REPORT

Bi-allelic Loss-of-Function *CACNA1B* Mutations in Progressive Epilepsy-Dyskinesia

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The occurrence of non-epileptic hyperkinetic movements in the context of developmental epileptic encephalopathies is an increasingly recognized phenomenon. Identification of causative mutations provides an important insight into common pathogenic mechanisms that cause both seizures and abnormal motor control. We report bi-allelic loss-of-function *CACNA1B* variants in six children from three unrelated families whose affected members present with a complex and progressive neurological syndrome. All affected individuals presented with epileptic encephalopathy, severe neurodevelopmental delay (often with regression), and a hyperkinetic movement disorder. Additional neurological features included postnatal microcephaly and hypotonia. Five children died in childhood or adolescence (mean age of death: 9 years), mainly as a result of secondary respiratory complications. *CACNA1B* encodes the pore-forming subunit of the presynaptic neuronal voltage-gated calcium channel $Ca_v2.2$/N-type, crucial for SNARE-mediated neurotransmission, particularly in the early postnatal period. Bi-allelic loss-of-function variants in *CACNA1B* are predicted to cause disruption of Ca^{2+} influx, leading to impaired synaptic neurotransmission. The resultant effect on neuronal function is likely to be important in the development of involuntary movements and epilepsy. Overall, our findings provide further evidence for the key role of $Ca_v2.2$ in normal human neurodevelopment.

The developmental and epileptic encephalopathies (DEEs) are a heterogeneous group of complex disorders characterized by severe early-onset seizures that are typically refractory to medication and associated with neurodevelopmental delay, regression, and often multiple comorbidities.[1–3] To date, advances in next-generation sequencing have facilitated the identification of more than 150 monogenic causes of DEE. A broad range of pathophysiological processes, including disturbance of synaptic function, impaired neurotransmitter release, ion

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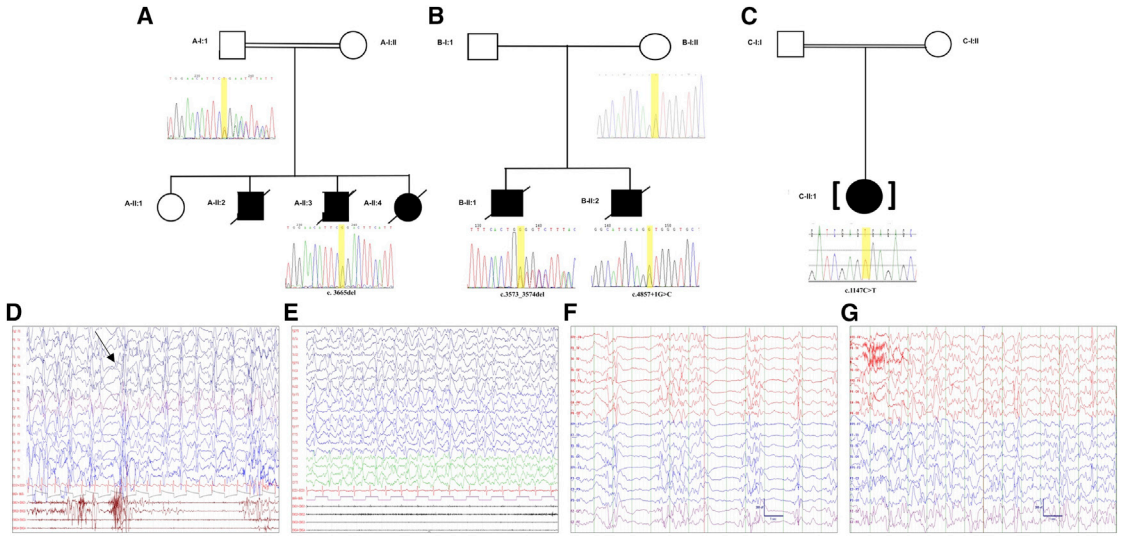

Figure 1. Molecular Genetic Investigation and Electroencephalogram Features of Affected Individuals with Bi-allelic *CACNA1B* Variants

(A) Segregation of *CACNA1B* c.3665del variant in family A shows all three affected children to be homozygous for the variant and parents to be heterozygous carriers of this variant.

