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A Male Child with Infantile Epilepsy due to a Mosaic Missense Variant of PCDH19

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Abstract
Background: Pathogenic variants of PCDH19, located on the X-chromosome (Xq22.1), cause a rare epileptic encephalopathy with speech and development delay, seizures, behavioral and psychiatric problems. The specific underlying pathogenic mechanism is known as "cellular interference" that results in affected heterozygous females, normal hemizygous males and affected mosaic males but its functioning is not yet clear. ***Objectives:*** Reporting new cases of affected males is considered useful to a deeper insight. ***Subject and Method:*** We present the case of a three-year-old boy with early-onset seizures at 3 months of age, mild cognitive impairment, partial control of seizures with levetiracetam, normal brain imaging. ***Results:*** The patient has a mosaic pathogenic variant c.698A>G (p.Asp233Gly) in PCDH19 assessed by Next Generation Sequencing analysis. We have compared his characteristics with the genotypes and phenotypes of 34 PCDH19 mosaic males earlier reported in the literature. Finally, we have summarized today's knowledge about phenotype-genotype correlation and pharmacological response in these patients. ***Conclusions:*** Our report confirms that the clinical picture of mosaic affected males, resembling that of females, can show a wide variability in severity of disease and underlines a stringent need to improve therapeutic approaches and to collect data on long-term follow-up.

Introduction

Developmental and epileptic encephalopathies are characterized by early-onset seizures (often refractory to antiseizure medications [ASMs]), electroencephalographic abnormalities and developmental delay/regression or intellectual disability (ID). Genetic heterogeneity of epileptic disorders is broad, with a high number of underlying pathogenic mutations in many distinct genes [1] including PCDH19 [2]. According to Human Gene Mutation Database (HGMD), about 300 pathogenic variants in this gene cause epilepsy that

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usually starts in the first months of life, defined Infantile Epilepsy Type 9 (OMIM #300460), previously known as "Epilepsy and mental retardation limited to females" (EIEE 9, OMIM #300088), which develops only in a peculiar "mosaic" cellular condition. PCDH19 is located on the X-chromosome (Xq22.1), consists of six exons, and encodes an 1,148 amino acid transmembrane protein called Protocadherin-19 [3].

The first exon encodes the extracellular, the transmembrane, and a part of the intracellular domain, while exons 2–6 encode the remaining intracellular part of the protein [4]. PCDH19 protein is a calcium-dependent adhesion molecule belonging to the δ2-protocadherin subclass and is expressed in developing human and mouse central nervous system, mainly in the hippocampus and cortex. It contributes to neuronal development and to the maintenance of the optical tectum architecture in zebrafish. Its extracellular domain has a role in cellular interaction, and its absence leads to the loss of cell-cell cohesion and an aberrant cell proliferation rate. Moreover, PCDH19 modulates signaling pathways inside the neurons and it is important for dendrite morphology and neuronal migration. Its intracytoplasmic region binds the GABAAR alpha subunits regulating the receptor surface availability that is crucial in balancing excitatory and inhibitory transmission in neuronal circuits [5].

Its variants are primarily located in the first exon of the gene and only a few specific variants are recurrent [6].

Subject and Methods

We report a 3-year-old boy with epilepsy and mild ID. He was born at term by spontaneous delivery after an uneventful pregnancy, obtained with assisted reproductive technology. Meconium-stained amniotic fluid was noted during labor. Auxological parameters at birth were: weight 3,280 g (33rd centile), length 52 cm (75th centile). He suffered from temporary respiratory distress from meconium aspiration. Development milestones were normal: the sitting position was achieved at about 6–7 months, independent walking at 18 months, babbling at 6–7 months, first words at about 12 months. At 3 months of age, the patient presented the first hypertonic crisis. At onset, these episodes were monthly, disappeared between the 7th and the 10th month of age, and started again at about 1 year with a different presentation (clusters of 2–3 episodes/day). Therapy with levetiracetam was undertaken at 2 years of age with a good response for 2 months, then the crisis started again. For this reason, he was hospitalized and the dosage of ASM was increased. At the last evaluation at 3 years, he still had few episodes of cluster seizures. The neuropsychiatric evaluation showed a mild cognitive impairment and a medium impairment in the linguistic/communicative, affective/relational area, and in the area of personal and social autonomy. Cerebral MRI was normal. Polygraphic EEG recordings showed a wake and sleep pattern without epileptiform anomalies and no recordings of a clear critical nature. When he was 2 years and 10 months old, the patient was 92 cm of height (10–25th centile), weighed 17 kg (75–90th centile), and his OFC was 50 cm (25–50th centile). No dysmorphological features were noticed. Next-generation sequencing analysis, through a multi-gene panel of 43 epilepsy-associated genes, identified a missense variant, namely c.698A>G (p.Asp233Gly), in the first exon of PCDH19 (NM_001184880). The variant was in a mosaic state with a variant allelic frequency (VAF) of 80% in a peripheral blood sample. No other tissues were available. Sanger sequencing confirmed the presence of the PCDH19 mosaic variant and excluded the variant on maternal DNA from a peripheral blood sample.

