Dystonia and Contractures are Potential Early Signs of *CACNA1E*-Related Epileptic Encephalopathy

Nelmar V. Ortiz Cabrera^a Anna Duat Rodríguez^b Bárbara Fernández Garoz^c
Beatriz Bernardino Cuesta^b María Jiménez Legido^b Verónica Cantarín
Extremera^b Juan J. García Peñas^b

^aClinical Genetics, Hospital Infantil Universitario Niño Jesús, Madrid, Spain; ^bNeurology Department, Hospital Infantil Universitario Niño Jesús, Madrid, Spain; ^cDepartment of Laboratory Medicine, Hospital Infantil Universitario Niño Jesús, Madrid, Spain

Established Facts

- Pathogenic variants in *CACNA1E* are related to early infantile epileptic encephalopathy with macrocephaly, hypotonia, and hyperkinetic movements.
- Most of the variants described in *CACNA1E* are gain of function variants, and the role of haploinsufficiency has yet to be clarified.

Novel Insights

- Early-onset dystonia with global developmental delay and areflexia may precede the appearance of epileptic encephalopathy.
- A genotype-phenotype correlation could exist for different locations of pathogenic missense variants in this gene.

Keywords

CACNA1E · Exome sequencing · Intellectual disability · Early onset epileptic encephalopathy

Abstract

Epileptic encephalopathy related to *CACNA1E* has been described as a severe neurodevelopmental disorder presenting with early-onset refractory seizures, hypotonia, macrocephaly, hyperkinetic movements, and contractures and is

associated with an autosomal dominant inheritance pattern. Most pathogenic variants described to date are missense variants with a gain of function effect, and the role of haploinsufficiency has yet to be clarified. We describe 2 cases of *CACNA1E* encephalopathy. Notable findings include congenital contractures and movement disorders predating onset of epilepsy, particularly dystonia. We further compared the key phenotypic features depending on variant location. In conclusion, the appearance of congenital contractures, areflexia, and movement disorders before the onset of epi-

lepsy may provide key guidance in the diagnosis of epileptic *CACNA1E* encephalopathy. A genotype-phenotype correlation was found between the presence of movement disorders and severe intellectual disability and the location of the variant in the *CACNA1E* gene.

© 2020 S. Karger AG, Basel

Introduction

In 2018, Helbig et al. [2018] described epileptic encephalopathy related to *CACNA1E* (OMIM #618285), studying 30 patients with 14 de novo pathogenic variants in the *CACNA1E* gene (MIM*601013; 1q25). In this channelopathy, heterozygous variants in the *CACNA1E* gene cause a severe neurodevelopmental disorder presenting as early-onset refractory seizures, hypotonia, macrocephaly, hyperkinetic movements, and contractions. *CACNA1E* is inherited according to an autosomal dominant pattern, although, due to the severity of the phenotype, all patients described have a de novo variant [Helbig et al., 2018]. The vast majority of pathogenic variants are missense variants with an apparent gain of function effect and cluster within the cytoplasmic ends of all 4 S6 segments [Carvill, 2019], with the recurrent variant c.1054G>A; p.Gly352Arg found to be present in 9 out of 30 (60%) patients in the study [Helbig et al., 2018]. The role of haploinsufficiency has not been clarified to date.

This paper describes 2 unrelated patients with early infantile epileptic encephalopathy due to *CACNA1E*, comparing their genotype and phenotypic to all cases published up to July 2020 in order to determine a possible phenotype-genotype correlation.

Case Presentation

Case 1. A 2-year-4-month-old male born to healthy and nonconsanguineous parents with no relevant family history presented with congenital talipes equinovarus and subtle shortening of the long bones. At 2 weeks of age, he began to experience episodes of dystonia several times a day with a tendency toward opisthotonos, spinal hyperextension, internal rotation of the upper limbs, and irritability. He presented global developmental delay, poor eye contact, noncongenital macrocephaly (head circumference at birth 36.6 cm + 1 SD; head circumference at 2 months 42 cm + 2.7 SD), and severe axial hypotonia (Fig. 1). At 6 months of age, the patient developed epileptic spasms with hypsarrhythmia. His symptoms evolved into dystonic cerebral palsy with areflexia and a profound

developmental delay with no motor or social milestones achieved at 2 years of age, and he required a gastrostomy due to a feeding disorder. His epilepsy was refractory to multiple antiepileptic drugs (i.e., vigabatrin, corticosteroids, valproic acid, zonisamide, topiramate), and he also received pyridoxine and was initiated on a ketogenic diet (KD). Manifestations consisted of spasms and tonic seizures. Facial features included a flattened philtrum, frontal bossing, features consistent with hypotonic facies, epicanthus, and preauricular pits (Fig. 1a). Dystonia (twisting and repetitive movements) progressed, and the patient presented with choreoathetoid movements that were more noticeable with voluntary movement. The frequency and intensity of his dystonia symptoms and irritability have decreased over time.

