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BRIEF COMMUNICATION

The second point mutation in *PREPL*: a case report and literature review

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Abstract

Prolyl endopeptidase-like (PREPL) deficiency (MIM# 616224) is a rare autosomal recessive inherited congenital myasthenic syndrome characterized by neonatal hypotonia, feeding problems, mild dysmorphism, and neuromuscular symptoms, followed by hyperphagia and obesity in later childhood. Some patients also exhibit growth deficits, sexual hormone deficiency, and cognitive impairments. This syndrome is caused by biallelic mutations in *PREPL*. To date, only one nucleotide deletion and seven small microdeletions in *PREPL* have been reported. Here we report a female patient with a novel homozygous frameshift mutation in *PREPL* (NM_006036.4, c.342delA:p.Val115Leufs*39). Her clinical features are similar to those of previously reported cases. The mutation is the first homozygous point mutation reported in humans.

Prolyl endopeptidase-like (*PREPL*; MIM*609557, NM_006036.4) encodes PREPL protein, a serine oligopeptidase involved in the filling of acetylcholine into synaptic vesicles [1]. Historically, PREPL deficiency was described as part of a recessive contiguous gene deletion syndrome involving *PREPL* and *SLC3A1*, also known as hypotonia-cystinuria syndrome (HCS, MIM#606407) [2–5]. Because cystinuria in HCS is caused by *SLC3A1* deficiency, the other symptoms (severe neonatal hypotonia, growth impairment, and cognitive problems) were thought to arise from PREPL deficiency [3, 6]. Isolated PREPL deficiency causes an autosomal recessive inherited congenital myasthenic syndrome (MIM# 616224) characterized by severe neonatal hypotonia, muscular weakness, feeding problems, and failure to thrive. These symptoms tend to improve spontaneously after the first year of life, with subsequent development of obesity due to hyperphagia in late childhood (6–11 years) [7, 8]. Patients also present with facial dysmorphism and motor developmental delay, and some show growth and sexual hormone deficiencies as well as cognitive deficits [7]. To date, only seven patients with isolated PREPL deficiency have been reported, and homozygous point mutations have never been reported [7–9]. Here we report a Chilean female with a novel homozygous *PREPL* mutation.

The proband is a 10-year-old female born to healthy parents as an only child (Fig. 1). Her nonconsanguineous parents originated from the same small part of Chiloé Island off the coast of southern Chile. She was born after an uneventful pregnancy at 38 weeks of gestation with Apgar scores of 9 and 10 at 1 and 5 min, respectively. At birth, her weight, length, and occipitofrontal circumference were 2950 g (−0.73 SD), 49 cm (−0.33 SD), and 34 cm (−0.41 SD), respectively. Dysmorphic features were noted at birth, including dolichocephaly, prominent ears, long palpebral fissures with palpebral ptosis, and high-arched palate. She presented with neonatal hypotonia and feeding problems with severe failure to thrive during the first 3 years of life (Fig. 1), requiring nutritional supplements and a nasogastric tube. Neurological evaluation at 10 months of age revealed global hypotonia, muscle weakness, and nystagmus, which is currently absent. After the first year of life, hypotonia and muscular weakness showed spontaneous improvement. She showed predominantly motor developmental delay, walking independently at 27 months of age. After 1 year of age, she spoke single words with difficulty and a remarkable nasal voice.

