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The phenotypic spectrum of *PCDH12* associated disorders - five new cases and review of the literature

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Declaration of competing interest

The authors have no potential conflicts of interest to disclose.

Appendix

Videos of Patients #4 and #5 demonstrating dystonia during phases of the gait cycle, most evident in the affected brother (Patient #4) as mild camptocormia, variable stride-length, and dystonic posturing of the hands and feet.

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Abstract

PCDH12 is a member of the non-clustered protocadherin family of calcium-dependent cell adhesion proteins, which are involved in the regulation of brain development and endothelial adhesion.

To date, only 15 families have been reported with *PCDH12* associated disease. The main features previously associated with PCDH12 deficiency are developmental delay, movement disorder, epilepsy, microcephaly, visual impairment, midbrain malformations, and intracranial calcifications.

Here, we report novel clinical features such as onset of epilepsy after infancy, episodes of transient developmental regression, and dysplasia of the medulla oblongata associated with three different novel truncating *PCDH12* mutations in five cases (three children, two adults) from three unrelated families. Interestingly, our data suggests a clinical overlap with interferonopathies, and we show an elevated interferon score in two pediatric patients.

This case series expands the genetic and phenotypic spectrum of *PCDH12* associated diseases and highlights the broad clinical variability.

Keywords

PCDH12; epilepsy; movement disorder; brain malformation; intracranial calcification; interferonopathy

1. Introduction

PCDH12 (protocadherin 12) is located on chromosome 5q31 and encodes a calcium-dependent cell adhesion protein, belonging to the protocadherin superfamily. Given that protocadherins are involved in the development and maintenance of neuronal circuits, their genetic alteration can lead to neurodevelopmental and neuropsychiatric disorders [1]. To date, bi-allelic truncating *PCDH12* variants have been reported in 15 families only and were associated with varying clinical conditions including the diencephalic-mesencephalic junction dysplasia syndrome 1 (DMJDS1, OMIM #251280) [1–6]. Here, we review the literature on previously reported cases and present 5 additional cases harboring 3 novel *PCDH12* mutations. We illustrate the broad genotypic and phenotypic spectrum and expand the clinical spectrum by adding novel clinical and radiological features.

PCDH12 is ubiquitously expressed with a particular focus on the central nervous system and endothelial cells [1]. The exact role within the nervous system as well as the pathomechanism in PCDH12 deficiency remain not well understood. As the phenotype includes midbrain malformation, intracranial calcifications, and recurrent episodes of sterile

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fever with transient developmental regression, we suspected a potential overlap with type I interferonopathies and thus investigated the interferon score in two pediatric cases.

2. Methods

In this study, we report the clinical course, imaging data, and the genetic findings of three pediatric and two adult patients with homozygous truncating mutations in *PCDH12* (pedigrees are provided in the supplement). Informed consent of the parents and patients for genetic testing and publication was obtained. Genetic diagnoses were identified by next-generation sequencing methods and confirmed by Sanger sequencing. For details regarding the whole exome sequencing (WES) applied in Patients #1, #4, and #5, we refer to previously published studies [7,8]. The *PCDH12* variant in Patient #2 was identified by panel sequencing (panel EPI02, early-onset epileptic encephalopathies), the variant of Patient #3 – the brother of Patient #2 – was confirmed by Sanger sequencing.

Possible systemic activation of type I interferon was assessed by expression analysis of interferon-stimulated genes in peripheral blood lymphocytes and calculation of the so-called interferon score as described earlier [9]. At the time of blood sampling and in the previous days the patients did not show symptoms of a febrile infection. An interferon score of >12.49 indicating the mean of 10 healthy controls plus 2 SD is considered to be pathologic.

