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Title: Recurrence of Developmental and Epileptic Encephalopathy 9 (DEE9) in Two Siblings Due to Parental Germline Mosaicism of PCDH19 Mutation and Review of the Literature

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Abstract

Introduction: Developmental and epileptic encephalopathy 9 (DEE9) is caused by pathogenic variants in the *PCDH19* gene. The clinical features include early-onset seizures that are often provoked by fever and display clustered seizures, mild to profound intellectual disability, autistic traits, and behavioral disturbances. DEE9 is characterized by an unusual X-linked pattern where heterozygous females or rarely mosaic hemizygous males are affected, but hemizygous males and homozygous females are asymptomatic. In recent years, an increasing number of female and male patients with *PCDH19*-related epilepsy and symptoms have been reported.

Methods & Results: Here, we report two additional female patients with DEE9 who are siblings. Whole exome sequencing revealed that these patients have a heterozygous frameshift variant (NM_001184880.2: c.1091delC, p.P364Rfs*4) in the *PCDH19* gene. We also reviewed previously reported cases with this mutation in detail.

Conclusion: This is the first report of germline mosaicism in the *PCDH19* gene in the Iranian population and expanded the phenotypic spectrum of DEE9. Genetic testing has become an effective way of determining the diagnosis. Parental germline mosaicism should be considered when providing genetic counseling for X-linked/autosomal dominant disorders. This report also provides an emphasis on the importance of considering prenatal diagnosis (PND) in such cases.

Key words: Developmental and epileptic encephalopathy 9; Epilepsy; *PCDH19;* Genetic, Germline mosaicism

1. Introduction:

Epilepsy is one of the most common neurological conditions among children and its highest incidence is in the first year of life (Fine & Wirrell, 2020). There are a heterogeneous group of disorders characterized by the triad of early-onset epileptic seizures, abnormal EEG activity, and developmental impairment which are called "developmental and epileptic encephalopathies" (DEEs). DEEs may be caused by both genetic and non-genetic etiologies (Guerrini et al., 2023). We have different types of DEEs and one of them is "developmental and epileptic encephalopathy 9" (DEE9) that results from pathogenic variants in *PCDH19* gene (Kolc et al., 2019). Several names have been adopted to signify the clinical characterization of this disorder, including ''Girls Clustering Epilepsy'' (PCDH19- GCE) (Kolc et al., 2019), ''Early Infantile Epileptic Encephalopathy-9'' (EIE19) or "Developmental and epileptic encephalopathy 9" (DEE9), and ''PCDH19 Clustering Epilepsy'' (PCDH19-CE) (Depienne et al., 2009; Kolc et al., 2020). Here, we use DEE9 (OMIM #300088) to refer to the disorder.

Symptoms of DEE9 patients evolve in early infancy, mostly within the first year of life. It is often provoked by fever, and present clustered seizures, different degrees of cognitive impairment, and behavioral problems such as autism spectrum disorder (ASD), hyperactivity, attention deficit, and aggression (Kolc et al., 2020). The clinical characteristics may be similar to those of Dravet syndrome (Depienne et al., 2009). The disorder is defined by a distinctive pattern of X-linked inheritance where heterozygous females or rarely mosaic hemizygous males are affected, but hemizygous males and homozygous females are asymptomatic. *PCDH19* is located in the region exposed to X-chromosome inactivation. Random X-inactivation and consequent tissue mosaicism is posited to be implicated in the pathogenesis of heterozygous females (Depienne et al., 2009; Depienne & Leguern, 2012). Although the exact mechanism remains unclear, a mechanism known

4

as 'cellular interference' has been proposed to clarify this unusual inheritance pattern (Depienne et al., 2009; Depienne & Leguern, 2012; Samanta, 2020; Thiffault et al., 2016). The 'cellular interference' hypothesis postulates that the existence of a variety of cells in the brain, expressing either mutant or wild-type PCDH19 protein, results in abnormal neurodevelopment and is therefore the cardinal cause of the clinical presentations (Depienne et al., 2009).

The causative gene of DEE9 was mapped to X-chromosome in 1997 (Ryan et al., 1997) and then determined to be *PCDH19* in 2008 (Dibbens et al., 2008). *PCDH19* (protocadherin 19) has been identified as the second most common mutated gene in epilepsy after *SCN1A* which leads to Dravet Syndrome (Depienne & Leguern, 2012; Niazi, Fanning, Depienne, Sarmady, & Abou Tayoun, 2019; Perez, Hsieh, & Rohena, 2017). *PCDH19* (OMIM: 300460) is located at Xq22.1 and composed of six exons that encode protocadherin-19. Protocadherin-19, an 1148 amino-acid protein, contains six extracellular cadherins (EC) domains with conserved calcium-binding sequences, a transmembrane domain, and also a cytoplasmic domain (Hulpiau & Van Roy, 2009). Most of the pathological variants occur within the first and the largest exon, encoding the six extracellular cadherin domains and a transmembrane domain. Exons 2–6 encode the intracellular domain of the protein (Depienne & Leguern, 2012; Duszyc, Terczynska, & Hoffman-Zacharska, 2015; Lyons, Marnane, Reavey, Williams, & Costello, 2017). This protein belongs to non-clustered (delta 2) protocadherin subclass of the cadherin superfamily, that is greatly expressed in nerve tissues and at various developmental periods (Dibbens et al., 2008; Gaitan & Bouchard, 2006; Wolverton & Lalande, 2001). PCDH19 partakes in calcium dependent cell-to-cell adhesion contributing to the regulation of neuronal connection and signal transduction in the early phases of neurodevelopment (Dibbens et al., 2008). Besides, during the early stages of postnatal life, PCDH19 contributes to the development of synaptic connections and also the maintenance of

synaptic connections during adulthood (Kim et al., 2010). It seems that this protein regulates gamma-aminobutyric acid type A receptors (GABAAR). This adhesion molecule mediates the differentiation of neuronal progenitors, neuronal migration, and maturation by inducing GABAergic signaling (Bassani et al., 2018).