Discussion

The first family with epilepsy and ID linked to the X chromosome with an atypical X-linked inheritance was described about 50 years ago. In this family, there were affected females and unaffected "transmitting" males [7]. PCDH19 pathogenic variants were later identified as the cause of this condition [8]. This unusual clinical expression was explained considering that, in mutated females, the random X-inactivation produces tissue mosaicism with the presence of 2 cell populations, one expressing wild-type and the other one the mutated allele. It has been hypothesized that the copresence of cell populations expressing normal and mutated PCDH19 alleles would be pathogenic because this may interfere with cell-cell communication. This was called "cellular interference" but the precise pathogenic mechanism is not clearly understood yet [8].

There usually is no pathogenic effect if a homogeneous cell population, either wild-type or mutated, is present, as it happens in males with a hemizygous PCDH19 variant where neurons with the normal protein are absent. In mosaic males, however, there are 2 cell populations and, according to this hypothesis, they are expected to be affected. The clinical features of PCDH19-mutated females have been thoroughly described. Their phenotype includes seizures of different type, that usually occur in

Table 1. Summary of phenotypic and genetic data of our case and of total reported affected males

	Our case	35 cases including our one
Development before seizures onset	Normal	Usually normal
Age at seizures onset, months	3	Within 3 and 96 (median 8)
Seizures types	Tonic	Focal 23, GTCS 11, tonic 10, ms 5, clonic 2, abs 2, atypical abs 1, atonic 1
Triggered by fever	No	20/28 (71%)
Cluster occurrence	Yes	1
Status epilepticus	No	8/35 (23%)
D.D./I.D.	Yes	24 ID (mild 7, moderate 6, moderate-severe 2 severe 6, severity rate N.R. 3), borderline 5, normal 6
Psychiatric problems/behavioural disturbance	No	18/33 (54%)
Effective AEDs	No	Data available of 28/35 patients: VPA 11/25, LEV 10/15, CLB 4/10, CLN 3/6, PHB 4/6, OXC 3/14, ZNS 1/2, CPZ 1/6, TPM 2/16
Seizures free for ≥1 year before last follow up	No	5/31 (16%)
Age at last follow up	2 y + 10 m	From 1 y + 11 m to 25 y (mean 6 y, median 6 y, 5 m)
Interictical epileptic discharges (EEG)	Normal	Abnormal in 20/27 (74%)
Cerebral MRI	Normal	Abnormal in 3/17 (18%)
Types of PCDH19 variant	Missense	Missense 15, Truncating 18, Splice site variant 1, Whole gene deletion 1
% mutated cells	80% (blood)	From 0–100% in blood or other tissues

AEDs, anti-epileptic-drugs; CLB, clobazam; CLN, clonazepam; D.D., developmental delay; GTCS, generalized tonic-clonic seizures; I.D., intellectual disability; LEV, Levetiracetam; M, months; MRI, magnetic resonance imaging; MS, myoclonic seizures; NR, not reported; OXC, oxacarbazepine; PHB, phenobarbital; SG, secondary generalization; TPM, topiramate; VPA, valproate acid; y, years; ZNS, zonisamide.

clusters and are triggered by fever, ID from mild to severe, behavioral problems such as autism spectrum disorder (ASD), aggressive behavior, depression, and psychiatric symptoms [9].

However, the phenotype is variable, ranging from absent or minimal behavioral and/or neurodevelopmental issues to a severe clinical picture [10].

The male phenotype varies if a mutation is present in all the cells as in the "normal transmitting males" or in patients with somatic mosaicism. The "normal transmitting males" are generally unaffected, do not have epilepsy but could show a rigid personality and other symptoms of ASD, mild neuropsychiatric features, and ID [11].

We report a young boy with a c.698A>G (p. Asp233Gly) variant in PCDH19 present in a mosaic state (VAF 80%) in peripheral blood. This variant was already reported in a young female patient and in another subject (sex not specified) both affected by severe myoclonic epilepsy [12, 13]. It is not reported in the GnomAD database, it is considered pathogenic by in silico predictors such as Polyphen2 and Sift, and can be classified as pathogenic (class 5) according to ACMG guidelines (PM1 strong, PP3 strong, PM5 moderate, PM2 supporting). The phenotype of this child is characterized by very early onset of seizures (at 3 months), that occurred in cluster but were poorly fever sensitive, an initially normal neurodevelopment followed by a delay after the beginning of symptoms, mild ID, no psychiatric or behavioral problems, a recent good but partial response to ASMs and negative MRI and intercritical EEG. This child's anomalies are part of the clinical variability previously reported i.e., in other 34 mosaic males [14–17]. Phenotypic and genetic data of our case compared to other ones are shown in Table 1.