3. Results

3.1 Case reports

3.1.1 Pediatric cases—Patient #1 is the 1st child of healthy non-consanguineous parents of Afghan origin. One younger sibling is healthy. Pregnancy was unremarkable, birth at term without complications (weight 3540 g [P40], length 54 cm [P70], head circumference 34.5 cm [P20]). The boy presented during the first year of life with delayed motor development, intermittent opisthotonus, and squinting. On follow-up a severe global developmental delay, progressive secondary microcephaly, truncal hypotonia, and myopia became evident. Additionally, the child developed a movement disorder characterized by dystonic posturing of the arms and dyskinesia of the trunk on voluntary movements. At the age of 2.5 years, he was hospitalized several days after a febrile upper respiratory tract infection because of his first epileptic seizure that led to a status epilepticus. In the following months, epilepsy became a prominent feature with recurrent prolonged tonic seizures, absence seizures, focal tonic seizures of the upper extremity with impaired awareness, and drop attacks. Antiepileptic medication with levetiracetam and lamotrigine was only moderately effective, cannabidiol led to a distinct improvement of seizure frequency and intensity. Remarkably, the patient suffered from recurrent episodes of fever without any clinical or biochemical signs of acute infections. After generalized seizures and febrile episodes, he showed a transient loss of motor skills lasting a few weeks. Despite these intercurrent episodes of regression, he showed slow, but ongoing improvement of his development as he learned to walk independently and to speak a few single words by the age of six years. Ophthalmological examination at six years of age revealed progressive myopia, astigmatism, esotropia, and bilateral optic atrophy. Retinal pathology was excluded

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by fundoscopy. At the age of 5 years his weight was 21.5 kg [P76], his height 107.5 cm [P16], and his head circumference 48 cm [<P3, −2.9z].

Patient #2 is a currently 5-year-old girl of Syrian origin who was born as the first child to healthy first-degree consanguineous parents. Body length, weight and head circumference at birth were not available. While pregnancy was unremarkable, birth was protracted and the girl developed therapy refractory epileptic seizures on the second day of life. Her only brother is Patient #3. Under the current anti-epileptic treatment with levetiracetam, topiramate, and valproic acid, seizure frequency ranges between one and three self-limiting focal seizures of varying semiology per day. EEG showed multifocal epileptic discharges with occasional secondary generalization as well as background slowing. In her first year of life, the girl showed global developmental delay. She was able to sit independently at 12 months of age. According to the parents, she presented a neurodegenerative clinical course during the following months resulting in loss of acquired motor skills and severe spastic cerebral palsy. Bilateral atrophy of the optic nerves and exotropia were observed. Flash visual evoked potentials using light-emitting diode goggles were not elicitable. At the age of 5.7 years her weight was 18 kg [P20], her length 109 cm [P10], and her head circumference 43.5 cm [<P3, −7z].

Patient #3 is the younger brother of Patient #2, he was born in Germany to consanguineous parents of Syrian origin. Pregnancy and birth were unremarkable. A primary microcephaly was noticed (40 + 2 weeks of gestation; birth weight 2840 g [P3], birth length 50cm [P 10], birth head circumference 32.5 cm [<P3, −2.3z]; APGAR 8/9). Focal epileptic seizures occurred on the 1st day of life. Repeated EEGs showed multifocal sharp waves with predominance in the right hemisphere. On follow-up, the patient showed occasional focal seizures (1x/month under monotherapy with levetiracetam). Psychomotor development was severely delayed, yet the boy did not show a neurodegenerative course at any time. At 1.8 years of age, the child had not acquired head control and was not able to turn from supine to prone position or vice versa. Clinical examination revealed truncal hypotonia and spastic hypertonia of the extremities with intermittent dystonic posturing of the right arm. Ophthalmologic investigations at 5 months of age showed bilateral atrophy of the optic nerve (right > left) and exotropia of the right eye. At latest follow-up at the age of 2.3 years his weight was 11.6 kg [P18], his length 83 cm [P2], and his head circumference 43 cm [<P3, −5.3z].

3.1.2 Adult cases—In addition, we report two adult siblings, a 27-year-old male (Patient #4) and an 18-year-old female (Patient #5). The siblings are born to healthy consanguineous parents of Iranian origin. Body length, weight and head circumference at birth were not available.

They first presented to us in adulthood, showing mild intellectual disability and microcephaly. As depicted in Figure 1, Patients #4 and #5 exhibited dysmorphic facial features. Neurologic examination showed mild spasticity. Also, mild ataxia and dystonic posturing of the hands and feet with variable stride-length while walking, as well as mild camptocormia were observed, most evident in the affected brother (see also supplementary videos). The movement disorder was without clear progression in both over the last years.

