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Short communication

PCDH19 mutations in female patients from Southern Italy

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ABSTRACT

Purpose: Mutations in PCDH19, encoding protocadherin 19 on chromosome X, cause familial epilepsy and mental retardation limited to females or Dravet-like syndrome. We wished to explore the causative role of PCDH19 gene (Xq22) in female patients with epilepsy, from Southern Italy.
Methods: Direct sequencing of PCDH19 gene was conducted in 31 unrelated female patients with early onset (<1 year of age) epilepsy and a wide spectrum of phenotypes including febrile seizures, focal and generalized forms, with either sporadic or familial distribution.
Results: We identified two de novo heterozygous novel mutations of PCDH19 gene (p.Arg550Pro, Ile508ProfsX59) in two of 31 unrelated female patients. We also identified a novel silent mutation p.Ser856=.
Conclusions: The present findings confirm that PCDH19 is a major causative gene for infantile onset familial or sporadic epilepsy in female patients with or without mental retardation.

1. Introduction

Epilepsy and mental retardation limited to female (EFMR, MIM# 300088) is an X-linked disorder due to mutations in the PCDH19 gene, located on chromosome X and encoding the protocadherin 19. This disorder presents an unusual mode of inheritance as only females with heterozygous mutations are affected whereas males with hemizygous mutations are unaffected.[1–4] A plausible explanation of this peculiar pattern of inheritance is that the pathogenic mechanism underlying PCDH19 mutations results from cellular interference, so only subjects with a combination of PCDH19-negative and PCDH19 wild-type cells develop symptoms.[5] This mechanism of cellular interference is supported by the presence of affected mosaic males.

The protocadherin19 is a transmembrane protein that belongs to the cadherin family of calcium-dependent-cell adhesion molecules.[1,6] Most PCDH19 mutations are clustered in exon 1 probably because it is the biggest exon, corresponding to the extracellular domain,[1,5,7–12] but other exons may be also involved.[10] Missense and truncating mutations have been reported as well as deletions at the PCDH19 gene.[8,10,13] Most patients with PCDH19 mutations are sporadic or belong to families with few affected female patients, making the recognition of the inheritance pattern difficult. Moreover, whole gene deletions or partial deletion of PCDH19 gene were found in sporadic female patients,[10] suggesting that submicroscopic chromosomal abnormalities should be also investigated in patients negative for PCDH19 mutations.

The aim of the present study was to identify PCDH19 mutations in a series of 31 female patients with early onset (<1 year of age) epilepsy from Southern Italy. Multiplex ligation-dependent probe amplification (MLPA) was also applied to examine whether microdeletions and duplications of the PCDH19 gene may occur in such patients that were negative for PCDH19 mutations.

2. Subjects

We collected 31 Italian females with a wide spectrum of phenotypes including mainly DS patients, with febrile or afebrile

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Table 1
Summary of the clinical and genetic data of three female patients carrying de novo PCDH19 mutations.

Patient no.	Age at exam	Age at onset	Type of the first seizure	Type of seizures	SE	SZ in cluster	Pho sen.	Language	Intellectual disability	PCDH19 de novo mutations
1	8y	7 m	FS	Febrile and afebrile sGTCS Absences	Yes	Yes	No	Normal	Severe with autistic features	Ile508ProfsX59
2	9y	8 m	FS	Febrile and afebrile sGTCS Absences	Yes	Yes	No	Normal	Severe with autistic features	Arg550Prof
3	9y	9 m	FS	sGTCS Absences	Yes	Yes	No	Normal	Severe with autistic features	Ser856=

y = years; m = months; FS: febrile seizure; sGTCS = secondarily generalized tonic clonic seizures.