In recent years, a growing number of female and male patients with *PCDH19*-related epilepsy and symptoms have been reported. Here, we report two additional female patients with DEE9 who are siblings and both have a heterozygous frameshift variant in the *PCDH19* gene. Although there is a report of *PCDH19* polymorphism in the Iranian population (Asadi, goodarzi, Zahiri, & jaafarnia, 2022), but there is no report of disease-causing variants. This is the first report of *PCDH19* mutation in the Iranian population. We have also expanded the phenotypic spectrum of DEE9. Moreover, this is a report of parental germline mosaicism which emphasizes on the clinical importance of considering prenatal diagnosis (PND) in the X-linked/autosomal dominant disorders.

Case presentation

Here, we describe two female patients with DEE9 who are siblings. Their developmental milestones in motor (sitting, neck holding, rolling over, and walking), and social interactions (first social smile and responding to name) were normal for their age. After seizure onset, they showed developmental regression and some other conditions. Karyotype analyses were normal. Their perinatal history, pregnancy, and birth history were all unremarkable. There is history of febrile seizures in some relatives with normal development. (Fig. 1). There was no family history of recurrent abortions, congenital anomalies, intellectual disability, and genetic disorders.

Case 1:

This patient is a 5-year-old girl. She is the first child of consanguineous parents, from a 21-year-old mother and a 22-year-old father. She was born at full-term via normal vaginal delivery with a 2.5 kg birth weight. At the age of 5.5 months, she presented afebrile tonic seizures with lateral gaze lasting for 15-20 seconds and repeated 15 times in a day. Three days later, a second seizure happened with identical characteristics, and then she had seizures every 2 months. At 14 months, she had recurrent seizures (4-5 times in a week). At 1 year and 3 months of age, she exhibited speech regression and behavioral changes including irritability, excessive crying, screaming, and attentional problems. At 2 years and 9 months of age, she had afebrile cluster seizures which were mixed types including tonic with lateral gaze, tonic-clonic, and myoclonic. Now, she still has refractory seizures, hypertelorism, hypoplastic midface, normal motor development, intellectual disability, impaired speech development, aggressive behavior, and autistic features (avoiding eye contact, impaired cognitive and learning skills, inattentive behavior, Unresponsive to smile, getting very upset if she does not like certain clothing or sound), and constipation. Her current seizures are clustered, afebrile, and mostly tonic with lateral gaze. Brain magnetic resonance imaging (MRI) was unremarkable. The analysis of electroencephalography (EEG) showed normal background without any asymmetry between hemispheres and some interictal epileptiform discharges (IEDs) in right temporal region. Her seizures were partially controlled with anti-seizure medications (ASMs). Her previous medication regimen includes Sodium valproate, Nitrazepam, Carbamazepine, Levetiracetam, Primidone, Topiramate, and Phenobarbital. The last ASM regimen includes Topiramate and Lacosamide, but she still suffers from recurrent seizures approximately every 2 months.

Case 2:

The patient is the second child of consanguineous parents, from a 24-year-old mother and a 25-year-old father. She is a 2.5-year-old girl and was born at full-term via normal vaginal delivery. Birth weight was 2.55 kg, length was 48 cm, and HC at birth was 33 cm. At 6 months of age, she had febrile seizures provoked by vaccination, described as tonic seizures with lateral gaze lasting 20-25 seconds and repeated 3-4 times in a day. Now, her seizures are well controlled with medication. She has normal motor development, mild intellectual disability, and autistic features (repetitive movement, hyperactivity, and delayed speech and language skills). Brain computed tomography scan (CT) was normal, and EEG showed normal background without any asymmetry between hemispheres and some IEDs as bilaterally spikes in the occipital regions.

She has been on various ASMs. Her first episodes of seizures at 6 months of age were well controlled by Levetiracetam and Phenobarbital. Approximately at 7 months, she had adverse drug reactions and the ASM was switched to Clobazam. After a seizure-free period lasting 5 months, seizures recurred at age 1 year due to arbitrarily discontinuing Clobazam. Her next drug regimen included Levetiracetam, Topiramate, and Phenytoin. Now, her seizures are controlled by the three ASMs including Levetiracetam, Oxcarbazepine, and Clobazam.

[Fig. 1 here]

2. Methods:

3.1. Participants, whole-exome sequencing, bioinformatics analysis, and sanger sequencing

Peripheral blood samples were collected from the proband, her sister and parents after informed consent. Genomic DNA from blood samples was extracted using Zistagen DNA extraction kit according to the manufacturer's guidelines. For the proband, whole-exome sequencing (WES) was

8

performed by capturing the targeted regions using Agilent SureSelect ^{XT2} V7 (per manufacturer's instructions). Then, sequencing performed on Illumina NovaSeq6000 platform (Illumina, San Diego, CA, USA) with 100 bp paired-end reads at an average sequencing depth of 100×. Sequence reads were aligned to the GRCh37/hg19 human genome assembly using Burrows-Wheeler Aligner (BWA). Variant calling was done using SAMTools and Genome Analysis Toolkit (GATK v 3.7) (Li & Durbin, 2010; Li et al., 2009; McKenna et al., 2010). In addition, ANNOVAR software annotated and filtered the variants. For more filtrations, all pathogenic variants described in HGMD, and also variants with minor allele frequency (MAF) less than 0.01% measured against gnomAD, ExAC, 1000Genome project, dbSNP138, ESP6500, NHBL Exome Variant Server (EVS), and Iranome. The biological effects of candidate variant genes were determined in silico by predictor tools including MutationTaster, SIFT, Polyphen2, and CADD software. Eventually, sequencing result was also filtered based on phenotype, inheritance pattern, and variant type. Then, Sanger sequencing was carried out using ABI Prism3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The primer sequences for PCR in Sanger sequencing are listed as follows: forward primer: 5′- GGTGAGCGTGCCAGAAAAC -3′, reverse primer: 5′- GTCAGTGATGAGCACGGTAAAG -3′.