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Both patients had experienced a sudden loss of vision, affecting the right eye in Patient #4, and both eyes in Patient #5, visualized as rhegmatogenous retinal detachment.

Somatic data at last visit were as follows: Patient #4 – body weight 69 kg [P42], body length 168 cm [P5], head circumference 52 cm [<P3, −2.8z]; Patient #5 – body weight 65 kg [P68], body length 160 cm [P18], head circumference 50 cm [<P3, −3.4z].

3.2 Neuroimaging

Magnetic resonance imaging (MRI) of Patient #1 at age 4.9 years and 7.1 years showed mild progressive hyperintensity of both hippocampi with pronunciation of the left amygdala suggestive of hippocampal sclerosis without any signs of the “butterfly” appearance typical of diencephalic-mesencephalic junction dysplasia (DMJD) or signs of vasculopathy (Figure 2A). On cranial computed tomography (CT) scans at the age of 2.5 years, one 0.5 mm hyperdense spot (Hounsfield units 101 SI) in the left thalamus suggestive of a small isolated calcification was found (Figure 2A).

Cranial MRI of Patient #2 at 19 months of age revealed leukoencephalopathy, hypoplasia of cortex, corpus callosum and pons, dysplasia of the medulla oblongata, and bilateral atrophy of the optic nerve (Figure 2B). Additionally, bilateral T2-hyperintensity within the occipital lobes was reported. A Blake’s pouch cyst was found as an incidental finding (Figure 2B).

In Patient #3 early cranial MRI on postnatal day one showed a broad communication between the fourth ventricle and the posterior fossa, and an abnormally thin corpus callosum. Follow-up ultrasound investigations of the brain at the age of five months showed increased echogenicity with sonic shadow in the basal ganglia and periventricular white matter, suggestive of calcifications. A follow-up cranial MRI at the age of 17 months (Figure 2C) showed bilateral optic atrophy, general cortical and subcortical hypoplasia, delayed myelination, reduced diameter of the corpus callosum, and a Dandy Walker continuum comprising a cystic enlargement of the fourth ventricle and the posterior fossa, and a mild vermian hypoplasia with cranial rotation, and – in consistency with the older sister – a dysplastic medulla oblongata, yet no dysplasia of the midbrain (Figure 2C).

For the adult cases, only cranial CT scans were available showing no abnormalities, especially no signs of calcifications nor brainstem malformation.

3.3 Genetic diagnostics

In Patient #1, WES identified a novel homozygous truncating variant (c.1176G>A; p.Trp392*) in *PCDH12* (NM_016580.3). Sanger sequencing confirmed the variant, mother and father were heterozygous carriers. By panel sequencing, a novel homozygous pathogenic variant (c.2424G>A; p.Trp808*) in *PCDH12* was identified in Patient #2. Following the diagnosis in the older sister, the same mutation was confirmed by Sanger sequencing in Patient #3. The parents have declined to be tested themselves for this variant. In Patients #4 and #5, WES revealed the novel homozygous *PCDH12* variant c.2437C>T (p.Gln813*). All variants were classified as pathogenic according to the ACMG guidelines (supporting criteria: PVS1, PM1, PM2, PM3) [10].

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3.4 Interferon signature

We assessed the interferon signature of Patients #1, #2 and #3 at 7.7 years, 1.6 years and 4.9 years of age, respectively. While Patient #2 showed no evidence of an elevated interferon signature, Patient #1 had a highly elevated interferon score of 2666.1, whereas Patient #3 showed a mildly elevated score of 23.73 (threshold <12.49), consistent with systemic type I interferon activation.

4. Discussion

4.1 Phenotypic spectrum

PCDH12 deficiency is a rare disorder and several clinical conditions have been reported in association with biallelic loss-of-function mutations. Here we present five new cases of *PCDH12* associated neurological disease and a review of the clinical and imaging features of the previously reported 24 patients. These data highlight that PCDH12 deficiency causes a broad and continuous phenotypic spectrum of overlapping features rather than separated disease entities (see Table 1 and Supplementary Table 1 for further details) [1–6].

