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Case Report

Prolyl Endopeptidase-like (PREPL) Deficiency Associated with Growth Hormone Deficiency: Case Report

Sayol-Torres et al. PREPL and GH deficiency: Case Report

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What is already known on this topic?
Prolyl endopeptidase-like (PREPL) deficiency (MIM#616224) is a rare congenital disorder characterized by neonatal hypotonia and feeding difficulties, growth hormone (GH) deficiency and hypergonadotropic hypogonadism. This syndrome is an autosomal recessive disease resulting from mutations in the *PREPL* gene (MIM#609557).

What this study adds?
This report describes a novel mutation that has not been described in the literature to date. Also we describe a typical presentation of the syndrome, with early growth impairment in infancy due to growth hormone deficiency and a good response to growth hormone treatment. The description of new patients with PREPL deficiency syndrome is essential to better delineate the phenotypic and genotypic spectrum of the disease.

Abstract
Prolyl endopeptidase-like (PREPL) deficiency (MIM#616224) is a rare congenital disorder characterised by neonatal hypotonia and feeding difficulties, growth hormone (GH) deficiency and hypergonadotropic hypogonadism. This syndrome is an autosomal recessive disease resulting from mutations in the PREPL gene (MIM#609557). Herein we report a 7-year-old female patient with biallelic mutations in PREPL (c.1528C>T in one allele and a whole gene deletion in the other) with early growth impairment in infancy. Growth hormone deficiency was confirmed at 20 months of life. Recombinant growth hormone treatment was introduced with a good response. Her clinical features were similar to those of previously reported cases. The description of new patients with PREPL deficiency syndrome is essential to better delineate the phenotypic and genotypic spectrum of the disease.
Keywords: Prolyl endopeptidase-like, growth hormone deficiency, genetics

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Introduction
The prolyl endopeptidase-like gene (*PREPL*) is ~43 kb long, located in 2p21 and encodes the PREPL protein which is a cytoplasmatic serine hydrolase belonging structurally to an oligopeptidase family [1]. Historically PREPL deficiency was described as part of a recessive contiguous gene deletion syndrome involving *PREPL* and *SLC3A1*, known as hypotoniacystinuria syndrome (HCS). While cystinuria in HCS is caused by SLC3A1 deficiency, the other symptoms (neonatal hypotonia, growth impairment and cognitive problems) arise from PREPL deficiency [2]. This second isolated PREPL deficiency is also known as congenital myasthenic syndrome 22 (MIM#616224).
To date, only fourteen mutations have been described in the *PREPL* gene that are associated with HCS or congenital myasthenic syndrome [3]. Here we report a female child with isolated PREPL deficiency, with a single nucleotide variant (c.1528 C>T) in *PREPL* and a 0.031Mb deletion in 2p21 (including *PREPL* only).
Most of the available literature about PREPL deficiency focuses on neurological symptoms. In this report, we outline the hormonal disorders associated with this syndrome.

Case Report
The proband is a 7-year-old female born to nonconsanguineous Caucasian healthy parents, with a healthy younger brother. Pregnancy was appropriately monitored and without major medical problems, teratogenic exposures or hospitalizations. The patient was born by C-section for breech presentation at 39 weeks of gestation. At birth, her weight was 2.855g (-0.9 SD), length

47cm (-1.5 SD) and cranial perimeter 34.5cm (+0.2 SD). Dysmorphic features were noted at birth, including broad nasal root, microretrognatia, mild thenar hypoplasia and bilateral 5th finger clinodactyly. She presented with neonatal hypotonia and was poorly reactive to stimulus. She suffered from neonatal hypoglycaemia due to feeding problems, so she required a nasogastric tube for nutrition for the first month after birth. Among the diagnostic possibilities for the neonatal hypotonia infection, cardiopathy and toxics were discarded. Additionally, she had a normal metabolic workup, no cystinuria and a normal EKG and brain MRI at birth. Electromyography at 4 months of age was also normal. Muscle biopsy was not performed, but levels of creatine-kinase were normal. Thyroid hormones were also normal thus, having ruled out other causes, array-CGH and also MLPA for Prader-Willi syndrome were performed with neither revealing any alterations.