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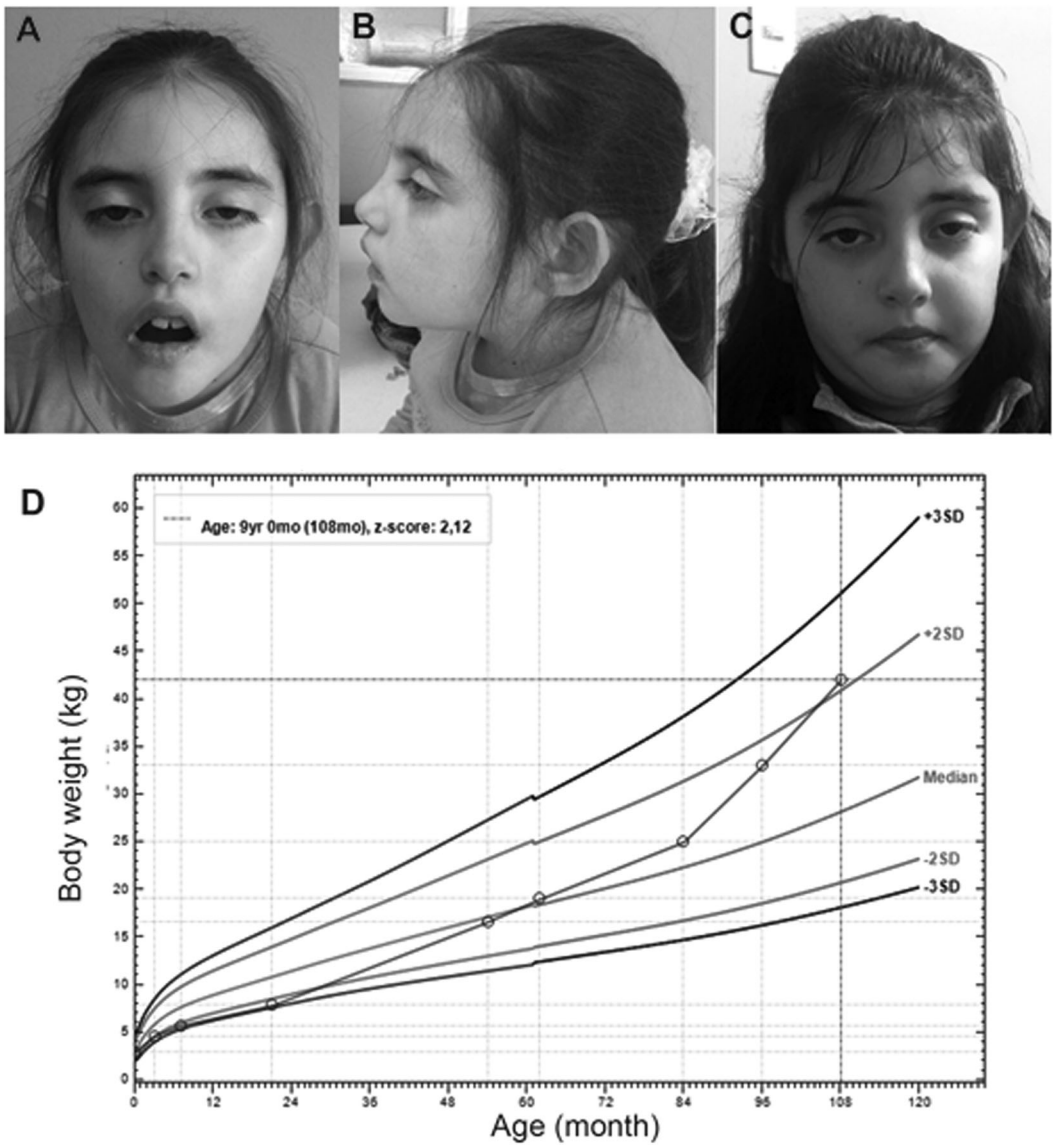

Fig. 1 Clinical features of the patient. Facial photographs of the patient at the age of 7 years (**a**, **b**) and 9 years (**c**), and her growth curve (**d**)

Serum creatine kinase was normal in her medical records. Electromyography and nerve conduction velocity were normal at 3 and 6 years of age. Muscle biopsy had not been performed. Brain magnetic resonance imaging at 6 years was unremarkable and serum amino-acid profile was normal. At 7 years, her intelligence quotient was 49, requiring special educational support. At 9 years, serum insulin-like growth factor-1 (IGF-1; 199 ng/ml, normal range: 175–445 ng/ml) and IGF-binding protein-3 (IGFBP-3; 3188 ng/ml, normal range: 3100–4544 ng/ml) were normal, and bone age was concordant with her chronological age. Growth hormone and sexual hormones were not measured. Her karyotype is 46,XX.

