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RESEARCH ARTICLE

Biallelic *CACNA2D2* variants in epileptic encephalopathy and cerebellar atrophy

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Abstract

Objective: To characterize the molecular and clinical phenotypic basis of developmental and epileptic encephalopathies caused by rare biallelic variants in *CACNA2D2*. **Methods**: Two affected individuals from a family with clinical features of early onset epileptic encephalopathy were recruited for exome sequencing at the Centers for Mendelian Genomics to identify their molecular diagnosis. GeneMatcher facilitated identification of a second family with a shared candidate disease gene identified through clinical gene panel-based testing. **Results**: Rare biallelic *CACNA2D2* variants have been previously reported in three families with developmental and epileptic encephalopathy, and one family with congenital ataxia. We identified three individuals in two unrelated families with novel homozygous rare variants in *CACNA2D2* with clinical features of developmental and epileptic encephalopathy and cerebellar atrophy. Family 1 includes two affected siblings with a likely damaging homozygous rare missense variant c.1778G>C; p.(Arg593Pro) in *CACNA2D2*. Family 2 includes a proband with a homozygous rare nonsense variant c.485_486del; p.(Tyr162Ter) in *CACNA2D2*. We compared clinical and molecular findings from all nine individuals reported to date and note that cerebellar atrophy is shared among all. **Interpretation**: Our study supports the candidacy of *CACNA2D2* as a disease gene associated with a phenotypic spectrum of neurological disease that include features of developmental and epileptic encephalopathy, ataxia, and cerebellar atrophy. Age at presentation may affect apparent penetrance of neurogenetic trait manifestations and of a particular clinical neurological endophenotype, for example, seizures or ataxia.

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Introduction

Developmental and epileptic encephalopathies (DEEs) encompass a heterogenous group of early-onset clinically severe disorders characterized by intractable seizures, abundant epileptiform activity on EEG, and developmental impairment or regression.[1]

A substantial number of DEE patients have been shown to have an underlying genetic etiology, with de novo heterozygous variants playing an important role.[2–5] With rapid advancements in molecular biology and genetic techniques, >100 genes have been associated with DEEs in the last 20 years, revolutionizing our understanding of their molecular etiology.[6] These genes have been linked to pathways involved in synaptic transmission, ion channels, transcriptional regulation, DNA damage repair, chromatin remodeling, and metabolism.[3] Pathogenic variants in genes involved in ion channel function represent perhaps one of the most common causes of DEEs, also called channelopathies, and their identification and molecular diagnostic specification in individuals with EE can have important therapeutic implications.[7] Pathogenic variants in genes encoding subunits of voltage-gated calcium channels (VGCCs), that is, *CACNA1A*, *CACNA2D2*,[8–11] and *CACNA2D1*[12] have been linked to epileptic encephalopathy (EE) and/or ataxia phenotypes [MIM#617106, 108500]. *CACNA2D2* encodes the $\alpha 2\delta$-2 auxiliary subunit of high VGCCs, is highly expressed in the cerebellar cortex, and is reportedly a receptor for the antiepileptic drugs pregabalin and gabapentin.[13] Auxiliary $\alpha 2\delta$ subunits support calcium channels in their assembly and trafficking to form complexes, increase channel density, and modulate channel kinetic properties.[14] Biallelic pathogenic variants in *CACNA2D2* have been reported in three families with DEEs and one family with congenital ataxia.[8–11] In this study, we present an additional two families with novel homozygous variants in *CACNA2D2* causing DEE and cerebellar atrophy. We also compared the molecular and clinical features of all previously reported cases, thus providing further evidence to support *CACNA2D2* as a disease-causing gene for DEE and a syndromic neurological trait that includes ataxia as a manifestation of the neurobiology of cerebellar dysfunction.

Patients and Methods

Participants and ethical approval

Informed consent was obtained from all family members in Family 1 in accordance with the Baylor-Hopkins Center for Mendelian Genomics (BHCMG) research protocol (Baylor College of Medicine IRB protocol number: H-29697). A second family, Family 2, was identified and recruited via GeneMatcher following informed consent.

Sequencing, variant interpretation, and bioinformatics

Exome sequencing (ES) was performed on two affected siblings (BH9685-1 and BH9685-4) of Turkish descent who were born to consanguineous parents in Family 1 using our standard sequencing and variant prioritization workflow that has been previously described, including BafCalculator for determination of absence of heterozygosity (AOH), potentially reflecting identity-by-descent (IBD), from exome variant data.[15] The proband in Family 2 had commercial panel testing from Invitae (San Francisco, California), the Early Infantile Epileptic Encephalopathy Panel (gene content enumerated alphabetically in Table S1), which includes *CACNA2D2*. The *CACNA2D2* variants in Family 1 and 2 have been added to ClinVar under the accession numbers SCV000920875 and SCV000551913.2, respectively. Both *CACNA2D2* variants are absent from the public variant databases gnomAD, ExAC, ARIC, and ESP. Bioinformatic analyses were used to predict potential variant effects on protein function (SIFT, PolyPhen2, MutationTaster, Phylop, CADD) and assess predicted (NMDEscPredictor) premature termination codons (PTCs).

Variant confirmation

Potential disease-causing variants in *CACNA2D2* were examined for segregation in all family members with available DNA for both families using standard PCR and Sanger sequencing techniques.