3.2. Search strategy

A search of studies about "PCDH19" OR "Protocadherin-19" OR "KIAA1313" AND "Mosaicism" was conducted in PubMed (until December 20, 2022). We included only English articles. A total of 41 potentially relevant records were found and screened, 11 records were cell line or animal studies. Then, 8 records were excluded because of being review articles. We also searched for previous reports of cases with c.1091delC (NM_001184880.2) in *PCDH19*, and 3 records were identified. Finally, 36 articles were selected for studying in detail.

3. Results

In consistence with the phenotype, the proband was detected to be heterozygous for c.1091delC (p.P364Rfs*4) in *PCDH19* (RefSeq accession numbers NM_001184880.2 and NP_001171809.1). The number of alternate alleles/total read depth at this base position was 177/424 reads. This mutation was afterward confirmed by Sanger sequencing, and segregation study was carried out. The proband and her sister were found to be heterozygous for the variant but it was not detected in the parents, therefore it was supposed it could be due to germline mosaicism (Fig. 2). In this variation, a cytosine (C) at coding position 1091 was deleted, and the mutation subsequently resulted in the change of amino acid at position 364 and ending in the fourth amino acid after the change (p.Pro364ArgfsTer4). Indeed, the variant disrupts the open reading frame and creates a premature stop codon in the mRNA of *PCDH19*. This presumably produces degradation of the mRNA in affected cells. According to the guide of The American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015), this gene mutation was considered pathogenic (PVS1 + PP5 + PM2). It was characterized as disease causing in MutationTaster prediction tool (https://www.mutationtaster.org/). It was also reported as pathogenic in ClinVar. At the time of reporting, the variant does not exist in controls from gnomAD Aggregated and has a frequency of <0.01% in ExAC databases.

[Fig. 2 here]

4. Discussion:

With the broad use of next-generation sequencing technologies, numerous genes have been increasingly identified as the etiology of epilepsy. *PCDH19* is the second most common genetic cause of epilepsy (Duszyc et al., 2015; Symonds & McTague, 2020). Pathogenic variants in

PCDH19 lead to developmental and epileptic encephalopathy 9. This disorder has extremely variable clinical manifestations including early onset of different types and frequency of recurrent seizure clusters that are noticeably fever sensitive, mild to profound intellectual disability, autistic characteristics, and behavioral problems (Depienne & Leguern, 2012; Depienne et al., 2011; Higurashi et al., 2015; Perez et al., 2017; Steel, Symonds, Zuberi, & Brunklaus, 2017; M. Trivisano et al., 2016; Van Harssel et al., 2013). The phenotypes of DEE9 and Dravet syndrome (OMIM #607208) can overlap, however *PCDH19*-associated epilepsy has some specific features such as later seizure onset, absence of photosensitivity, raised seizure cluster frequency, and satisfactory response to steroid treatment. Its inheritance is also distinct from the autosomal dominant inheritance in Dravet syndrome, and selectively affects heterozygous females and mosaic hemizygous males (Depienne et al., 2009; Depienne & Leguern, 2012; Samanta, 2020). In DEE9 patients, the neuropsychiatric features in mosaic male patients resemble female phenotypes (De Lange et al., 2017; Kolc et al., 2020; Yang et al., 2020). In addition to the "cellular interference" hypothesis as a key pathogenic mechanism of DEE9 (Depienne et al., 2009; Depienne & Leguern, 2012), the other hypotheses also include decreased GABAA receptor function (Bassani et al., 2018; Serratto et al., 2020), allopregnanolone deficiency in females (Tan et al., 2015; Marina Trivisano et al., 2017), and blood–brain barrier impairment (Higurashi et al., 2015). In order to shed light on the related mechanisms, some animal studies have been done recently. Lim et al. (2019) indicated that hemizygous *Pcdh19* knockout (KO) male mice have autistic traits. So additional studies will help to understand whether both mosaic loss and complete loss of *PCDH19* lead to autism-like phenotypes (Lim, Ryu, Kang, Noh, & Kim, 2019). Furthermore, Rakotomamonjy et al. (2020) investigated seizure susceptibility and progression in the *Pchd19* mouse model. They observed increased susceptibility in *Pcdh19* KO females which proposes

further mechanisms other than cellular interference have a role in *PCDH19*-associated epilepsy. (Rakotomamonjy, Sabetfakhri, McDermott, & Guemez- Gamboa, 2020). The study implemented by Robens et al. (2022) in mosaic and non-mosaic *pcdh19* mutant zebrafish challenged the theory that mosaicism is responsible for all *PCDH19*-related phenotypes. They suggested interneuron-mediated mechanisms govern these phenotypes (Robens et al., 2022). Despite these experiments, the exact mechanisms underlying the disorder are still unclear. Hence, additional future studies are required for understanding and clarifying the involved mechanisms.

In the pedigree depicted in figure 1, there are multiple members with childhood febrile seizures that lasted only up to 5 or 7 years of age without any medications and all of them had normal development. The proband's father also had this kind of seizures, but the variant detected in the proband was absent in her father. Therefore, it seems that there are other seizure susceptibility loci in this family and further genetic studies should be performed in these individuals to identify these loci.

