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

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## **Abstract**

The occurrence of non-epileptic hyperkinetic movements in the context of developmental epileptic encephalopathies is an increasingly 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 Ca<sub>v</sub>2.2/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<sup>2+</sup> 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<sub>v</sub>2.2 in normal human neurodevelopment.



## Main Text

The developmental and epileptic encephalopathies (DEE) are a heterogeneous group of complex disorders characterised by severe early-onset seizures that are typically refractory to medication and associated with neurodevelopmental delay, regression and often multiple co-morbidities.<sup>1,2,3</sup> 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.<sup>1</sup> 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.<sup>4,5</sup> Indeed, hyperkinetic movement phenotypes, such as dystonia and choreoathetosis, are now commonly reported in individuals with *FOXP1*- (MIM:164874), *GNAO1*- (MIM:139311), *SCN8A*- (MIM:600702) and *STXBP1*- (MIM: 602926) related epilepsy-dyskinesia syndromes.<sup>6-9</sup>

We report the identification of biallelic *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 babbled. 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.<sup>10</sup> 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.<sup>11</sup> 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

requested collaborating research groups to probe their whole exome and genome datasets (**Table S8**). 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.<sup>12</sup> 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-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 with a single affected proband (**Family C, Figure 1c**) was identified through GeneMatcher.<sup>13</sup> 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<sup>2+</sup> 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.<sup>14-20</sup> 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<sub>v</sub>1, Ca<sub>v</sub>2, and Ca<sub>v</sub>3). In neurons, the pre-synaptic Ca<sub>v</sub>2 channel family, comprising Ca<sub>v</sub>2.1, Ca<sub>v</sub>2.2, and Ca<sub>v</sub>2.3 isoforms (termed P/Q-type, N-type, and R-type calcium channels) are encoded by *CACNA1A*, *B* and *E* respectively.<sup>14–16</sup>

*CACNA1B* (Chr9:137,877,788-138,124,623 (GRCh38), MIM: 601012) encodes the calcium channel voltage-dependent, N-type,  $\alpha$ -1B subunit, the pore-forming subunit of presynaptic neuronal voltage-gated calcium channels (Ca<sub>v</sub>2.2). Ca<sub>v</sub>2.2 is expressed throughout the central nervous system including the cerebral white matter, cortex, hippocampus, basal ganglia and cerebellum (**Figure S4**).<sup>21–23</sup> 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,<sup>24</sup> given that all reported affected individuals had epilepsy and hyperkinesia as a major part of their clinical phenotype.

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

Given the key neuronal functions of Ca<sub>v</sub>2.1 and Ca<sub>v</sub>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).<sup>26</sup> This finding has not been replicated in subsequent studies.<sup>27</sup> Single nucleotide polymorphisms and heterozygous copy number variants involving *CACNA1B* have been described in individuals with

neurovascular disorders and schizophrenia.<sup>28-30</sup> 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.<sup>31-34</sup> 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.<sup>35</sup>

*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 (syniprint 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 Gβγ, protein kinase C (PKC) and PIP2.<sup>16,18,36</sup> 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 Gβγ (**Figure 2**).<sup>18</sup> 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<sub>v</sub>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.<sup>16,37-39</sup> Depolarisation of the pre-synaptic terminal initiates opening of Ca<sub>v</sub>2.2 and subsequent influx of Ca<sup>2+</sup> ions. A rise in intracellular Ca<sup>2+</sup> 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<sup>2+</sup> 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).<sup>8,40-43</sup>

*CACNA1B* is postulated to play a role in early brain development, as supported by the expression profile of Ca<sub>v</sub>2.2.<sup>20</sup> Ca<sub>v</sub>2.2 knockout murine models manifest a number of neurodevelopmental abnormalities including abnormal locomotor activity and memory impairment.<sup>44</sup> Future models of Ca<sub>v</sub>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.. Ingrid Scheffer 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; may accrue future revenue on pending patent WO61/010176 (filed: 2008): 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/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. John J Millichap reports honoraria as editor from 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 outside 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>

HSF, <http://www.umd.be/HSF/>

MaxEnt Scan, [http://genes.mit.edu/burgelab/maxent/Xmaxentscan\\_scoreseq.html](http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html)

MutationTaster, <http://www.mutationtaster.org>

NN Splice, [http://www.fruitfly.org/seq\\_tools/splice.html/](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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## 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 $Ca_v2.2$ with location of *CACNA1B* variants

The structure of  $Ca_v2.2$  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. G $\beta\gamma$ , G Protein  $\beta\gamma$  subunit; P, Binding site of PKC; PKC, Protein Kinase C.



