Journal of Human Genetics (2018) 63:749–753
https://doi.org/10.1038/s10038-018-0432-0

BRIEF COMMUNICATION

A case of new *PCDH12* gene variants presented as dyskinetic cerebral palsy with epilepsy

Sato Suzuki-Muromoto[1] · Keisuke Wakusawa[1] · Takuya Miyabayashi[1] · Ryo Sato[1] · Yukimune Okubo[1] · Wakaba Endo[1] · Takehiko Inui[1] · Noriko Togashi[1] · Atsuko Kato[2] · Hiroshi Oba[3] · Mitsuko Nakashima[4,5] · Hirotomo Saitsu[4] · Naomichi Matsumoto[5] · Kazuhiro Haginoya[1]

Received: 28 November 2017 / Revised: 7 February 2018 / Accepted: 8 February 2018 / Published online: 19 March 2018

Abstract

Here we report a Japanese patient with new compound heterozygous truncating variants in the *PCDH12* gene. As compared to the previously reported families who had congenital microcephaly, intrauterine growth retardation, intracranial calcification, and neonatal seizure associated with dysplasia of the midbrain-hypothalamus-optic tract, the present patient showed no midbrain-hypothalamus dysplasia or congenital/postnatal microcephaly, but dyskinetic cerebral palsy and severe intellectual disability as well as multifocal epilepsy. To understand phenotypic spectrum associated with *PCDH12* variants, more reports are needed.

Introduction

Protocadherins (PCDHs) are one of a group of calcium-dependent cell adhesion transmembrane protein belonging to the cadherin superfamily, and divided into clustered PCDH and non-clustered PCDH based on their genomic structures [1–3]. Both groups of the PCDHs are widely expressed in vertebrate nervous systems and play roles in neural development and neural circuit formation [1]. PCDH12 is a member of the non-clustered PCDH. However, its function in the central nervous systems (CNS) in human is yet to be clearly understood [2].

Aran et al. [4] first reported 10 Palestinian patients from four families who had loss of function variant of *PCDH12*. All four families shared the same homozygous variant in the *PCDH12* gene. They showed congenital microcephaly, hyper-echogenic brain foci in utero, and intrauterine growth retardation. After birth, progressive microcephaly, intracranial calcification [5], and neonatal seizure with virtually no developmental milestones were evident. Brain imaging showed dysplastic, elongated midbrain with poor distinction between the crus cerebri of midbrain, hypothalamus, and optic tract. Here we report a Japanese patient with compound heterozygous truncating variants of *PCDH12*, which is thought to be highly pathogenic and may expand clinical spectrum of *PCDH12*-related disorders.

Case report

This 14-year-old Japanese boy was fourth child of healthy and un-consanguineous parents. He was born after 38 weeks of pregnancy without asphyxia. The birth weight, body length, and occipito-frontal head circumference (OFC) were 3114 g (+0.3 SD), 50 cm (+0.5 SD), and 34 cm (+0.5 SD), respectively. At 2 months of age, he showed epileptic spasms, which appeared two to three times a day. On brain computed tomography, he had spot calcification affecting

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s10038-018-0432-0) contains supplementary material, which is available to authorized users.

✉ Sato Suzuki-Muromoto
sato.suzuki@kmf.biglobe.ne.jp

[1] Department of Pediatric Neurology, Miyagi Children's Hospital, Sendai 989-3126, Japan

[2] Department of Developmental Rehabilitation, Miyagi Children's Hospital, Sendai 989-3126, Japan

[3] Department of Radiology, Teikyo University Hospital, Tokyo 173-8605, Japan

[4] Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan

[5] Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan

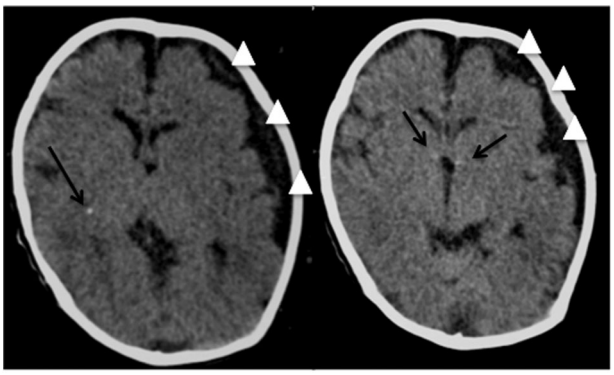

Fig. 1 Brain CT at 2.5 months old. White arrowheads pointed chronic subdural hematoma. Spot calcification in posterior limb of internal capsule and bilateral perithalamic regions are indicated by black arrows