(B) In family B, the two affected individuals carried the two rare variants, c.3573_3574del and c.4857+1G>C. The mother is heterozygous for one of the variants.

(C) For family C, the affected child is homozygous for the c.1147 C>T variant. Parental samples were not available.

(D–G) EEG traces from affected individuals. An EEG of individual A-II:3 shows epileptic encephalopathy at age 3.75 years (D), with bilateral high-amplitude spike and wave discharges with spasm (arrow), and at age 4.75 years (E), with bilateral continuous high-amplitude spike and wave discharges that were maximal over central regions. An EEG of individual B-II:2 at age 9 years (HF filter, 70 Hz; sensitivity, 15 μV/mm; timebase, 30 mm/s) shows a burst suppression pattern in sleep (F) and fairly continuous, high-amplitude, multi-focal spike and wave activity that was maximal over central regions during wakefulness (G).

channelopathies, dysregulation of gene transcription, abnormal DNA repair, peroxisomal defects, mitochondrial dysfunction, impaired transporter activity, and defective cell signaling and adhesion, have been identified.[1] The majority of mutations implicated in DEEs occur in genes that are widely expressed throughout the central nervous system and that have key roles in neuronal function. It is therefore not surprising that DEEs are commonly associated with additional disease features, including neurodevelopmental delay, intellectual disability, motor difficulties, microcephaly, autistic features and behavioral issues. More recently, non-epileptic movement disorders have been increasingly recognized in individuals with DEE.[4,5] Indeed, hyperkinetic movement phenotypes, such as dystonia and choreoathetosis, are now commonly reported in individuals with *FOXG1* (MIM: 164874), *GNAO1* (MIM: 139311), *SCN8A* (MIM: 600702), and *STXBP1* (MIM: 602926)-related epilepsy-dyskinesia syndromes.[6–9]

We report the identification of bi-allelic *CACNA1B* variants in six children from three families presenting with DEE associated with a severe hyperkinetic movement disorder (Figures 1A–1C). Over the last decade, we have recruited 494 children with DEE of unknown etiology for detailed endophenotyping and molecular genetic investigation. Of these, 61 had a prominent non-epileptic hyperkinetic movement disorder (Table S1) with dystonia, choreoathetosis, or generalized dyskinesia. Molecular genetic studies were approved by the local ethics committee (REC 13/LO/0168), and written informed consent was obtained from all participating families. Through multigene panel testing and whole-exome or whole-genome sequencing, an underlying genetic cause was identified in 20 of these individuals with DEE-dyskinesia phenotypes (Table S1).

Within the cohort of 41 unsolved cases, we identified a consanguineous family that was of Pakistani origin (first-cousin parents) and had three similarly affected children presenting with DEE and a hyperkinetic movement disorder (Family A, Table 1, Figure 1A). There was no history of neurological or metabolic disorders within the extended family. All children were born after an uncomplicated pregnancy and had a normal birth history. Affected individual A-II:2 had a period of normal development, and by 8 months of age, he babbled and was able to sit unsupported. Prior to the onset of seizures, there were some concerns regarding hypotonia, poor visual fixation, nystagmus, and slowing of developmental milestones. At age 10 months, he was having >100 epileptic spasms per day, and electroencephalogram (EEG) confirmed the presence of hypsarrhythmia. Seizures (tonic seizures, flexor spasms, and myoclonus) were refractory to medical treatment, and an EEG was consistent with Lennox-Gastaut syndrome. With the onset of seizures, there was concurrent regression of previously acquired skills, and the individual developed severe intellectual disability (ID), postnatal microcephaly, a hyperkinetic movement

Table 1. Key Clinical Characteristics of Individuals with Bi-allelic *CACNA1B* Mutations