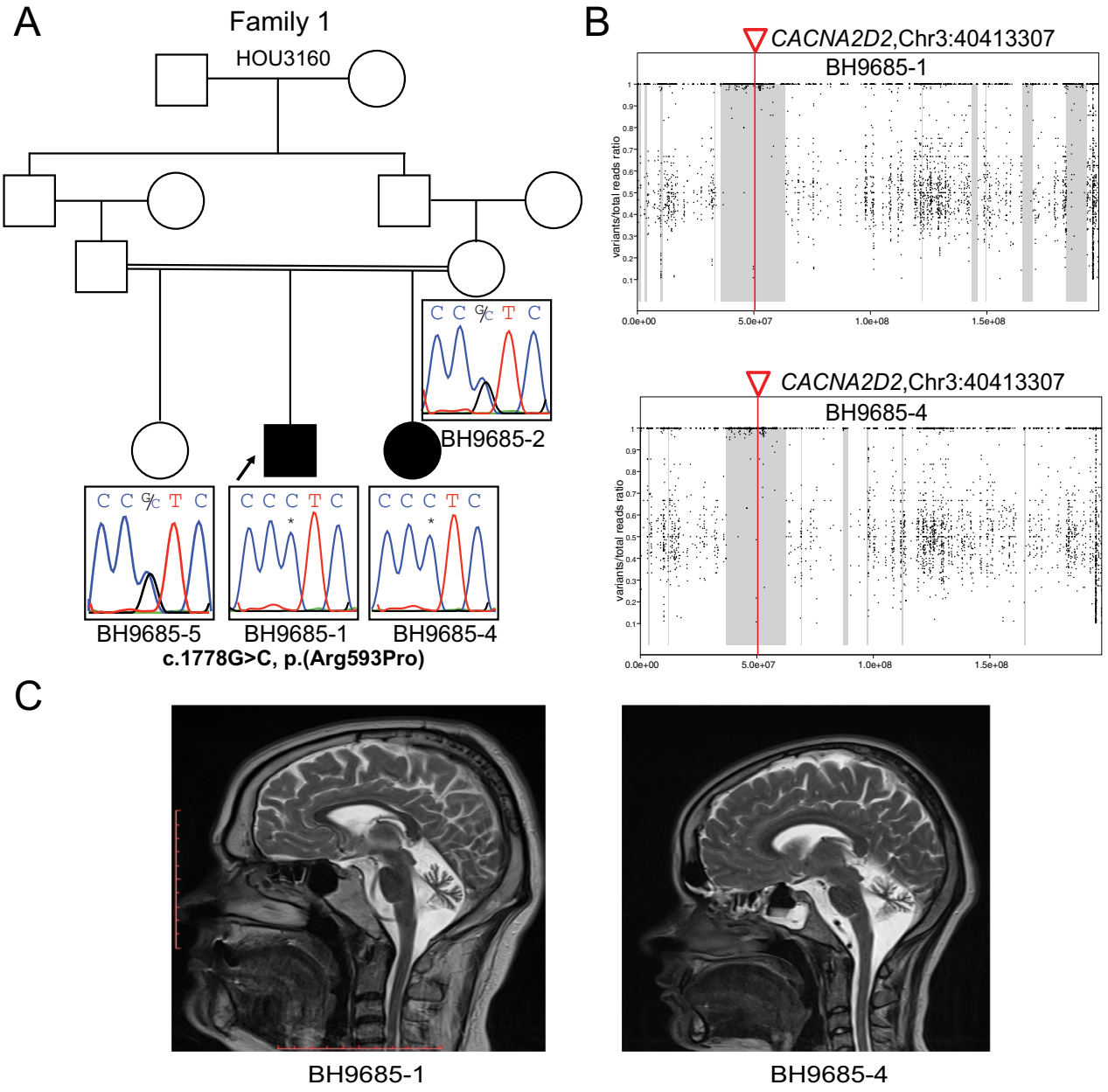

Figure 1. Biallelic variant in *CACNA2D2* in two siblings with developmental and epileptic encephalopathy (Family 1). (A) Pedigree diagram for Family 1 shows family structure and Sanger sequencing traces for the c.1778G>C, p.(Arg593Pro) *CACNA2D2* variant in family members with available DNA. The variant is homozygous in both affected siblings (BH9685-1 and BH9685-4) and heterozygous in the unaffected mother (BH9685-2) and unaffected sibling (BH9685-5) conforming to Mendelian expectations for an autosomal recessive (AR) disease trait. DNA from the unaffected father was not available for segregation studies. (B) Absence of heterozygosity (AOH) plots for Family 1 show that the *CACNA2D2* variant lies in a region of AOH in chromosome 3 that is represented by the gray shaded area for both affected siblings (BH9685-1 and BH9685-4). AOH regions are calculated using B-allele frequency data from exome data.[15] (C) T2-weighted brain MRI images for affected siblings BH9685-1 and BH9685-4 show cerebellar atrophy.