More than 270 variants of *PCDH19* have been identified in female patients (Chen et al., 2022). The present study reported a frameshift mutation (c.1091delC, p.P364Rfs*4) in exon 1 of *PCDH19* in two affected sisters, which occurred as a germline mosaicism. Among all exons in *PCDH19,* exon 1 includes the most numbers of pathogenic variants (Kolc et al., 2019; Wolverton & Lalande, 2001), and also has more pathogenic variants per base (5% per base) (Kolc et al., 2019). This suggests mutation in the extracellular domains of protocadherin-19 is poorly tolerated. Several studies in the *PCDH19* revealed that epilepsy onset time, mosaicism proportion, mutation types, and the affected domains are involved in the severity of encephalopathy (De Lange et al., 2017; Depienne et al., 2009; Kolc et al., 2019; Shibata, Ishii, Goto, & Hirose, 2021). In this study, the detected frameshift mutation affects a highly conserved amino acid residue (PhastCons ≈ 1) in

exon 1 at the extracellular cadherin 4 domain (UniProtKB- Q8TAB3), which contributes to calcium-dependent cell–cell interaction (Fig. 3) (Depienne & Leguern, 2012; Niazi et al., 2019).

[Fig. 3 here]

This variant had been previously reported in four female patients with epilepsy and neuropsychiatric manifestations (Table 1). Our two cases, like the majority of DEE9 cases had normal development prior to seizure onset and therefore speech delay/impairment, intellectual disability and neuropsychiatric symptoms observed after seizure onset. The 6 patients summarized in Table 1, share some clinical characteristics including early-onset seizures (5−18 months of age), and seizure clusters. In our cases (2 of 6 cases), the variant occurred as a germline mosaicism and in one case as a *de novo* mutation, the remaining cases had paternal inheritance. In all patients, intellectual disability was detected, except for Subject 6. Behavioral/psychiatric symptoms were found in 5 of 6 patients. Subject 5 had autistic features but it was not observed in her sister with the same mutation. Moreover, in spite of taking appropriate doses of ASMs, the subjects 1 and 5 had refractory seizures, but their sisters carrying the same mutation were seizure free for 1.5 years and 7 years respectively. In our 2 cases, although one sister had febrile seizures but the other did not experience any febrile seizures. Although dysmorphism and malformation are not commonly associated with *PCDH19* (Depienne et al., 2011), we observed them in our proband. Indeed, dysmorphic features were only reported in our proband, but not in her sister and other reported cases with c.1091delC mutation. In addition to the intra-familial phenotypic heterogeneity detected in our 2 patients, it also observed in subjects 5 and 6 who are dizygotic twin. Subjects 5 and 6 differ in some features including age at seizure onset, seizure type, behavioral/psychiatric problems, intellectual disability, and response to ASMs (A Liu et al., 2017). So, females with *PCDH19* mutations display extensive intra- and inter-familial phenotypic variability. This

13

diversity may be explained by different X-inactivation patterns in brain, genetic modifiers or mutation mosaicism in the brain of patients. This makes it difficult to predict the clinical course for unborn or young children (Scheffer et al., 2008). Females with heterozygous *PCDH19* mutation have incomplete penetrance (approximately 90%) (Aijie Liu et al., 2019). Although it is expected that epigenetic mechanisms particularly skewed X- inactivation have a pivotal role, but there is little data about the effect of X-chromosome inactivation on the penetrance of DEE9 (Hung et al., 2021).

There are also some other reports of *PCDH19* mutation recurrence in a family. Dibbens et al. (2011) reported two unrelated families with two affected sisters. They demonstrated that germline mosaicism of a *PCDH19* mutation in a parent is a crucial mechanism associated with the EFMR inheritance (Dibbens et al., 2011). Furthermore, mosaicism of *PCDH19* mutations have been founded in mildly affected or even unaffected mothers (Dibbens et al., 2011; Terracciano et al., 2012). This can be clarified by the random X-inactivation or the amount of mutant against wild-type protocadherin 19. Interestingly, Liu et al. (2017) reported an asymptomatic mosaic father with two affected girls (A Liu et al., 2017). *De novo* mutations can be transmitted from mosaic parents (Xu et al., 2015). With the consideration of asymptomatic mosaic fathers, it is proposed that the frequency of parental mosaicism is underestimated. Stosser et al. (2018) conducted a study for investigating the frequency of mosaicism identified by next-generation sequencing in genes related to epilepsy-associated neurodevelopmental disorders. Their results showed *PCDH19* and *CDKL5* had the highest frequency of mosaicism for a pathogenic or likely pathogenic variant (Stosser et al., 2018). Nevertheless, because of the technical limitations, mutations in mosaic parents could remain undiagnosed. Therefore, when there is a mutation in such genes in a patient, we recommend that genetic testing and PND should be considered for the next pregnancies of the family.

14

Negi et al. (2023) conducted a study to investigate the genetic explanation of Diphtheria, Tetanus, and whole-cell Pertussis (DTwP) vaccination-associated seizures or subsequent epilepsies. They performed genetic testing on 54 children with DTwP vaccination-associated seizures. Their study revealed 33 pathogenic variants in 12 genes, of which one variant has been detected in the *PCDH19* gene (Negi et al., 2023). Whole-cell pertussis (wP) vaccination is also used in Iran, increases the probability of vaccine-associated seizure and encephalopathy in individuals with a favorable genetic background. Moreover, in one of our cases with *PCDH19* mutation, seizures started following DPwT vaccination at age 6 months. Hence, regarding these issues and also the importance of diagnosis, prognosis, and potential individualized treatment, we recommend the use of next-generation sequencing in any child with vaccine-associated seizures. We also recommend educating healthcare professionals about the DPT vaccination's adverse effects.