Case 2. The patient is a 22-month-old female born to healthy and nonconsanguineous parents with no relevant family history. Prenatal development was uneventful except for talipes equinovarus. At 1 month of age, she presented with dystonic episodes with hyperextension of the trunk, internal rotation of the upper extremities, and progressive irritability. She also presented global developmental delay, poor eye contact, severe axial hypotonia, and oculogyric spasms not correlated with electroencephalographic recordings. At 4 months of age, the girl developed epileptic spasms and hypsarrhythmia. Her symptoms also evolved into dystonic cerebral palsy with areflexia, profound developmental delay (no motor or social milestones achieved), and epilepsy that was refractory to multiple antiepileptic drugs (valproic acid (VPA), levetiracetam, clobazam, vigabatrin, zonisamide, lamotrigine, and topiramate), and she also received pyridoxine and KD; her seizure types included spasms and tonic seizures. Other features were frontal bossing, hypotonic facies, microretrognathia, puffy cheeks, a flattened philtrum, and dolichocephaly without macrocephaly. The patient also required a gastrostomy due to a feeding disorder (Fig. 1b). Lastly, she also sustained an unexplained or stress fracture in the left femur and was diagnosed with osteopenia.

Laboratory tests of blood, urine, and spinal fluid were performed in both patients to study a) intermediate metabolism, e.g., amino acid disorders, organic acid disorders, carbohydrate metabolism, creatine, purines and pyrimidines, and neurotransmitters; b) defects of β -oxidation of fatty acids; c) congenital disorders of glycosylation (CDG); and d) peroxisomal diseases. All results were normal. Karyotype and CGH array 180Kb were normal. Cerebral and spinal magnetic resonance imaging (MRI) scans in both patients were normal at 2 months and when

Fig. 1. a. Phenotype of case 1 showing severe axial hypotonia, frontal bossing, low set ears, long and smooth philtrum, subtle rhizomelic shortening of limbs, and congenital talipes equinovarus. **b.** Phenotype of case 2 revealing microretrognathia, low set ears, frontal bossing, congenital talipes equinovarus, and severe hypotonia.

repeated at 6 months in case 1 and at 4 months in case 2. Eye fundus and visual and auditory evoked potentials were normal in both patients. Electromyogram and electroneurogram findings were also normal in both patients.

Results

In both patients, diagnostic singleton whole-exome sequencing (WES) was performed in 2 different commercial laboratories (CENTOGENE, Germany and IMEGEN, Spain), though the primary analysis was conducted on a list of genes related to CDG in case 1 and epileptic encephalopathies in case 2. No pathogenic variants were reported in either case. We subsequently performed an unbiased analysis of the WES data for both patients using phenotypes in accordance with the following Human Phenotype Ontology (HPO) terms: dystonia HP:0001332, global developmental delay HP:0001263, seizure HP:0001250, and talipes equinovarus HP:0001762. We observed the heterozygous missense pathogenic variant c.1054G>A; p.Gly352Arg in the *CACNA1E* gene in both patients. In case 1 the variant had a mutant allele fraction of 55% (22/40 reads) and in case 2 the variant had a mutant allele fraction of 45% (23/51 reads). Using Sanger sequencing, parental DNA was studied, revealing a de novo variant in *CACNA1E* in both patients.

We compared the clinical features described for the group studied by Heldig et al. [2018] with those of our 2 patients (Table 1) and attempted to identify potential genotype-phenotype correlations (Table 2). Using Fisher's exact test and a statistical significance level of 0.05, we compared every phenotypic feature for a specific domain against all other locations. A movement disorder was present in 43% of all patients and in all subjects with variants located at the cytoplasmatic topological domain between DI and DII. This location included the p. Gly352Arg variant, and movement disorders in this group of patients were statistically more frequent ($p < 0.05$) than in patients with variants in other protein locations. To the contrary, the presence of movement disorders were statistically less frequent ($p < 0.05$) in patients with variants located at DII, including the recurrent variant p.Ala702Thr located at the S6 transmembrane domain of DII.