Almost all patients present microcephaly (23/29 patients) and intellectual disability (24/26) of varying severity. Interestingly, all patients reported speech difficulties, regardless of the degree of cognitive impairment. Further common features are movement disorder – presenting as a varying combination of spasticity (18/26), ataxia (14/26) and dystonia (10/26), epilepsy (18/25), and variable cranio-facial dysmorphism (21/25).

Patients #2 and #3 illustrate the most severe end of the phenotypic spectrum, presenting during the first days of life with early-onset therapy-refractory epileptic encephalopathy with variable seizure semiology, comparable to the cases presented initially by Aran et al., and Guemez-Gamboa et al. [1,5]. On-follow up these patients developed a spastic-dystonic movement disorder, severe developmental delay, and progressive microcephaly. In all previously reported 15 patients with seizures as well as in Patients #2 and #3 of this study the onset of epilepsy was before the age of 3 months. Here we report an onset of epilepsy at the age of 2.5 years in Patient #1. This is the first case of epilepsy onset after infancy in patients with *PCDH12* mutations, thereby expanding the phenotypic spectrum.

In contrast, three families including Patients #4 and #5 of this study have been described with no or only mild to moderate intellectual disability [3,6]. In these patients, the phenotype is mainly characterized by movement disorder and retinopathy leading to visual loss in adolescence or adulthood. Between these two endpoints of the spectrum, some patients show an intermediate phenotype presenting with significant developmental delay and a movement disorder including ataxia and dystonia but lacking features such as epilepsy or intracranial calcifications [1,2].

4.2 Brain imaging

Brain malformations (18/22) and intracranial calcifications (13/18) are the most common imaging features. Other frequent features are generalized brain atrophy, thin corpus callosum (10/22), and ventriculomegaly (11/22).

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In a study by Guemez-Gamboa et al., brain malformations at the diencephalic-mesencephalic junction (DMJD) of variable expression were reported in 14 individuals from eight families harboring *PCDH12* mutations [1], i.e. the diencephalic-mesencephalic junction dysplasia syndrome 1 (DMJDS1, OMIM #251280). Aran et al., also described midbrain malformation and poor distinction between the crus cerebri of the midbrain, hypothalamus, and optic tract in four unrelated families [5]. More recently, three patients from two families were reported without any brain malformations [2,3]. In line with these cases, all five cases of our study had no signs of malformations of the midbrain or diencephalon. However, Patients #2 and #3 showed dysplasia of the medulla oblongata and a Dandy Walker continuum, reported for the first time in *PCDH12* patients. Furthermore, the three pediatric patients (#1, #2, #3) showed bilateral atrophy of the optic nerve. As retinopathy was excluded in two of our patients, we believe the optic atrophy to be caused directly by PCDH12 deficiency. *PCDH12* is highly expressed in neurons and has a distinct role in neural circuit formation and the formation of dendritic spines [1,5,11,12]. A defective growth due to loss of PCDH12 in dissociated neural progenitor cells further emphasizes the association of *PCDH12* and defective neurite growth [1]. In a 14-year-old patient from Japan with a severe phenotype including profound global retardation and early-onset epilepsy, asymmetric basal ganglia and hyperintensity of the central tegmental tract became evident on MRI [2]. In other published DMJD-patients tractography revealed an abnormal and shortened course of the corticospinal tract [13]. Patients #1–3 also showed bilateral streaky hyperintensity from the posterior horn of the ventricles to the occipital lobe. These findings could be indicative of impaired white matter structures as a result of neurodegeneration. Taken together, *PCDH12* patients present a variable spectrum of midbrain or brainstem malformations, potentially caused by disrupted axonal growth.

The pathogenesis of the calcifications remains unclear, but the occurrence of calcifications may correlate to some extent with the severity of the clinical phenotype. In our cohort, the CT scan of Patient #1 showed a small hyperintensity within the thalamus suggestive of a spot calcification, resembling calcification patterns comparable to previously reported cases, albeit significantly less pronounced[1,5].

