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Detection Of A Missense Variant In KCNB1 (-) (ENST00000371741.6) C.935G>A (P.Arg312His), Associated With Developmental And Epileptic Encephalopathy 26 (OMIM#616056)

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Abstract

A heterozygous missense variant (chr20:g.49374625C>T; Depth: 134x) in exon 2 of the KCNB1 gene, leading to the substitution of Histidine for Arginine at codon 312 (p.Arg312His; ENST00000371741.6), was identified. This variant, previously associated with Neurodevelopmental Disorders, causes loss-of-function by impeding channel opening within a physiological voltage range. Located in the Ion transport protein domain (PF00520) of KCNB1, it's classified as likely pathogenic/pathogenic in ClinVar. Absent in various databases, its frequency is 0.002% in our database. Predicted to be damaging by PolyPhen-2 (HumDiv), SIFT, LRT, and MutationTaster2, the variant's codon is conserved across species. Associated with Developmental and Epileptic Encephalopathy 26 (OMIM#616056), DEE26 presents with diverse seizure types in infancy or early childhood, alongside developmental delay, intellectual disability, and EEG abnormalities like hypsarrhythmia.

Keywords: KCNB1 gene, Neurodevelopmental Disorders, Developmental and Epileptic Encephalopathy 26, AARS1 gene

Introduction

Developmental and epileptic encephalopathy-26 (DEE26) manifests as a neurological disorder typified by the emergence of various seizure types during late infancy or early childhood. Affected individuals commonly exhibit developmental delay, intellectual disability, impaired speech, and behavioral anomalies. Electroencephalogram (EEG) assessments often reveal multifocal epileptic discharges, potentially accompanied by hypsarrhythmia (as described by Torkamani et al., 2014). This disorder is alternatively known as Early Infantile Epileptic Encephalopathy 26 (EIEE26), Epileptic Encephalopathy, Early Infantile, 26, and KCNB1-related epilepsy.

KCNB1 encephalopathy denotes a condition characterized by abnormal brain function (encephalopathy), recurrent seizures (epilepsy), and developmental delay. The seizure manifestations in individuals with this condition encompass myoclonic seizures, tonic seizures, tonic-clonic seizures, atonic seizures, drop attacks, or absence seizures. Moreover, some individuals may exhibit continuous spike and waves during slow-wave sleep (CSWS), an abnormal pattern of electrical activity in the brain observed during deep sleep.

Children affected by KCNB1 encephalopathy commonly experience delayed speech and motor skill development, potentially attributed to hypotonia. While many eventually achieve independent mobility and verbal communication, some individuals require assistance, and others may never develop these skills. Additionally, around half of those with KCNB1 encephalopathy may present with neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD) or autism spectrum disorder (ASD). Rarely, issues with vision, digestion, and sleep may also occur in this population.

The prevalence of KCNB1 encephalopathy remains undetermined, though scientific literature documents over 65 reported cases. The condition is caused by mutations in the KCNB1 gene, which encodes a component of a potassium channel known as Kv2.1. These channels play a crucial role in regulating neuronal activity and signal transmission in the brain. Mutations in the KCNB1 gene typically result in dysfunctional Kv2.1 channels, disrupting potassium ion flow within neurons and thereby impairing normal cell communication. This disruption leads to the characteristic features of KCNB1 encephalopathy, including seizures, intellectual disability, and encephalopathy.

KCNB1 encephalopathy follows an autosomal dominant inheritance pattern, requiring only one copy of the mutated gene to manifest the disorder. While most cases arise from new mutations, familial cases are also observed.

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

7 year old baby *(name not disclosed)*, born of a non-consanguineous marriage, presented with clinical indications of delayed milestones, right focal epilepsy, generalized tonic clonic seizures at one year of age, speech delay, CSWS and right partial ptosis. MRI suggestive of hypomyelination. EEG showed temporo occipital sharp waves and high amplitude slow waves. She has been evaluated for pathogenic variations.

TEST METHODOLOGY

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions of the genome/genes is performed. Variants identified in the exonic regions are generally actionable compared to variants that occur in non-coding regions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual.

DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean depth of >80-100X on Illumina sequencing platform. We follow the GATK best practices framework for identification of germline variants in the sample using Sentieon [Sentieon]. The sequences obtained are aligned to human reference genome (GRCh38) using BWA aligner [Sentieon, PMID:20080505] and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels [Sentieon]. Sentieon haplotype caller is then used to identify variants in the sample. The germline variants identified in the sample is deeply annotated using VariMAT pipeline. Gene annotation of the variants is performed using VEP program [PMID: 20562413] against the Ensembl release 99 human gene model [PMID: 29155950]. In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth method PMID: 22942019]. This algorithm detects CNVs based on comparison of the read-depths in the sample of interest with the matched aggregate reference dataset.

In addition, the sequences were aligned to revised Cambridge mitochondrial reference genome (rCRS) for identification of SNVs/Indel variants in the mitochondrial genome (NC_012920.1). Mitochondrial DNA contains 37 genes, all of which are essential for normal mitochondrial function.

Clinically relevant mutations in both coding and non-coding regions are annotated using published variants in literature and a set of diseases databases : ClinVar, MITOMAP,OMIM, HGMD, LOVD, DECIPHER (population CNV) and SwissVar [PMID: 26582918, 18842627, 28349240, 21520333, 19344873, 20106818, 9399813]. Common variants are filtered based on allele frequency in 1000Genome Phase 3,

gnomAD (v3.1 & 2.1.1), dbSNP (GCF_000001405.38), 1000 Japanese Genome, TOPMed (Freeze_8), Genome Asia,HmtDB and our internal Indian population database (MedVarDb v3.0) [PMID: 26432245, 32461613, 11125122, 26292667, 33568819, 31802016, 22139932]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Clinically significant variants are used for interpretation and reporting.

