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Case Report

Two unrelated girls with intellectual disability associated with a truncating mutation in the *PPM1D* penultimate exon

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Abstract

PPM1D truncating mutations in the last and penultimate exons of the gene have been associated with intellectual disability (ID) syndrome. Only 15 affected patients to-date have been reported with mild-to-severe ID, autistic behavior, anxiety and dysmorphic features. Here, we describe the clinical characteristics and underlying genetics of two unrelated girls with moderate developmental delay and dysmorphic features associated with novel mutations in *PPM1D* exon 5. The dysmorphic features demonstrated by these two patients are consistent with previously reported patients, including broad forehead, thin upper lip, brachydactyly, and hypoplastic nails. We identified a *de novo PPM1D* mutation in exon 5 of each patient (c.1250_1251insACCA p.V419Tfs*16 and c.1256_1257insCAAG p.S421Qfs*14) by panel sequencing for 4,813 disease-related genes. Both patients also had frameshift mutations (at different positions) that resulted in the same estimated termination codon at 434. These additional reports add to the growing literature on *PPM1D*-associated ID syndrome and help delineate the clinical phenotype and genetic basis.

Keywords: *PPM1D*; *De novo* mutation; Last exon; Dysmorphic; Intellectual disability

1. Introduction

Truncating variants around the last and penultimate exons cause various congenital anomalies [1–3]. Such variants have thus been proposed to escape nonsense-mediated mRNA decay. Jansen *et al.* reported 14 unrelated patients with intellectual disability (ID) syndrome in which *PPM1D* truncating mutations were identified in the last and penultimate exons (exon 5 and 6) [4]. *PPM1D* is a member of the PP2C family of Serine/Threonine protein phosphatases, encoding protein phosphatase Mg^{2+}/Mn^{2+}-dependent 1D [5]. PPM1D plays a role in the negative regulation of cellular stress response induced in p53-dependent manner [6]. Here, we report two unrelated girls with moderate developmental delay and dysmorphic features. Both patients had a novel mutation in exon 5 of *PPM1D*.

2. Clinical reports

2.1. Patient 1

Patient 1 is a 2 year-old girl and the first and only child of healthy and nonconsanguineous parents. She

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was delivered at 37 weeks gestation with vacuum extraction due to a decelerated fetal heart rate. Her birth weight was 2211 g (2−1.4 SD), length 45.5 cm (−1.3 SD), and occipital frontal circumference (OFC) 31.5 cm (0.8 SD). Newborn hearing screening testing was passed. She was referred to our hospital because of developmental delay at 11 month-of-age. Neurological examination revealed hypotonia. Blood amino acid, lactic acid, pyruvic acid, and thyroid hormone were normal.

At referral (11 months), her body weight was 7.3 kg (−1.4 SD), height 67 cm (−2.1 SD), and OFC 42 cm (−1.6 SD). Blood amino acid and She started to control her head at 4 months, rolling at 7 months, crawling at 15 months and sitting at 16 months. At 15 months, cranial MRI revealed hypoplasia of the ventral part of the pons but a normal level of myelination for her age (Fig. 1A). At 25 months, she had a moderate developmental delay (developmental quotient 50). At 31 months, her body weight was 10.0 kg (−1.6 SD), height 81.7 cm (−2.3 SD), OFC 46.0 cm (−1.3 SD). She did not walk unsupported or speak recognizable words, she showed a high pain threshold and had no sensitivity to sound. She had constipation and did not have periodic illness and recurrent infection. Dysmorphic features included a broad forehead, arched eyebrows, hypoplastic nasal columella, thin upper lip, pointed chin, absent middle phalanx of the right fifth finger, brachydactyly, and hypoplastic nails (Fig. 1B).

2.2. Patient 2

Patient 2 is a 5 year-old girl who is the first child of healthy and nonconsanguineous parents. She had two other siblings who were healthy. She was delivered at 38 weeks gestation by caesarian section due to cephalopelvic disproportion. Her birth weight was 3288 g (0.6 SD), length 48.0 cm (−0.6 SD), and OFC 32.0 cm (−0.8 SD). She was referred to our hospital because of signs of developmental delay at 11 month-of-age. She started controlling her head at 4 months, rolling at 9 months, crawling at 18 months, sitting at 16 months and walking at 3.5 years. She spoke recognizable words at 12 months, but still did not speak in sentences at 4 year-of-age. At 12 months, cranial MRI revealed enlarged cerebral ventricles, but findings from a follow-up MRI at 3 years were normal. She had a moderate developmental delay (intelligence quotient 49) at 5 years 9 months old. At this time, her body weight was 18.45 kg (−0.1 SD), height 104.1 cm (−1.6 SD) and OFC 48.2 cm (−1.7 SD). She showed a high pain threshold and high sensitivity to sound. She did not have periodic illness and recurrent infection. Dysmorphic features included a broad forehead, arched eyebrows, hypoplastic nasal columella, thin upper lip, pointed chin, brachydactyly, and hypoplastic nails (Fig. 1C).