In contrast, our milder affected adult cases showed no calcifications on CT scan, similar to the mildly affected patients presented by Vineeth et al [3]. It was hypothesized that the calcifications might be caused by vasculopathy, as *PCDH12* is highly expressed in endothelial cells and *PCDH12* knock-out in mice leads to morphological and functional alterations of arteries [14]. Additionally, in some of the previously reported cases, imaging findings included perithalamic hyperechogenicity suggestive of multiple tortuous vessels already visible on prenatal ultrasound [5]. In our patients, no signs of vasculopathy were noted.

4.3 Genotype-phenotype correlation

All reported *PCDH12* patients harbored bi-allelic loss-of-function mutations scattered over the complete protein (Figure 1A, B) [1–6]. Our data together with the published data do not allow any genotype-phenotype correlations, for example, Patients #2 and #3, representing the most severe end of the phenotypic spectrum, and the mildly affected adult cases (Patients

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#4 and #5) all harbor neighboring homozygous stop mutations in the cytoplasmic domain (p.W808* and p.Q813*, respectively). In contrast, the only previously reported mildly affected family with ataxia, dystonia, and retinopathy similar to Patients #4 and #5 had a mutation in Cadherin repeat six [3]. Nonetheless, the phenotype associated with the same variant within a family is similar, highlighting the potential role of genetic modifiers.
In our report we bring up the possibility of interferon mediated autoinflammation in the pathogenesis of *PCDH12* associated disease. In Patient #1 analysis of the WES data did not reveal mutations in known disease genes previously associated with interferonopathies, microcephaly or neurodevelopmental disorders. However, a potential effect of unknown genetic disease modifiers has to be considered. Since whole-exome and not whole genome sequencing was performed, it is not possible to exclude the low probability of a digenic inheritance based on deep intronic variants or structural variants in a second gene modifying the phenotype. For the remaining patients no NGS data was available.

4.4 Clinical overlap with interferonopathies and interferon score

Intracranial calcifications were reported in 12 previously published patients with *PCDH12* mutations as well as Patient #1 and #3 of our study [1,2,4], resembling similar patterns observed in neuroinfectious neonatal conditions like TORCH or Aicardi-Goutières syndrome (AGS), both resulting from the exposure of the brain to toxic levels of interferon-alpha [15]. Episodes of sterile fever have been reported in 9 patients harboring *PCDH12* mutations. We now report for the first time a *PCDH12*-case in which episodes of fever are associated with the temporary loss of previously acquired motor skills. Another important feature in *PCDH12* patients is visual impairment, caused by vascular retinopathy in the milder affected patients [3,6].

Type I interferonopathies are a clinically and genetically heterogeneous group of diseases caused by systemic autoinflammation due to constitutive activation of the type I interferon axis. Up to now, 23 genes have been assigned to this disease entity [9,16]. The most severe phenotype is early-onset Aicardi-Goutières syndrome (AGS), as the prototypic type I interferonopathy, showing basal ganglia calcifications and progressive cerebral atrophy, along with a clinical phenotype of movement disorder, epilepsy, and fever episodes leading to severe developmental delay and progressive microcephaly [17]. Interestingly, retinal vasculopathy has also been reported in association with the AGS gene *TREX1* as retinal vasculopathy with cerebral leukodystrophy (RVCL; OMIM #192315) [18]. Patients present progressive loss of vision in adolescence or adulthood and cerebrovascular disease [18].

Because of the clinical overlap, we hypothesized that type I interferon-mediated autoinflammation might be part of the pathomechanism in *PCDH12*-related disease. To further investigate this hypothesis, we measured the interferon score as a marker for systemic activation of the type I interferon axis in the pediatric cases. While the interferon score was unremarkable in Patient #3, Patient #1 and Patient #2 had elevated interferon scores. In patients with interferonopathies and patients with AGS in particular, the interferon score is generally significantly elevated [19]. Yet for some genes such as *RNASEH2B* or *PNPT1,* several cases with only mildly elevated or negative interferon scores have been reported [9,19]. In conclusion, these results might hint towards interferon-mediated

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inflammation in *PCDH12* patients but are not sufficient to conclusively verify or exclude this hypothesis. Further studies are needed to elucidate the mechanisms underlying *PCDH12* associated diseases.