Also, the presence of severe intellectual disability was significantly less common in the group of patients with variants located in the cytoplasmatic topological domain between DI-II and DIV than in individuals with variants located elsewhere in the protein (50 vs. 93%; Table 2).

Refractory epilepsy, contractures, and macrocephaly was present in 90, 52, and 40%, respectively. No differences in the frequency of these features were detected.

Table 1. Phenotypic description, image and electrophysiologic studies, sensory assessment, treatment, and response of published and available patients with early epileptic encephalopathy due to the variant c.1054G>A in the *CACNA1E* gene

Case	Gender	Phenotypic features			Brain and spine MRI	Electrophysiology		Sensory Assessment		Genetic studies	Treatment	Response to topiramate		
		Epilepsy ^a	Neurological features and development	Macrocephaly		Movement disorder	Congenital contractures	Miscellaneous	EMG, ENG, EKG				EEG	Eye fundus exam and visual evoked potentials
1	Male	Yes. Infantile spasms, 6 months. Axial tonic seizures, 12 months. Refractory course	6 Spastic-dystonic cerebral palsy with areflexia. Severe global developmental delay	Yes	Dystonia	Talipes equinovarus	Lack of visual contact, gastrostomy, episodes of hyperthermia. Shortened long bones	Normal	2 months: normal. 6 months: hypersarrhythmia. Follow up: multifocal	Normal	Normal	Singleton WES virtual panel CGD: negative. Reanalysis of WES: CACNA1E: c.1054G>A; Topiramate p.Gly352Arg de novo, heterozygous Ketogenic diet B6	None	
2	Female	Yes. Infantile spasms, 4 months. Axial tonic seizures, 15 months. Refractory course	4 Spastic-dystonic cerebral palsy with areflexia. Severe global developmental delay	No	Dystonia	Talipes equinovarus	Oculogyric spasms, osteopenia, lack of visual contact, gastrostomy	Normal	4 months: hypersarrhythmia. Follow up: multifocal	Normal	Normal	Singleton WES virtual panel CGD: negative. Reanalysis of WES: Valproic acid CACNA1E: c.1054G>A; Topiramate p.Gly352Arg de novo, heterozygous B6 Zonisamide Ketogenic diet	None	
Helbig et al. [2018]; individual 3	Female	Yes. Tonic seizures 36 months. Focal motor awareness, focal evolved to bilateral tonic clonic, gelastic seizures. Refractory course	Generalized hypotonia, developing to a spastic/dystonic quadriplegia. Severe intellectual disability	Yes	Dystonia	Talipes equinovarus	Cortical visual impairment; progressive scoliosis	NA	Focal epileptiform discharges in the frontal areas	NA	NA	WES, trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous Lamotrigine Topiramate Levetiracetam Stiripentol	Positive response but never seizure-free	
Helbig et al. [2018]; individual 4	Female	Yes. Tonic seizures, 47 months. Generalized tonic-clonic seizures. Refractory course	Axial hypotonia, spastic dystonic tetraplegia. Severe global developmental delay	Yes	Dystonia	None	Severe allergy to cow's milk	Normal at 24 months	Multifocal	NA	NA	WES trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous Levetiracetam Valproic acid Topiramate Clobazam Lamotrigine Rufinamide Zonisamide	None	
Helbig et al. [2018]; individual 5	Male	Yes. Infantile spasms, 10 months. Generalized tonic-clonic seizures. Refractory course	Severe hypotonia; Severe global developmental delay	Yes	Dystonia	None	Increased tracheal secretions and long-term difficulty with effective airway clearance; mild persistent asthma; osteopenia	Normal	NA	Generalized slow-spike wave pattern consistent with Lennox-Gastaut syndrome	NA	NA	WES trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous Unavailable	NA
Helbig et al. [2018]; individual 6	Male	Yes. Focal clonic seizures, 2 months. Infantile spasms. Refractory course	Poor visual fixing and following. Axial hypotonia. Severe global developmental delay	No	Dystonia	None	Decreased fetal movements, dysphagia	3 months: incidental Rathke's cleft cyst. 6 months: signs suggestive of atrophy	NA	Multifocal	NA	NA	WES trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous Topiramate Clobazam Levetiracetam Lacosamide Ketogenic diet Valproic acid	None
Helbig et al. [2018]; individual 7	Female	Yes. Infantile spasms, 4.5 months. No other type of seizures. Refractory course	Spastic-dystonic cerebral palsy. Severe global developmental delay	No	Dystonia	None	None	Initially normal with progressive atrophy	NA	Multifocal	NA	NA	WES trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous Vigabatrin Levetiracetam Oxcarbazepine Topiramate Corticotropin Valproic acid Lamotrigine Rufinamide Clobazam Ketogenic diet CBD	Positive response but never seizure-free

