Biallelic Loss-of-Function CACNA1B Mutations in Progressive Epilepsy-Dyskinesia


1. Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, London, WC1N 1EH, UK
2. Department of Neurology, Great Ormond Street Hospital, London, WC1N 3JH, UK
3. Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, CB2 OQQ UK
4. Epilepsy Center, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL 60611 USA
5. Departments of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
6. Division of Genetics, Birth Defects and Metabolism, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL 60611, USA
7. GeneDx, Gaithersburg, MD 20877, USA
8. Epilepsy Research Centre, Department of Medicine, University of Melbourne at Austin Health, Heidelberg 3084, VIC, Australia.
9. Department of Clinical Neurophysiology, Great Ormond Street Hospital, London WC1N 3JH, UK
10. Division of Genetics and Molecular Medicine, King’s College, London WC2R 2LS, UK
11. Department of General and Community Paediatrics, Birmingham Children’s Hospital, Birmingham B4 6NH, UK
12. Department of Neurology, Birmingham Children’s Hospital, Birmingham B4 6NH, UK
13. West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women’s and Children’s NHS Foundation Trust, B15 2TG, UK
14. Department of Human Genetics, Radboud University Medical Center, 6525 GA Nijmegen, Netherlands
15. Department of Neurology, Pediatrics and Biomedical Genetics, University of Rochester Medical Center, Rochester, NY 14642, USA
16. Department of Neurology and Neurophysiology, Children’s University Hospital, Temple Street, Dublin DO1 YC67, Ireland
17. Academic Centre on Rare Diseases, School of Medicine and Medical Science, University College Dublin, Dublin 4 Ireland
18. Clinical Neurosciences, Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, London WC1N 1EH, UK
CACNA1B and epilepsy-dyskinesia
Gorman et al., 2018

19. Epilepsy Genetics Program, Department of Neurology, Boston Children’s Hospital, Boston, MA 02115, USA
20. Department of Neurology, Harvard Medical School, Boston, MA 02115, USA
21. Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, WA 98195, USA
22. Florey Institute of Neuroscience and Mental Health, Parkville, VIC 3052, Australia
23. Department of Paediatrics, Royal Children’s Hospital, University of Melbourne, Parkville, VIC 3052, Australia
24. Institute of Medical Genetics and Applied Genomics, University of Tübingen, 72074 Tübingen, Germany
25. Department of Neurology, Royal Manchester Children’s Hospital, Manchester University Hospitals NHS Foundation Trust, Manchester M13 9WL, UK
26. DDD Study, Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK
27. UK10K, Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK
28. NIHR BioResource, Cambridge University Hospitals, Cambridge Biomedical Campus, Cambridge, CB2 0QQ, UK
29. Ken and Ruth Davee Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
30. Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University Hospitals, NHS Foundation Trust Manchester Academic Health Sciences Centre, Manchester M13 9WL, UK
31. Division of Evolution and Genomic Sciences, School of Biological Sciences, University of Manchester, Manchester M13 9NT, UK
32. Department of Medical Genetics, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, CB2 0QQ, UK
33. These authors contributed equally to this work

*Correspondence: Professor Manju Kurian, UCL Professor of Neurogenetics and NIHR Research Professor, Room 111, Level 1, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London, WC1N 1EH Email: manju.kurian@ucl.ac.uk Twitter: @DNP_ICh

Email Address of all authors
Kathleen M Gorman: k.gorman@ucl.ac.uk
Esther Meyer: esther.meyer@nbt.nhs.uk
Detelina Grozeva: dg417@cam.ac.uk
Egidio Spinelli: ESpinelli@luriechildrens.org
Amy McTague: a.mctague@ucl.ac.uk
Emily Bryant: embryant@luriechildrens.org
Adi Reich: areich@genedx.com
Amy Schneider: amy.schneider@unimelb.edu.au
Ronit Pressler: ronit.pressler@gosh.nhs.uk
Michael A Simpson: michael.simpson@kcl.ac.uk
Geoff D Debelle: geoff.debelle@bch.nhs.uk
Evangeline Wassmer: evangeline.wassmer@bch.nhs.uk
CACNA1B and epilepsy-dyskinesia
Gorman et al., 2018