5. Conclusions

As the spectrum of inherited epilepsy-dyskinesia syndromes is constantly expanding, these cases contribute to a further understanding of the genotypic and phenotypic spectrum associated with *PCDH12* mutations and emphasize the role of protocadherins during neural development. The phenotypic spectrum is broad, and *PCDH12* mutations should be considered in patients with various clinical conditions including intellectual disability, therapy-resistant epilepsy, and dyskinetic movement disorders including dystonic cerebral palsy [2,8], as well as a differential diagnosis to congenital TORCH-infections or AGS spectrum disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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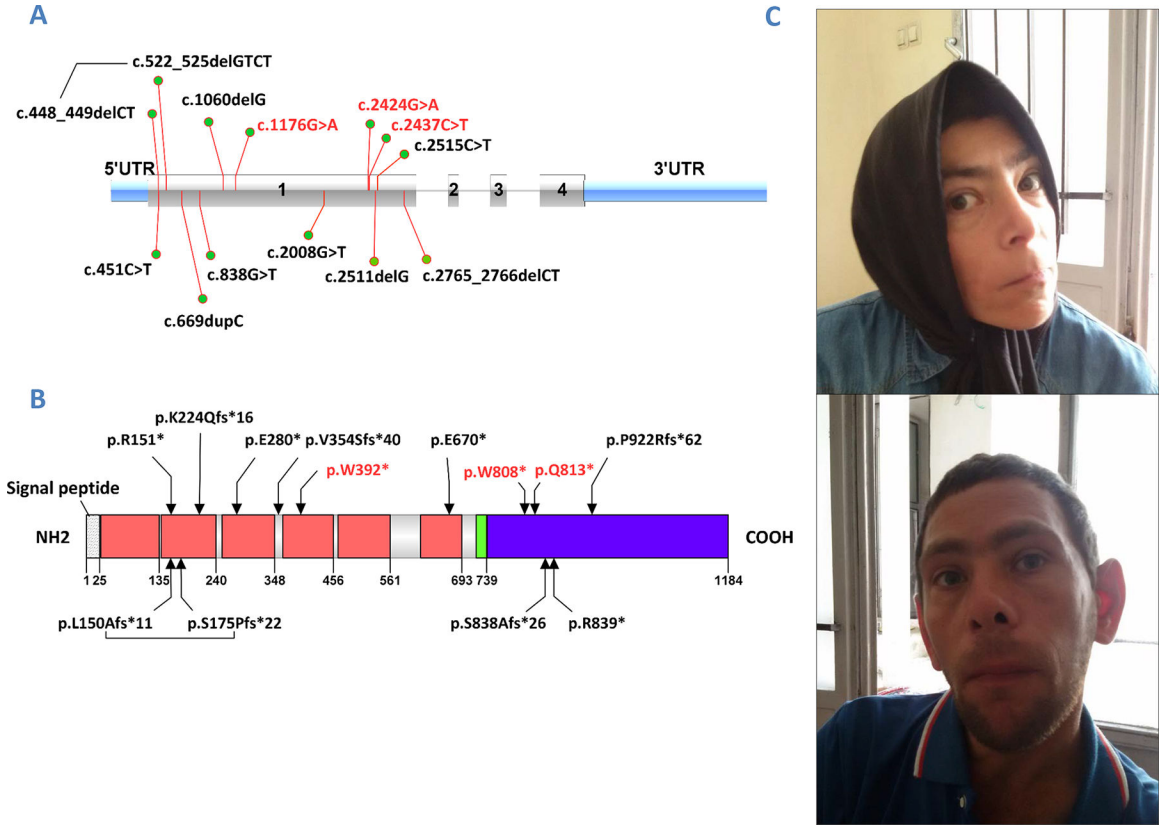

