Case report of a novel PCDH19 frameshift mutation in a girl with epilepsy and mental retardation limited to females

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Abstract
Rationale: Epilepsy with mental retardation limited to females (EFMR) is a rare type of X-linked epilepsy disorder, affecting heterozygous females disproportionately. The pathogenesis of EFMR has been identified as mutations in the protocadherin 19 (PCDH19) gene. To date, more than 60 different mutations in PCDH19 have been identified. Most of them are located at exon 1, but we describe a novel deletion mutation c.2468delT at exon 3 of PCDH19.

Patient concerns: The patient was an 11-year-old girl with onset of seizures at the age of 18 months and followed by progressive intellectual disability (ID) later.

Diagnosis: The girl was diagnosed as EFMR when a novel deletion mutation c.2468delT at exon 3 of PCDH19 was found. The deletion mutation c.2468delT was predicted to have caused a frameshift mutation of amino acid at position 823 (p.L823fs). There was no family history of seizures or ID. Her father was asymptomatic, but the mutation screening shows that he had a hemizygous deletion mutation c.2468delT at the same site of PCDH19. The secondary structure of PCDH19 (wide type) showed that the sequences undergoing frameshift mutations were located in the cytoplasm and contain 9 phosphorylation sites. The p.L823fs mutation caused a totally different amino sequence after position of 823, thereby resulting in the disappearance of phosphorylation sites. The frameshift mutation of amino acid at position 823 might affect its binding capability with GABAA receptor and results in migration and morphological maturation of hippocampal neurons.

Interventions: The patient has received antiepileptic treatments, including sodium valproate, carbamazepine, levetiracetam, topiramate and clonazepam et al.

Outcomes: The antiepileptic treatment effects were limited.

Lessons: This case report describes a novel PCDH19 gene mutation (c.2468delT) at exon 3 in a girl suffering from EFMR. The deletion mutation was predicted to cause a frameshift mutation-p.L823fs, which is highly conserved across different species.

Abbreviations: MB = bachelor of medicine, DS = Dravet syndrome, EEG = electroencephalography, EFMR = epilepsy with mental retardation limited to females, HGMD = Human Gene Mutation Database, ID = intellectual disability, IPPK = inositol 1,3,4,5,6-pentaphosphate 2 kinase, MD = doctor of medicine, MM = master of medicine, MRI = magnetic resonance imaging, MT = mutant-type, PCDH = protocadherin, SCN1A = sodium voltage-gated channel alpha subunit 1, WT = wide-type.

Keywords: c.2468delT, epilepsy with mental retardation limited to females, frameshift mutation, protocadherin 19

1. Introduction
Epilepsy with mental retardation limited to females (EFMR; MIM 300088) is a rare type of X-linked epilepsy disorder with a unique sex-limited expression pattern. It was first reported by Juberg and Hellman in 1971.[1] Different from typical X-linked disorders characterized by affected males and unaffected carrier females, EFMR is extremely rare in males and affects only carrier females. The hallmarks of EFMR include the early infantile onset of seizures followed by cognitive impairment, intellectual disturbances, and resistance to anti-epileptic drugs. There is nonspecific change in brain magnetic resonance imaging (MRI) in EFMR and the electroencephalography (EEG) is usually normal as well, except for focal, multifocal or bilateral independent multifocal epileptiform discharges with normal or slow backgrounds during episodes or intermissions. In EFMR, seizure is usually accompanied by fever and the types are diverse, including tonic, clonic or tonic-clonic, and partial seizures.