There is no specific pharmacological treatment for *PCDH19*-associated epilepsy (Moncayo et al., 2022). Therefore, a better understanding of the pathogenic mechanisms underlying DEE9 would pave the way for developing mechanism-based approaches. For optimizing these precision therapies, an important step is having more knowledge about *PCDH19* genetic variants and the biological processes disrupted by these mutations (Dell'Isola et al., 2022). For example, if the inhibitory neurons are reduced, modifying GABAergic activity would be an effective way of therapy. With precision therapies, there is hope that the prognosis of patients will improve in the future. Moreover, there is an immense need for performing research on novel advanced precision medicine approaches including different strategies of gene therapy. These novel approaches will have the potential to provide effective treatment for this rare genetic disorder in the future (Bertocchi, Cambiaghi, & Hasan, 2023).

In genetic epilepsies such as DEEs, diagnosis of a specific genetic defect could have multiple benefits for the patients and their families. For the patient, confirmation of a molecular etiology can be helpful in choosing the most appropriate treatment. In specific types of DEEs, there are targeted therapies that can improve seizure burden and neurodevelopmental outcomes in the affected children. In addition, molecular diagnosis will also impact on the genetic counseling of the patients and families and make them aware of the prognosis, recurrence risk, reproductive options, and prenatal diagnosis. Furthermore, a specific genetic diagnosis provides peace of mind and reduces the need for further diagnostic tests (Syrbe, 2022). We recommend considering genetic testing in the care of children with DEEs as a routine diagnostic approach.

Conclusion:

It is the first report of germline mosaicism in *PCDH19* gene in the Iranian population. Our study expanded the phenotypic spectrum of *PCDH19*-related developmental and epileptic encephalopathy (DEE9). Genetic testing has become an effective way of determining the diagnosis. Parental germline mosaicism should be taken into account when providing genetic counseling for X-linked/autosomal dominant disorders. This report provides an emphasis on the importance of considering PND in such cases.

[Table 1 here]

Highlights

- *PCDH19* is the second most common genetic cause of epilepsy.

- Developmental and epileptic encephalopathy 9, caused by *PCDH19* pathogenic variants, has extremely variable clinical manifestations.

- Parental germline mosaicism should be taken into account when providing genetic counseling for X-linked/autosomal dominant disorders.

- It is important to consider prenatal diagnosis (PND) in such cases.

Plain Language Summary

Genes are units of heredity which are transferred from a parent to offspring, and have instructions that our bodies use for many functions such as the control of growth and development. Developmental and epileptic encephalopathy 9 (DEE9) is a disorder that results from a change in a gene called *PCDH19*. It is speculated that this gene may have important roles during the early stages of brain development. Therefore, patients have early-onset seizures and may have intellectual disabilities, autistic characteristics, and behavioral problems. As genetic testing has become an effective way of determining diagnosis, so genetic counseling and appropriate test should be considered. Awareness of this disease and its genetic cause would have benefits for the patients and their families in different ways, such as treatment management or reproductive decisions.

Declarations

Compliance with ethical guidelines All ethical principles are considered in this article.

Consent for publication The patients' parents had written informed consent to publish this information.

Availability of data and materials The data are available on request from the first author.

Competing interests The authors declare no conflict of interest.

