Clinical Vignette

Truncation Variation in the Protocadherin 19 (*PCDH19*) Gene Exhibiting Mosaicism in a Manifesting Heterozygous Male

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Abstract

Epilepsy and intellectual disability limited to females (EFMR)/ early infantile epileptic encephalopathy-9 (EIEE9) is an unusual X-linked disorder in which obligate male carriers are not affected and females show severe epilepsy with cognitive impairment. In the present study, a male child who presented with cortical dysplasia, gray matter heterotopias and seizures at two months of age was evaluated using clinical exome sequencing. Clinical exome analysis revealed a mosaic truncation variant NM_020766.2: c.462 C>G in exon 1 of the protocadherin 19 gene (*PCDH19*). This variant was further confirmed by Sanger sequencing, which revealed mosaicism in peripheral blood as well as saliva DNA in the proband. This variant was not detected in the Sanger sequencing of the parents. The *PCDH19* gene located at the chromosome Xq22.1 locus, encodes for protocadherin delta-2 protein with 1148 amino acids and is involved in calcium dependent cell-cell adhesion. The present result can be explained using cellular interference as the disease mechanism and is well supported by previous research studies.

Keywords: *PCDH19*, epilepsy, X-linked, mosaicism, truncation variant

Introduction

PCDH19 gene located at the chromosome Xq22.1 locus, spanning six exons and encoding for protocadherin 19 protein (PCDH19), belongs to delta-2 protocadherin subclass of the cadherin superfamily. The *PCDH19* gene is predominantly expressed in the brain and several different pathogenic variants have been identified causing epilepsy and mental retardation limited to females (EFMR). EIEE9 is characterized by febrile or afebrile tonic-clonic, myoclonic or atonic seizures, starting in the early first year of life, and in some cases, is associated with intellectual disability. *PCDH19* gene is the second known gene related to epilepsy subsequent to *SCN1A* gene. The EFMR disorder was first reported in 1971. Later, Dibbens et al identified *PCDH19* as the disease-associated gene in 2008 (Dibbens et al., 2008). EFMR was known to affect only female carriers and spare hemizygous males. However, affected hemizygous mosaic males were later reported to have clinical features similar to those in affected females (Depienne et al., 2009). Moreover, a person with sex chromosome abnormality such as Klinefelter syndrome (47, XXY) or trisomy X syndrome (47, XXX) with pathogenic variant in *PCDH19* gene is also known to develop the phenotype (Romasko et al., 2018). In addition, there are reports of asymptomatic mosaic males and mutant allele fractions (MAFs) of 4.16%–37.38% and 1.27%–19.13% in different tissues, hypothesizing 50% of MAFs for disease manifestation (Liu et al., 2019).

We report on clinical exome sequencing in a four-year-old male child who presented with cortical dysplasia, gray matter heterotopias and seizures since two months of age which revealed mosaic variant in the *PCDH19* gene.

Patient and methods

This male patient initially presented at 2 months of age with generalized tonic-clonic seizures without fever. The seizures were controlled with one drug initially but subsequently the patient needed multiple antiepileptic drugs. Patient had global developmental delay and at 4 years of age, he could walk without support, run, write a few letters, and could communicate with the parents.

The patient was born to non-consanguineous parents and there was no family history of seizures. MRI of brain revealed cortical dysplasia, gray matter heterotopias and thickened gray matter. The patient's family consented for conducting the study and the same was also approved by the Institutional Ethics Committee.

Genomic DNA was extracted from the peripheral blood and saliva for the proband and the parents using the phenol-chloroform and salting out method, respectively. Clinical exome sequencing was performed on the genomic DNA and sequenced to mean coverage of 100X on the Illumina platform (Centogene, Germany). The reads were mapped against human reference genome assembly (hg19/GRCh37) using Burrows-Wheeler Aligner (BWA-MEM) and variants were identified through the Genome Analysis Toolkit (GATK) pipeline. The variants annotated using Annovar were filtered with 1% minor allele frequency (MAF) against population databases including 1000 genomes, Exome Variant Server (EVS), Exome Aggregation Consortium (ExAC), Genome Aggregation database (gnomAD), 69 Genome data (Cg69), Great Middle East (GME_all) and in-house databases. The functional impact of the variants identified was predicted using bioinformatics tools like PolyPhen2, SIFT and MutationTaster and known mutation databases like ClinVar, OMIM etc.

Specific genomic primers were designed and PCR was performed on the genomic DNA of proband and parents from blood and saliva, using the Qiagen Fast Cycling kit (Qiagen, Germany). Sanger sequencing was performed to confirm the identified variants on the ABI 3130 genetic analyzer machine (Thermo Fisher Scientific, USA).

Results and Discussion

The quality metrics analyses of the clinical exome sequence revealed a mean depth of ~135X with 97% of the reads having more than or equal to 20X coverage. The total number of variants was 32,607, which was reduced to 1333 variants after filtering against population databases. Further, only variants of exonic and splicing regions were analysed for variant types such as non-synonymous SNV, frameshift deletion, frameshift insertion, and stop gain variants. A hemizygous variant NM_020766.2(PCDH19):c.462C>G (p.Tyr154*) was identified in exon 1 of the *PCDH19* gene in the proband. This variant is absent in 1000G, ExAC, gnomAD, Complete genomics (cg69), Great middle east (GME) and in-house Indian databases. At the variant position, the read depths for C and G nucleotides were 16 and 64 respectively (Figure 1A). The identified variant was confirmed by Sanger sequencing in both the blood and saliva genomic DNA in the proband (Figure 1B). Parental segregation analysis revealed a *de novo* mechanism for the variant (Figure 1). It is also interesting to observe that the mutant G allele showed predominance over the reference C allele in the targeted Sanger testing. Moreover, the ClinVar database classified the identified variant as likely pathogenic with the accession ID: VCV000619130.3 from multiple submitters including the submission from the present study. The other submitters reported on mosaic variants found in five males showing severe symptoms.