Figure 1: PCDH12 mutations and facial dysmorphic features

Schematic representation of the genomic (A) and protein structure (B) of *PCDH12*, and the localization of the identified mutations. All identified mutations are located within the first exon of *PCDH12*. The PCDH12 protein contains three different types of domains, six cadherin repeats (red), a transmembrane domain (green), and the intracellular domain (violet) at the C-terminus. Mutations identified in the current study are indicated in red. One loss of function mutation (p.W392*) is located in the cadherin repeat 3, the other two loss of function mutations (p.W808*, p.Q813*) affect the intracellular domain. The adult Patients #4 and #5 presented facial dysmorphism (C) consisting of elongated faces, long flat philtrum, thin upper lip, and anteverted nares.

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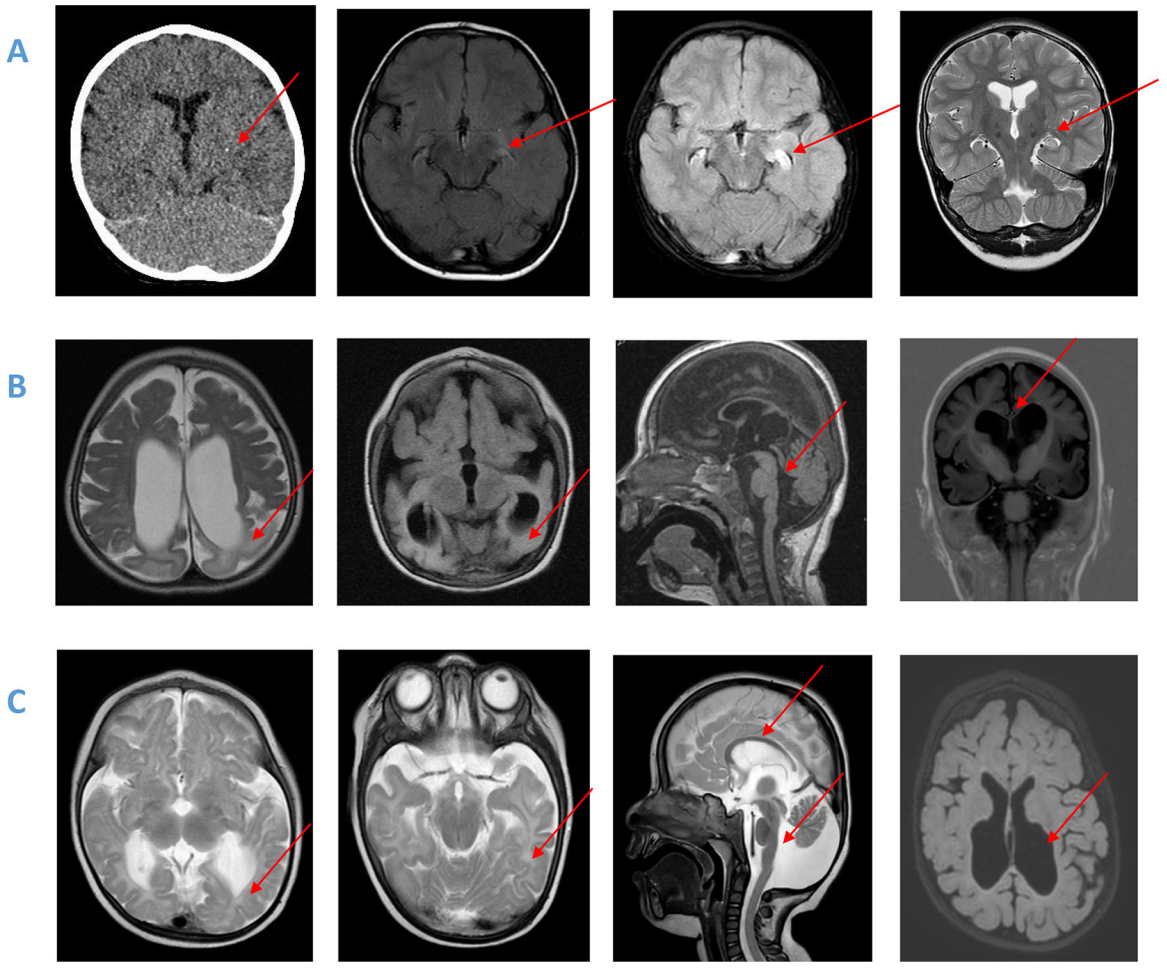