3. Results

The study design was approved by the Kanagawa Children's Medical Center Review Board and Ethics Committee. Written informed consent was obtained from the patients' parents.

We performed panel sequencing for 4813 genes with known associated clinical phenotypes (TruSight One Sequencing Panel (Illumina Inc., San Diego, CA)). The mean depth of coverage over all samples was 52.97 and 62.17 per base, and bases covered by at least 10 reads were 97.0% and 96.2% of coding regions, respectively. The data were analyzed using BWA (version 6) and the GATK pipeline (Broad Institute) as described previously [7]. Copy-number variations (CNV) were called based on log-ratio analysis and the z-score of the read depth on each exon. Here, we identified a *PPM1D* mutation at exon 5 (c.1250_1251insACCA p.V419Tfs*16) in Patient 1 and exon 5 (c.1256_1257insCAAG p.S421Qfs*14) in Patient 2. The *PPM1D* variants were selected as only one truncating variant from total 354 and 344 heterozygous variants according to the pipelines (Fig. 2A). The *PPM1D* variants were confirmed by Sanger sequencing as a *de novo* mutation (Fig. 2B, C). Neither mutation was present in an in-house database including genetic data from 528 individuals or various reference databases (see Supplementary Material and Fig. 2A).

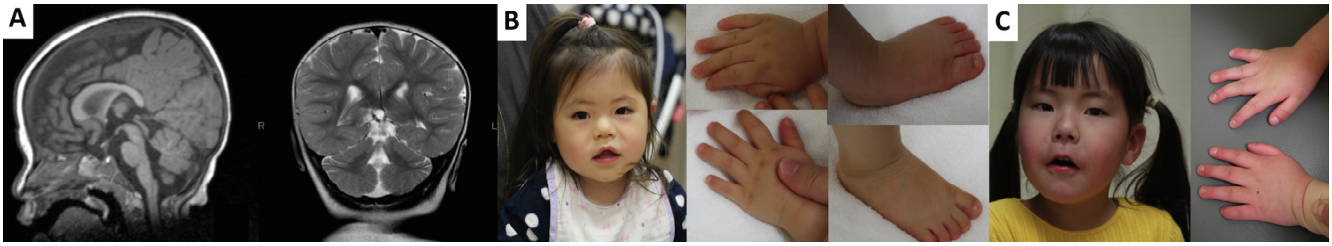

Fig. 1. Patient photographs showing dysmorphic features and cranial MRI. (A) Cranial MRI of Patient 1 at 15 month-of-age (left: sagittal, right: coronal) (B) Photograph of Patient 1 at 2-year-of-age and (C) Photograph of Patient 2 at 5 year-of-age. Patient 1 and 2 had consistent dysmorphic feature, including a broad forehead, arched eyebrows, hypoplastic nasal columella, prominent philtrum, thin upper lip, pointed chin, brachydactyly and hypoplastic nails. Written consent to publish photographs was obtained from each family.

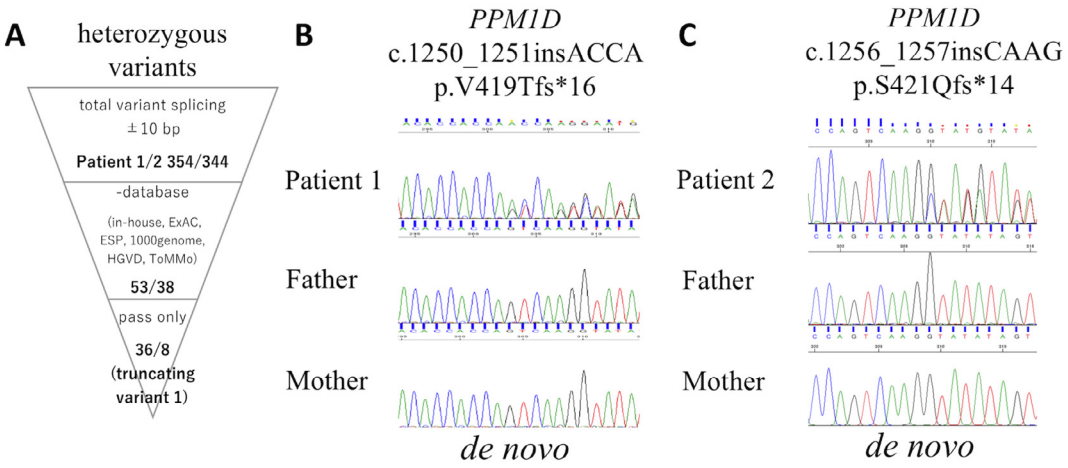

Fig. 2. Proband-only targeted next generation sequencing (NGS) and Sanger sequencing of Patient 1 and 2. (A) The detected variants with targeted NGS (a panels of 4813 disease-related genes) and filtering process of heterozygous variants according to the pipelines. (B, C) Sanger sequencing confirmed the *de novo PPM1D* variants.