Clinical evaluation

Family 1

BH9685-1 and BH9685-4 are affected siblings born to consanguineous Turkish parents with no known clinical history of seizures, and have an unaffected elder female sibling (Fig. 1A). BH9685-1 is currently a 29-year-old male who was born at term via C-section after an uncomplicated pregnancy. His seizures started at 1 month of life and quickly became refractory to several antiepileptic

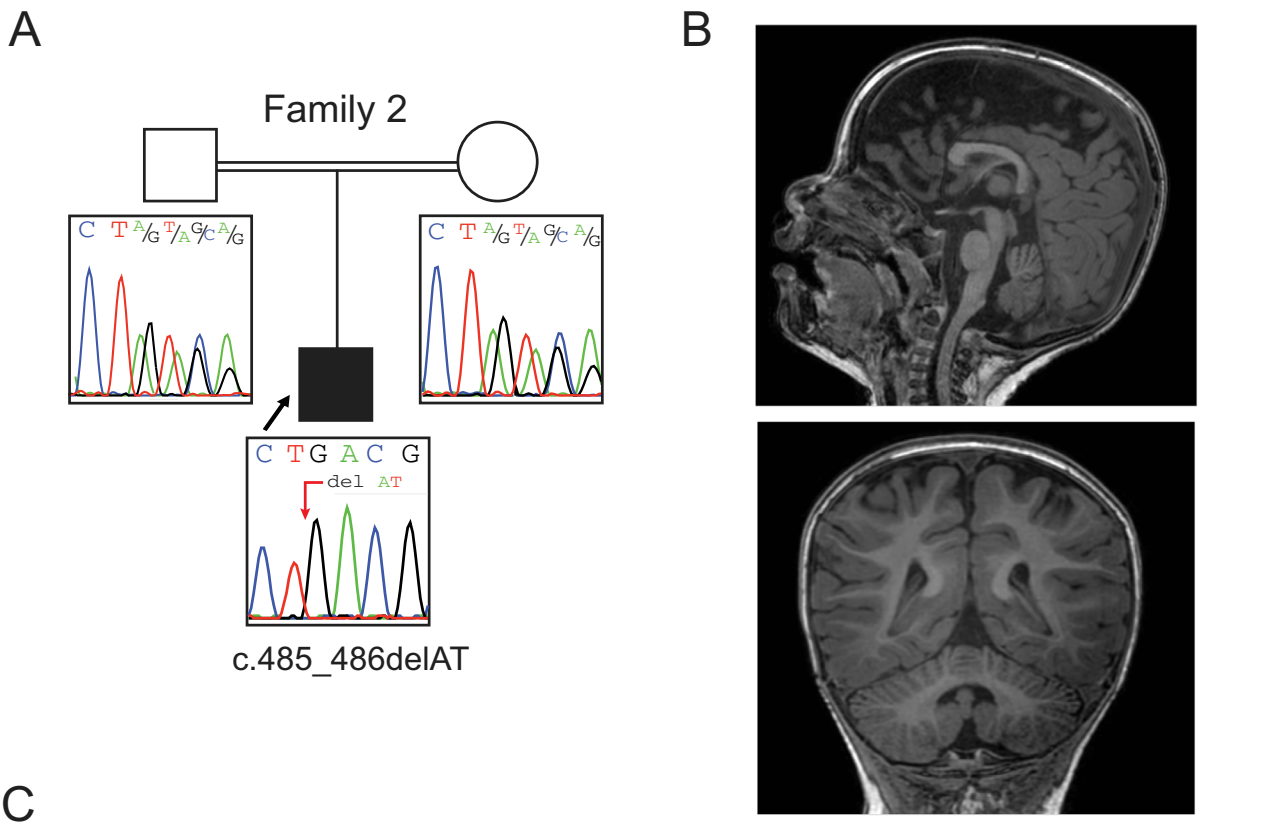

Figure 2. Biallelic variant in *CACNA2D2* in proband with epileptic encephalopathy (Family 2). (A) Pedigree diagram and Sanger sequencing validation in Family 2 for *CACNA2D2* variant c.485_486del, p.(Tyr162Ter) show heterozygosity for the variant in both parents, and homozygosity for the variant in the affected proband, consistent with an AR disease pattern. (B) T1-weighted brain MRI images for affected proband show cerebral and cerebellar atrophy. (C) Routine EEG recording at 13 months of age captured an episode of unresponsiveness and eye-blinking lasting for 8 seconds associated with 3 Hz generalized spike and slow wave activity consistent with early-onset absence seizure [Settings: Low frequency filter at 1 Hz; high frequency filter at 70 Hz; sensitivity at 15 μV/mm; paperspeed 30 mm/sec].

drugs (AEDs) including levetiracetam, oxcarbazepine, topiramate, and clonazepam. His most prominent clinical seizure type is tonic-clonic. He had global developmental delay (GDD) and currently has intellectual disability (ID).

Table 1. Molecular features of individuals with rare biallelic *CACNA2D2* variants.

	Family 1		Family 2	Edvardson et al.			Pippucci et al.	Valence et al.	Butler et al.
Ethnicity	Turkish		Afghani	Arab-Palestinian			Italian	Portuguese	Unknown
Gender	M	F	M	M	M	F	M	M	M
Age at last evaluation	29 years	24 years	5 years	7 years	4 years	–	10 years	20 years	5 years
Parental consanguinity	Yes		Yes	Yes			Yes	unknown	No
Zygosity	Hom		Hom	Hom			Hom	Hom	Compound het
cDNA (NM_006030.3)	c.1778G>C		c.485_486delAT	c.3119A>G			c.1295delA	c.2971G>A	c.782C>T, c.3137T>C
Exon (of 38)	Exon 20		Exon 5	Exon 36			Exon 13	Exon 34	Exon7, Exon 36
Protein	p.(Arg593Pro)		p.(Tyr162Ter)	p.(Leu1040Pro)			p.(Asn432fs)	p.(Asp991Asn)	p.(Pro261Leu), p.(Leu1046Pro)
Variant information	Missense		Nonsense/potential LoF	Missense			Frameshift/potential LoF	Missense	Missense + missense
Testing	ES	ES	Epilepsy panel	ES			ES	ES	ES

Abbreviations: ES, exome sequencing; F, female; het, heterozygous; hom, homozygous; LoF, loss of function; M, male.