## Tables

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

<sup>a</sup> SNP array revealed ~30% areas of homozygosity, possibly suggestive of consanguinity

Abbreviations: ACTH, adrenocorticotrophic hormone; AED, anti-epileptic drug; BIO, biotin; CBM, clobazam; CPM, clonazepam; CVI, cortical visual impairment; FOL, folic 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

	A-II:2	A-II:3	A-II:4	B-II:1	B-II:2	C-II:1
<b>Biallelic variants</b>	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*
<b>Consanguinity</b>	Yes, Parents 1 <sup>st</sup> cousins	Yes, Parents 1 <sup>st</sup> cousins	Yes, Parents 1 <sup>st</sup> cousins	No	No	Unknown <sup>a</sup>
<b>Ethnicity</b>	Pakistani	Pakistani	Pakistani	European descent	European descent	European descent
<b>Age of Death (years)</b> <b>Cause of Death</b>	3y Respiratory infection	7y Meningitis End organ failure	14y Respiratory infection	17y Respiratory infection	5y Respiratory infection	Alive – 6y
<b>Sex</b>	Male	Male	Female	Male	Male	Female
<b>Pregnancy</b> <b>Birth</b>	Normal Term, normal	Normal Term, normal	Normal 36 weeks, normal	Normal Term, normal	Normal Term, normal	Unknown Unknown
<b>Best</b> <b>Neurodevelopmental</b> <b>I stage (age)</b>	Sat with support, babbled and smiled (8m)	Sat unsupported, reached for objects and babbled (9m)	Sat with support and smiled (8m)	Sat unsupported, 1 word (2y)	Always delayed Never sat/babbled	Always delayed Never sat
<b>Age of regression</b>	10m	10m	8m	2y	Always delayed	Unknown
<b>Age of seizure onset</b>	10m	9m	12m	30m	21m	Unknown
<b>Seizure type</b> <b>At presentation →</b> <b>evolution over time</b>	Epileptic spasms (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
<b>Medications tried</b> <b>(medications with</b> <b>some beneficial</b> <b>effect underlined)</b>	NPM, PHY, Steroids, VB6, VBN, VPA  Refractory to AED	<u>CBM</u> , <u>VBN</u> , VPA  Periods of seizure freedom on CBM and VBN	<u>ACTH</u> , CBM, LAC, <u>LEV</u> , NPM, PHB, Steroids, RUF, TOP, VBN, <u>VPA</u> 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, <u>PHB</u> , <u>RUF</u> , Steroids  6m seizure free on PHB and RUF
<b>Movement disorder</b>	Myoclonus Dystonia Episodic exacerbations	Myoclonus Dystonia	Myoclonus Dystonia Oromotor dyskinesia	Myoclonus Dystonia Choreoathetosis Dyskinesia Frequent exacerbations	Myoclonus Dystonia Choreoathetosis Hand-wringing stereotypies	Myoclonus Choreoathetosis

<b>Other features</b>						
Head size	Postnatal microcephaly (OFC 0.4 <sup>th</sup> centile)	Postnatal microcephaly (OFC 0.4 <sup>th</sup> centile)	Postnatal microcephaly (OFC 3 <sup>rd</sup> centile)	Postnatal microcephaly (OFC <0.4 <sup>th</sup> centile) CVI	Postnatal microcephaly (OFC 2 <sup>nd</sup> centile)	Microcephaly (not known whether congenital or postnatal) (OFC 2 <sup>nd</sup> centile)
Vision	Strabismus Nystagmus		Left strabismus CVI		Divergent strabismus Congenital nystagmus CVI	
Central and peripheral tone	Central hypotonia and brisk limb reflexes	Central hypotonia with increased peripheral tone L>R	Generalised hypotonia	Generalised hypotonia	Generalised hypotonia	Generalised hypotonia
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
<b>Dysmorphic features</b>	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