The PCDH19 gene, located at Xq22, consists of six exons and encodes the 1148-amino-acid protein belonging to the proto-
cadherin (PCDH) 82 subgroup within the cadherin superfamily of homophilic cell-adhesion proteins. The PCDH family is expressed predominantly in the nervous system and the biological roles remain uncertain. PCDH might play a role in the development of neuronal connections and in signal transduction in the brain. In 2008, Dibbens et al. first identified PCDH19 gene mutations located at Xq22 in EFMRI patients. PCDH19 gene has 6 exons, of which the first exon encodes the entire extracellular domain of the protein, which is essential for the normal functioning of the original cadherin function. Mutations in PCDH19 gene have been identified as the cause of EFMRI. Till now, the reported PCDH19 gene mutations were most commonly located at exon 1, and no mutations were found at exon 2. The mutations are extremely rare in exon 3 to 7 days frequently. Her EEG showed nonspecific slow waves. MRI was normal. She was diagnosed as having been attacked by epilepsy when she was 2 years old, then received antiepileptic treatments, including sodium valproate, carbamazepine, levetiracetam, topiramate, and clonazepam et al. However, the treatment effects were limited. The longest period of remission was just 10 months.

The girl was born at full term with a birth weight of 3.5 kg. She had normal early developmental milestones and regressed in infancy, but later exhibited features of autism and intellectual disability (ID). She was the first and only child of unrelated Chinese parents. There was no family history of seizures or ID. The pedigree of the family is shown in Figure 1A.

2.2. Mutation screening and bioinformatics analysis

Genomic DNA from peripheral blood leukocytes derived from the affected individual and her parents were extracted using a MagBead Blood DNA Kit (CoWin Biosciences, Beijing, China). Gene capture and high-throughput screening were performed using GenCap (MyGenostics, Beijing, China). The enrichment libraries were captured and sequenced using an Illumina HiSeq 2000 sequencer.

Short read mapping and alignment were performed using Burrows-Wheeler Aligner software. All human genome reference sequences were based on the NCBI37/hg19 assembly of the human genome. Sequence changes were verified via Sanger sequencing on the affected daughter and her parents.

3. Results

Mutation screening in the affected patient identified a novel heterozygous deletion change of thymine (T) at exon 3 of PCDH19 (c2468delT), resulting in a frameshift mutation at position 823 of amino acid sequences (p.L823fs (Fig. 1B)). No previous report has examined a mutation lacking thymidine at exon 3 of PCDH19 gene. The Human Gene Mutation Database (HGMD) has not classified the mutation and states that it occurs at a relatively low-frequency in the population. Mutation screening of her parents showed a hemizygous mutation in the same site of her father who was clinically asymptomatic. No mutations were found in her mother. The mutation was further confirmed by Sanger sequencing (Fig. 1B).

According to the NCBI RefSeq database, there are 3 known transcripts of PCDH19, NM_001105243, NM_020766, and NM_001184880 (Fig. 2A). The mutation was categorized into PV51 according to the ACMG guidelines. Compared to wide-type (WT), the amino acid sequence in human mutants (MT) is totally changed after p.823. Leucine at p.823 is highly conserved in PCDH19 across different species according to Multiple Sequence Alignment in Phylogenetic Analysis (Fig. 2B). The c.2468delT mutation is predicted to cause frame shift of the Leucine (L) amino acid at position 823 (p.823) (Fig. 2C). This suggests that the highly conserved amino acid sequence must have functional value. We further predicted the secondary structure of PCDH19 in human WT, which contains 6 cadherin domains. The red frame shows the sequences undergoing frameshift mutations. Frameshift mutation causes a totally different amino acid sequence after p.823, resulting in the disappearance of phosphorylation sites (green sites) located in the sequences (Fig. 2D).

4. Discussion

The child was diagnosed as being afflicted by EFMRI according to the clinical manifestations, genetic testing report and the typical pedigree. EFMRI shares some features with the Dravet syndrome (DS) which was mainly caused by mutations in sodium-channel gene-sodium voltage-gated channel alpha subunit 1 (SCN1A), including early onset in infancy, fever sensitivity, cognitive impairment as well as resistance to antiepileptic treatment. Moreover, PCDH19 mutation was determined to be the causing gene of DS and was reported to account for about 16% of the reported SCN1A-negative DS patients. However, DS affects males as well as females. It has an earlier onset at around 6 months of age, which points to an onset of EFMRI (mean age of 14 months). In addition, seizures in DS are mainly isolated and longer in duration. Myoclonus is also common in DS.