Neurological evaluation at 5 months revealed persistence of global hypotonia with axial dominance, with apparent improvement over time. She acquired autonomous standing at one year of life and began autonomous ambulation at 18 months. She needed motor rehabilitation and stimulation in a specialized centre.

Stunted growth became evident with a height of 72cm (-3.8 SD) at 20 months of life. The serum insulin-like growth factor (IGF-1) level was low (27ng/mL), as was the binding protein IGFBP-3 level (1.78 mg/L). Pharmacological testing with glucagon showed no response, with the highest peak of growth hormone at the start (3.22ng/mL) and 0.48ng/mL at 60 minutes. Additionally, she presented a delayed bone age (9 months of bone-age at a chronological age of 14 months). Celiac disease and hypothyroidism were discarded as part of the study of GH deficiency and a hypothalamic-hypophyseal MRI did not reveal any alteration. Being diagnosed with GH deficiency, substitutive treatment was started at an age of 2 years and 8 months. She rapidly responded to GH treatment, significantly increasing growth velocity from 7cm/year to 11cm/year.

Currently, at 7 years and 5 months old, she is still under GH treatment presenting a good response (Fig1), with a weight of 18 kg (-1.75 SD), a height of 116.5 cm (-1.8 SD) and a prepuberal Tanner (P1S1). Her bone-age is still younger than her chronological age. She eats all kinds of food in small quantities without dysphagia. On physical examination, she only presents left ptosis associated with fatigue, no hypomimia, and a normal axial tone. Dysmorphologic evaluation shows epicanthus, mandibular retrognathia, ogival palate, a notch in the earlobe and mild clinodactyly of the 5th finger with small but proportionate feet and hands. Ligament laxity is also evident. She has nasal voice. Social development and educational attainment are normal. Her motor exam revealed that the patient has a normal muscular axial tone and correctly aligned rachis, with normal osteotendinous reflexes and tendency to toe walking.

To identify the genetic condition of this patient, we first performed a Next-Generation Sequencing study including genes *IGF2, IGF1R, IGF1, NPR2, GH1, GHR, GHRHR, IGFALS, STAT5B, CCDC8* and *GHSR* without revealing any pathogenic variant. Furthermore, methylation analysis of Silver-Russel syndrome region was also normal. Therefore, a whole-exome sequencing was carried out after obtaining informed consent from the patient's family. We identified an apparently homozygous variant in *PREPL* c.1528C>T, recognized as pathogenic in VarSome [4]. The progenitor direct genetic study revealed that this was from paternal inheritance. Although the explanation for this apparently homozygous state could be an isodisomy, given that deletion of *PREPL* has been frequently described, an array-CGH (with exonic coverage of *PREPL*) was performed and it showed a 0.031Mb deletion in 2p21 chromosome (including *PREPL* gene), classified as pathogenic with a recessive inheritance. The deletion was inherited from the mother. Therefore, the PREPL deficiency in the patient was due to a point mutation in one allele and a whole gene deletion in the other.

Discussion

Hypotonia in early infancy may be a sign of a central nervous disorder, a primary neuromuscular disorder or a genetic syndrome associated with hypotonia. However these signs most frequently occur as a consequence of common neonatal conditions, such as congenital infections, hypothyroidism or drug toxicity. In our case, these more common conditions were excluded, so genetic syndromes were considered.

Hypotonia-cystinuria syndrome (HCS) has been described as a disorder with cystinuria and congenital myasthenic resulting from the recessive deletions in SLC3A1 and PREPL [2, 5-8]. To date, only 11 patients [2, 3, 5, 6, 8, 10] with isolated PREPL deficiency have been reported. Isolated PREPL deficiency causes an autosomal recessive inherited congenital myasthenic syndrome characterized by severe neonatal hypotonia that improves spontaneously with age, and endocrinology problems like growth hormone deficiency and hypergonadotropic hypogonadism. In late childhood (6-11 years) obesity can appear due to hyperphagia. Patients also show mild facial dysmorphism [10].