Individual	A-II:2	A-II:3	A-II:4	B-II:1	B-II:2	C-II:I
Bi-allelic variants	p.Leu1222Argfs*29/ p.Leu1222Argfs*29	p.Leu1222Argfs*29/ p.Leu1222Argfs*29	p.Leu1222Argfs*29/ p.Leu1222Argfs*29	p.Gly1192Cysfs*5/ c.4857+1G>C	p.Gly1192Cysfs*5/ c.4857+1G>C	p.Arg383*/p.Arg383*
Consanguinity	yes, parents 1st cousins	yes, parents1st cousins	yes, parents 1st cousins	no	no	unknown[a]
Ethnicity	Pakistani	Pakistani	Pakistani	European descent	European descent	European descent
Age of death	3 years	7 years	14 years	17 years	5 years	alive; 6 years
Cause of death	respiratory infection	Meningitis, end organ failure	respiratory infection	respiratory infection	respiratory infection	-
Sex	male	male	female	male	male	female
Pregnancy	normal	normal	normal	normal	normal	unknown
Birth	term, normal	term, normal	36 weeks, normal	term, normal	term, normal	unknown
Best neurodevelopmental stage (age)	sat with support, babbled and smiled (8 months)	sat unsupported, reached for objects and babbled (9 months)	sat with support and smiled (8 months)	sat unsupported, 1 word (2 years)	always delayed, never sat or babbled	always delayed, never sat
Age of regression	10 months	10 months	8 months	2 years	always delayed	unknown
Age of seizure onset	10 months	9 months	12 months	30 months	21 months	unknown
Seizure type at presentation → evolution over time	epileptic spams (100 cluster spasms/day) → tonic, myoclonic, flexor spasms	epileptic spasms → GTC, myoclonic, tonic	epileptic spasms → myoclonic, GTC, flexor spasms, focal daily clustering	myoclonic → focal, GTC, myoclonic daily episodes	myoclonic, focal, GTC daily	epileptic spasms tonic
Medications tried (medications with some beneficial effect underlined)	NPM, PHY, steroids, VB6, VBN, VPA; refractory to AED	CBM, VBN, VPA; periods of seizure freedom on CBM and VBN	ACTH, CBM, LAC, LEV, NPM, PHB, steroids, RUF, TOP, VBN, VPA; non-sustained response to some drugs, generally refractory	CBM, FOL, LAC, LEV, LTG, PIR, TOP, VB6, VPA; refractory to AED	BIO, CPM, FOL, LEV, LTG, PIR, VB6, VPA; refractory to AED	CBM, LEV, PHB, RUF, steroids; 6 months seizure free on PHB and RUF
Movement disorder	myoclonus dystonia, episodic exacerbations	myoclonus dystonia	myoclonus dystonia, oromotor dyskinesia	myoclonus dystonia, choreoathetosis, dyskinesia, frequent exacerbations	myoclonus dystonia, choreoathetosis, hand-wringing stereotypies	myoclonus, choreoathetosis
Other eatures						
Head size	postnatal microcephaly (OFC 0.4th centile)	postnatal microcephaly (OFC 3rd centile)	postnatal microcephaly (OFC 3rd centile)	postnatal microcephaly (OFC <0.4th centile)	postnatal microcephaly (OFC <2nd centile)	microcephaly (not known whether congenital or postnatal)
Vision	strabismus, nystagmus	-	left strabismus, CVI	CVI	divergent strabismus, congenital nystagmus, CVI	-
Central and peripheral tone	central hypotonia and brisk limb reflexes	central hypotonia with increased peripheral tone L>R	generalized hypotonia	generalized hypotonia	generalized hypotonia	generalized hypotonia

(*Continued on next page*)

Table 1. *Continued*

Individual	A-II:2	A-II:3	A-II:4	B-II:1	B-II:2	C-II:1
Gastrointestinal	enteral feeding	enteral feeding	enteral feeding, GER	enteral feeding	enteral feeding, GER	enteral feeding, GER
Respiratory	recurrent respiratory infections	recurrent respiratory infections	recurrent respiratory infections	recurrent respiratory infections	recurrent respiratory infections, stridor	NR
Other	NR	NR	conductive deafness	periods of agitation	Bruxism	NR
Dysmorphic features	right talipes at birth	NR	dislocated L hip at birth	small testis Anteverted nares Thickened gums Slim hands/feet 2/3 syndactyly	small testis	NR