right posterior limb of internal capsule and bilateral perithalamic regions as well as chronic subdural hematoma of the left hemisphere (Fig. 1). Brain magnetic resonance imaging (MRI) at the same period showed chronic subdural hematoma with normal myelination pattern. Along with increased subdural hematoma, his seizures changed to left hemiconvulsion with eye deviation to the right. The interictal electroencephalography (EEG) showed beta range rhythmic activity in bilateral parietal area independently. Anticonvulsants (valproic acid, later switched to zonisamide) were started on the diagnosis of focal epilepsy, which resulted in partial control. At 3 months old he received On Meyer reservoir placement to treat increased subdural effusion. After reservoir placement, his seizure was well controlled.

Because of severe developmental delay, he was referred to our hospital for physiotherapy at the age of 9 months, when he showed no head control, eye contact, or purposeful hand use, characterized with asymmetrical tonic neck reflex and opisthotonic posture in some occasions. He was diagnosed as cerebral palsy (CP) with dyskinetic quadriplegia and severe psychomotor retardation. Brain MRI at 3 years old showed asymmetric basal ganglia and lateral ventricle and high intensity of central tegmental tract (CTT) on T2WI (Fig. 2). There were no abnormalities in infratentorial structures (Fig. 2). His EEG at 7 years old showed frequent spikes in the occipital and frontal areas independently during sleep. At the age of 5 and 7, he had status epilepticus, which started with vomiting and eye deviation to the right, followed by left hemiconvulsion. Despite of those episodes, his seizure was completely controlled by using topiramate and phenobarbital.

He had swallowing difficulty, which was evident on video fluorography and likely caused by dyskinetic quadriplegia, and nasogastric tube feeding was administered since 7 years old. He gained social smile and visual pursuit in late childhood.

At present, he had profound psychomotor retardation with no meaningful words, epilepsy with some sporadic seizures, dyskinetic quadriplegia characterized with axial hypotonia, variable tonus, occasional opisthotonic posture, and athetotic movement as well as growth failure; his body weight, height, and OFC were 16 kg (−3.3 SD), 116 cm (−5.7 SD), and 47.2 cm (−5.3 SD), respectively. He showed unstable head control, no achievement of sitting alone, self-standing, or purposeful hand use. He did not done any trachea surgery and his pharyngeal strider was lasting. He could step when seated in the posture control walker. He had some dysmorphic features (hypertelorism, broad nasal bridge, large mouth, and widely spaced teeth).

To clarify the etiology of the patient, we performed trio-based whole-exome sequencing and identified five possible candidate variants, including one de novo variant in the *GPI* gene and four compound heterozygous variants in the *PCDH12* and *MYH4* genes. Among them, compound heterozygous frameshift variants in *PCDH12*, c.448_449del:p.(Leu150Alafs*11) and c.522_525del:p.(Ser175Profs*22), were predicted to be deleterious (Table S). Segregation of *PCDH12* variants was confirmed by Sanger sequencing. These two variants were absent in 575 in-house Japanese control exomes and public databases, including dbSNP137 data, ESP6500, 1KJPN, and The Exome Aggregation Consortium. We evaluated the pathogenicity of these two *PCDH12* variants in accordance with the ACMG variant classification guideline. Both variants were classified as pathogenic.

Discussion

The present patient had new compound heterozygous truncating variants in the *PCDH12* gene, associated with dyskinetic CP, severe intelligence disability, multifocal epilepsy, and severe growth failure. Brain imaging showed asymmetric basal ganglia and lateral ventricle, and high intensity of CTT on T2WI.

Several studies mentioned about the function of non-clustered PCDHs; axonal growth in striatal neurons (*PCDH10*) or amygdala (*PCDH17*), synapse function in hippocampal (*PCDH8*) or basal ganglia (*PCDH17*), cell migration in gastrulation (*PCDH8*) or ventral telencephalon (*PCDH10*), and cortical formation (*PCDH19*) [1, 2, 6]. These findings show that non-clustered PCDHs play roles as the wiring of neural circuit formation with region-specificity and spatiotemporal diversity, and this motility depends on the cell adhesion [1, 2, 6, 7].