At present, he is unable to walk unassisted, has a limited vocabulary of a few words and some behavioral issues including restlessness and sleep disturbance. He has subtle dysmorphic craniofacial features including thick eyebrows and a prominent nose not observed in his parents. Brain MRI at age 26 years showed marked cerebellar and mild cerebral atrophy (Fig1C).

BH9685-4 is BH9685-1's 24-year-old sister. She was delivered by C-section at term, following an uncomplicated pregnancy. Her seizures started at 2 months of life, and quickly became refractory to multiple AEDs. Her seizures spontaneously resolved following puberty, and currently she is in remission (seizure-free). Additional clinical features are similar in severity and character to those of her affected brother. Brain MRI at age 21 years showed marked cerebellar and mild cerebral atrophy similar to her affected brother.

Family 2

The proband of Family 2 was a 3-year-old male born premature at 31 weeks of corrected gestational age to first cousin consanguineous parents of Afghani descent (Fig. 2A). Parents did not report any personal history of seizures. He was noted to have GDD, was able to sit with support but had an unsteady posture, showed dysmetria, axial hypotonia, and ataxia. He showed pincer grasp and reciprocal smile. He produced a few simple words and had not yet walked. His brain MRI demonstrated global cerebral and cerebellar atrophy (Fig. 2B). He was first noted to have seizures at 7 months, and these became refractory to several antiseizure medications including amantadine, ethosuximide, valproic acid, levetiracetam, and clobazam. He had several seizure types including typical absence, generalized tonic-clonic, generalized myoclonic and focal sensory seizures with decreased level of awareness. A routine EEG performed at 13 months of age captured two typical absence seizures associated with 3Hz spike and slow wave activity lasting for 8 seconds (Fig. 2C). A 48-hour EEG at 3 years of age showed background slowing, focal and generalized epileptiform discharges, and captured a single 12-second typical absence seizure. He eventually underwent placement of a vagal nerve stimulator (VNS) passing away less than 48 hours after insertion due to broncho-aspiration.

Results

Genetic analyses

In Family 1, ES analyses showed a homozygous rare missense variant c.1778G>C, p.(Arg593Pro) in *CACNA2D2* (NM_006030.3) in both affected siblings. This variant is

predicted to be disease causing (SIFT, Polyphen-2, MutationTaster), conserved (PhyloP), and has a CADD score of 34. In Family 2, an epilepsy gene panel test showed a homozygous rare nonsense variant c.485_486del, p.(Tyr162Ter) in *CACNA2D2* (NM_006030.3) in the proband. This variant is predicted to result in a truncated protein by producing a stopgain at amino acid residue position 162 in exon 5 of this 38-exon gene. Sanger sequencing confirmed the variants and segregation in accordance with Mendelian expectations for an autosomal recessive (AR) disease trait, showing heterozygosity for the identified *CACNA2D2* variant in the mother and unaffected older sister in Family 1 and heterozygosity for the *CACNA2D2* variant in both parents in Family 2 (Figs. 1A and 2A).

Analysis of absence of heterozygosity (AOH) from exome variant data in Family 1 demonstrated overlapping regions of AOH in both siblings, measuring ~29.9 Mb and ~25.8 Mb in size (Fig. 1B). Notably, these regions of AOH were not the largest AOH interval in either sibling (~37 Mb and ~30.6 Mb), and the siblings demonstrated a total AOH of ~380 Mb and ~393 Mb consistent with the reported history of parental consanguinity. The rare likely damaging variant in *CACNA2D2* found in the ~30 Mb and ~26 Mb regions of AOH was the only such variant found in any AOH interval. For Family 2, a SNP array was carried out and it demonstrated that the rare *CACNA2D2* variant is in ~27.1 Mb region of AOH, which also happens to be the largest AOH interval.

CACNA2D2 has a high probability of being loss-of function intolerant, that is, pLI (probability of being loss-of-function intolerant) score of 1, and analysis using the NMDEscPredictor tool indicates that it has a noninformative transcript for predicting escape of nonsense-mediated decay (NMD).[16]

Comparison of clinical and molecular features

The principal molecular and clinical features of all individuals reported including the two families (three individuals) in our study and four families reported earlier are shown in Tables 1 and 2 and Table S2. Affected individuals in all five DEE families show different seizure types and clinically severe epilepsy, as demonstrated by drug resistance to AEDs, in 7/8 individuals. Typical absence seizures with early onset (before 4 years of age) were observed in three individuals (Family 2, Pippucci et al., Butler et al.), and treatment with ethosuximide (ESM) improved absence seizure frequency in one individual (Pippucci et al.). The individual reported with congenital ataxia (Valence et al.) has only had one seizure, interpreted as a febrile seizure, and no features of intellectual disability (ID) or developmental delay (DD), distinct from the DEE individuals for whom recurrent seizure activity and severe DD were both present. Available MRI images of all individuals were compared by a pediatric neuroradiologist (JVH). All individuals (9/9) show cerebellar atrophy by MRI. Of note is the remarkable consistency of the MRI patterns from the affected siblings of Family 1 (Fig1C). Three individuals with DEE also showed moderate or truncal ataxia. Although ataxia was not mentioned in the clinical features for the other two families, these individuals showed intermittent choreiform movements (Edvardson et al.) and dyskinesia (Pippucci et al.). The positions of the biallelic variants within the transcript and protein domains have been mapped and are shown in Figure 3.