Jenny Morton: jenny.morton@bwhct.nhs.uk
Diana Sieciechowicz: DSieciechowicz@luriechildrens.org
Eric Jan-Kamsteeg: Erik-Jan.Kamsteeg@radboudumc.nl
Alex Paciorkowski: Alex_Paciorkowski@URMC.Rochester.edu
Mary D King: mary.king@cuh.ie
J Helen Cross: h.cross@ucl.ac.uk
Annapurna Poduri: Annapurna.Poduri@childrens.harvard.edu
Heather C Mefford: hmefford@uw.edu
Ingrid E Scheffer: i.scheffer@unimelb.edu.au
Tobias B Haack: Tobias.Haack@med.uni-tuebingen.de
Gary McCullagh: Gary.McCullagh@cmft.nhs.uk
DDD: contact@sanger.ac.uk
UK10K Consortium: info@uk10k.org
NIHR BioResource: nbr@bioresource.nihr.ac.uk
John J Millichap: JMillichap@luriechildrens.org
Gemma C Carvill: gemma.carvill@northwestern.edu
Jill Clayton-Smith: jill.clayton-smith@mft.nhs.uk
Eamonn R Maher: erm1000@medschl.cam.ac.uk
F Lucy Raymond: flr24@cam.ac.uk
Manju A Kurian: manju.kurian@ucl.ac.uk
Abstract

The occurrence of non-epileptic hyperkinetic movements in the context of developmental epileptic encephalopathies is an increasing recognised phenomenon. Identification of causative mutations provides an important insight into common pathogenic mechanisms that cause both seizures and abnormal motor control. We report biallelic loss-of-function CACNA1B variants in six children from three unrelated families presenting 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 due to secondary respiratory complications. CACNA1B encodes the pore-forming subunit of the pre-synaptic neuronal voltage-gated calcium channel CaV2.2/N-type, crucial for SNARE-mediated neurotransmission, particularly in the early postnatal period. Biallelic 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 CaV2.2 in normal human neurodevelopment.
Main Text

The developmental and epileptic encephalopathies (DEE) 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 co-morbidities.\textsuperscript{1,2,3} To date, advances in next-generation sequencing have facilitated the identification of over 150 monogenic causes of DEE. A broad range of pathophysiological processes have been identified, including disturbance of synaptic function, impaired neurotransmitter release, ion channelopathies, dysregulation of gene transcription, abnormal DNA repair, peroxisomal defects, mitochondrial dysfunction, impaired transporter activity and defective cell signalling and adhesion.\textsuperscript{1} The majority of mutations implicated in DEE occur in genes widely expressed throughout the central nervous system, with key roles in neuronal function. It is therefore not surprising that DEE is commonly associated with additional disease features, including neurodevelopmental delay, intellectual disability, motor difficulties, microcephaly, autistic features and behavioural issues. More recently, non-epileptic movement disorders have been increasingly recognised in individuals with DEE.\textsuperscript{4,5} Indeed, hyperkinetic movement phenotypes, such as dystonia and choreoathetosis, are now commonly reported in individuals with \textit{FOXG1} (MIM:164874), \textit{GNAO1} - (MIM:139311), \textit{SCN8A} - (MIM:600702) and \textit{STXBP1} - (MIM: 602926) related epilepsy-dyskinesia syndromes.\textsuperscript{6-9}