PCDH12, previously called vascular endothelial cadherin-2, was first identified in mouse endothelial cells [8]. Studies in mice showed PCDH12 expression in angiogenic

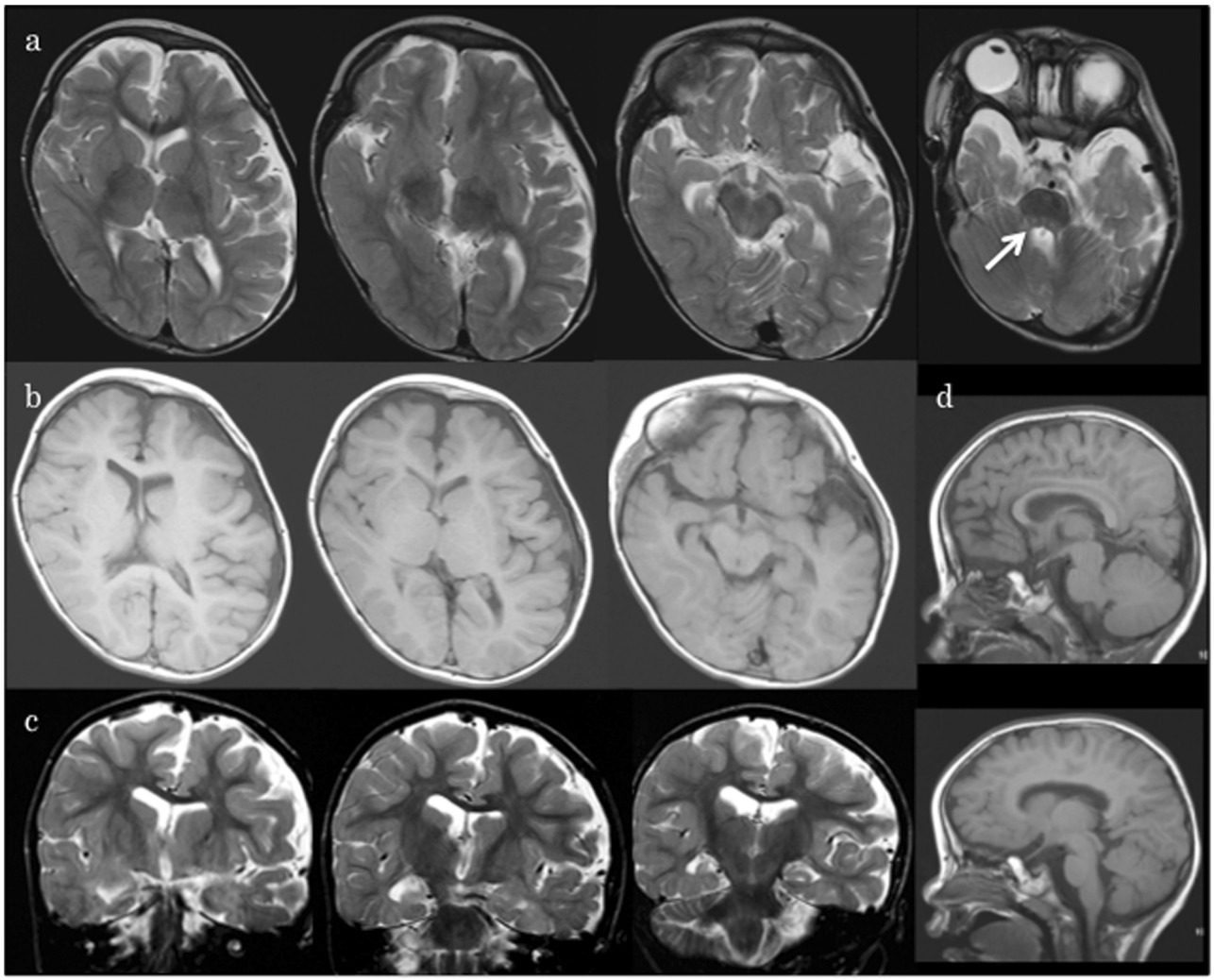

Fig. 2 Brain MRI at 3 years old, axial T2WI (**a**), FLAIR (**b**), coronal T2WI (**c**), and sagittal T1WI (**d**). Asymmetric basal ganglia and lateral ventricle are indicated. High intensity of the central tegmental tract on T2WI was shown with white arrow. He had no midbrain-hypothalamus dysplasia. The infratentorial structures, optic nerve, and corpus callosum are considered as normal

endothelium, placental glycogen cells, and messangial cells of renal glomeruli but barely detectable in brain [8, 9]. *PCDH12*-deficient mice showed reduced placental and embryonic size compared with wild-type mice, but no sign of intelligence disability or seizures. It did not affect survival or postnatal growth [9]. Human *PCDH12* is located on chromosome 5q31 and encodes a protein of 1184 amino acid, with 81% sequence identity with the mouse [3]. In human, same as mice, PCDH12 showed high levels of expression in the placenta, but it has not been detected in the brain [10]. However, taken together with previous report [4], it is evident that PCDH12 has an important role on the development of CNS, which needs further investigation.