Discussion

Inherited channelopathies represent a heterogenous group of disorders caused by mutations in genes encoding ion channel subunits or their regulators, and account for a substantial proportion of Mendelian epilepsy syndromes.[17] Genes encoding voltage-gated calcium channels (VGCCs) have been linked to both epilepsy and/or ataxia neurological phenotypes (Table 3). Gene expression pattern of these five genes (*CACNA1A*, *CACNA1G*, *CACNB4*, *CACNA2D1*, and *CACNA2D2*) reveals high expression in the brain with a pattern of higher expression in the cerebellum in genes that present with symptoms of ataxia or cerebellar atrophy/hypoplasia on brain MRI, that is, *CACNA1A*, *CACNA1G*, *CACNB4*, and *CACNA2D2* (Fig. 4). Cerebellar Purkinje cell impairment is a pathological hallmark of ataxia and may result in a wide spectrum of motor symptoms.[18] *CACNA2D1* has been proposed as a candidate gene for intellectual disability and epilepsy, and affected individuals have been reported to show features of cortical atrophy without any cerebellar involvement, concordant with its expression pattern in the brain (Fig. 4).[12]

CACNA2D2 was first described as a candidate DEE gene in 2013[8,9], however, its role as a disease gene remained ambiguous as both studies also reported rare homozygous variants (rs149614835, rs587777163) in *CELSR3* that segregated with disease in both families. Recently, there have been two additional reports describing biallelic variants in *CACNA2D2,* rare homozygous variants in *CELSR3* were absent in both individuals reported.[10,11] Valence et al. describe an individual with congenital ataxia and cerebellar atrophy, this clinically milder neurologic phenotype was attributed to the nature of the rare homozygous splice variant observed that was shown by RT-PCR to produce a leaky splicing defect, impacting only a proportion of the transcripts.[10] Butler

Table 2. Clinical features of individuals with rare biallelic *CACNA2D2* variants.

	Family 1		Family 2	Edvardson et al.	Pippucci et al.	Valence et al.	Butler et al.
Age at onset	1 month	2 months	7 months	1–2 months	5 months	-	7 months
Seizure types	Tonic-clonic	Tonic-clonic	Tonic-clonic, typical absence, myoclonic, focal sensory	Atonic, clonic, tonic-clonic	Clonic, clonic-tonic, typical absence	Single febrile seizure at 1 year of age	Typical absence, atonic, tonic, tonic-clonic
Epilepsy	+	+	+	+	+	-	+
	DR	DR	DR	DR	DR	-	Controlled
AED treatment & response	LEV, OCBZ, TPM, CLN		Amantadine, ESM, VPA, CLB; VNS placement	VGB, CLN, TPM, CLB PB, VPA	PB, BZD, VPA, LEV, LTG, ESM (effective)	n/a	VPA, ESM, CLN (effective), ketogenic diet
ID/GDD	ID, GDD		Severe GDD	Severe GDD	Severe GDD	-	GDD
Brain MRI findings	Mild supratentorial WM loss, thinning of CC, marked; diffuse CA (at ages 21 years, 26 years)		Normal WM, foreshortened CC, mild CA (at 1.1 years)	Normal WM, borderline lower to normal CC, mild-moderate; diffuse CA (at 8 years)	Prominent ventral WM loss, thinning and slight foreshortening of CC, moderate-marked; diffuse CA (at 7 years)	Mild WM loss, slight foreshortening of CC, moderate CA; localized to superior vermis (at 18 years)	Prominent ventral WM loss, normal CC, marked; diffuse CA (at 2.4 years)
Ataxia	Truncal ataxia		Axial ataxia	-	-	Congenital ataxia	Moderate ataxia
Other neurological features	Mild behavioral problems, wheelchair dependent, speech limited to a few words		Nonambulatory, absent speech, axial hypotonia, dysmetria	Intermittent choreiform movements, axial hypotonia, absent speech	Dyskinesia, tremors, myoclonic jerks, hypertonia, axial hypotonia, oculomotor apraxia, recurrent SE	Motor delay, dysmetria, dysarthria	Nonambulatory, absent speech, hypotonia, recurrent SE

Abbreviations: AED, antiepileptic drugs; BZD, benzodiazepine; CA, cerebellar atrophy; CC, corpus callosum; CLB, clobazam; CLN, clonazepam; DR, drug resistant; EEG, electroencephalogram; ESM, Ethosuximide; GDD, global developmental delay; GSSW, generalized spike and slow waves; ID, intellectual disability; LEV, levetiracetam; LTG, lamotrigine; OCBZ, oxcarbazepine; PB, phenobarbital; SE, status epilepticus; TPM, topiramate; VNS, vagal nerve stimulator; VGB, vigabatrin; VPA, valproic acid; WM, white matter.