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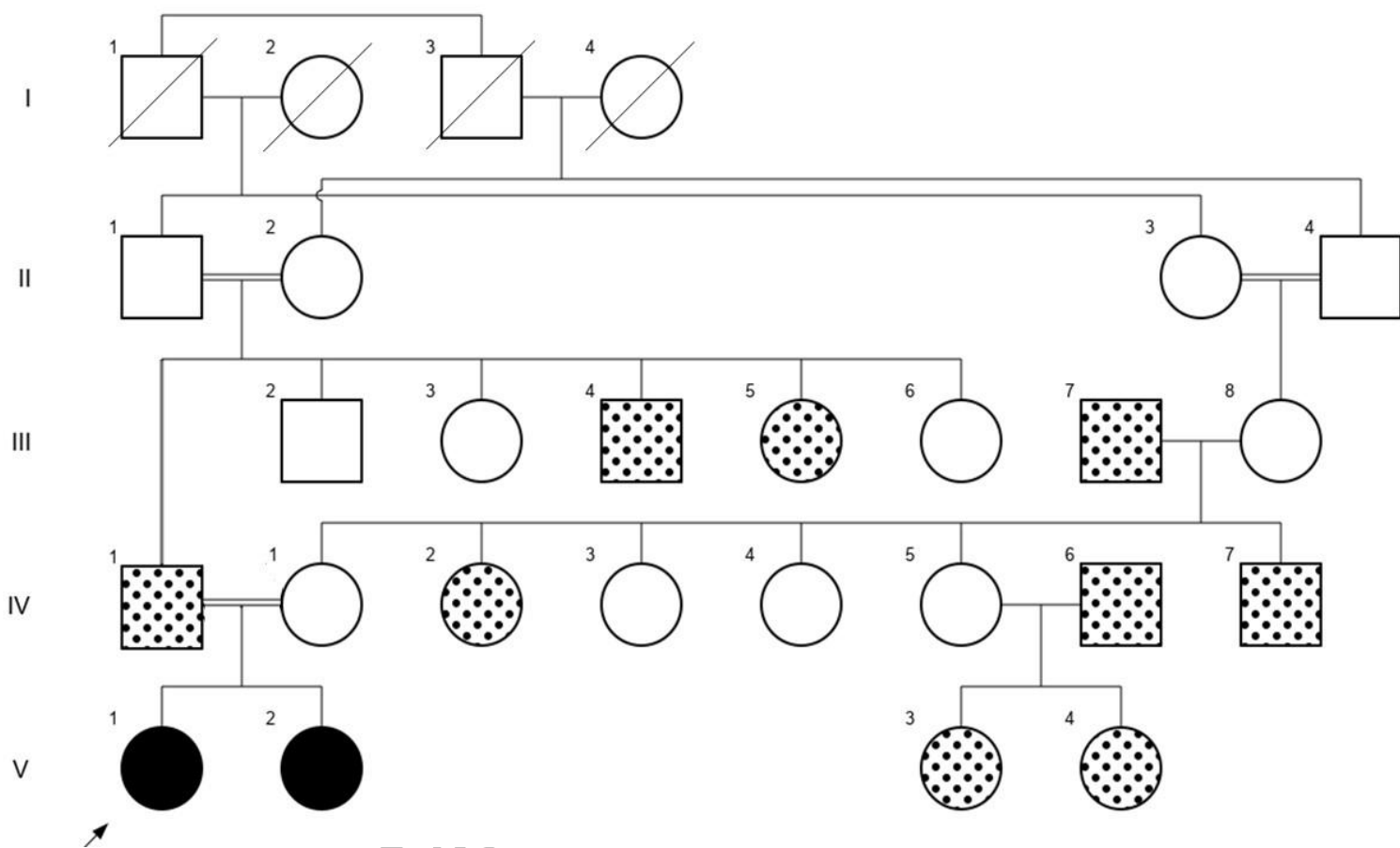
Fig. 1 Pedigree of the family. Black filled symbols represent members with DEE9; dotted filled symbols represent members with febrile seizures only up to 5 or 7 years of age while having normal development.
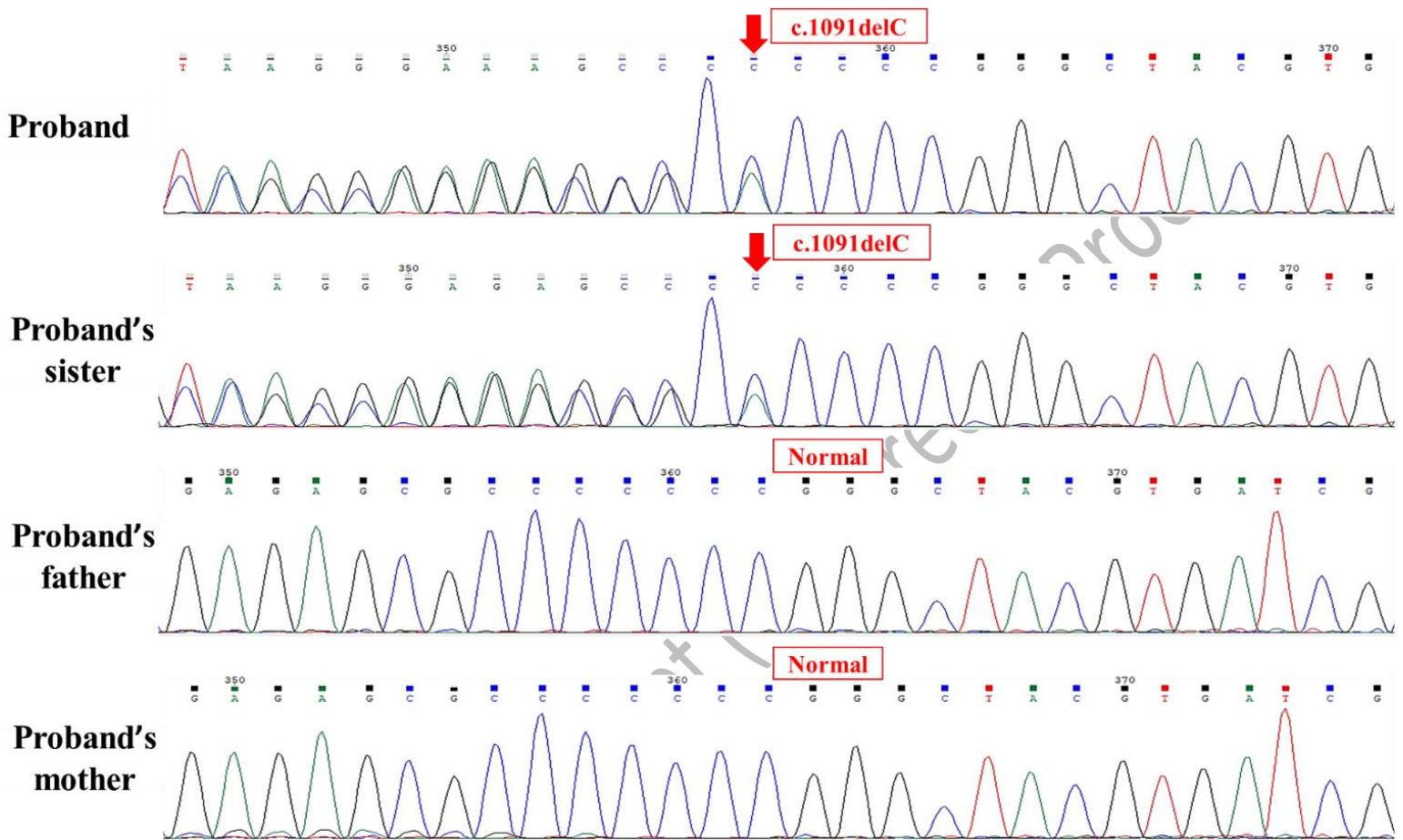
Fig. 2 Sanger sequencing chromatograms. Sanger confirmed the heterozygous variant c.1091delC (p.P364Rfs*4) in exon 1 of *PCDH19* (NM_001184880.2) in the proband and her sister. The variant was not found in the parents. The red arrows show the location of the identified mutation. Sequences were generated using a reverse primer (GTCAGTGATGAGCACGGTAAAG).