The present patient had some particular findings compared with previous report (Table 1). He had no congenital or postnatal microcephaly and intrauterine growth retardation. His severe growth failure may be explained as a result of hypothalamic pituitary hormonal deficiency, suggesting a continuum of the phenotypic spectrum.

At brain imaging, midbrain-hypothalamus dysplasia was not evident in this patient, while he had high intensity of CTT. CTT represents extrapyramidal tract connecting the red nucleus and inferior olivary nucleus. High intensity of CTT on T2WI has been detected in several disorders, e.g., CP, hypoxic ischemic encephalopathy, and congenital metabolic disorders [11–13]. Autopsy analysis of CTT demonstrated fiber loss, gliosis, and increased vacuoles in tegmental tracts [11]. Derinkuyu et al. reported high intensity of CTT on T2WI was most frequently detected in dyskinetic CP [12]. From these findings, high intensity of CTT may show the results of tegmental fiber injury and be caused by complex pathomechanism, including secondary degeneration [11–13].

Table 1 Detailed clinical summary and neuroimaging findings of the patient and previously reported patients

	Present case	Aran et al.			
		Case 1	Case 2	Case 3	Case 4
Sex	Male	Female	Male	Female	Female
Intrauterin growth reterdation	−	+	+	+	+
Brain calcification	+	ND	+	ND	ND
Brain imaging (age studied)	Asymmetric basal ganglia, high intensity of central tegmental tract (3 y)	Dysplasia of the midbrain-hypothalamus-optic tract (6 months)	Dysplasia of the midbrain-hypothalamus, ventriculomegaly, HCC (day 26)	ND	Dysplasia of the midbrain-hypothalamus (32 w, GA)
Seizure onset	2 months	Day 21	Day 4	Day 7	NA
Progressive microcephaly	−	+	+	+	NA
Growth failure	+	NC	NC	NC	NA
Dysmorphism	Hypertelorism, broad nasal bridge, large mouth, widely spaced teeth	−	−	NC	NA
Axial hypotonia	+	+	+	+	NA
Dystonia/athetosis	+	+	+	+	NA
Spasticity	+/−	−	−	+	NA
Hearing loss	−	−	−	−	NA
Visual impairment	−	+	+	+	NA
Developmental milestones (age examined)	Social smile, visual pursuit, unstable head control (14 y)	Rare social smile, inconsistent eye contact (3 y)	Social smile, laugh (5 y)	Social smile, laugh, rolls over (26 y)	NA
Mutation of *PCDH12*	c.448_449del (p.Leu150Alafs*11) c.522_525del (p.Ser175Profs*22)	c.2515C>T p.Arg839* (homozygous)	c.2515C>T p.Arg839* (homozygous)	c.2515C>T p.Arg839* (homozygous)	c.2515C>T p.Arg839* (homozygous)

ND not done, *NA* not applicable, *NC* not commented, *GA* gestational age, *HCC* hypoplasia of corpus callosum

His truncation mutation of both alleles suggests severe functional damage enough to be pathogenic and the variation of mutation site may partly explain clinical differences. As another explanation, epigenetic dysregulation of *PCDH12* is possible, because several studies reported alterations in DNA methylation patterns of PCDHs, which was identified in postmortem brains of individuals with various brain disorders such as chromosome disorders, psychiatric or autism spectrum disorders, child maltreatment, and prenatal alcohol exposure [14].

Previous report highlighted congenital microcephaly and intracerebral calcification as a feature of *PCDH12* gene variants. From our experience, however, we need to consider *PCDH12* gene variants as a differential diagnosis for patients with dyskinetic CP associated with nonspecific MRI findings. Besides *PCDH12* gene variants, several gene variants were previously identified as a candidate for dyskinetic CP, most of which were complicated with epilepsy and intellectual disability [15]. Further case presentations are required to elucidate clinical spectrum of this gene variants.

