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BRIEF COMMUNICATION

Homozygous *PCDH12* variants result in phenotype of cerebellar ataxia, dystonia, retinopathy, and dysmorphism

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Abstract

We report on a sib pair of Indian origin born of a consanguineous parentage with a novel phenotype of distinct facial dysmorphism, cerebellar ataxia, dystonia, and exudative retinopathy due to homozygous *PCDH12* nonsense variations. cDNA studies showed >90% reduction in transcript levels in both patients, indicating nonsense-mediated decay and loss of function as the probable causative molecular mechanism of the phenotype.

Introduction

Protocadherin 12 (PCDH12) is an adhesion protein belonging to the cadherin family and has previously been implicated in a severe infantile pseudo-TORCH-like phenotype with distinctive neuroimaging findings (MIM: 251280) [1]. Pathogenic variations in other proteins of the cadherin family are reported to be causative for infantile epilepsy and cone rod dystrophy, whereas polymorphisms in various cadherin molecules are associated with complex neurological phenotypes [2]. We report a sib pair of Indian origin born of third-degree consanguineous parentage harboring homozygous *PCDH12* variants, who presented with a distinctive facial gestalt and neurological phenotype, quite different from the initially reported phenotype of *PCDH12* variations but consistent with the biological function of this protein and overlapping with the recently reported phenotype in a Japanese patient [3].

Materials and methods

A sib pair was brought for clinical genetics evaluation in view of movement disorder. The genetics consultation was sought for the younger sibling, an 8-year-old boy who had gait difficulties with frequent falls since the past 1 year, involuntary jerky movements of the whole body, and history of visual impairment due to a retinal pathology. School performance was good, although there were speech difficulties since early childhood. There were no significant perinatal issues and the parents felt that the symptoms were largely non-progressive. There was no history of seizures. Examination revealed facial dysmorphism in the form of an elongated face, prognathism, thick lips, flared nostrils with hypoplastic alae nasi, and anteverted nares. Neurological examination revealed normal higher mental function, stuttering of speech, and truncal and neck dystonia but no cerebellar signs. The elder sibling was a female aged 15 years at the time of evaluation, who was on treatment for seizure disorder and a retinal pathology. The parents reported an unclear speech but appropriate school performance and no other significant issues. Neurological assessment revealed normal higher mental function, stuttering speech with difficulty in initiation, presence of truncal and neck dystonia, and cerebellar signs in the form of wide-based gait, difficulty in tandem walk, and mild dysdiadokokinesia. There was significant facial dysmorphism in the form of elongated face, prognathism, thick lips, and