Table 1 (continued)

Case	Gender	Phenotypic features			Brain and spine MRI	Electrophysiology		Sensory Assessment		Genetic studies	Treatment		
		Epilepsy ^a	Neurological features and development	Macrocephaly disorder		Movement disorder	Congenital contractures	Miscellaneous	EMG, ENG, EKG			Eye fundus exam and visual evoked potentials	Auditory evoked potentials
Helbig et al. [2018]; individual 8	Female	Yes. Focal tonic seizures, 14 months. Refractory course.	NA. Severe global hypotonia. No developmental delay	NA	Dystonia	Talipes equinovarus	Possible lactose intolerance	NA	At onset: bioelectrical status epilepticus. Follow up: NA	NA	WES trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous	Lamotrigine Valproic acid Sulthiame	NA
Helbig et al. [2018]; individual 9	Female	Yes. Focal clonic seizures, 6 months. Focal seizures with impairment of awareness, tonic seizures. Refractory course	Dyskinetic motor pattern, generalized hypotonia. Severe global developmental delay	No	Dystonia	Talipes equinovarus	Lack of visual contact, gastrostomy, episodes of hyperthermia	NA	Epileptic encephalopathy with multifocal epileptiform activity	NA	WES trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous	Valproic acid Lamotrigine Topiramate Levetiracetam Stiripentol Clobazam Diazepam Gabapentin	None
Helbig et al. [2018]; individual 10	Male	Yes. Infantile spasms, 4.5 months. Tonic seizures, generalized tonic-clonic seizures, and focal seizures with impairment of awareness. Refractory course	Generalized hypotonia. Severe intellectual disability	No	Dystonia, orofacial dyskinesia	Talipes equinovarus, multiple joint contractures	Plagiocephaly, inconsistent visual fixation or tracking, scoliosis, pectus excavatum, suspected autonomic dysregulation	EMG: At 4.5 months: epileptic encephalopathy with incomplete hypsarhythmia. At 5.5 months: continuous spike and polyspike-wave in generalized distribution with posterior predominance	Normal	WGS trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous	Normal	Vigabatrin Hydrocortisone Valproic acid Nitrazepam Levetiracetam	NA
Helbig et al. [2018]; individual 11	Female	Yes. NA	Axial hypotonia, stereotypies. Severe global developmental delay	Yes	Dystonia	Bilateral wrists, ulnar deviation	Strabismus	Normal at 2 months	NA	WES trio: CACNA1E: c.1054G>A; p.Gly352Arg de novo, heterozygous	NA	Clobazam	NA

^a Type and age at onset (months), other type of seizures, outcome. NA, not available; CDG, congenital disorder of glycosylation; WES, whole exome sequencing; CBD, cannabidiol.

^a Type and age at onset (months), other type of seizures, outcome. NA, not available; CDG, congenital disorder of glycosylation; WES, whole exome sequencing; CBD, cannabidiol.

Table 2. Different frequencies of the cardinal phenotypic features related to *CACNA1E* in terms of the location of the pathogenic variant along the protein domain