From the age of 4 years, she developed hyperphagia (Fig. 1). Her current body mass index at 10 years is 27.6 kg/m^2 (+2.8 SD) based on weight (46 kg, +1.9 SD) and height (129 cm, −1.6 SD). She had facial weakness with bilateral palpebral ptosis, open mouth with tented upper lip, and large front teeth (Fig. 1). Social development is normal. Salivation is normal, and her nasal voice persists. Her motor exam revealed that the patient has global muscle weakness and it is more prominent at the proximal muscles (deltoid and psoas muscles), with reduced osteotendinous reflexes and Gower sign. Her gait is normal and mild scoliosis is recognized, and she is accordingly receiving physiotherapeutic intervention. There is no history of fatigability or muscle wasting induced by exercise or fluctuation in peripheral weakness. Facial weakness is occasionally seen and irregularly improves transiently together with mild attenuation of palpebral ptosis and open mouth.

To identify the genetic cause of this patient, we performed whole-exome sequencing as reported previously [10] after obtaining informed consent from the patient's family. This study was approved by the Institutional Review Board of Yokohama City University School of Medicine. We identified a homozygous frameshift mutation in *PREPL* (c.342delA:p.Val115Leufs*39) (Fig. 2). Sanger sequencing revealed that her mother was heterozygous for the same mutation. Unfortunately, the paternal sample was unavailable. Quantitative PCR at two positions within *PREPL* (exon1: chr2:44586650–44586808, 159 bp; and exon 2 involving this variant: chr2:44573281–44573508, 228 bp) in the proband ruled out the presence of a deletion (Fig. 1c). In addition, two different copy number detection programs using exome data, eXome-Hidden Markov Model [11] and depth of coverage methods [12], did not reveal any copy number changes involving *PREPL*. Thus, the possibility of a microdeletion around *PREPL* was ruled out. The 15.4-Mb homozygous region including this variant was highlighted by HomozygosityMapper (http://www.homozygositymapper.org/) using exome data. This variant is not registered in ExAC, Exome Variant Server, or our in-house exome database ($n = 575$). This frameshift was located in protein-coding exon 2 (of a total of 14 exons), which might be subjected to nonsense-mediated mRNA decay. Given the reported loss of function in *PREPL* mutants [7, 8], the homozygous *PREPL* mutation in the proband is likely pathogenic based on ACMG guidelines [13].

To date, 7 patients with isolated *PREPL* deficiency [7–9] and 21 HCS families (contiguous deletion, including *SLC3A1* and *PREPL* genes) have been reported [2–5, 7, 9, 14–16]. All deletions are homozygous or compound heterozygous. The clinical features of all reported families whose detailed clinical data were provided and our patient are summarized in Table 1. The characteristic features are neonatal hypotonia, feeding difficulty with later hyperphagia and obesity, dysmorphic traits, nasal voice, and delayed independent walking. Growth deficiency is frequently reported, and some cases are accompanied by growth hormone deficiency, with a good response to growth hormone therapy [3, 7, 8]. Our patient's stature is normal (approximately −1.6 SD) with normal serum IGF-1 and IGFBP-3.

Of note, moderate intellectual disability (ID) in the current patient attracted our attention. Reported patients usually require special support in school, but ID is infrequent and usually mild if observed [7, 8]. This finding that a homozygous point mutation results in moderate ID strongly suggests that biallelic *PREPL* mutations alone (without involvement of other genes) can cause ID.

In conclusion, we report the first homozygous *PREPL* point mutation in a girl with typical PREPL deficiency. This syndrome should be considered in the differential diagnosis of hypotonic neonates exhibiting myasthenic symptoms, hyperphagia, and various degrees of ID.

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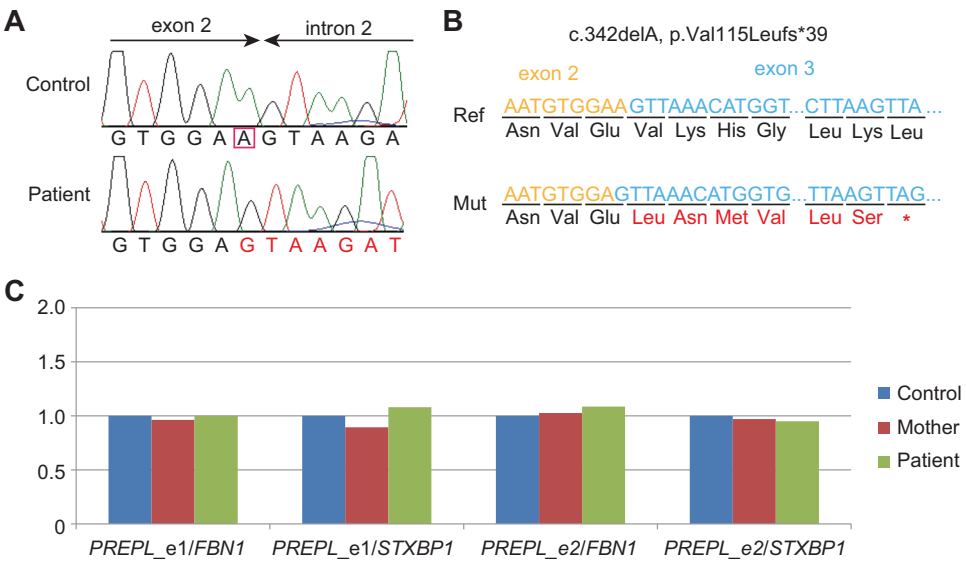