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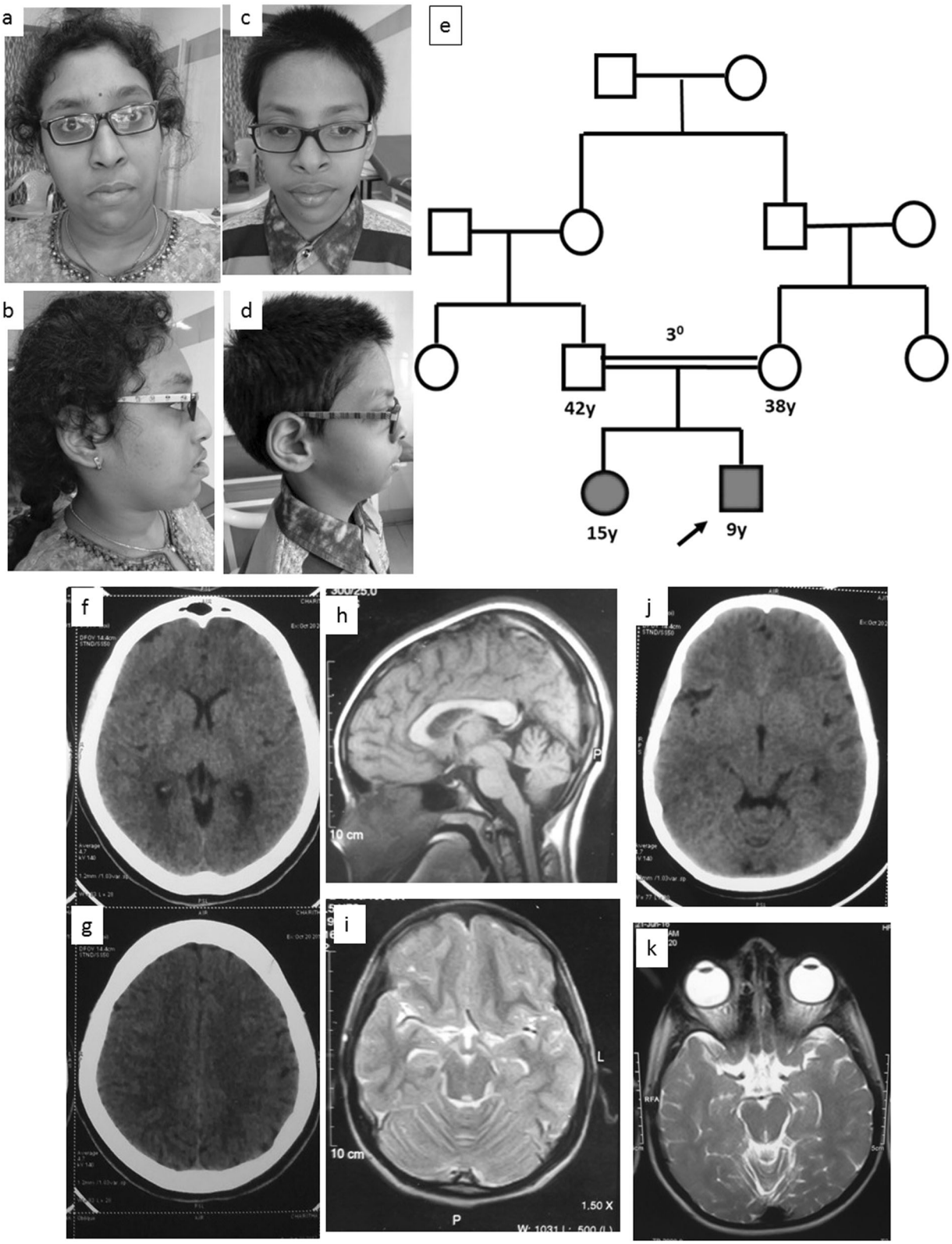

Fig. 1 **a**–**d** shows the facial gestalt of the two siblings. **e** shows the family pedigree. **f**, **g** CT scan of the elder sibling showing unremarkable axial planes at the level of cortex and basal ganglia. **h**, **i** MRI brain of elder sibling showing vermis and cerebellar atrophy with largely unremarkable midbrain and optic tracts. **j** CT brain of younger sibling with axial section showing unremarkable thalami. **k** MRI brain of younger sibling with axial sections showing unremarkable midbrain

Table 1 List of final 10 homozygous variants remaining in this patient after filtering

Gene	Transcript id	Variant (cDNA; protein)	OMIM phenotype (id)	dbSNP code	CADD score	ACMG 2015 guidelines interpretation	Reasons to not consider as likely disease causing
PCDH12	NM_016580	c.2008G>T; p.Glu670*	Microcephaly, seizures, spasticity, and brain calcification (605622)	rs531630376	35	Uncertain significance	Considered as likely disease causing in our patient
ESYT3	NM_031913	c.1249C>T; p.Arg417Trp	NR	rs200238751	34	Uncertain significance	Earlier knock-out mice model studies showed that it does not affect normal mouse development and viability [Herdman et al. [7]]
GRK7	NM_139209	c.1204G>A; p.Asp402Asn	NR	rs150840377	28.3	Uncertain significance	7 homozygotes were reported in ExAC database
SH3TC2	NM_024577	c.1597C>T; p.Leu533Phe	Charcot–Marie–Tooth disease, type 4C (601596)		25.9	Uncertain significance	Associated with a phenotype in OMIM not related to our patient
SEC16B	NM_033127	c.1820C>T; p.Thr607Met	NR		25.5	Uncertain significance	10 homozygotes were reported in ExAC database
HSD17B13	NM_001136230	c.68T>G; p.Val23Gly	NR	rs571845258	25.2	Uncertain significance	9 homozygotes were reported in ExAC database
COL1A1	NM_000088	c.4387T>C; p.Phe1463Leu	OI, type I (166200); type II (166210); type III (259420); type IV (166220)	rs577626107	24.1	Benign	Variant is classified as benign
NUP214	NM_005085	c.2293A>G; p.Ile765Val	Leukemia, T-cell acute lymphoblastic, somatic (613065)	rs61756081	23.6	Uncertain significance	15 homozygotes were reported in ExAC database and also associated with a phenotype in OMIM not related to our patient
DNAJC13	NM_015268	c.6305G>A; p.Arg2102Gln	Parkinson disease 21, (616361); Tourette Syndrome (137580)	rs754120262	23.4	Uncertain significance	Associated with a phenotype in OMIM not related to our patient
ULK2	NM_001142610	c.1963C>T; p.Arg655Trp	NR	rs557671381	22.9	Likely benign	Variant is classified as likely benign