Average sequencing depth (x)	Average on-target sequencing depth (x)	Percentage target base pairs covered		
		0x	≥5x	≥20x
249	111.9	1.12	98.14	96.84

Results

VARIANT INTERPRETATION AND CLINICAL CORRELATION

VARIANT 1 (KCNB1 gene):

Variant description: A heterozygous missense variant in exon 2 of the ***KCNB1*** gene (chr20:g.49374625C>T; Depth: 134x) that results in the amino acid substitution of Histidine for Arginine at codon 312 (p.Arg312His; ENST00000371741.6) was detected (Table). The observed variant has previously been reported in patients affected with Neurodevelopmental Disorders and resulting in loss-of function due to impaired channel opening in a physiological voltage range [PMID: 31600826]. The variant lies in the Ion transport protein domain of the ***KCNB1*** protein (PF00520). This variant has been classified as likely pathogenic/pathogenic in the ClinVar database. This variant has not been reported in the 1000 genomes, gnomAD (v3.1), gnomdAD (v2) and topmed databases and has a minor allele frequency of 0.002% in our internal database respectively. The *in silico* predictions# of the variant are probably damaging by PolyPhen-2 (HumDiv) and damaging by SIFT, LRT and MutationTaster2. The reference codon is conserved across species.

OMIM phenotype: Developmental and epileptic encephalopathy 26 (OMIM#616056) is caused by heterozygous mutations in the *KCNB1* gene (OMIM*600397). DEE26 is a neurologic disorder characterized by onset of variable types of seizures late in infancy or in the first years of life. Affected children show developmental delay with intellectual disability, poor speech, and behavioral abnormalities. EEG shows multifocal epileptic discharges, and may show hypsarrhythmia [PMID: 18842627].

Gene# Transcript	KCNB1 (-) (ENST00000371741.6)	AARS1 (-) (ENST00000261772.13)	
Location	Exon 2	Exon 8	Exon7
Variant	c.935G>A (p.Arg312His)	c.1001A>T (p.Tyr334Phe)	c.959G>A (p.Arg320His)
Zygosity	Heterozygous	Likely compund Heterozygous	
Disease (OMIM)	Developmental and epileptic encephalopathy 26 (OMIM#616056)	Developmental and epileptic encephalopathy 29 (OMIM#616339)	
Inheritance	Autosomal dominant	Autosomal recessive	
Classification	Pathogenic (PS3,PM1,PM2,PP3,PP5)	Uncertain Significance (PM2)	Uncertain Significance (PM2,PP3)

Based on the above evidence$, ***this KCNB1 variation is classified as a likely pathogenic variant and has to be carefully correlated with the clinical symptoms.***

VARIANTS 2 AND 3 (AARS1 gene)

Variant description: Likely compound heterozygous variants were detected in the ***AARS1*** gene (Table).

Variant 2: A heterozygous missense variant in exon 8 of the ***AARS1*** gene (chr16:g.70268341T>A; Depth: 115x) that results in the amino acid substitution of Phenylalanine for Tyrosine at codon 334 (p.Tyr334Phe; ENST00000261772.13) was detected (Table). This variant has been classified as a variant of uncertain significance in the ClinVar database. This variant has not been reported in the topmed databases and has a minor allele frequency of 0.03%, 0.002%, 0.01% and 0.05% in the 1000 genomes, gnomAD (v3.1), gnomdAD (v2.1) and in our internal database respectively. The *in silico* prediction# of the variant is damaging by MutationTaster2. The reference codon is conserved across species.

Based on the above evidence$, this ***AARS1*** **variation is classified as a variant of uncertain significance.**

Variant 3: A heterozygous missense variant in exon 7 of the ***AARS1*** gene (chr16:g.70269621C>T; Depth: 83x) that results in the amino acid substitution of Histidine for Arginine at codon 320 (p.Arg320His; ENST00000261772.13) was detected (Table). This variant has been classified as a variant of uncertain significance in the ClinVar database. This variant has a minor allele frequency of 0.03%, 0.005%, 0.02%, 0.003% and 0.05% in the 1000 genomes, gnomAD (v3.1), gnomdAD (v2.1), topmed and in our internal database respectively. The *in silico* predictions# of the variant are probably damaging by PolyPhen-2 (HumDiv) and damaging by SIFT, LRT and MutationTaster2. The reference codon is conserved across species.

Based on the above evidence$, this ***AARS1*** **variation is classified as a variant of uncertain significance. These *AARS1* variations are considered to be likely compound heterozygous variants and must be carefully correlated with the clinical symptoms.**

OMIM Phenotype: Developmental and epileptic encephalopathy 29 (DEE29) (OMIM#616339) is caused by homozygous or compound heterozygous mutations in the *AARS1* gene (OMIM*601065). DEE29 is a neurologic disorder characterized by the onset of refractory myoclonic seizures in the first months of life. Affected individuals have poor overall growth, congenital microcephaly with cerebral atrophy and impaired myelination on brain imaging, spasticity with abnormal movements, peripheral neuropathy, and poor visual fixation [PMID: 18842627].

Summary

Summary and Conclusion

The *AARS1* gene has pseudogenes in the human genome. Validation of the variant by an alternate technique is recommended to rule out false positives.

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