Congenital ataxia, mental retardation, and dyskinesia associated with a novel CACNA1A mutation, J. Child Neurol. 25 (7) (2010) 892–897.

[4] V. Humbertclaude, B. Krams, E. Nogue, N. Nagot, D. Annequin, B. Tourniaire, et al., Benign paroxysmal torticollis, benign paroxysmal vertigo, and benign tonic upward gaze are not benign disorders, Dev. Med. Child Neurol. 60 (12) (2018) 1256–1263, https://doi.org/10.1111/dmcn.13935.

[5] R.W. Baloh, Q. Yue, J.M. Furman, S.F. Nelson, Familial episodic ataxia: clinical heterogeneity in four families linked to chromosome 19p, Ann. Neurol. 41 (1) (1997) 8–16, https://doi.org/10.1002/ana.410410105.

[6] L. Lacinová, Voltage-dependent calcium channels, . *Gen Physiol Biophys* 24 (Suppl 1) (2005) 1–78.

[7] K. Dunlap, J. Luebke, T.J. Turner, Exocytotic Ca2+ channels in mammalian central neurons, Trends Neurosci. 18 (2) (1995) 89–98, https://doi.org/10.1016/0166-2236(95)80030-6.

[8] R.A. Ophoff, G.M. Terwindt, M.N. Vergouwe, R. Van Eijk, P.J. Oefner, S.M.G. Hoffman, et al., Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4, Cell 87 (3) (1996) 543–552, https://doi.org/10.1016/S0092-8674(00)81373-2.

[9] F. Trettel, E. Mantuano, V. Calabresi, L. Veneziano, A.S. Olsen, A. Georgescu, et al., A fine physical map of the CACNA1A gene region on 19p13.1-p13.2 chromosome, Gene 241 (1) (2000) 45–50, https://doi.org/10.1016/S0378-1119(99)00470-9.

[10] O. Zhuchenko, J. Bailey, P. Bonnen, T. Ashizawa, D.W. Stockton, C. Amos, et al., Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α(1A)-voltage-dependent calcium channel, Nat. Genet. (1997) 62, https://doi.org/10.1038/ng0197-62.

[11] K. Reinson, E. Õiglane-Shlik, I. Talvik, U. Vaher, A. Õunapuu, M. Ennok, et al., Biallelic CACNA1A mutations cause early onset epileptic encephalopathy with progressive cerebral, cerebellar, and optic nerve atrophy, Am. J. Med. Genet. 170 (8) (2016) 2173–2176, https://doi.org/10.1002/ajmg.a.37678.

[12] E. Mantuano, S. Romano, L. Veneziano, C. Gellera, B. Castellotti, S. Caimi, et al., Identification of novel and recurrent CACNA1A gene mutations in fifteen patients with episodic ataxia type 2, J. Neurol. Sci. 291 (1–2) (2010) 30–36, https://doi.org/10.1016/j.jns.2010.01.010.

[13] K. Scoggan, J. Friedman, D. Bulman, CACNA1A mutation in a EA-2 patient responsive to acetazolamide and valproic acid, Can. J. Neurol. Sci. 33 (1) (2006) 68–72, https://doi.org/10.1017/S0317167100004728.

[14] S. Rajakulendran, T.D. Graves, R.W. Labrum, D. Kotzadimitriou, L. Eunson, M.B. Davis, et al., Genetic and functional characterisation of the P/Q calcium channel in episodic ataxia with epilepsy, J. Physiol. 588 (11) (2010) 1905–1913, https://doi.org/10.1113/jphysiol.2009.186437.

[15] E. Fernández-Alvarez, Transient benign paroxysmal movement disorders in infancy, Eur. J. Paediatr. Neurol. 22 (2) (2018) 230–237, https://doi.org/10.1016/j.ejpn.2018.01.003.

[16] L. Salvador-Carulla, J.C. García-Gutiérrez, M. Ruiz Gutiérrez-Colosía, J. Artigas-Pallarès, J. García Ibáñez, J. González Pérez, et al., Borderline intellectual functioning: consensus and good practice guidelines, Rev. Psiquiatía Salud Ment. 6 (3) (2013) 109–120, https://doi.org/10.1016/j.rpsmen.2012.12.002.

[17] M.I. Bahamonde, S.A. Serra, O. Drechsel, R. Rahman, A. Marcé-Grau, M. Prieto, et al., A single amino acid deletion (ΔF1502) in the S6 segment of CaV2.1 domain III associated with congenital ataxia increases channel activity and promotes Ca2+ influx, PloS One 10 (12) (2015) 1–28, https://doi.org/10.1371/journal.pone.0146035.

[18] A. Tonelli, M. D'Angelo, R. Salati, L. Villa, Early onset, non fluctuating spino-cerebellar ataxia and a novel missense mutation in CACNA1A gene, J. Neurol. Sci. 241 (1–2) (2003) 13–17, https://doi.org/10.1016/j.jns.2005.10.007.