NR not reported, *OI* osteogenesis imperfecta

Table 2 Comparative detail of phenotypes observed among the siblings and previously reported patients

Patient characteristics	Aran et al. [1] Palestinian families						Suzuki-Muromoto et al. [3]	Our patients		Fraction of individuals (percentage)
Gender	Female	Male (fetus)	Male	Male (fetus)	Female	Female (fetus)	14 years, male	13 years, female	11 years, male	
Developmental delay	Present	Not applicable	Present	Not applicable	Present	Not applicable	Present	Absent	Absent	4/6 (66.7%)
Seizures	Present	Not applicable	Present	Not applicable	Present	Not applicable	Multi-focal epilepsy	Present	Absent	5/6 (83%)
Visual impairment	Severe	Not applicable	Severe	Not applicable	Severe	Not applicable	Visual pursuit in late childhood	Bilateral exudative retinopathy	Bilateral exudative retinopathy	6/6 (100%)
Hearing impairment	Absent	Not applicable	Absent	Not applicable	Absent	Not applicable	Not mentioned	Absent	Absent	0/5 (0%)
Neurological symptoms	Axial hypotonia, peripheral dystonia	Not applicable	Axial hypotonia, severe peripheral dystonia	Not applicable	Axial hypotonia, peripheral dystonia, and spasticity	Not applicable	Dyskinetic quadriplegia and severe psychomotor retardation	Cerebellar Ataxia, Truncal and Neck dystonia	Cerebellar Ataxia, Truncal and Neck dystonia	6/6 (100%)
Cranio-facial dysmorphism	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Present	3/9 (33.3%)
Microcephaly	Present	Present	Present	Absent	Present	Present	Present	Absent	Absent	6/9 (66.7%)
MRI brain	Midbrain dysplasia	Not done	Midbrain dysplasia, mild ventriculomegaly, Hypoplastic corpus callosum	Not done	Not done	Midbrain dysplasia	Chronic subdural hematoma of the left hemisphere	Diffuse cerebellar atrophy	Diffuse cerebellar atrophy	3/6 (50%) with midbrain dysplasia
Brain calcification/ hyperechogenicity	Perithalamic	Periventricular and perithalamic	Periventricular	Perithalamic	Not done	Perithalamic	Posterior limb of internal capsule and bilateral perithalamic	Absent	Absent	6/8 (75%)