Abbreviations are as follows: ACTH, adrenocorticotropic hormone; AED, anti-epileptic drug; BIO, biotin; CBM, clobazam; CPM, clonazepam; CVI, cortical visual impairment; FOL, folinic acid; GER, gastro-esophageal reflux; GTC, generalized tonic clonic; L, left; LAC, lacosamide; LEV, levetiracetam; NPM, nitrazepam; NR, nil reported; OFC, occipitofrontal circumference; PHY, phenytoin; PIR, piracetam; R, right; RUF, rufinamide; TOP, topiramate; VBN, vigabatrin; VB6, vitamin B6 (pyridoxine); and VPA, sodium valproate. [a]SNP array revealed ~30% areas of homozygosity, possibly suggestive of consanguinity.

disorder, and bulbar dysfunction. The hyperkinetic movement disorder was characterized by a combination of dystonia and severe non-epileptic myoclonus, with frequent exacerbations. His siblings (A-II:3 and A-II:4) followed an almost identical course, with onset of epilepsy and developmental regression at the age of 9 and 10 months, respectively (Table 1 and Figure 1A). Extensive diagnostic neurometabolic work-up failed to identify an underlying cause (Table S2). Electroencephalogram showed changes consistent with epileptic encephalopathy (Figures 1D and 1E). Brain magnetic resonance imaging (MRI) showed non-specific findings of cerebral atrophy in affected individual A-II:2 (age 12 months) and asymmetry of temporal horns and white-matter signal changes in individual A-II:3 (age 24 months). Neuroimaging was normal in individual A-II:4 at age 14 months.

The affymetrix 250K Sty1 SNP mapping array was used for genome-wide linkage studies in family A (individuals II:2, II:3, and II:4). Genotype data were processed with Genomestudio (Illumina) and subsequently analyzed with both HomozygosityMapper (see Web Resources) and manually in Microsoft Excel.[10] Eight common regions of homozygosity (>2 Mb) were initially identified (Table S3). These regions were further evaluated in all family members through the use of microsatellite markers. Linkage to two regions on chromosomes 14 and 21 were excluded by detection of similarly homozygous alleles in unaffected individuals, leaving six potential disease loci (Table S4 and Figures S1 and S2). Whole-exome sequencing was performed on affected individual A-II:4 with SureSelect All Exon 50 Mb Target Enrichment System and SureSelect human All Exon kit (v2; Agilent Technologies), according to the manufacturer's recommendations. Data were analyzed according to the Genome Analysis Toolkit's (GATK) Best Practices. A total of 23,158 variants were identified, and these were further prioritized as follows: (1) those within the six regions of homozygosity; (2) non-synonymous, frameshift, splice-site, and nonsense changes; (3) those that were absent or only observed at a very low frequency in control populations (variants with a minor-allele frequency of >0.01% in publicly available databases, including dbSNP, 1000 Genomes, the Exome Variant Server [EVS], and gnomAD, were excluded); (4) those affecting highly conserved amino acids; and (5) missense changes predicted to be damaging by at least one prediction program (PolyPhen-2, SIFT, PROVEAN, or MutationTaster). According to these criteria, three homozygous variants were identified as follows: *CACNA1B* (GenBank: NM_000718.4; c.3665del [p.Leu1222Argfs*29], chr9: 140943722), *TSHB* (GenBank: NM_000549.3; c.223A>G [p.Arg75Gly]), and *DPP7* (GenBank: NM_00013379; c.1343+5G>A) (Genome Reference Consortium Human Build 37 [GRCh37]/hg19) (Table S5). The *TSHB* variant was predicted to be benign by multiple *in silico* programs. MutationTaster predicted the *DDP7* variant to be a polymorphism that has minimal effect on splicing (MaxEnt Scan, NN Splice, human splicing finder [HSF]).

The *CACNA1B* variant, a homozygous 1 bp deletion predicted to cause a frameshift and premature truncation, was predicted to be deleterious. The variant was absent in gnomAD, 1000 Genomes, EVS, and in-house exomes (n = 250). In the ExAC database, *CACNA1B* is predicted to be extremely intolerant of loss of function and has a pLi score of 0.98.[11] Direct Sanger sequencing confirmed whole-exome sequencing findings and showed appropriate segregation of the mutation in the family (Figure 1A). An unaffected sibling (A-II:1) was not sequenced. Whole-exome sequencing data from individual A-II:4 were also probed for 154 DEE-related genes, but no potentially pathogenic variants were identified (Tables S6 and S7).