[19] A. Quade, A. Thiel, I. Kurth, M. Holtgrewe, M. Elbracht, D. Beule, K. Eggermann, U. Scholl, M. H€ausler, Paroxysmal tonic upgaze: a heterogeneous clinical condition responsive to carbonic anhydrase inhibition, Eur. J. Paediatr. Neurol. 25 (2019) 181–186, https://doi.org/10.1016/j.ejpn.2019.11.002.

[20] A. Roubertie, B. Echenne, J. Leydet, S. Soete, B. Krams, F. Rivier, et al., Benign paroxysmal tonic upgaze, benign paroxysmal torticollis, episodic ataxia and CACNA1A mutation in a family, J. Neurol. 255 (10) (2008) 1600–1602, https://doi.org/10.1007/s00415-008-0982-8.

[21] E.M. Tantsis, D. Gill, L. Griffiths, S. Gupta, J. Lawson, N. Maksemous, et al., Eye movement disorders are an early manifestation of CACNA1A mutations in children, Dev. Med. Child Neurol. 58 (6) (2016) 639–644, https://doi.org/10.1111/dmcn.13033.

[22] M. Vila-Pueyo, G.G. Gené, M. Flotats-Bastardes, X. Elorza, C. Sintas, M.A. Valverde, et al., A loss-of-function CACNA1A mutation causing benign paroxysmal torticollis of infancy, Eur. J. Paediatr. Neurol. 23 (10) (2015) 430–433, https://doi.org/10.1016/j.ejpn.2013.12.011.

[23] V. Humbertclaude, F. Riant, B. Krams, V. Zimmermann, N. Nagot, D. Annequin, et al., Cognitive impairment in children with CACNA1A mutations, Dev. Med. Child Neurol. (2019) 17–23, https://doi.org/10.1111/dmcn.14261.

[24] O. Carreño, M.T. García-Silva, A. García-Campos, A. Martínez-De Aragón, B. Cormand, A. MacAya, Acute striatal necrosis in hemiplegic migraine with de novo CACNA1A mutation, Headache 51 (10) (2011) 1542–1546, https://doi.org/10.1111/j.1526-4610.2011.02014.x.