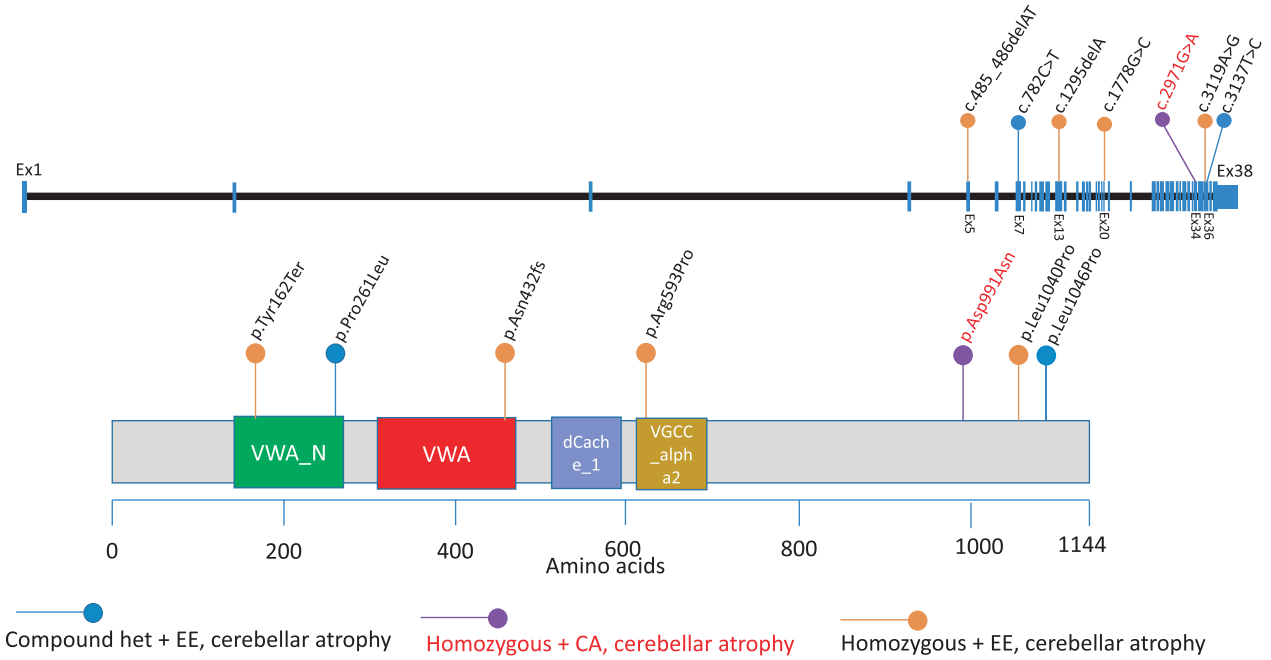

Figure 3. Exonic and protein map positions of *CACNA2D2* variant alleles showing potential protein domains mutated. Lollipop diagram showing biallelic variants reported in this study (2 families) and reported previously in the literature (4 families) mapped across the transcript and protein domain structures of CACNA2D2. The orange lollipops indicate the positions of homozygous variants in *CACNA2D2* from four families (Edvardson et al.[8], orange, Pippucci et al.[9], orange, Family 1, orange, and 2, orange, in this report) with developmental and epileptic encephalopathy (DEE) and cerebellar atrophy. The blue lollipops show the positions of reported compound heterozygous variants inherited *in trans* in a patient with DEE and cerebellar atrophy (Butler et al.[11], blue). The purple lollipop and red text indicate the position of a homozygous variant reported to cause a milder phenotype of congenital ataxia (CA) and cerebellar atrophy (Valence et al.[10], purple). The genomic structure of *CACNA2D2* represented here is based on information from the UCSC genome browser (https://genome.ucsc.edu) using transcript NM_006030; note the *CACNA2D2* transcript is encoded by the minus strand and has been flipped here for representational purposes. The protein domain structures were visualized and variants mapped using the Mutation Mapper portal (http://www.cbioportal.org/mutation_mapper). Protein domains: VWA_N: VWA N-terminal (141–265), VWA: von Willebrand factor type A domain (291–454), dCache_1: Cache domain (485–573), VGCC_alpha2: Neuronal voltage-dependent calcium channel alpha 2acd (580–662).

et al. reported an individual with compound heterozygous variants in *CACNA2D2* and a phenotype of DEE with cerebellar atrophy.[11] Rare variants in *CELSR3* are absent in Family 1 in this report (Family 2 had gene panel testing that did not include *CELSR3*), providing further evidence for *CACNA2D2* as an independent disease-causing gene.

Figure 3 represents all biallelic variants mapped to the transcript and protein domain structures of CACNA2D2 showing that the variants are not limited to a specific protein domain. Of the two missense variants that are shown to occur in protein domains, the homozygous missense variant p.Arg593Pro in Family 1 lies in the neuronal voltage-dependent calcium channel alpha-2 domain that is thought to play a role in calcium channel subunit assembly.[19] The heterozygous missense variant reported in Butler et al. (compound heterozygous variants) is predicted to be in the von Willebrand factor type A, N-terminus (VWA_N) domain which may be involved in the trafficking and function of VDCC subunits.[20] The other missense variants are not associated with known protein domains and thus their functional impact is uncertain; however, Edvardson et al. performed functional studies in *Xenopus laevis* oocytes and observed that the *CACNA2D2* p.(Leu1040Pro) variant resulted in the reduction of current density and slower inactivation of calcium channels compared to wildtype.[8] The identification of two frameshifting variants predicted to undergo nonsense-mediated decay (NMD) suggests a loss-of-function mechanism for the association between rare variation in *CACNA2D2* and DEE.

Additional support for *CACNA2D2* as a disease gene is seen in its mouse models. The spontaneous autosomal recessive (AR) mouse mutant *ducky* (*du*) is a model for absence epilepsy, and is characterized by spike-wave seizures and cerebellar ataxia.[21] Mutations in *Cacna2d2* were identified in the original *du* strain and a new allele du^{2J}, both resulted in loss of the full-length protein and were

Table 3. Genes encoding voltage-gated Calcium channels that are linked to epilepsy and/or ataxia phenotypes.