We report the identification of biallelic \textit{CACNA1B} variants in six children from three families presenting with DEE associated with a severe hyperkinetic movement disorder (Figure 1a-c). Over the last decade, we have recruited 494 children with DEE of unknown aetiology for detailed endophenotyping and molecular genetic investigation. Of these, 61 had a prominent non-epileptic hyperkinetic movement disorder (Table S1) with dystonia, choreoathetosis, or generalised 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 of Pakistani origin (first cousin parents) with 3 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 following 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 was able to sit unsupported and babble. There were some concerns immediately prior to the onset of seizures with regards to hypotonia, poor visual fixation, nystagmus, and slowing of developmental milestones. At age 10 months, he had onset of epileptic spasms with >100 episodes per day, and electroencephalogram (EEG) confirmed the presence of hypsarrhythmia. Seizures were refractory to medical treatment, (tonic seizures, flexor spasms and myoclonus) and EEG consistent with Lennox-Gastaut syndrome. With the onset of seizures, there was concurrent regression of previously acquired skills, with development of severe intellectual disability (ID), postnatal microcephaly, a hyperkinetic movement 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, Figure 1a). Extensive diagnostic neurometabolic work-up failed to identify an underlying cause (Table S2). Electroencephalogram showed changes consistent with epileptic encephalopathy (Figure 1d,e). 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.

Genome-wide linkage studies were undertaken in Family A (II:2, II:3, II:4) using Affymetrix 250K Sty1 SNP mapping array (Affymetrix, Inc., Santa Clara, CA). Genotype data was processed with Genomestudio (Illumina Inc.) and subsequently analyzed using both homozygosity mapper and manually in Microsoft Excel.10 Eight common regions of homozygosity (>2Mb) were initially identified (Table S3). These regions were further evaluated in all family members using microsatellite markers. Linkage to two regions on chromosome 14 and 21 were excluded by detection of similarly homozygous alleles in unaffected individuals, leaving six potential
disease loci (Table S4, Figure S1,2). Whole exome sequencing was performed on affected individual A-II:4 using SureSelect All Exon 50Mb Target Enrichment System/SureSelect human All Exon kit (v2; Agilent Technologies), according to manufacturer’s recommendations. Data were analysed following Genome Analysis Toolkit’s (GATK) Best Practices. A total of 23,158 variants were identified, that were further prioritized as follows: (i) those within the six regions of homozygosity; (ii) non-synonymous, frameshift, splice site, and nonsense changes; (iii) absent or only observed at a very low frequency in control populations (exclusion of variants with minor allele frequency of >0.01% in publically available databases, including dbSNP, 1000 Genomes, Exome Variant Server (EVS) and gnomAD; (iv) affecting highly conserved amino acids; and (v) missense changes predicted to be damaging by at least one prediction program (PolyPhen-2, SIFT, PROVEAN or MutationTaster). Using these criteria, 3 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) (GRCh37/hg19) (Table S5). The TSHB variant was predicted to be benign by multiple in-silico programs. The DDP7 variant was predicted to be a polymorphism in MutationTaster with minimal effect on splicing (MaxEnt Scan, NN Splice, human splicing finder [HSF]). The CACNA1B variant, a homozygous 1bp deletion predicted to cause a frameshift and premature truncation, was predicted to be deleterious. The variant was absent in gnomAD, 1000 genomes, Exome Variant Server (EVS), and in-house exomes (N=250). In the ExAC database, CACNA1B is predicted to be extremely intolerant of loss-of-function, with a pLi score of 0.98. Direct Sanger sequencing was performed, confirming whole exome sequencing findings, with appropriate segregation of the mutation in the family (Figure 1a). Unaffected sibling (A-II:1) was not sequenced. Whole exome sequencing data from individual A-II:4 was also probed for 154 DEE-related genes, but no potentially pathogenic variants were identified (Tables S6,7).

The remaining affected individuals in the epilepsy cohort were screened for CACNA1B variants by either analysis of available whole exome/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
CACNA1B and epilepsy-dyskinesia
Gorman et al., 2018

requested collaborating research groups to probe their whole exome and genome datasets (Table 58). Through these routes, we identified two further families with biallelic CACNA1B variants.