[25] N.J. Giffin, Benign paroxysmal torticollis of infancy: four new cases and

linkage to CACNA1A mutation, Dev. Med. Child Neurol. 44 (2002) 490–493, https://doi.org/10.1017/S0012162201002407.
[26] S. Naik, K. Pohl, M. Malik, A. Siddiqui, D. Josifova, Early-onset cerebellar atrophy associated with mutation in the CACNA1A gene, Pediatr. Neurol. 45 (5) (2011) 328–330, https://doi.org/10.1016/j.pediatrneurol.2011.08.002.
[27] L. Travaglini, M. Nardella, E. Bellacchio, A. D'Amico, A. Capuano, R. Frusciante, et al., Missense mutations of CACNA1A are a frequent cause of autosomal dominant nonprogressive congenital ataxia, Eur. J. Paediatr. Neurol. 21 (3) (2017) 450–456, https://doi.org/10.1016/j.ejpn.2016.11.005.
[28] E. Bertini, G. Zanni, E. Boltshauser, Nonprogressive congenital ataxias, Handb. Clin. Neurol. 155 (2018) 91–103, https://doi.org/10.1016/B978-0-444-64189-2.00006-8.
[29] R. Brandsma, C. Verschuuren-Bemelmans, D. Amrom, N. Barisic, P. Baxter, E. Bertini, L. Blumkin, A clinical diagnostic algorithm for early onset cerebellar ataxia, Eur. J. Paediatr. Neurol. 23 (5) (2019) 692–706, https://doi.org/10.1016/j.ejpn.2019.08.004.
[30] M. Steinlin, Non-progressive congenital ataxias, Brain Dev. 20 (4) (1998) 199–208, https://doi.org/10.1016/S0387-7604(98)00019-9.
[31] Z. Gao, B. Todorov, C. Barrett, S. Dorp, M. Ferrari, A. Maagdenberg, C. Zeeuw, F. Hoebeek, Cerebellar ataxia by enhanced Ca (V) 2.1 currents is alleviated by Ca2+-dependent K+-channel activators in Cacna1a (S218L) mutant mice, *Journal of Neuroscience* 15533–15546 (2012) (44) 32 , https://doi.org/10.1523/JNEUROSCI.2454-12.2012.
[32] T. Freilinger, N. Ackl, A. Ebert, C. Schmidt, B. Rautenstrauss, M. Dichgans, A. Danek, A novel mutation in CACNA1A associated with hemiplegic migraine, cerebellar dysfunction and late-onset cognitive decline, J. Neurol. Sci. 300 (1–2) (2011) 160–163, https://doi.org/10.1016/j.jns.2010.09.032.
[33] E. Indelicato, W. Nachbauer, E. Karner, A. Eigentler, M. Wagner, I. Unterberger, et al., The neuropsychiatric phenotype in CACNA1A mutations: a retrospective single center study and review of the literature, Eur. J. Neurol. 26 (1) (2019), https://doi.org/10.1111/ene.13765. 66-e7.
[34] A. Allen, S. Berkovic, P. Cossette, et al., De novo mutations in epileptic encephalopathies, Nature 217–21 (2013), https://doi.org/10.1038/nature12439.
[35] F. Hamdan, C. Myers, P. Cossette, et al., High rate of recurrent de novo mutations in developmental and epileptic encephalopathies, Am Soc Hum Genet 101 (5) (2017) 664–685, https://doi.org/10.1016/j.ajhg.2017.09.008.
[36] T. Hayashida, Y. Saito, A. Ishii, H. Yamada, A. Itakura, T. Minato, T. Fukuyama, Y. Maegaki, S. Hirose, CACNA1A-related early-onset encephalopathy with myoclonic epilepsy: a case report, Brain Dev. 40 (2) (2018) 130–133. 10.1016/j.braindev.2017.08.006.
[37] X. Jiang, P.K. Raju, N. D'Avanzo, M. Lachance, J. Pepin, F. Dubeau, et al., Both gain-of-function and loss-of-function de novo CACNA1A mutations cause severe developmental epileptic encephalopathies in the spectrum of Lennox-Gastaut syndrome, Epilepsia 60 (9) (2019) 1881–1894, https://doi.org/10.1111/epi.16316.
[38] Jouvenceau A., Eunson L., Spauschus A.,Ramesh V., Zuberi S., Kullmann D., Hanna M., Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. Lancet 200;358(9284):801-807.
[39] S. Rajakulendran, Neuronal P/Q-type calcium channel dysfunction in inherited disorders of the CNS, Nat. Rev. Neurol. 8 (2012) 86–96, https://doi.org/10.1038/nrneurol.2011.228.
[40] Y. Zhang, M. Mori, D.L. Burgess, J.L. Noebels, Mutations in high-voltage-activated calcium channel genes stimulate low-voltage-activated currents in mouse thalamic relay neurons 22 (15) (2002) 6362–6371, https://doi.org/10.1523/JNEUROSCI.22-15-06362.2002.
[41] J.W. Dreier, J. Li, Y. Sun, J. Christensen, Evaluation of long-term risk of epilepsy, psychiatric disorders, and mortality among children with recurrent febrile seizures: a national cohort study in Denmark, JAMA Pediatr 173 (12) (2019) 1164–1170, https://doi.org/10.1001/jamapediatrics.2019.3343.
[42] F.F. Ghasia, G. Wilmot, A. Ahmed, A.G. Shaikh, Strabismus and micro-opsoclonus in machado-joseph disease, Cerebellum 15 (4) (2016) 491–497, https://doi.org/10.1007/s12311-015-0718-0.
[43] D. Geschwind, S. Perlman, K. Figueroa, J. Karrim, Spinocerebellar ataxia type 6: frequency of the mutation and genotype-phenotype correlations, Neurology 49 (5) (1997) 1247–1251, https://doi.org/10.1212/WNL.49.5.1247.
[44] M. Maschke, G. Oehlert, T. Xie, S. Perlman, S. Subramony, N. Kumar, et al., Clinical feature profile of spinocerebellar ataxia type 1–8 predicts genetically defined subtypes, Mov. Disord. 20 (11) (2005) 1405–1412, https://doi.org/10.1002/mds.20533.
[45] M. Shin, L.M. Douglass, J.M. Milunsky, N. Paul Rosman, The genetics of benign paroxysmal torticollis of infancy, J. Child Neurol. 31 (8) (2016) 1057–1061, https://doi.org/10.1177/0883073816636226.
[46] S. Kimura, A. Nezu, Electromyographic study in an infant with benign paroxysmal torticollis, Pediatr. Neurol. 19 (1998) 236–238, https://doi.org/10.1016/s0887-8994(98)00044-7.
[47] C. Angelini, J. Van Gils, A. Bigourdan, P.S. Jouk, D. Lacombe, P. Menegon, et al., Major intra-familial phenotypic heterogeneity and incomplete penetrance due to a CACNA1A pathogenic variant, Eur. J. Med. Genet. 62 (6) (2019), https://doi.org/10.1016/j.ejmg.2018.08.011.
[48] R.J. Sinke, E.F. Ippel, C.M. Diepstraten, et al., Clinical and molecular correlations in spinocerebellar ataxia type 6: a study of 24 Dutch families, Arch. Neurol. 58 (11) (2001) 1839–1844, https://doi.org/10.1001/archneur.58.11.1839.