The remaining affected individuals in the epilepsy cohort were screened for *CACNA1B* variants by either analysis of available whole-exome or whole-genome data or through targeted *CACNA1B* sequencing with a custom amplicon array (TruSeq). No further cases were identified. We submitted the variant to GeneMatcher and requested that collaborating research groups probe their whole-exome and whole-genome datasets (Table S8). Through these routes, we identified two further families with bi-allelic *CACNA1B* variants.

A second British family (family B, Figure 1B) with two affected children harboring compound heterozygous variants in *CACNA1B* was identified from the UK10K Genome Project.[12] Both children had a 2 bp deletion creating a frameshift (c.3573_3574del [p.Gly1192Cysfs*5], chr9: 140941880) and a splice-site variant in the donor splice site of intron 34 (c.4857+1G>C, chr9:140968519). MaxEnt Scan, NN Splice, HSF and BDGP fruit fly, all predict 100% loss of donor site, resulting in skipping of exon 34. Both variants are absent from control databases (gnomAD, 1000 Genome, and EVS). No other variants in known genes causing neurological disorders were identified. Sanger sequencing confirmed the two variants in both children (Figure 1B). The c.4857+1G>C variant was detected as a heterozygous change in the mother. Paternal DNA was unavailable for genetic testing. The children from family B were found to have a phenotype that was clinically similar to that of children in family A (Table 1). Both boys were born to non-consanguineous parents of European descent and had no pertinent family history, and both were born after an uneventful antenatal and birth history. Affected individual B-II:1 had pre-existing developmental delay prior to onset of epilepsy at age 2.5 years; there was regression of previously acquired skills before the onset of epilepsy. He had a number of different seizure types, including myoclonic, focal, and generalized tonic-clonic (GTC) seizures, which were refractory to multiple anti-seizure medications. A complex hyperkinetic movement disorder, characterized by dystonic posturing, choreoathetosis, and dyskinesia, emerged at 2.5 years. The movement disorder was drug resistant and was associated with frequent exacerbations, leading to significant impairment of daily living activities and quality of life. His younger brother (individual B-II:2) had a similar presentation, including severe developmental delay, before the onset of epilepsy at age 21 months. Epilepsy was refractory to conventional anti-seizure medications. EEG abnormalities were seen as a burst-suppression pattern in sleep and high-amplitude multi-focal spike and wave activity when awake. (Figures 1F and 1G). At age 21 months, he developed a prominent complex hyperkinetic movement disorder with features of dystonia, choreoathetoid movements, non-epileptic myoclonus, and hand-wringing stereotypies. Both children had additional neurological features, including microcephaly, hypotonia, visual impairment, and severe cognitive difficulties. Both died, at age 17 years and 5 years, respectively, from secondary respiratory complications.

A third family (family C, Figure 1C) with a single affected proband was identified through GeneMatcher.[13] A homozygous variant (c. 1147 C>T [p. Arg383*], chr9: 140850226) in *CACNA1B* was identified through a commercial clinical exome and confirmed by Sanger sequencing (Figure 1C). The proband (C-II:1) of Bulgarian origin was adopted, and familial segregation studies were not possible. This variant was located within an area of extended SNP homozygosity (Table S9). A variant in *MMACHC* (GenBank: NM_015506.2, c. 506T>C [p. Ile169Thr], chr1: 45974544) was also identified but was excluded because the proband's serum homocysteine and urine organic acids were normal and because multiple *in silico* programs predicted the variant to be benign (Table S10). No other candidates were identified from the clinical exome, despite targeted analysis of 117 DEE genes (Table S11). Details of the birth, early medical history, and developmental milestones are unavailable. She was first reviewed by pediatric neurology services at aged 4 years, at which time she presented with refractory epilepsy (epileptic spasms and tonic seizures), a hyperkinetic movement disorder (non-epileptic myoclonus and chorea), and global neurodevelopmental delay. EEG at first review, when she was 4 years, was consistent with an epileptic encephalopathy; high amplitude, disorganized background (with no normal awake architecture), frontally dominant sharp slow waves of 1–2 Hz, tonic seizures, and epileptic spasms were captured. Magnetic resonance imaging of the brain showed subtle asymmetry of the frontal lobes and a unilateral deep and linear-appearing sulcus of the anterior left frontal lobe (Figure S3). Now aged 6 years, she has developed microcephaly, hypotonia, and severe intellectual disability and is fed via gastrostomy (Table 1).