A second British family with two affected children (Family B, Figure 1b) harbouring compound heterozygous variants in CACNA1B was identified from the UK10K Genome project. Both children had a 2bp 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 clinically similar phenotype to those in Family A (Table 1). Both boys were born to non-consanguineous parents of European descent with no pertinent family history, both 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, with regression of previously acquired skills before the onset of epilepsy. He had a number of different seizure types including myoclonic, focal and generalised tonic-clonic (GTC) seizures, which were refractory to multiple anti-seizure medications. A complex hyperkinetic movement disorder, characterised by dystonic posturing, choreoathetosis and dyskinesia emerged at 2.5 years. The movement disorder was drug-resistant, and 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, with 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. (Figure 1f,g). At age 21 months, he developed a prominent complex hyperkinetic movement disorder with features of dystonia, choreoathetoid movements, non-epileptic myoclonus and hand-wrinking 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 with a single affected proband (Family C, Figure 1c) was identified through GeneMatcher. A homozygous variant (c. 1147 C>T, p. Arg383*, Chr9:140850226) in CACNA1B was identified through a commercial clinical exome and confirmed on 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 as the proband’s serum homocysteine and urine organic acids were normal and 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 Paediatric Neurology services at aged 4 years, presenting 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, aged 4 years, was consistent with an epileptic encephalopathy with features of high amplitude, disorganised background (with no normal awake architecture), frontally dominant sharp slow waves of 1-2Hz, tonic seizures and epileptic spasms captured. Magnetic resonance imaging of the brain showed subtle asymmetry of the frontal lobes with a unilateral deep and linear appearing sulcus of the anterior left frontal lobe (Figure S3). Now aged 6 years, she has developed microcephaly, hypotonia, severe intellectual disability, and is fed via gastrostomy (Table 1).

We have identified biallelic 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 (VGCC) have a key role in neurons, mediating Ca\(^{2+}\) ion influx into excitable cells in response to membrane depolarisation, and thereby regulating a number of calcium-dependent processes, including neurotransmitter release, gene transcription, calcium-dependent enzymes and muscle contraction. To date, ten subtypes of VGCC have been identified, differentiated by varying voltage and pharmacological properties. VGCC are classified into
three subfamilies by sequence similarity (\(Ca_{1}\), \(Ca_{2}\), and \(Ca_{3}\)). In neurons, the pre-synaptic \(Ca_{2}\) channel family, comprising \(Ca_{2.1}\), \(Ca_{2.2}\), and \(Ca_{2.3}\) isoforms (termed P/Q-type, N-type, and R-type calcium channels) are encoded by \(CACNA1A\), \(B\) and \(E\) respectively.\(^{14-16}\)

\(CACNA1B\) (Chr9:137,877,788-138,124,623 (GRCh38), MIM: 601012) encodes the calcium channel voltage-dependent, N-type, \(\alpha_{1}B\) subunit, the pore-forming subunit of presynaptic neuronal voltage-gated calcium channels (\(Ca_{2.2}\)). \(Ca_{2.2}\) is expressed throughout the central nervous system including the cerebral white matter, cortex, hippocampus, basal ganglia and cerebellum (\textbf{Figure S4}).\(^{21-23}\) The expression pattern, especially in the basal ganglia and cerebellum, may 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.

\(Ca_{2.1}\) and \(Ca_{2.2}\) synergistically modulate presynaptic \(Ca^{2+}\) levels, thereby regulating SNARE-mediated release of neurotransmitters (monoamines, glutamate, GABA, serotonin).\(^{21}\) \(Ca_{2.2}\) is also postulated to have a role in synaptic plasticity, synaptogenesis, gene transcription, neuronal survival and migration of immature neurons.\(^{19,25}\) Expression of \(CACNA1B\) is thought to be crucial for neurotransmission in the early postnatal period, as \(Ca_{2.2}\) channels are replaced by \(Ca_{2.1}\) in mature synapses within the thalamus, cerebellum, and auditory brainstem.\(^{18,20}\)