Gene	Disease	Phenotype MIM#	Inheritance	Gene expression
CACNA1A	Epileptic encephalopathy, early infantile, 42	617106	AD	Cerebellum, caudate, brain, other
	Episodic ataxia, type 2	108500	AD	
	Migraine, familial hemiplegic, 1	141500	AD	
	Migraine, familial hemiplegic, 1, with progressive cerebellar ataxia	141500	AD	
	Spinocerebellar ataxia 6	183086	AD	
CACNA1G	Spinocerebellar ataxia 42	616795	AD	Cerebellum, caudate, brain, retina, ovary, uterine, other
	Spinocerebellar ataxia 42, early-onset, severe, with neurodevelopmental deficits	618087	AD	
CACNB4	Episodic ataxia, type 5	613855	AD	Cerebellum, caudate, brain, hippocampus, other
	{Epilepsy, idiopathic generalized, susceptibility to, 9}	607682	AD	
	{Epilepsy, juvenile myoclonic, susceptibility to, 6}	607682	AD	
CACNA2D1	Intellectual disability and epilepsy (Vergult et al., 2015)[12]	–	AD	Skeletal muscle, hippocampus, brain, caudate, smooth muscle, thyroid, pituitary gland, other
CACNA2D2	Early-onset epileptic encephalopathy (Edvardson et al.[8], Pippucci et al.[9], Butler et al.[11])	–	AR	Lung, cerebellum, retina, caudate, other
	Congenital ataxia (Valence et al.[10])	–	AR	

Abbreviations: AD, Autosomal dominant; AR, Autosomal recessive. Note that gene expression in major tissues (>5% total expression in all tissues) from humans was incorporated from Seitter et al.[28] and the GTEx database.

associated with decreased calcium channel current in cerebellar Purkinje cells.[22,23] *Du* mice also showed an ataxic wide-based gait, dyskinesia, reduced size and did not survive beyond 35 days; neuropathology showed dysgenesis of the cerebellum, medulla, and spinal cord.[24]

Another spontaneous AR mouse model "*entla*" with mutations in *Cacna2d2* was observed with neurological features of epilepsy and ataxia. *Entla* (*ent*) mice result from an in-frame duplication in *Cacna2d2* and resemble the phenotype of *ducky* mice except that *entla* mice do not show neuroanatomical or central nervous system abnormalities.[25] Ligand-binding assays using gabapentin on cerebellar Purkinje cells isolated from *entla* mice showed >60% reduced binding to the membrane compared to wildtype and heterozygous mice demonstrating that α2δ-2 contributes to gabapentin binding.

Targeted knockout of *Cacna2d2* resulted in *Cacna2d2*^{tmINCIF} mice with ataxic gait, growth retardation, seizure susceptibility, and cardiac abnormalities.[13] Cerebellar degeneration was observed in *Cacna2d2*^{tmINCIF} mice with initial loss of the granule cell layer followed by gradual depletion of Purkinje cells. The cerebellar pathology observed was less severe than the *ducky* mice and the authors hypothesized this may be due to elimination of longer segments of the α2δ-2 protein in *ducky* mutants.

Overall, the studies in mice display a robust link between *Cacna2d2* and neurological disorders with features including epilepsy and ataxia. Pippucci et al. noted that the individual with a biallelic, loss-of-function (LoF) *CACNA2D2* variant in their study more closely resembled the absence epilepsy phenotype of the LoF *ducky* mouse compared to individuals with biallelic, missense variants.[9] The proband in family 2 with a biallelic, potential LoF *CACNA2D2* variant also shows absence seizures indicating that biallelic LoF variants may result in a slightly different phenotype.

Antiepileptic drugs gabapentin and pregabalin have been observed to bind to α2δ-1 and α2δ-2, high VGCCs encoded by *CACNA2D1* and *CACNA2D2,* respectively, with similar affinity.[14,24] Ataxia is reported as a side effect of gabapentin that may potentially be due to α2δ-2 binding in the cerebellum.[26] Notably, individuals with *CACNA2D2*-associated DEE and drug-resistant seizures have not been treated with gabapentin. Although treatment with gabapentin in these cases has not been objectively explored, it is possible that rare pathogenic variation in *CACNA2D2* may impact the efficacy of gabapentin as an AED.

To date, biallelic rare variants in *CACNA2D2* have been reported in a total of three unrelated families with DEE, notably, one additional individual has been described with a milder phenotype of congenital ataxia and cerebellar atrophy by MRI, which lies within the phenotypic spectrum of clinical features associated with *CACNA2D2*. Genotype–phenotype associations in another VGCC encoding gene, *CACNA1A*, have indicated that functional effects (gain-of-function, loss-of-function, hypomorphic) can impact phenotypic expression of disease, and may have