We have identified bi-allelic loss-of-function variants of *CACNA1B* in six children from three families with DEE associated with a severe hyperkinetic movement disorder. Voltage-gated calcium channels (VGCCs) play a key role in neurons by mediating Ca^{2+} ion influx into excitable cells in response to membrane depolarization and thereby regulating a number of calcium-dependent processes, including neurotransmitter release, gene transcription, calcium-dependent enzymes, and muscle contraction.[14–20]

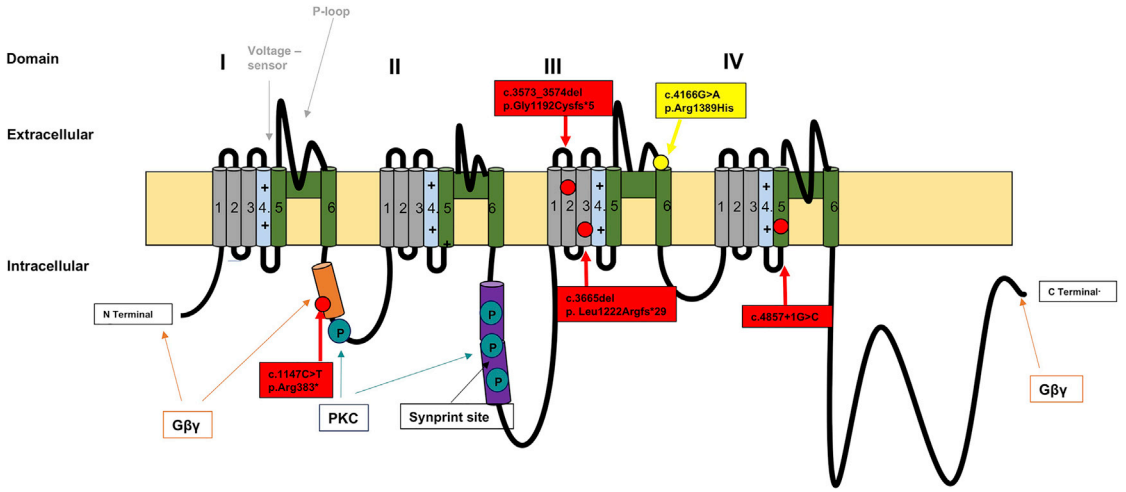

Figure 2. Schematic Representation of $Ca_v2.2$, Showing Location of *CACNA1B* Variants
The structure of $Ca_v2.2$ consists of four homologous repeats (domains I–IV), each containing six transmembrane α-helices (S1–S6) and a p loop between S5 and S6. The S5 and S6 helices and the p loop represent the pore domain of the channel (green). The fourth segment (S4) of each domain is the voltage sensor for activation. Gene variants identified in families A, B, and C are indicated in red (these are loss-of-function variants). The previously reported heterozygous missense variant associated with myoclonus dystonia is highlighted in yellow. Abbreviations are as follows: Gβγ, G protein βγ subunit; P, binding site of PKC; PKC, protein kinase C.

To date, ten VGCC subtypes, differentiated by varying voltage and pharmacological properties, have been identified. VGCCs are classified into three subfamilies by sequence similarity (Ca_v1, Ca_v2, and Ca_v3). In neurons, the pre-synaptic Ca_v2 channel family, comprising $Ca_v2.1$, $Ca_v2.2$, and $Ca_v2.3$ isoforms (termed P/Q-type, N-type, and R-type calcium channels) are encoded by *CACNA1A, CACNA1B*, and *CACNA1E*, respectively.[14–16]

CACNA1B (chr9: 137,877,788–138,124,623 [GRCh38), MIM: 601012) encodes the calcium-channel-voltage-dependent, N-type, α-1B subunit ($Ca_v2.2$), the pore-forming subunit of presynaptic neuronal voltage-gated calcium channels. $Ca_v2.2$ is expressed throughout the central nervous system, including the cerebral white matter, cortex, hippocampus, basal ganglia, and cerebellum (Figure S4).[21–23] The expression pattern, especially in the basal ganglia and cerebellum, might bear relevance to the observed clinical manifestations associated with genes encoding calcium-channel subunits,[24] given that all reported affected individuals had epilepsy and hyperkinesia as a major part of their clinical phenotype.