Given the key neuronal functions of \(Ca_{2.1}\) and \(Ca_{2.2}\), 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 DEE 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 with 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 organised in 4 homologous domains (DI-IV), each containing a motif of 6 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 (synprint site). The intracellular linkers between domains I-II, II-III, N and C termini are important for channel regulation and interaction with other proteins, including G\(\beta\gamma\), protein kinase C (PKC) and PIP2.\(^{16,18,36}\) Variants reported in Family A and B are both located within DIII. The p. Arg380* (Family C) is in the intracellular linker between domain I-II, key for binding of G\(\beta\gamma\) (Figure 2).\(^{18}\) All variants are predicted to cause loss-of-function through nonsense-mediated decay and/or protein truncation. Identification of further CACNA1B-mutation positive cases will determine mutation hot spots and any genotype-phenotype correlation.

Ca\(_{2.2}\) channels have 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.\(^{16,37-39}\)

Depolarisation of the pre-synaptic terminal initiates opening of Ca\(_{2.2}\) and 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 may 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 recognised, including STXBP1 (EIEE MIM:612164, hyperkinetic movement disorder), STX1B (generalised epilepsy with febrile seizures MIM: 616172), GOSR2 (progressive myoclonic epilepsy MIM: 614018), SYT-1 (early-onset dyskinesia and intellectual disability) and SNAP25 (epilepsy and intellectual disability).

CACNA1B is postulated to play a role in early brain development, as supported by the expression profile of Ca$_2$.2 knockout murine models manifest a number of neurodevelopmental abnormalities including abnormal locomotor activity and memory impairment. Future models of Ca$_2$.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 characterised 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 includes five figures and 11 tables.

Declaration of Interest

Adi Reich is an employee of GeneDx, Inc., a wholly owned subsidiary of OPKO Health, Inc..
All other authors declare no competing interests.

Acknowledgments

We thank our patients and their families for participating in this study. MAK is funded by an NIHR Research Professorship, and receives funding from the Wellcome Trust, Great Ormond Street Children’s Hospital Charity (GOSHCC) and Rosetrees Trust. EM received funding from the Rosetrees Trust (CD-A53), and Great Ormond Street Hospital Children’s Charity. KG received funding from Temple Street Foundation. FLR and DG are funded by Cambridge Biomedical Research Centre, KC is funded by NIHR Bioresource for Rare Diseases and the UK10K was funded by the Wellcome Trust. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003], a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Trust Sanger Institute [grant number WT098051]. We acknowledge support from the UK Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy’s and St. Thomas’ National Health Service (NHS) Foundation Trust in partnership with King’s College London. This research was also supported by the NIHR Great Ormond Street Hospital Biomedical Research Centre. JHC is in receipt of an NIHR Senior Investigator Award. The research team acknowledges the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, Department of Health or Wellcome Trust. ERM acknowledges support from NIHR Cambridge Biomedical Research Centre, a NIHR Senior Investigator Award and the University of Cambridge has received salary support in respect of ERM from the NHS in the East of England through the Clinical Academic Reserve.

Web Resources

The URLs for data presented herein are as follows:

BDGP, http://www.fruitfly.org/
Braineac, http://braineac.org
EVS, http://evs.gs.washington.edu/EVS/
ExAC Browser, http://exac.broadinstitute.org/
GeneMatcher https://genematcher.org
GnomAD Browser, http://gnomad.broadinstitute.org
MaxEnt Scan, http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html
MutationTaster, http://www.mutationtaster.org
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
PROVEAN, http://provean.jcvi.org
SIFT, http://sift.jcvi.org/
References


STXBP 1 mutations. Epilepsia 52, 1828–1834.


17


Figure Legends

Figure 1: Molecular genetic investigation and electroencephalogram features of affected individuals with biallelic 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, 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. EEG A-II:3 showing epileptic encephalopathy; aged 3.75 years (d) with bilateral high-amplitude spike and wave discharges with spasm (arrow) and (e) aged 4.75 years with bilateral continuous high-amplitude spike and wave discharges maximal over central regions. EEG B-II:2 aged 9 years (HF filter, 70Hz; sensitivity, 15uV/mm; timebase, 30mm/sec) showing burst-suppression pattern in sleep (f) and fairly continuous, high amplitude, multi-focal spike and wave activity maximal over central regions during wakefulness (g).