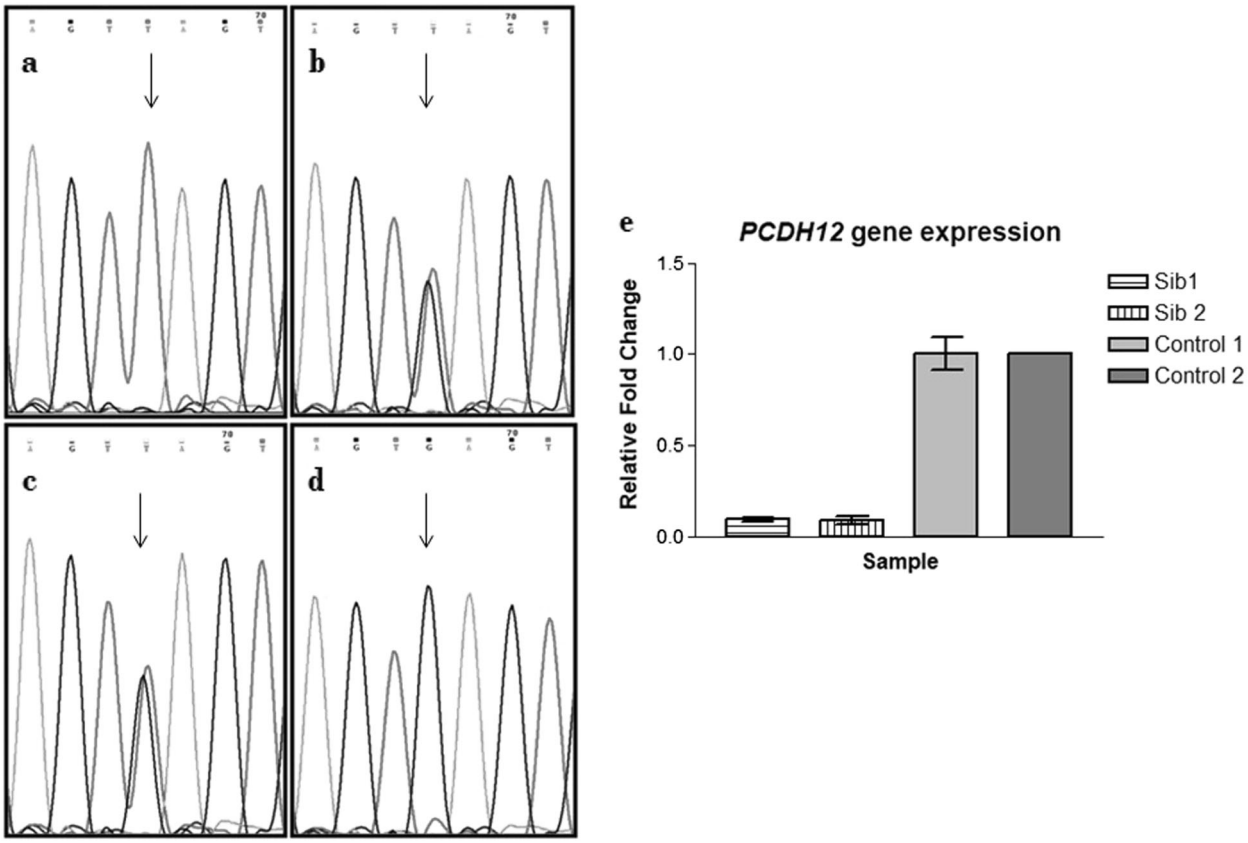

Fig. 2 Representative Sanger sequencing chromatogram of c.2008G>T nonsense variant in (**a**) Sibling 1 (T/T) (**b**) Father (G/T) (**c**) Mother (G/T) (**d**) Control (G/G). **e** Quantitative PCR (qPCR) assay results showing *PCDH12* gene expression (±SE) levels in the siblings along with two unrelated controls. $2^{-\Delta\Delta Ct}$ method was used to validate the relative fold change in gene expression. *GAPDH* was used as reference gene for normalization

hypoplastic alae nasi with anteverted nares. Figure 1a–d depicts the facial gestalt of the patients and Fig. 1e indicates the family pedigree. Rest of systemic examination was unremarkable for both siblings. Ophthalmic evaluation indicated exudative chorio-retinopathy in both patients. There was a history of laser coagulation for the younger sibling 3 years back. Nerve conduction studies also revealed sensorimotor neuropathy in the male sib.

Magnetic resonance imaging of the brain showed diffuse cerebellar atrophy involving especially vermis, paravermian areas, and the dentate nuclei in both siblings, while computed tomographic scan did not reveal any foci of calcification. Figure 1f–k shows the neuroimaging findings. Testing for Friedreichs ataxia and spinocerebellar ataxia type 3, 7 and 17 was non-contributory. As no clinical diagnosis was apparent for the siblings, whole-exome sequencing was planned. The study protocol was approved by the Institutional Ethics Committees of respective institutes.