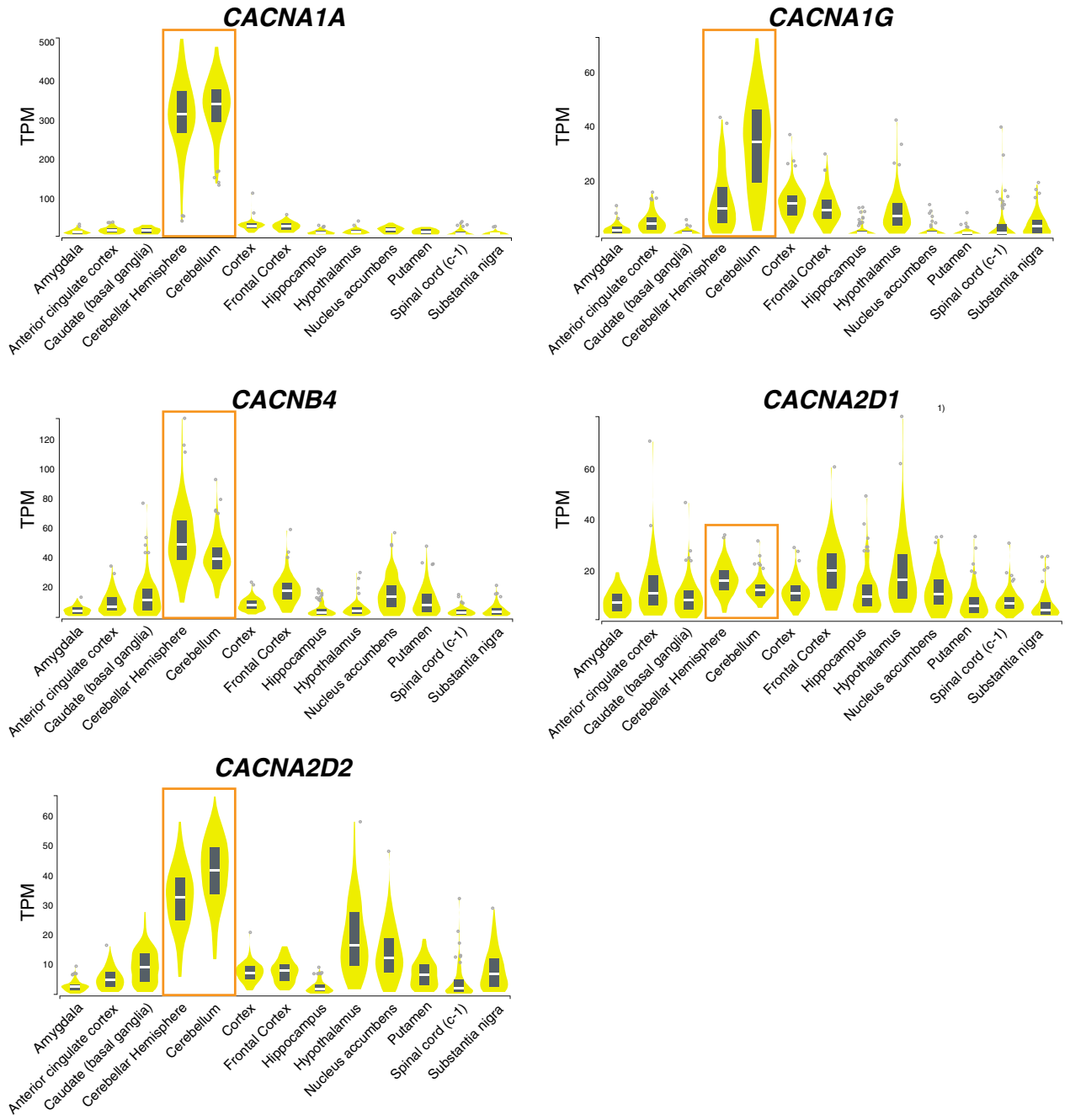

Figure 4. Gene expression in brain tissues of genes encoding voltage-gated calcium channels linked to epilepsy and/or ataxia. Gene expression pattern in all brain tissues from GTEx portal for the genes encoding voltage-gated calcium channels *CACNA1A, CACNA1G*, *CACNB4*, *CACNA2D1*, and *CACNA2D2*. The orange box highlights expression in the specific brain regions of cerebellar hemisphere and cerebellum. TPM: Transcripts Per Million, Data Source: GTEx Analysis Release V7 (dbGaP Accession phs000424.v7.p2), accessed on 30th March, 2019.

important clinical consequences, including the response to therapeutic treatment by AED.[27] Detailed studies of additional individuals with *CACNA2D2* rare biallelic variants will help illuminate the penetrance of neurological endophenotypes at this locus. The potential effectiveness of gabapentin as an AED of choice in treating DEE due to *CACNA2D2* pathogenic biallelic variants remains to be explored. *CACNA2D2* should be considered as part of the differential for early onset absence seizure conditions such as GLUT1 deficiency syndrome (MIM#606777). Rapid

advances in medical genetics and clinical genomics are predicated on establishing robust genotype/phenotype correlations; constructing allelic series at every locus will be a prerequisite for such analyses. Progress in the field of epilepsy continues to be made by employing a gene/variant-based model to inform epilepsy classification and therapeutics, driving the way for epilepsies to serve as a model for future precision-based medicine.

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Author Contributions

Study concept and design: JEP, RAG, JRL; Acquisition of data: JP, EK, AG, DP, SNJ, REL, JPA, AMI; Data analyses and interpretation: All authors; Drafting and revision of manuscript: All authors; Study supervision: JEP, JRL.

Study funding: RAG, JRL.

Conflict of Interest

JVH receives royalties for a chapter on pediatric neuroimaging in *UpToDate*. JRL has stock ownership in 23andMe, is a paid consultant for Regeneron, and is a coinventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting.

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Supporting Information

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

Table S1. List of genes tested using the commercial Early Infantile Epileptic Encephalopathy Panel for proband of Family 2.
Table S2. Detailed clinical features of individuals with rare biallelic *CACNA2D2* variants.