Figure 2: Schematic representation of Ca_{v}2.2 with location of CACNA1B variants

The structure of Ca_{v}2.2 consists of four homologous repeats (domain I-IV) each containing 6 transmembrane alpha-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 (loss-of-function variants). The previously reported heterozygous missense variant associated with myoclonus-dystonia is highlighted in yellow. Gβγ, G Protein βγ subunit: P, Binding site of PKC; PKC, Protein Kinase C.
Tables

Table 1 (overleaf): Key clinical characteristics of individuals with biallelic CACNA1B mutations

* SNP array revealed ~30% areas of homozygosity, possibly suggestive of consanguinity

Abbreviations: 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, generalised tonic clonic; L, left; LAC, lacosamide; LEV, levetiracetam; m, months; NPM, nitrazepam; NR nil reported; OFC, occipitofrontal circumference; PHY, phenytoin; PIR, piracetam; R, right; RUF, rufinamide; TOP, topiramate; VBN, vigabatrin; VB6, vitamin B6 (pyridoxine); VPA, sodium valproate; y, years
<table>
<thead>
<tr>
<th></th>
<th>A-II:2</th>
<th>A-II:3</th>
<th>A-II:4</th>
<th>B-II:1</th>
<th>B-II:2</th>
<th>C-II:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consanguinity</td>
<td>Yes, Parents 1st cousins</td>
<td>Yes, Parents 1st cousins</td>
<td>Yes, Parents 1st cousins</td>
<td>No</td>
<td>No</td>
<td>Unknown*</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Pakistani</td>
<td>Pakistani</td>
<td>Pakistani</td>
<td>European descent</td>
<td>European descent</td>
<td>European descent</td>
</tr>
<tr>
<td>Age of Death (years)</td>
<td>3y Respiratory infection</td>
<td>7y Meningitis</td>
<td>14y Respiratory infection</td>
<td>17y Respiratory infection</td>
<td>5y Respiratory infection</td>
<td>Alive – 6y</td>
</tr>
<tr>
<td>Cause of Death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Pregnancy Birth</td>
<td>Normal Term, normal</td>
<td>Normal Term, normal</td>
<td>Normal 36 weeks, normal</td>
<td>Normal Term, normal</td>
<td>Normal Term, normal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Best Neurodevelopmental stage (age)</td>
<td>Sat with support, babbled and smiled (8m)</td>
<td>Sat unsupported, reached for objects and babbled (9m)</td>
<td>Sat with support and smiled (8m)</td>
<td>Sat unsupported, 1 word (2y)</td>
<td>Always delayed</td>
<td>Always delayed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Never sat/babbled</td>
<td>Never sat</td>
</tr>
<tr>
<td>Age of regression</td>
<td>10m</td>
<td>10m</td>
<td>8m</td>
<td>2y</td>
<td>Always delayed</td>
<td>Unknown</td>
</tr>
<tr>
<td>Age of seizure onset</td>
<td>10m</td>
<td>9m</td>
<td>12m</td>
<td>30m</td>
<td>21m</td>
<td>Unknown</td>
</tr>
<tr>
<td>Seizure type</td>
<td>Epileptic spasms (100 cluster spasms/day)</td>
<td>Epileptic spasms GTC, myoclonic, tonic</td>
<td>Epileptic spasms Myoclonic, GTC, flexor spasms, focal Daily clustering</td>
<td>Myoclonic Focal, GTC, myoclonic Daily episodes</td>
<td>Myoclonic, focal, GTC Daily</td>
<td>Epileptic spasms Tonic</td>
</tr>
<tr>
<td>At presentation evolution over time</td>
<td>Tonic, myoclonic, flexor spasms</td>