Fig. 2 Genetic analysis. Electropherogram of the proband (**a**), and cDNA and predicted amino-acid sequences in the wild-type (Ref) and mutant (Mut) (**b**). The cDNA regions corresponding to exons 2 and 3 are colored orange and light blue, respectively. Amino acids altered by the mutation are colored red. **c** q-PCR using calibration curve methods was performed. The amplicons of *FBN1* and *STXBP1* were used as internal controls

Table 1 Clinical features of patients with isolated PREPL deficiency and HCS (*SLC3A1* and *PREPL*)

Diseases/deleted genes	PREPL deficiency	PREPL deficiency	PREPL deficiency	HCS/ *SLC3A1-PREPL*	HCS/*SLC3A1-PREPL*	HCS/ *SLC3A1-PREPL*	HCS/ *SLC3A1-PREPL*	HCS/ *SLC3A1-PREPL*
Reference	Present case	Regal et al. [7]	Regal et al. [8]	Regal et al. [7]	Eggermann et al. [14]	Regal et al. [5]	Martens et al. [4]	Jaeken et al. [3]
Number of patients	1	5 (5 families)	1	3 (2 families)	1	2 (2 families)	4 (4 families)	11 (9 families)
Gender (female:male)	1:0	3:2	1:0	1:2	0:1	1:1	1:3	5:6
Age at diagnosis	9 y	1–25 y	NA	3 m–31 y	NA	NA	3–16 y	4–42 y
Perinatal period								
Neonatal hypotonia	+	+ (5/5)	+	+ (3/3)	+	+ (2/2)	+ (4/4)	+ (11/11)
Feeding difficulty	+	+ (4/5)	+	+ (3/3)	+	+ (2/2)	+ (4/4)	+ (11/11)
Growth deficiency	–	+ (2/5)	NA	+ (3/3)	+	+ (1/2)	+ (3/4)	+ (8/9)
GH deficiency	ND	+ (3/5)	+	+ (1/1)	NA	NA	NA	+ (6/8)
Hypergonadotropic hypogonadism	ND	+ (2/2)	NA	+ (1/2)	NA	NA	NA	+ (4/11)
Childhood overweight	+	+ (2/5)	NA	+ (2/3)	NA	NA	NA	+ (11/11)
Intellectual disability	+ moderate	+ (1/5) mild	NA	+ (1/3) mild	NA	+ (1/2) mild	NA	NA
Dysmorphic features	+	+ (5/5)	+	+ (3/3)	+	+ (2/2)	+ (3/4)	+ (11/11)
Neurological features								
Age at walking (months)	27	21 (19–24)	17	32 (26–36)	15	NA	17 (14–23)	15–36
Nasal voice	+	+ (5/5)	NA	+ (3/3)	NA	+ (1/2)	+ (3/4)	+ (11/11)
Facial weakness	+	+ (5/5)	+	+ (3/3)	NA	NA	NA	NA
Proximal weakness	+	+ (1/5)	+	+ (1/2)	NA	NA	NA	NA
Fluctuating weakness	+ (facial)	+ (3/5)	+	+ (3/3)	NA	NA	NA	NA
EMG-NCS	Normal	Normal (1/1)	Abnormal[a]	NA	NA	NA	Normal	Normal

EMG-NCS electromyography-nerve conduction study, *HCS* hypotonia-cystinuria syndrome, *GH* growth hormone, *NA* not available, *ND* not done, + present, − absent

[a] Myasthenic features on electrophysiologic study

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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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