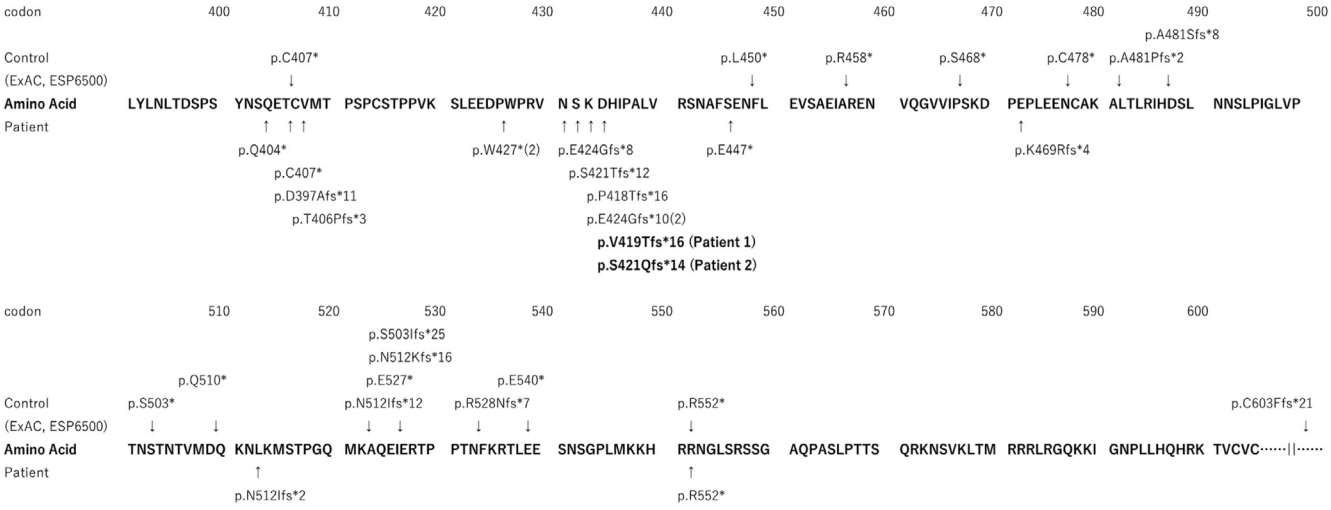

Fig. 3. Genetic mapping of termination codon for *PPM1D*-reported variants in patients and controls. The upper and lower rows indicate the variants present in the control population (ExAC and ESP6500) and patients, respectively. Arrows indicate the termination codon for each variant. The variants showed a different distribution among two populations.

4. Discussion

Here we report of two unrelated patients demonstrating moderate developmental delay and distinctive dysmorphic features. Both patients had an underlying *PPM1D* truncation mutation in the penultimate exon. *PPM1D* truncating mutations in the last and penultimate exons are known to cause ID syndrome, resulting from escaped nonsense-mediated mRNA decay. *PPM1D* amplification or gain-of-function mutation have been detected in the breast, ovarian, colon and lung cancer [8,9]. Cancer predisposition have not been reported in the patients with *PPM1D*-related ID syndrome. Only 15 patients have been reported to-date with *PPM1D*-associated ID [4,10]. Our study thus adds to the clinical phenotype information of affected patients. Present and previous reported patients exhibited clinical features that were characterized by ID, anxiety disorder, autism spectrum disorder, high pain threshold, and sensitivity of sounds. They also presented with dysmorphic features consistent with each other and the previously reported patients, including a broad forehead, thin upper lip, and brachydactyly. Their dysmorphic features delineate the clinical features associated with *PPM1D*-related ID syndrome.

Truncation mutations at specific positions in the final two exons of *PPM1D* are proposed to underlie ID pathogenesis. Here, both patients had *PPM1D* frameshift mutations creating the same termination codon at 434. According to ExAC and EPS6500 databases, *PPM1D* truncating mutations around the last and penultimate exons (exon 5 and 6) are also present in the control population. *PPM1D* showed a low loss-of-function intolerance (pLI) score, meaning that *PPM1D*

truncating mutations are present in the healthy population but are not associated with any clinical phenotypes. *PPM1D* truncating variants around exon 5 and 6 were also present in the reported patients, but the distributions between patients and the control population are different (Fig. 3). Combining the data from the present and previous studies, truncating mutations at codon position 418–427 (boundary between exon 5 and 6) have now been identified in nine of 17 reported patients with ID. The locations of these estimated termination codons were concentrated to codon 432–434 in eight of these nine patients.

In conclusion, we have identified and characterized two patients with *PPM1D*-associated ID syndrome. *PPM1D* truncating mutations in specific regions - namely at the exon 5–6 boundary – seem to lead to ID with distinctive clinical features. Such truncating mutations might make the most important contribution to ID pathogenesis.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.02.007.

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