<td>Epileptic spasms Myoclonic, GTC, flexor spasms, focal Daily clustering</td>
<td>Myoclonic, focal, GTC, myoclonic Daily episodes</td>
<td>Myoclonic, focal, GTC Daily</td>
<td>Myoclonic, focal, GTC Daily</td>
<td>Epileptic spasms Tonic</td>
</tr>
<tr>
<td>Medications tried</td>
<td>NPM, PHY, Steroids, VB6, VBN, VPA</td>
<td>CBM, VBN, VPA</td>
<td>ACTH, CBM, LAC, LEV, NPM, PHB, Steroids, RUF, TOP, VBN, VPA</td>
<td>CBM, FOL, LAC, LEV, LTG, PIR, TOP, VBN, VPA</td>
<td>BIO, CPM, FOL, LEV, LTG, PIR, VB6, VPA</td>
<td>CBM, LEV, PHB, RUF, Steroids</td>
</tr>
<tr>
<td>(medications with some beneficial effect underlined)</td>
<td>Refractory to AED</td>
<td>Periods of seizure freedom on CBM and VBN</td>
<td>Non-sustained response to some drugs, generally refractory</td>
<td>Refractory to AED</td>
<td>Refractory to AED</td>
<td>6m seizure free on PHB and RUF</td>
</tr>
<tr>
<td>Movement disorder</td>
<td>Myoclonus Dystonia Episodic exacerbations</td>
<td>Myoclonus Dystonia</td>
<td>Myoclonus Dystonia Oromotor dyskinesia</td>
<td>Myoclonus Dystonia Choreoathetosis Dyskinesia Frequent exacerbations</td>
<td>Myoclonus Dystonia Choreoathetosis Hand-wringing stereotypies</td>
<td>Myoclonus Choreoathetosis</td>
</tr>
<tr>
<td>Other features</td>
<td>Head size</td>
<td>Vision</td>
<td>Central and peripheral tone</td>
<td>Gastrointestinal</td>
<td>Respiratory</td>
<td>Other</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>--------</td>
<td>-----------------------------</td>
<td>------------------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>Postnatal microcephaly (OFC 0.4th centile)</td>
<td>Strabismus Nystagmus</td>
<td>Central hypotonia and brisk limb reflexes</td>
<td>Enteral feeding</td>
<td>Recurrent respiratory infections</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>Postnatal microcephaly (OFC 0.4th centile)</td>
<td>Left strabismus CVI</td>
<td>Central hypotonia with increased peripheral tone L&gt;R</td>
<td>Enteral feeding GER</td>
<td>Recurrent respiratory infections</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>Postnatal microcephaly (OFC 3rd centile)</td>
<td></td>
<td>Generalised hypotonia</td>
<td>Enteral feeding</td>
<td>Recurrent respiratory infections</td>
<td>Conductive deafness</td>
</tr>
<tr>
<td></td>
<td>Postnatal microcephaly (OFC &lt;0.4th centile)</td>
<td>Left strabismus CVI</td>
<td>Generalised hypotonia</td>
<td>Enteral feeding GER</td>
<td>Recurrent respiratory infections</td>
<td>Periods of agitation</td>
</tr>
<tr>
<td></td>
<td>Postnatal microcephaly (OFC 2nd centile)</td>
<td></td>
<td>Generalised hypotonia</td>
<td>Enteral feeding GER</td>
<td>Recurrent respiratory infections</td>
<td>Minimalist deformities</td>
</tr>
<tr>
<td></td>
<td>Microcephaly (not known whether congenital or postnatal) (OFC 2nd centile)</td>
<td></td>
<td>Generalised hypotonia</td>
<td>Enteral feeding GER</td>
<td>Recurrent respiratory infections</td>
<td>Stridor</td>
</tr>
<tr>
<td></td>
<td>Postnatal microcephaly (OFC 2nd centile)</td>
<td></td>
<td>Generalised hypotonia</td>
<td>Enteral feeding GER</td>
<td>Recurrent respiratory infections</td>
<td>Minimalist deformities</td>
</tr>
</tbody>
</table>