$Ca_v2.1$ and $Ca_v2.2$ synergistically modulate presynaptic Ca^{2+} levels and thereby regulate SNARE-mediated release of neurotransmitters (monoamines, glutamate, GABA, and serotonin).[21] $Ca_v2.2$ is also postulated to have a role in synaptic plasticity, synaptogenesis, gene transcription, neuronal survival, and the migration of immature neurons.[19,25] Expression of *CACNA1B* is thought to be crucial for neurotransmission in the early postnatal period as $Ca_v2.2$ channels are replaced by $Ca_v2.1$ channels in mature synapses within the thalamus, cerebellum, and auditory brainstem.[18,20]

Given the key neuronal functions of $Ca_v2.1$ and $Ca_v2.2$, over time there has been great interest regarding the potential role of these channels in neurological diseases. Our work now implicates a role for biallelic mutations of *CACNA1B* in DEEs and movement disorders. Previously, a heterozygous *CACNA1B* missense variant (c.4166G>A [p.Arg1389His], rs184841813) was reported in a single Dutch family with five affected individuals presenting with adult-onset myoclonus-dystonia and cardiac arrhythmia (DYT-23, MIM: 614860).[26] This finding has not been replicated in subsequent studies.[27] Single-nucleotide polymorphisms and heterozygous copy-number variants involving *CACNA1B* have been described in individuals with neurovascular disorders and schizophrenia.[28–30] Notably, heterozygous variants in the related gene, *CACNA1A* (MIM: 601011), are now an established cause of early infantile epileptic encephalopathy (EIEE) (MIM: 617106), episodic ataxia type 2 (MIM: 108500), familial hemiplegic migraine type 1 (MIM: 141500), and spinocerebellar ataxia type 6 (MIM: 183086). Biallelic mutations in *CACNA1A* have also been reported in a single family whose affected members have severe DEE associated with progressive cerebral, cerebellar, and optic atrophy.[31–34] More recently, heterozygous missense mutations in *CACNA1G* (MIM: 604065) have been reported in childhood-onset cerebellar atrophy with EIEE, providing further evidence that disruption of calcium channels is a key pathogenic mechanism in DEE-related syndromes.[35]

CACNA1B is organized in four homologous domains (DI–IV), each containing a motif of six transmembrane helices (S1–S6) and a P-loop between S5 and S6 (Figure 2). The S5 and S6 segments and the P-loop represent the pore domain of the channel. The fourth segment (S4) of each domain is the voltage sensor for activation. SNARE-complex proteins interact directly through a specific synaptic protein site in the large intracellular loop connecting

domains II and III (syniprint site). The N and C termini and the intracellular linkers between domains I and II and between domains II and III are important for channel regulation and interaction with other proteins, including Gβγ, protein kinase C (PKC), and PIP2.[16,18,36] Variants reported in families A and B are both located within DIII. p.Arg380* (family C) is in the intracellular linker between domains I and II; this linker is key for the binding of Gβγ (Figure 2).[18] All variants are predicted to cause a loss of function through nonsense-mediated decay and/or protein truncation. Identification of further *CACNA1B*-mutation-positive individuals will determine mutation hot spots and any genotype-phenotype correlation.