DNA isolated from blood (~1 ug) was used to perform exome capture using the Nextera Rapid Capture Exome v1.2 Kit (Illumina, San Diego, CA) following the manufacturer's protocol. The libraries were sequenced to >100× coverage on Illumina HiSeq2000 platform (Illumina, San Diego, CA). Exome sequencing and data analysis pipelines were described earlier [4]. Human genome issue hg37 was used for mapping and annotation. Targeted PCR was performed using gene-specific primers and Sanger sequencing was done using ABI 3130 Genetic analyzer (Life Technologies, Carlsbad, CA). Pathogenicity of the variant was tested using prediction tools like MutationTaster2 [5] and CADD [6].

Total RNA was extracted using the RNeasy Mini Kit (QIAGEN, Valencia, CA) and reverse transcription was carried out using SuperScriptTM III First-Strand cDNA Synthesis assay system (Invitrogen, Carlsbad, CA) following the manufacturer's protocol.

Quantitative PCR (qPCR) was performed with the Power SYBR Green PCR Master Mix (Takara Bio Inc., Tokyo, Japan) on an Applied Biosystems 7500 Real-Time PCR System (Life Technologies, Carlsbad, CA) following the manufacturer's protocol. Relative gene dosage was assessed

by $2^{-\Delta\Delta Ct}$ method calculating the difference in cycle number (ΔCt). The primer sets used for PCR, Sanger sequencing, and qPCR are provided in supplementary table S1.

The case details were submitted to the Phenomcentral database, ID FAM0000984.

Results

The quality information of exome sequencing raw data and the variant filtering strategy are outlined in supplementary table S2 and table S3, respectively. Stringent filtering of variants for CADD score ≥20 left 10 shared variants between the two siblings (Table 1) [7]. After detailed clinical correlation, one likely pathogenic nonsense variant NM_016580.3:c.2008G>T [p.Glu670*] with highest CADD score of 35 was identified in the exon 1 of *PCDH12* gene. The variant was not present in homozygous state in 1000 Genomes project, Exome Variant Server, ExAC browser, gnomAD browser, and in our in-house exome database of unrelated individuals from the local population. However, it was present in the heterozygous state in 1/16,402 South Asian individuals (minor allele frequency 0.00006097), with overall frequency of 1/120,614 (minor allele frequency 0.00000829) in the ExAC database and 1/30,768 South Asian individuals (minor allele frequency 0.00003250) with overall frequency of 1/246,122 (minor allele frequency 0.000004063) in the gnomAD database. Heterozygous variants shared by the siblings were analyzed to look for any possible variants that can explain few or all of the phenotypes observed. None of the variants and the associated disorders fit the phenotype of the siblings. A list of common heterozygous variants that passed the filtering criteria is provided in supplementary table S4. Comparative details of the phenotype observed among all the patients reported in the literature along with the siblings of this study are depicted in Table 2.

Sanger sequencing of target amplicon among family members confirmed the siblings to harbor homozygous nonsense variant NM_016580.3:c.2008G>T [p.(Glu670*)] and both parents to be the carriers of the same variant [Fig. 2a–d].

qPCR results revealed significantly low levels of *PCDH12* transcripts in the patient blood cells indicating nonsense-mediated decay (NMD) of most of the transcripts (>90%) [Fig. 2e].

Discussion

We report a sibling pair with homozygous nonsense variant in *PCDH12* that leads to almost complete NMD and presents with a phenotype of dysmorphism, cerebellar ataxia, dystonia, and exudative retinopathy with preserved intelligence. *PCDH12* (or Vascular Endothelial Cadherin 2; MIM: 605622) is a member of non-clustered protocadherin group of cadherins and is demonstrated to promote endothelial adhesion [8]. In humans, *PCDH12* is expressed at comparable levels in several tissues and is not neuronal specific (Biogps.org). Very recently, biallelic nonsense and frameshift pathogenic variants in *PCDH12* have been implicated to cause microcephaly, seizures, and brain calcifications in four consanguineous families of Palestinian origin and dyskinetic cerebral palsy in a child of Japanese ethnicity, respectively [1, 3]. The phenotype of the sib pair reported by us is significantly discordant as compared to previous literature reports. Both the children were intellectually normal, with no growth issues, and primarily presented with movement disorder and ophthalmic issues. This is in contrast to the severe phenotypes of the previously reported individuals with biallelic *PCDH12* variants. However, the findings of exudative retinopathy and cerebellar involvement in the siblings are concordant with the known expression profile and function of this group of proteins [2, 8, 9]. Dystonia has also been a common feature of the previously reported patients with both biallelic and heterozygous *PCDH12* variants [1, 3, 10]. Intracranial calcification, which has been reported in individuals with *PCDH12* variants, was not documented in our patients [10].