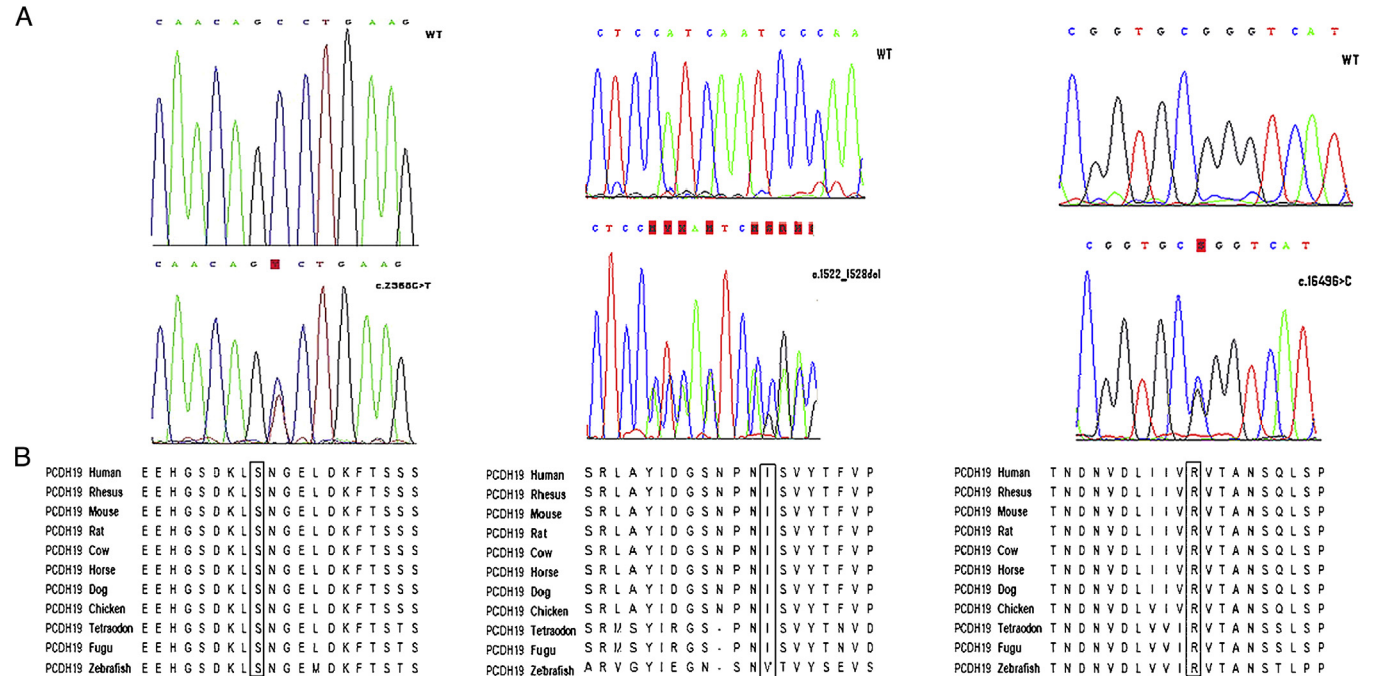

Fig. 1. Three different heterozygous de novo novel variants of PCDH19 gene. (A) Sequence electropherograms of the mutations identified (c.2568C > T/p.Ser856=, c.1522_1528del/p.Ile508ProfsX59, c.1649G > C/p.Arg550Pro). (B) Alignment of the regions surrounding the mutations (indicated in the box) in orthologous and paralogous proteins, showing the high conservation of each affected amino acid in vertebrates and in the delta protocadherin paralogous genes.

seizures, normal MRI, and with or without mental retardation. All patients had an age at onset of epilepsy during the first year of life. The local Ethics Committee approved the study, and written informed consent was obtained from all individuals involved.

3. Methods

Genomic DNA was extracted from peripheral blood by standard methods. The presence of mutations of the SCN1A gene was previously excluded by sequencing analysis and MLPA method (SALSA MLPA P137-B2 SCN1A probemix). The primers flanked all 5 exons and intron–exon boundaries of PCDH19 (Entrez Gene, Gene ID: 57526, Accession Number: EF676096.1) were designed using the web primer SGD (Saccharomyces Genome Database). The purified PCR products were sequenced on an ABI 3130 XL AVANT sequencer (Applied Biosystems and Life Technologies, Carlibad, CA, USA). The new identified PCDH19 mutations were checked in a control population of 200 ethnically matched controls.