Location of the variant (protein level)	Missense variant	Severe developmental delay		Refractory epilepsy		Contractures		Movement disorder (dystonia or choreoathetoid movements)		Macrocephaly	
		Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
DI from p90 to p.350	Tr. S5 c.683T>C; p.(Leu228Pro)	2	0	2	0	0	2	1	1	0	2
	Tr. S6 c.1042G>C; p.(Gly348Arg)										
Cytoplasmic topological domain between DI and DII from p.351 to p.476	c.1054G>A; p.(Gly352Arg)	11	0	11	0	7	4	11**	0	5	5
	Cat. S4-S5 c.1807A>C; p.(Ile603Leu)	14	0	11	2	6	7	3	11**	6	7
DII from p.477 to p.703	Tr. S6 c.2069G>A; p.(Gly690Asp)										
	c.2093T>C; p.(Phe698Ser)										
	c.2098G>A; p.(Ala700Thr)										
	c.2101A>G; p.(Ile701Val)										
	c.2104G>A; p.(Ala702Thr)										
	c.2104G>C; p.(Ala702Pro)										
Cytoplasmic topological domain between DIII and DIV from p.1423 to p.1479	c.4264A>T; p.(Ile1422Phe)	2	2**	3	1	2	2	0	4	1	3
	c.4274C>A; p.(Thr1425Asn)										
	c.4288G>A; p.(Gly1430Arg)										
DIV from p.1480 to p.1724	Tr. S6 c.5159C>G; p.(Ala1720Gly)	1	0	1	0	1	0	0	1	0	1
%		93	7	90	10	52	48	43	57	40	60

** p<0.05 (Fisher's test) in patients with variants in that protein domain compared to the rest of the patients. NA, not available.; Cyt, cytoplasmatic domain; Tr, transmembrane. Protein sequence based on entry Q15878 from Uniprot [The UniProt Consortium, 2018].

Discussion

Early-onset epileptic encephalopathy (EOEE) represents a heterogeneous group of disorders in which epilepsy appears in the first 6 months of life and is usually associated with a severe neurodevelopmental disorder, among other symptoms [Heyne et al., 2018; Carvill, 2019]. Currently, a genetic etiology can be identified in about 30% of cases [Mercimek-Mahmutoglu et al., 2015; Balciuniene et al., 2019], mainly as pathogenic variants in genes encoding neuronal ion channels, proteins involved in synaptic transmission, or transcriptional regulatory proteins [Mercimek-Mahmutoglu et al., 2015; Heyne et al., 2018; Balciuniene et al., 2019].

Calcium channels are critical for neuronal function and different types of channels have different functions. High voltage-activated (HVA) channels open in response to large membrane depolarizations [Simms and Zamponi, 2014]. HVA channels have a complex, tissue-specific structure where the main components are a) α subunits, the primary pore-forming subunits of the calcium channel; b) β subunits linked to the α subunit in the intracytoplasmic environment regulating channel opening; c) the $\alpha 2\delta$ subunit attached to the extracellular leaflet of the plasma membrane via a glycoposphatidylinositol (GPI), which augments channel cell surface density; and d) the γ subunit, which includes transmembrane domains, the function of which is less clear [Simms and Zamponi, 2014; Wu et al., 2015].

The α subunits are encoded by the following 10 genes: *CACNA1A*, *CACNA1B*, *CACNA1C*, *CACNA1D*, *CACNA1E*, *CACNA1F*, *CACNA1G*, *CACNA1H*, *CACNA1I*, and *CACNA1S* [Carvill, 2019]. These subunits are made up of 4 homologous domains with 6 transmembrane domains each, where the transmembrane segments 5 (S5) and 6 (S6) line the inner pore of the channel [Carvill, 2019]. Each gene has a cell- and tissue-specific expression pattern. *CACNA1E* is highly expressed in the central nervous system and encodes the $\alpha 1$ -subunit of the HVA, rapidly inactivating the R-type calcium channel that initiates synaptic transmission [Helbig et al., 2018].

The pathogenic variant in *CACNA1E* found in our patients, c.1054G>A; p.(Gly352Arg), was first described by Helbig et al. [2018] in 9 patients (Table 1). Raybaud et al. [2006] demonstrated that this variant, which is located at the cytoplasmic topological domain between DI and DII, causes a gain of function that favors voltage-dependent activation and diminishes the rate of voltage-dependent inactivation. The role of haploinsufficiency has yet to be clarified.

Some aspects of *CACNA1E* encephalopathy are phenotypically similar to other early epileptic encephalopathies, including early-onset refractory epileptic seizures (primarily spasms and axial tonic seizures), in whom the disease tends to be refractory and presents with severe developmental delay and abnormalities in muscle tone. However, *CACNA1E* encephalopathy has unique features:

a) Dystonia is a common symptom in patients with *CACNA1E* encephalopathy, especially those carrying the c.1054G>A; p.(Gly352Arg) recurrent variant, as in the case of both patients described in this paper and all patients in Helbig's group [Helbig et al., 2018]; b) Congenital contractures are present in 52% (16/31) of patients with *CACNA1E* (Table 2); c) Macrocephaly was evident in 40% (12/30) of patients (Table 2); d) Deep tendon areflexia, as presented by both patients described here, with normal peripheral electroneurogram.