Figure 2: Neuroimaging of Patients #1 and #2

A, serial imaging of Patient #1, left to right: CCT axial at 2y: Isolated unilateral hyperdensity within the left thalamus, suspicious of small calcification. MRI Fluid attenuated inversion recovery (FLAIR) axial at 2.4y and 4.9y and MRI T2 coronal at 4.9y: Progressive bilateral hyperintensities of the hippocampi with volume loss, slightly more pronounced on the left amygdala between ages. MRI T2 axial at 7.1y: Constant atrophy of hippocampi (left > right).

B, MRI imaging of Patient #2, left to right (at 19 months of age): T2 transverse and FLAIR transverse: Hyperintensity as a sign of delay of myelination with parieto-occipital predominance; microcephaly. T1 multiplanar reformation (MPR) 3D sagittal: Dysplasia of the medulla oblongata; Dandy Walker continuum with an opening of the fourth ventricle towards the cisterna cerebello-medullaris. T1 turbo inversion recovery (TIR) coronal: Hypoplastic corpus callosum, generalized cerebral hypoplasia.

C, MRI imaging of Patient #3, left to right (at 17 months of age): T2 transverse: Hyperintensity as a sign of delayed myelination with parieto-occipital predominance; microcephaly. T2 sagittal: Hypoplastic corpus callosum; Dandy Walker continuum with a cystic enlargement of the fourth ventricle and vermian hypoplasia with cranial rotation;

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dysplastic medulla oblongata. FLAIR axial: Dysmorphic lateral ventricles, generalized cerebral atrophy

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Table 1:

Clinical and radiological features of PCDH12 associated patients, comparing the previously reported cases with the five new cases of our study. (CC: corpus callosum; CT: computed tomography).

	previous published cases	our cases	total (% of cases with available data)
Cases	**n=24**	**n=5**	**n=29**
Intellectual disability	91% (19/21)	100% (5/5)	92% (24/26)
Speach impairment	100% (21/21)	100% (5/5)	100% (26/26)
independent ambulation	16% (3/19)	60% (3/5)	25% (6/24)
Autistic features	28% (4/14)	0% (0/3)	24% (4/17)
Microcephaly	75% (18/24)	100% (5/5)	79% (23/29)
Seizures	75% (15/20)	60% (3/5)	72% (18/25)
Cranio-facial dysmorphism	90% (18/20)	60% (3/5)	84% (21/25)
Vasomotor instability	53% (8/15)	0% (0/3)	44% (8/18)
Recurrent unexplained fever	53% (9/17)	33% (1/3)	50% (10/20)
Movement Disorder	100% (21/21)	100% (5/5)	100% (26/26)
Spasticity	71% (15/21)	60% (3/5)	69% (18/26)
Ataxia	43% (9/21)	100% (5/5)	54% (14/26)
Dystonia	39% (6/21)	80% (4/5)	39% (10/26)
Axial hypotonia	38% (8/21)	60% (3/5)	42% (11/26)
Dysphagia	31% (5/16)	20% (1/5)	29% (6/21)
Strabism/squinting	44% (7/16)	0% (0/5)	33% (7/21)
Visual impairment	64% (7/11)	100% (5/5)	56% (14/25)
Retinopathy	100% (3/3)	66% (2/3)	80% (5/6)
Optic atrophy	NA	60% (3/5)	60% (3/5)
Brain MRI features			
Brain malformation	84% (16/19)	66% (2/3)	82% (18/22)
Thinned CC	42% (8/19)	66% (2/3)	46% (10/22)
Ventriculomegaly	47% (9/19)	66% (2/3)	50% (11/22)
Other	15% (3/20)	100% (3/3)	26% (6/23)
Brain calcification/hyperechogenicity (CT or ultrasound)	80% (12/15)	33% (1/3)	72% (13/18)