We used the Exome Variant Server (http://evs.gs.washington.edu/EVS/) to ascertain that the PCDH19 identified was not present in variants databases. We assessed amino acid conservation in orthologs using UCSC (http://genome.ucsc.edu). The pathogenic potential of the identified variant was predicted using Mutation Taster Server (http://www.mutationtaster.org).

In the present study, MLPA analysis was also performed to detect PCDH19 rearrangements. The probes used for MLPA reaction were designed to hybridize to each region of the exons.

4. Results

A total of three different variants, all novels, were identified at the heterozygous state in 3 out of 31 (9.6%) female patients (Table 1, Fig. 1). Two heterozygous mutations consisted of a frameshift mutation p.Ile508ProfsX59 (c.1522_1528delATCAATC) and a missense mutation p.Arg550Pro (c.1649G > A) respectively, which were located in the large exon 1. In the third patient, we identified a novel silent mutation p.Ser856 = (c.2568C > T) that was located in the exon 4. The corresponding mutations were absent in both parents of all three affected members, indicating that they always arose de novo. Both missense variants affected amino acids of the extracellular domain of protocadherin 19. All the three novel variants occurred at reasonably conserved amino acid (Fig. 1). The identified PCDH19 alterations were not found in a control population of 200 healthy Italian individuals.

5. Discussion

The results of this study indicate that PCDH19 mutations are a relatively frequent cause of epilepsy in female patients from Southern Italy (6.4% (2/31) carried PCDH19 mutations) with early onset epilepsy before one year of age. This relatively high rate of PCDH19 mutations probably comes from the analysis of a particular and rather specific phenotype where the likelihood of finding PCDH19 mutation is higher than just selecting female patients with early onset epilepsy and with a broader phenotype.

In all three female patients, the clinical spectrum associated with PCDH19 mutations was severe with early onset seizures, which generally occurred in cluster and were especially likely to occur with fever. Of note, these clinical features are very similar to those of previously reported patients carrying PCDH19 mutations: clusters of seizures during febrile illnesses. Conversely, they differed slightly from classical DS phenotype, as none of them have myoclonic jerks/atypical absences.

Cadherins play a crucial role in neuronal cell adhesion. In fact, the Arg550Pro and Ile508ProfsX59 mutations identified in this study fall in the large exon 1 of the gene, corresponding to the extracellular cadherin domains of protocadherins that are crucial for normal function. These mutations affect residues belonging to conserved calcium binding activity or alter the PCDH19 extracellular structure. Amino acid substitutions in the cytoplasmic domain appear to have less or no deleterious effects on protein function. The novel heterozygous silent mutation, p.Ser856=, was located in exon 4. In exons 3–5 a relatively small number of only truncating mutations are found. In the literature, indeed, four distinct mutations in the exon 4 (framshift, nonsense, and splicing site mutation) were already reported.[7–9] The exon 4 encodes to the intracellular cadherin domains of the PCDH19 protein. It interacts with cytoskeleton and with signal transmission pathway. Although this alteration is not located in on EC domain, the cytoplasmic domains of protocadherins do show a high level of conservation.[7]

Previous studies claimed that chromosomal rearrangements may occur, so screening of PCDH19 should always include a specific method to search for rearrangements in addition to direct sequencing of the whole coding sequence. Nonetheless, we failed to depict chromosomal rearrangements involving the PCDH19 gene in our series.

These two novel mutations are damaging according to prediction program (Mutation Taster) and suggest a loss of function of the protein in both cases. The affected amino acids are extremely conserved in the evolution of the PCDH19 homologues, suggesting an important functional role.

In summary, we have identified two novel mutations in the PCDH19 gene further confirming that it is a major causative gene for infantile-onset familial or sporadic epilepsy in female patients with or without mental retardation. Genotype–phenotype correlation was largely consistent with the previous studies, a molecular diagnosis results important for patient's management and genetic counseling.

Conflicts of interest

The authors declare no financial or other conflict of interests.

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