Collected data show that subjects' age at the last follow up ranged from 1 year 11 months to 25 years with a mean of about 6 years and 6.5 of median. Development before the onset of seizures was usually normal, although speech delay was present in few cases. Seizures started within 3 and 96 months (median 8 months but in 83% in the first year of life). Seizures' type was heterogenous, i.e., generalized tonic clonic or clusters of focal or complex partial seizures, with possible changes over time.

All these patients presented cluster seizures and only 8/35 (23%) showed status epilepticus. Furthermore, seizures could be triggered by fever in 20/28 (71%) cases. Finally, the response to ASM (data available for 28/35 patients) was at least partially satisfactory in about half of the reported subjects, with only 5 patients who were seizure-free since a year before last follow up. VPA and LEV were the most efficient drugs.

Interictical epileptic discharges were present in 20/27 patients (74%). Brain MRI, reported in 17 patients, was usually normal: expansion of periventricular spaces and widened peripheral subarachnoid spaces were reported in 3 unrelated patients. Regarding cognitive assessment, 24 patients (one with a diagnosis of ID based only on a clinical evaluation) showed ID ranging from mild to severe, 5 were borderline and 6 were normal. Behavioral/psychiatric disturbances, like aggressiveness, depression, and attention deficit hyperactivity were present in 18/33 (54%) subjects. Minor dysmorphic features were described in one single patient.

Variants' type of the 35 patients were: 15 missense, 18 truncating, 1 splice site variant and 1 whole gene deletion with VAF in peripheral blood ranging from 10 to 100%. Genotype-phenotype correlations are not yet clear and, when considering type of variant and VAF, different studies come partially to different conclusions. In the largest available study on mosaic males [15], patients with missense mosaic variants were more frequently found with high VAF and had an earlier onset of disease. Patients with truncating low VAF (i.e., nearer to 50%) variants were more likely to achieve a seizure-free status.

This would be in line with the patient we report here, who carries a missense variant with high VAF, had an early onset of seizures and only partial control with ASM. However, the variant features do not hold as yet a clear prognostic value.

According to some authors, the early onset of the first symptoms in females, especially seizures, often triggered by fever, is a negative prognostic factor [18]. However, in mosaic males, who usually have early-onset seizures, response to treatment still needs further observation. More data are needed about the evolution of the clinical phenotype in mosaic males, because a long-term follow up was available for a very small number of patients [17].

Affected females are treated with different types of ASMs, the most effective ones being bromide and clobazam [19]. However, they often tend to have recurrent seizures and, in some cases, evolve to status epilepticus. Generally, corticosteroids improve seizures, but the benefit is usually transient. Clinical trials using ganaloxone, a synthetic analogue of the neurosteroid allopregnanolone, recently started following a study on affected females, in which reduced values of such hormone were observed. This study gave evidence that allopregnanolone, which is a GABA receptor modulator, could be a therapeutic target in relation to PCDH19 pathophysiology [20].

In affected females, there is often a clinical improvement with a reduced frequency of epileptic seizures after the age of 20. Described mosaic males are still too few and young and will deserve long-term follow-up to verify if age improvement holds true.

Conclusions

We report a rare case of a child affected by early infantile epilepsy due to a mosaic variant of PCDH19, adding his clinical and genetic data to those of other 34 mosaic males previously described. His clinical picture is within the phenotypic variability of the other subjects and similar to that of affected females. Our case confirms that it is not easy to predict the severity of the clinical spectrum of this epileptic encephalopathy even if the age of onset of epilepsy, VAF and the type of pathogenic variant could have some prognostic value. Finally, there is a stringent need to improve therapeutic approaches and to collect data on long-term follow-up.

Statement of Ethics

Ethics approval was not required for this study in accordance with local guidelines. An informed written consent form was obtained from the participants (or their parent/legal guardian/next of kin) for publication of the details of their medical case.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Parmeggiani Giulia, Graziano Claudio, Seri Marco, Laura Licchetta and Francesca Bisulli contributed to conception an design of the manuscript. Minardi Raffaella contributed to the

molecular data acquisition, analysis and their interpretation. Antonella Boni, Antonella Pini and Pruccoli Jacopo contributed to NPI data acquisition. All the authors participated in drafting and revising the manuscript. All authors confirm that the manuscript was read and approved for publication.

Data Availability Statement

All data generated or analysed during this study are included in this. Further enquiries can be directed to the corresponding author.

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