a

| Exon 1 | 2 | 3 | 4 | 5 | Exon 6 |

NH2 SP EC1 EC2 EC3 EC4 EC5 EC6 TM CM1 CM2 COOH

1 23 129 130 238 239 346 350 453 454 563 569 672 679 699 906 923 957 973 1148

p.P364Rfs*4

b

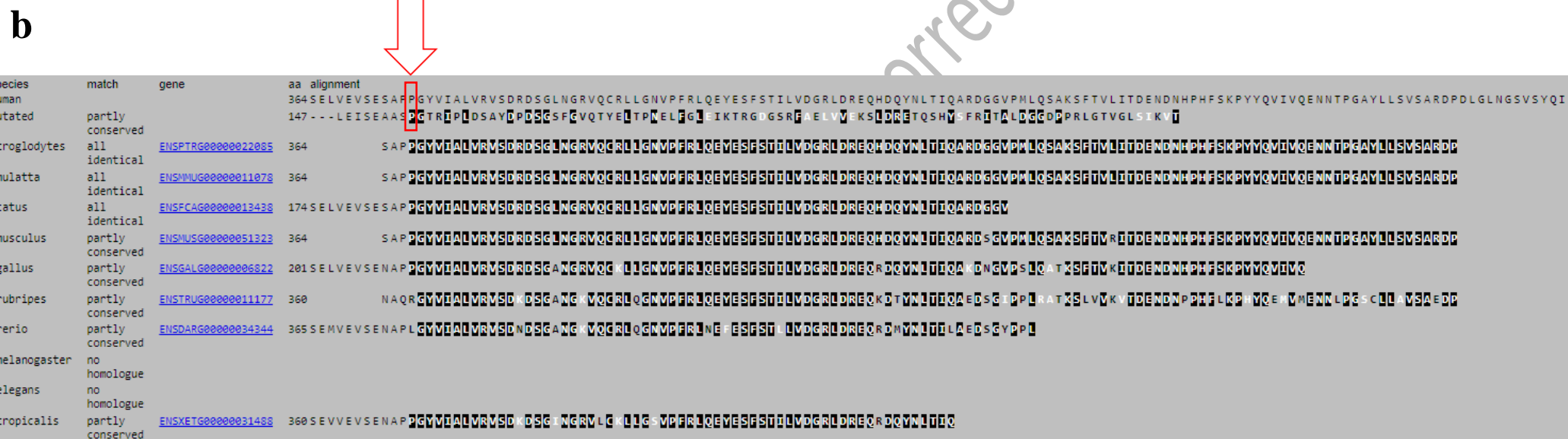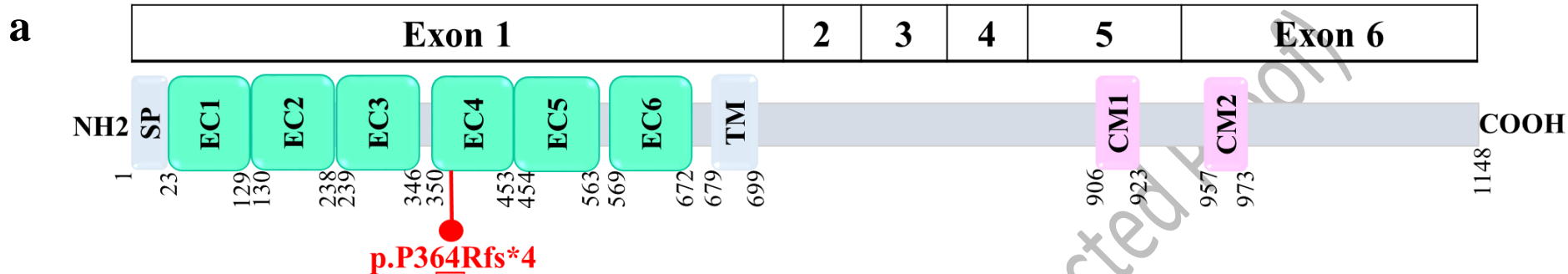| species	match	gene	aa	alignment
Human			364	SELVEVSESAPPGYVIALVRVSDRDSGLNGRVQCRLLGNVPFRLQEYESFSTILVDGRLDREQHDQYNLTIQARDGGVPMLQSAKSFTVLITDENDNHPHFSKPYYQVIVQENNTPGAYLLSVSARDPDLGLNGSVSYQI
mutated	partly conserved		147	---LEISEAASPGTRIPLDSAYDPDSGSFGVQTYELTPKELFGLEIKTRGDGSRFAELVVEKSLDRETQSHYSFRITALDGGDPPRLGTVGLSIKYT
Ptroglodytes	all identical	ENSPTRG00000022085	364	SAPPGYVIALVRVSDRDSGLNGRVQCRLLGNVPFRLQEYESFSTILVDGRLDREQHDQYNLTIQARDGGVPMLQSAXSFTVLITDENDNHPHFSKPYYQVIVQENNTPGAYLLSVSARDP
Mmulatta	all identical	ENSMMUG00000011078	364	SAPPGYVIALVRVSDRDSGLNGRVQCRLLGNVPFRLQEYESFSTILVDGRLDREQHDQYNLTIQARDGGVPMLQSAXSFTVLITDENDNIPHFSKPYYQVIVQENNTPGAYLLSVSARDP
Fcatus	all identical	ENSFCAG00000013438	174	SELVEVSESAPPGYVIALVRVSDRDSGLNGRVQCRLLGNVPFRLQEYESFSTILVDGRLDREQHDQYNLTIQARDGGV
Mmusculus	partly conserved	ENSMUSG00000051323	364	SAPPGYVIALVRVSDRDSGLNGRVQCRLLGNVPFRLQEYESFSTILVDGRLDREQHDQYNLTIQARDSGVPMLQSAXSFTVRITDENDNHPHFSKPYYQVIVQENNTPGAYLLSVSARDP
Ggallus	partly conserved	ENSGALG00000006822	201	SELVEVSENAPPGYVIALVRVSDRDSGANGRVQCKLLGNVPFRLQEYESFSTILVDGRLDREQRDQYNLTIQAKDNGVPSLQATXSFTVKITDENDNHPHFSKPYYQVIVQ
Trubripes	partly conserved	ENSTRUG00000011177	360	NAQRGYVIALVRVSDKDSGANGKVQCRLQGNVPFRLQEYESFSTILVDGRLDREQKDTYNLTIQAEDSGIPPLRATXSLVYKVTDENDNPPHFLKPHYQEIVMENNLPGSCLLIVSAEDP
Drerio	partly conserved	ENSDARG00000034344	365	SEMVEVSENAPLGYVIALVRVSDNDSGANGKVQCRLQGNVPFRLNEFESFSTILVDGRLDREQRDMYNITILAEDSGYPPL
Dmelanogaster	no homologue			
Celegans	no homologue			
Xtropicalis	partly conserved	ENSXETG00000031488	360	SEVVEVSENAPPGYVIALVRVSDKDSGINGRVLCKLLGSVPFRLQEYESFSTILVDGRLDREQRDQYNLTIQ