In the 9 patients with the c.1054G>A; p.(Gly352Arg) variant described by Helbig et al. [2018], no deep tendon reflexes are mentioned. However, 1 patient with the variant c.683T>C; p.(Leu228Pro) had brisk reflexes and 1 with the variant c.1807A>C; p.(Ile603Leu) presented hyporeflexia, while the rest of the patients were described as having normal reflexes or no information of reflexes was provided.

Although we describe only 2 patients, both were phenotypically very similar and presented with the movement disorder and contractures at early stages. We believe these 2 features could aid in identifying this group of patients early in the course of the disease.

In case 2, the cause of osteopenia has not been elucidated, though its relation to *CACNA1E* seems unlikely and could be explained by a multifactorial complication of chronic immobility, the effect of drugs, and KD [Bachrach and Gordon, 2016].

Conclusion

Epileptic encephalopathies continue to pose a significant challenge for etiology and diagnosis, and a direct genetic approach is difficult. It is of great interest to identify early and specific phenotypic data in those entities that could be relevant. Congenital contractures and presence of a movement disorder prior to the onset of epilepsy may be the key to guiding the diagnosis of epileptic *CACNA1E* encephalopathy. In this study, we found a genotype-phenotype correlation between movement disorders and the presence of severe intellectual disability with a variant in the *CACNA1E* gene.

Acknowledgements

We thank the children and their families who participated in the study.

We thank the Biomedical Research Foundation Niño Jesús and O. Shaw for the assistance in reviewing and editing the present manuscript.

Statement of Ethics

This work was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All patients (or their parents or guardians) have given written informed consent for the use of patient clinical data and images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

References

- Bachrach LK, Gordon CM. Section on Endocrinology. Bone Densitometry in Children and Adolescents. *Pediatrics*. 2016; 138(4): e20162398.
- Balciuniene J, DeChene ET, Akgumus G, Romascko E, Cao K, Dubbs H, et al. Use of a dynamic genetic testing approach for childhood-onset epilepsy. *JAMA Netw Open*. 2019;2(4): e192129.
- Carvill GL. Calcium channel dysfunction in epilepsy: Gain of CACNA1E. *Epilepsy Curr*. 2019;19(3):199–201.
- Helbig KL, Lauerer RJ, Bahr JC, Souza IA, Myers CT, Uysal B, et al. De novo pathogenic variants in CACNA1E cause developmental and epileptic encephalopathy with contractures, macrocephaly, and dyskinesias. *Am J Hum Genet*. 2018;103(5):666–78.
- Heyne H, Singh T, Stamberger H, Abou Jamra R, Caglayan H, Craiu D, et al. De novo variants in neurodevelopmental disorders with epilepsy. *Nat Genet*. 2018;50(7):1048–53.
- Mercimek-Mahmutoglu S, Patel J, Cordeiro D, Hewson S, Callen D, Donner EJ, et al. Diagnostic yield of genetic testing in epileptic encephalopathy in childhood. *Epilepsia*. 2015; 56(5):707–16.
- Raybaud A, Dodier Y, Bissonnette P, Simoes M, Bichet DG, Sauvé R, et al. The role of the GX9GX3G motif in the gating of high voltage-activated Ca²⁺ channels. *J Biol Chem*. 2006;281(51):39424.
- Simms BA, Zamponi GW. Neuronal voltage-gated calcium channels: structure, function, and dysfunction. *Neuron*. 2014;82(1):24–45.
- The UniProt Consortium. UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Research*. 2018;47(D1):D506–D515.
- Wu J, Yan Z, Li Z, Yan C, Lu S, Dong M, et al. Structure of the voltage-gated calcium channel Cav1.1 complex. *Science*. 2015;350(6267): aad2395.

Funding Sources

Whole exome sequencing performed on the patients' DNA samples and all other analyses were funded by non-discretionary grants.

Author Contributions

All authors have substantially contributed to the study. N.V.O.C., A.D.R., and B.F.G. designed the study, performed the literature search, and drafted the initial manuscript. B.B.C., V.C.E., and M.J.L. participated in patient recruitment and gathering of clinical data. J.J.G.P., N.V.O.C., and A.D.R. conceptualized the study and reviewed the manuscript.