The OMIM database has reported *PCDH19* gene related phenotype as inherited in an unusual X-linked pattern (OMIM number. #300088). This unusual mode of inheritance is explained by cellular interference, where the co-existence of different cellular populations distorts the cell sorting event in male mosaic. The heterozygous females are affected as a result of random X-inactivation. The cellular interference mechanism was reported from the study of Depienne et al, 2009, which revealed a deletion in *PCDH19* gene in a male patient with similar phenotype of early infantile epileptic encephalopathy-9 (EIEE9) or 'epilepsy and mental retardation limited to females' (EFMR). EFMR has been known to affect only female patients and pathogenic variation in *PCDH19* gene in hemizygous male does not result in the disease. The *PCDH19* gene encodes for the protocadherin-19 protein which is involved in cell-cell adhesion (Juberg et al., 2009). Hence, cellular interference was hypothesized for the affected females, comprising of two different cell populations existing as PCDH19-negative and PCDH19-wild type cells due to X inactivation disrupting the cell sorting event. This cellular interference has also been reported for craniofrontonasal syndrome, caused by pathogenic variations in the *EFBN1* gene. The heterozygous females were observed to be more severely affected than the hemizygous males. The mosaic males with this condition suffered from a more severe outcome in X-linked dominant disorder which supports the cellular interference mechanism (Twigg et al., 2013).

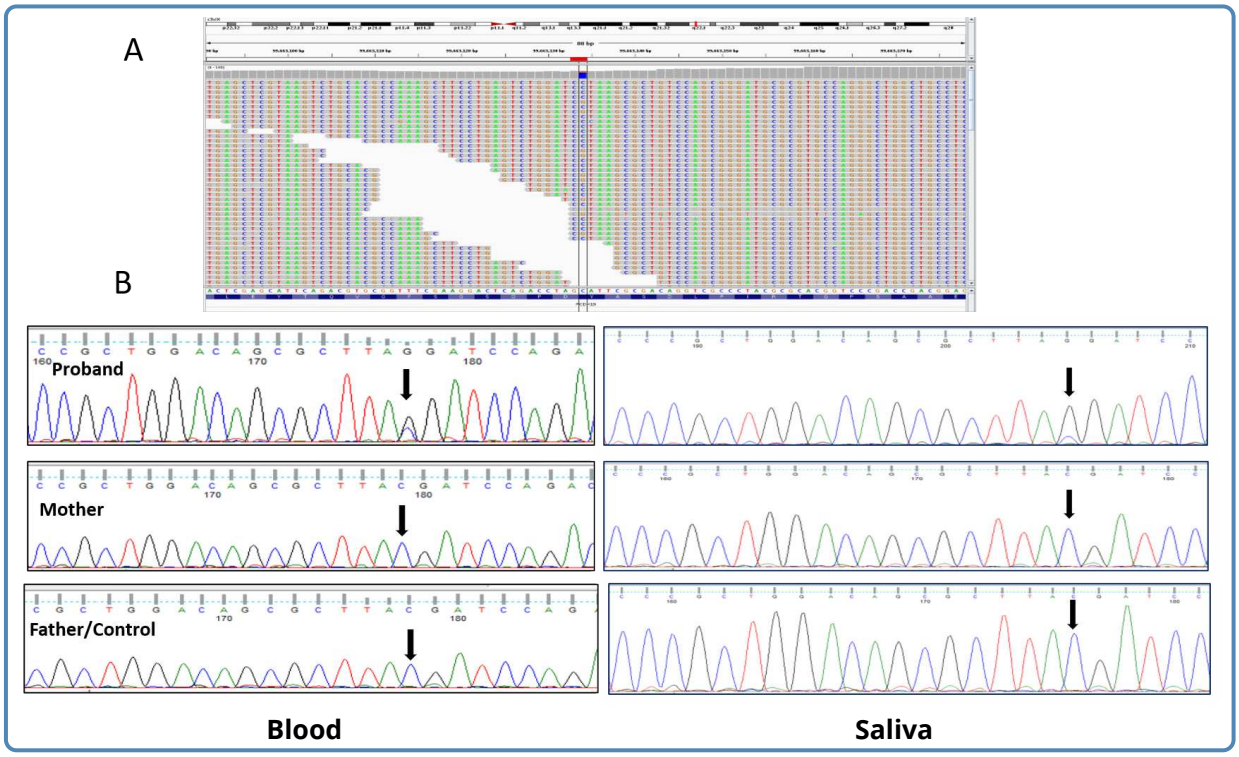

Figure 1 A) Integrative Genomics Viewer (IGV) screenshot showing hemizygous variant c.462C>G in *PCDH19* gene in the proband; read count for reference allele C is 16 and alternate allele G is 64. B) Sanger sequencing on genomic DNA isolated from blood (left panel) and saliva (right panel) showing mosaicism for the variant in the proband.

Conclusion

Early infantile epileptic encephalopathy-9 (EIEE9) or Epilepsy and mental retardation limited to females (EFMR) is an epileptic disease characterized by early onset of seizures with or without intellectual disability. The identified variant NM_020766.2:c.462 C>G in *PCDH19* gene in proband is classified as likely pathogenic according to the American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/ AMP) guidelines. The aim of this report is to highlight this neurologic disorder which is inherited through X-linked inheritance with a distinct disease mechanism known as cellular interference.

References

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