Fig. 3 a Schematic representation of the frameshift mutation identified in *PCDH19* gene. Top bar indicates exon structure of NM_001184880.2. Second bar represents its corresponding protein domains. The frameshift mutation is shown in red. SP: signal peptide; EC: extracellular cadherin domain; TM: transmembrane domain; CM1 and CM2; cytoplasmic domains 1 and 2. **b** Partial sequence alignment of *PCDH19* orthologs is depicted. The frameshift mutation caused by c.1091delC in *PCDH19* leads to a completely different amino acid sequence after p.P364. Orthologs of *PCDH19* show a high degree of conservation of the Proline 364 residue. Identical residues are shown in black. The location of amino acid changes in human is shown in red box. (https://www.mutationtaster.org/)

Table 1. Clinical characteristics of patients with c.1091delC mutation in *PCDH19*.

(NM_001184880.2, c.1091delC; NP_001171809.1, p.P364Rfs*4; rsID, rs758946412; GRCh37/hg19 Genomic Coordinate, chrX-99662511-G-).

	This study		Van Harssel et al. (2013) (Van Harssel et al., 2013)	Zhang et al. (2015) (Zhang et al., 2015)	Liu et al. (2017) (A Liu et al., 2017)	
Subjects ID	Subject 1*	Subject 2	Subject 3	Subject 5	Subject 5	Subject 6*
Sex	F	F	F	F	F	F
Age at study (years)	5	2.5	16	9	11	11
Age at seizures onset (months)	5.5	6	12	5	5	18
Transmission	Germline mosaicism	Germline mosaicism	De novo	Paternal inheritance	Paternal inheritance	Paternal inheritance
Seizure type (at onset & later)	Onset: tonic seizures with lateral gaze Later: tonic seizures with lateral gaze, tonic-clonic seizures; and myoclonic seizures	Tonic seizures with lateral gaze	Onset: febrile, tonic seizures Later: febrile, tonic, tonic–clonic, myoclonic seizures, complex partial/(atypical) absences, clusters	Partial seizures	Focal Seizures	Generalized tonic–clonic seizures
Seizure clusters	+	+	+	N/A	+	+
Febrile seizures	−	+	+	N/A	+	+
Development prior to seizure onset	Normal	Normal	N/A	N/A	N/A	N/A
Motor delay or impairment	−	−	N/A	N/A	N/A	N/A
Speech delay or impairment	+ (Unable to speak)	+ (can say only a few words)	N/A	N/A	N/A	N/A
Intellectual disability	+ (Moderate)	+ (mild)	+ (Moderate)	+ (Severe ID/DD)	+	−
Behavioral/Psychiatric problems	+ (irritability, aggression, excessive crying, screaming, attentional problems & autistic features)	+ (autistic features)	+ (autistic features)	N/A	+ (aggression, autism)	+ (aggression, attention deficit)
Dysmorphisms	Hypertelorism, hypoplastic midface	−	N/A	N/A	N/A	N/A
EEG	Normal background without any asymmetry between hemispheres and some IEDs as right temporal spikes during tracing	Normal background without any asymmetry between hemispheres and some IEDs as spikes in the occipital regions bilaterally during tracing	N/A	N/A	N/A	N/A

Imaging findings	Normal Brain MRI	Normal Brain CT scan	Normal MRI	N/A	N/A	N/A
Previously used ASMs	Sodium valproate, Nitrazepam, Carbamazepine, Levetiracetam, Primidone, Topiramate, and Phenobarbital	Levetiracetam, Phenobarbital, Clobazam, Topiramate & Phenytoin	N/A	N/A	Sodium valproate, Topiramate,	Lamotrigine, Levetiracetam
Current ASMs	Topiramate & Lacosamide	Levetiracetam, Oxcarbazepine, & Clobazam	N/A	N/A	Clonazepam, Lamotrigine, Levetiracetam	Sodium valproate
Persistence of seizures in spite of treatment	+ (Refractory to ASMs)	− (seizure free for 1.5 years)	+	N/A	+ (yearly clusters)	− (seizure free for 7 years)
Tissue sample DNA	Peripheral blood	Peripheral blood	Whole blood cells	Peripheral leukocytes	Peripheral blood lymphocytes	Peripheral blood lymphocytes
Diagnostic genetic test	Whole-exome sequencing & Sanger sequencing	Only confirmation by Sanger sequencing	Sanger sequencing, MLPA & Multiplex Amplicon Quantification (MAQ)	Targeted next-generation sequencing & Sanger sequencing	Sanger sequencing & MLPA	Sanger sequencing & MLPA

* Subject 1 is the elder sister of subject 2; Subject 6 is the dizygotic twin sister of subject 5.

ASMs, anti-seizure medications; N/A, not available; EEG, electroencephalography; MRI, magnetic resonance imaging; IEDs, interictal epileptiform discharges; CT scan, computed tomography scan; MLPA, multiplex ligation-dependent probe amplification; ID/DD, intellectual/developmental disabilities; F, female; Het, heterozygous; +, presence; −, absence.