$Ca_v2.2$ channels play a key role in normal synaptic function. Soluble N-ethylmaleimide-sensitive-factor-activating protein receptor (SNARE) complexes (syntaxin, SNAP-25, VAMP, and synaptobrevin) are key elements of vesicle trafficking, docking, and presynaptic vesicle recycling in neuronal membranes.[16,37–39] Depolarization of the presynaptic terminal initiates the opening of $Ca_v2.2$ and the subsequent influx of Ca^{2+} ions. A rise in intracellular Ca^{2+} concentration is detected by synaptotagmin-1 (SYT1), triggering fusion and subsequent exocytosis of the neurotransmitter vesicles through primed SNARE-protein complexes (Figure S5). We postulate that loss-of-function mutations in *CACNA1B* impair Ca^{2+} flux and normal synaptic transmission. Effects on monoamine and GABA/glutamatergic networks might influence the development of epilepsy and abnormal motor control in affected children. Dysfunctional presynaptic vesicle recycling is emerging as a key cellular mechanism underlying epilepsy-dyskinesia phenotypes. Indeed, disease-causing variants in other SNARE or SNARE-regulatory proteins are increasingly recognized; such variants include *STXBP1* (EIEE, MIM:612164, hyperkinetic movement disorder), *STX1B* (generalized epilepsy with febrile seizures, MIM: 616172), *GOSR2* (progressive myoclonic epilepsy, MIM: 614018), *SYT-1* (Baker-Gordon syndrome, MIM: 618218, early-onset dyskinesia and intellectual disability), and *SNAP25* (epilepsy and intellectual disability).[8,40–43]

CACNA1B is postulated to play a role in early brain development, as supported by the expression profile of $Ca_v2.2$.[20] $Ca_v2.2$ knockout murine models manifest a number of neurodevelopmental abnormalities, including abnormal locomotor activity and memory impairment.[44] Future models of $Ca_v2.2$ dysfunction will be integral in further understanding the neurodevelopmental role of this protein.

In summary, we report six affected individuals with biallelic loss-of-function variants in *CACNA1B* and a neurodevelopmental disorder characterized by developmental and epileptic encephalopathy, postnatal microcephaly, and a complex hyperkinetic movement disorder. Identification of further cases will provide more insight into the spectrum of neurological diseases associated with *CACNA1B* variants, as well as potential genotype-phenotype correlations. The identification of *CACNA1B* further expands genetic heterogeneity in severe childhood epilepsy-dyskinesia syndromes.

Supplemental Data

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2019.03.005.

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Declaration of Interests

A.R. is an employee of GeneDx, a wholly owned subsidiary of OPKO Health. I.S. has served on scientific advisory boards for UCB, Eisai, GlaxoSmithKline, BioMarin, Nutricia and Xenon Pharmaceuticals; editorial boards of the Annals of Neurology, Neurology and Epileptic Disorders; might accrue future revenue on pending patent WO61/010176 (filed in 2008) for Therapeutic Compound; has received speaker honoraria from GlaxoSmithKline, Athena Diagnostics, UCB, BioMarin, Eisai, and Transgenomics; has received funding for travel from Athena Diagnostics, UCB, Biocodex, GlaxoSmithKline, Biomarin and Eisai; and receives or has received research support from the National Health and Medical Research Council of Australia, National Institutes of Health, Australian Research Council, Health Research Council of New Zealand, CURE, and March of Dimes. J.J.M. reports honoraria as an editor from the American Academy of Neurology; royalties from Up-To-Date and BMJ Best Practice, honoraria for speaking for Invitae,

BioMarin, Greenwich, Sunovion, and Mallinkrodt; consulting for Esai, Xenon, and Ionis; research grants from UCB, NIH, and Citizens United for Research in Epilepsy; all of these are outside of the current work. All other authors declare no competing interests.

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Web Resources

The URLs for data presented herein are as follows:
BDGP, http://www.fruitfly.org/
Braineac, http://braineac.org
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/Database
EVS, http://evs.gs.washington.edu/EVS/
ExAC Browser, http://exac.broadinstitute.org/
GeneMatcher, https://genematcher.org
GnomAD Browser, http://gnomad.broadinstitute.org
HomozygosityMapper, http://www.homozygositymapper.org/
HSF, http://www.umd.be/HSF/
MaxEnt Scan, http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html
MutationTaster, http://www.mutationtaster.org
NN Splice, http://www.fruitfly.org/seq_tools/splice.html/
OMIM, http://www.omim.org/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
PROVEAN, http://provean.jcvi.or
SIFT, http://sift.jcvi.org/

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