EFMRI predominantly affects females. Males with hemizygous PCDH19 mutations are asymptomatic. The unique sex-limited expression pattern may have been caused by one possible mechanism, which was called “cellular interference”. In this mechanism, the PCDH19 was considered to be non-functional. A gain of function at tissue level occurs because of abnormal interactions between “mutated” and “normal” cells when they are coexistant. Terracciano et al reported 2 symptomatic males with 2 mosaic PCDH19 point mutations, which supported the hypothesis of “cellular interference”. In this case report, the father was asymptomatic because of hemizygous mutation in PCDH19.

Mutations in PCDH19 cause EFMRI. To date, more than 60 different mutations in PCDH19 have been identified, most of which have been missense mutations and nonsense mutations. There are some other types of mutations, including small nucleotide deletions and insertions, mutations altering the splice sites and intragenic or whole gene deletions, most of which are located at exon 1 of PCDH19 gene. This case report is the first one to report an EFMRI patient with a frameshift mutation of
Leucine at p.823 caused by a c2468delT mutation at exon 3 of PCDH19 (c2468delT). The Leucine at p.823 is highly conserved in PCDH19 across multiple species, indicating that the DNA sequence has been maintained by evolution despite speciation and has functional values. Silvia Bassani et al demonstrated that PCDH19 is a new GABAAR-binding partner that regulates GABAergic transmission as well as migration and morphological maturation of hippocampal neurons.[12] Interestingly, in this case report, the mutation at p.823 belongs to the intracellular domain, which plays an important part in the interaction of PCDH19 with GABA receptor.[12] In this patient, the frameshift mutation totally changed the amino acid sequence after p.823, resulting in an incomplete protein with disappearance of phosphorylation sites. Recently, Huttlin et al reported that IPPK (inositol 1,3,4,5,6-pentaphosphate 2 kinase) interacts directly with PCDH19 and phosphorylates PCDH19.[13] The frameshift mutation observed in this patient may have caused EFMR by affecting phosphorylation in PCDH that eventually influenced the activation of cell signaling pathways.

5. Conclusion
Gene testing plays a vital role in the diagnosis of EFMR because of its atypical symptoms, which are difficult to be differentiated from other types of epilepsy. This case report is the first to report a female EFMR patient with a novel variation located at exon 3 of PCDH19 gene (c.2468delT). The mutation was predicted to cause a frame shift of Leucine at position p.823, which remained highly conserved across different species.

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Figure 1. A novel mutation in the affected female. (A) Pedigree of the family; black symbol represents the affected daughter. (B) Sanger sequencing shows that the sequence chromatograms of PCDH19 exon 3 illustrating the c.2468delT mutation in unaffected "carrier" father. This was the reason the heterozygous mutation was detected in the affected daughter. PCDH19 = protocadherin 19.
Figure 2. The frameshift mutation in the affected female. (A) The 3 PCDH19 transcripts caused by c.2468delT mutation; (B) A frameshift mutation at p.823 in the MT causes a totally different amino sequence after p.823. Orthologues of PCDH19 from 6 available species show a high degree of conservation of the Leucine 823 residue. (C-D) The secondary structure of PCDH19 shows that it contains 6 cadherin domains. The red frame shows the sequences undergoing frameshift mutations, which are located in cytoplasm, resulting in the disappearance of 9 phosphorylation sites (green sites). MT = mutant-type, PCDH19 = protocadherin 19.

Author contributions
All authors confirmed they have all made contributions to this paper. Xinying Zhang, Na Chen and Xueyu Wang designed the study, collected the data and drafted the manuscript; Aihua Ma contributed her founding which is from Shandong Nature Science Foundation (Ref: ZR2017MH021). Wenxiu Sun performed molecular analysis; Yuxing Gao recruited all the involved subjects and specialized medical examinations.
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