Acknowledgements We thank the patient and the patient's family for the participation in this study. This study was supported partly by a Grants from Research on Measures for Intractable Diseases (NM), Comprehensive Research on Disability Health and Welfare (NM), the Strategic Research Program for Brain Science (NM), the Initiative on Rare and Undiagnosed Diseases in Pediatrics (NM), the Initiative on Rare and Undiagnosed Diseases for Adults (NM) from the Japanese Agency for Medical Research and Development; a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grants-in-Aid for Scientific Research (A and C), and challenging Exploratory Research from the Japan Society for the Promotion of Science (JSPS); the fund for the Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems (NM) from the Japanese Science and Technology Agency; and the Takeda Science Foundation (NM).

Author contributions SS-M: study conceptualization; design; and manuscript preparation. KW, RS, TM, YO, WE, TI, NT, and AK: clinical evaluation and rehabilitation. MN, HS, NM: gene analysis and interpretation of genetic analysis data. HO: interpretation of neuroradiological data. KH: study concept; critical revision of the manuscript; and study supervision.

Compliance with ethical standards

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

References

1. Hayashi S, Takeichi M. Emerging roles of protocadherins: from self-avoidance to enhancement of motility. J Cell Sci. 2015;128:1455–64.
2. Kim SY, Yasuda S, Tanaka H, Yamagata K, Kim H. Non-clustered protocadherin. Cell Adh Migr. 2011;5:97–105.
3. Wu Q, Maniatis T. Large exons encoding multiple ectodomains are a characteristic feature of protocadherin genes. Proc Natl Acad Sci USA. 2000;97:3124–9.
4. Aran A, Rosenfeld N, Jaron R, Renbaum P, Zuckerman S, Fridman H, et al. Loss of function of PCDH12 underlies recessive microcephaly mimicking intrauterine infection. Neurology. 2016;86:2016–4.
5. Nicolas G, Sanchez-Contreras M, Ramos EM, Lemos RR, Ferreira J, Moura D, et al. Brain calcifications and *PCDH12* variants. Neurol Genet. 2017;3:e166.
6. Kurian M, Korff CM, Ranza E, Bernasconi A, Lübbig A, Nangia S, et al. Focal cortical malformations in children with early infantile epilepsy and *PCDH19* mutations: case report. Dev Med Child Neurol. 2018;60:100–5.
7. Kim SY, Chung HS, Sun W, Kim H. Spatiotemporal expression pattern of non-clustered protocadherin family members in the developing rat brain. Neuroscience. 2007;147:996–1021.
8. Telo' P, Breviario F, Huber P, Panzeri C, Dejana E. Identification of a novel cadherin (vascular endothelial cadherin-2) located at intercellular junctions in endothelial cells. J Biol Chem. 1998;273:17565–72.
9. Rampon C, Bouillot S, Climescu-Haulica A, Prandini MH, Cand F, Vandenbrouck Y, et al. Protocadherin 12 deficiency alters morphogenesis and transcriptional profile of the placenta. Physiol Genomics. 2008;34:193–204.
10. Bouillot S, Tillet E, Carmona G, Prandini MH, Gauchez AS, Hoffmann P, et al. Protocadherin-12 cleavage is a regulated process mediated by ADAM10 protein: evidence of shedding up-regulation in pre-eclampsia. J Biol Chem. 2011;286:15195–204.
11. Shioda M, Hayashi M, Takanashi J, Osawa M. Lesions in the central tegmental tract in autopsy cases of developmental brain disorders. Brain Dev. 2011;33:541–7.
12. Derinkuyu BE, Ozmen E, Akmaz-Unlu H, Altinbas NK, Gurkas E, Boyunaga O. A magnetic resonance imaging finding in children with cerebral palsy: symmetrical central tegmental tract hyperintensity. Brain Dev. 2017;39:211–7.
13. Yoshida S, Hayakawa K, Yamamoto A, Aida N, Okano S, Matsushita H, et al. Symmetrical central tegmental tract (CTT) hyperintense lesions on magnetic resonance imaging in children. Eur Radiol. 2009;19:462–9.
14. El. Hajj N, Dittrich M, Haaf T. Epigenetic dysregulation of protocadherins in human disease. Semin Cell Dev Biol. 2017;69:172–82.
15. Kobayashi Y, Tohyama J, Kato M, Akasaka N, Magara S, Kawashima H, et al. High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders. Brain Dev. 2016;38:285–92.