Interestingly, the initial report of four consanguineous Palestinian families, with a very severe presentation of prenatal onset and a specific midbrain-optic tract dysplasia, had revealed all families as being homozygous for the same nonsense variant in exon 1. This variant was also present in heterozygous form in the common population of 7000 individuals, from which these families hailed. Also, this study involved exome seqeucning of only one individual, while for other families homozygosity mapping was done to identify the common shared homozygous region containing the *PCDH12* variant [1]. In view of the extremely severe phenotype of these cases and the closed population structure with consanguinity, we feel that the phenotype of these individuals could probably completely or partially be explained by another recessive variant, which was missed as exome sequencing was not performed in the remaining three families. The *PCDH12* variant could be only partly contributory towards the phenotype.

The second report of biallelic *PCDH12* variant in a Japanese individual involves two compound heterozygous frameshift variants in proximal part of exon 1, which are likely to result in almost complete loss of the functional protein [3]. This individual also did not have the midbrain dysplasia or the severe prenatal presentation of the Palestinian families. Although the authors have not done any functional studies, the nature of the variants suggest severe loss of function, possibly more than the Palestinian variant,

but the concomitant milder phenotype, with no characteristic midbrain dysplasia, further supports our theory of *PCDH12* variants as being unlikely the sole cause of the severe phenotype of the Palestinian patients. The Japanese individual is also reported to be dysmorphic with hypertelorism, broad nasal bridge, large mouth, and widely spaced teeth, although no photos were provided. No ophthalmic findings have been provided in any of these two reports. Hence, the phenotype of this individual, compound heterozygous for more proximal and presumably more deleterious variants, overlaps more with the siblings reported by us. The variant in our patients is located in the cytoplasmic domain very near to the Palestinian variant, and with similar functional effect on the transcript, but the mild clinical phenotype is completely discordant. Also, as whole-exome sequencing has been performed for both the siblings in our study and stringent filtering criteria did not reveal any other potential pathogenic variant, the *PCDH12* variant is the only likely cause of the phenotype.

A comparison of the clinical and neurimaging findings of all the reported patients with biallelic *PCDH12* variants shows movement disorder as the most consistent symptom with all patients demonstrating dystonia, and in addition, the siblings reported here had cerebellar ataxia. Seizures are also seen in a significant majority, with the male sibling reported by us being the only seizure-free individual. Other common findings appear to be developmental delay, microcephaly, and intracranial calcifications, all of which were conspicuous by their absence in the siblings reported here. The siblings had additional finding of diffuse cerebellar atrophy, which has not been reported previously. Visual impairment is seen in all patients; however, in the absence of fundus findings in previous patients, it is not possible to ascertain whether it is in view of cortical involvement or retinopathy. As discussed above, midbrain dysplasia may not possibly be consequent to *PCDH12* defects. Facial dysmorphism was appreciated in our patients and Japanese case, and a larger case series would be needed before any specific dysmorphism can be attributed to *PCDH12* involvement.

Hence, we propose that *PCDH12* biallelic variants result in a primary phenotype of movement disorder including cerebellar ataxia and dystonia, facial dysmorphism, with or without cognitive involvement, seizures, intracranial calcification, and exudative retinopathy. The initial report of the pseudo-TORCH phenotype with characteristic midbrain dysplasia is probably due to effect of another variant, with possible modifier effects of the *PCDH12* variant.

This report emphasizes the need to identify further affected individuals to understand the complete clinical spectrum and genotype–phenotype correlation for *PCDH12* variants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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