In this case, we found a novel variant in heterozygous c.1528C>T p.(Arg510Ter) in one allele associated with a whole gene deletion of 0.031Mb in 2p21 in the other. Further analysis showed that the novel mutation c.1528C>T p.(Arg510Ter) inherited from the father results in a change of an arginine to a premature-stop-codon, resulting in a trunked protein or the absence of it, thus leading to a loss of function. This variant has not been identified until now in the public databases consulted (1000genomes, Exome Variant Server, Exome Aggregation Consortium). The other variant, mother-inherited, is a 0.031Mb deletion in 2p21 implying the *PREPL* gene (Fig2).

Fig2. Study of the genetic condition. The whole-exome sequencing identified a homozygous variant in *PREPL* c.1528C>T from paternal inheritance. The array-CGH (with exonic coverage of *PREPL*) showed a 0.031Mb deletion in 2p21 chromosome (including *PREPL* gene) inherited from the mother. In the array-CGH, the DNA from the patient is signalled with CY3 red, whereas the DNA from the mother is signalled with CY5 blue.

As stated, patients with PREPL deficiency often present with growth deficiency and growth hormone therapy has a positive effect on the cohort of cases that exhibit growth hormone deficiency [2, 5, 6]. In our case, the patient is under treatment with GH with good response. However, previous research has not found the mechanism that explains the GH deficiency in PREPL deficiency.

The *PREPL* gene is located in 2p21 and encodes the cytoplasmic PREPL protein which is ubiquitously expressed, with highest levels in brain, kidney, and skeletal muscle, in descending order [11]. *PREPL* encodes a putative serine peptidase from the prolyl peptidase family [12]. Prolyl peptidases cleave peptides on the C-terminal side of proline residues, modulating the levels of different peptides and hormones. Nonetheless, substrates for PREPL have not been determined yet and its exact cellular function remains unknown [13]. Some clues might be found in the literature based on the function of its homologues PREP (prolyl oligopeptidase) and OpdB (oligopeptidase B) which suggest a proteolytic activity can be expected of PREPL. However, PREP seems to have important cellular and physiological effects besides peptide cleavage (such as a role in growth cone development and acting as a binding partner of tubulin and influencing protein secretion), which are primarily caused by protein-protein interactions [14].

Prolyl peptidases have the potential to participate in a wide range of cellular regulatory processes, as their substrates are involved in regulating different signalling pathways [15]. Based on the clinical observation that patients with isolated PREPL deficiency exhibit growth hormone deficiency, it has been hypothesized that PREPL might be involved in the secretion and/or processing of peptide hormones. It is possible that PREPL plays a role in signalling pathways, leading to, for instance, GH secretion. In addition, normal downstream signalling of the GH receptor is apparent from the good response of these patients to GH administration in the literature.

Patients with PREPL deficiency often develop obesity due to hyperphagia in late childhood; currently, at 7 years and 5 months, our patient presents low intake and a normal BMI (13.2; p8, -1.5 SD). Although hypergonadotropic hypogonadism has been observed in some patients with isolated PREPL deficiency [2], sexual hormones have not yet been tested in our proband because she has not reached the puberal age.

Previous studies [3, 10] described moderate intellectual disability (ID) in those patients. *Silva et al* observed that biallelic PREPL mutations alone (without involvement of other genes) can cause ID. Besides the motor delay present in early infancy our patient does not present developmental delay and has only needed some logopaedic therapy for diction problems. She also presented the common phenotype associated with PREPL deficiency, including neonatal hypotonia and feeding problems during the first months after birth.

Further follow-up of this patient is needed to reveal new outcomes and evaluate GH treatment response and the final height attained in adulthood.

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Figure 1.

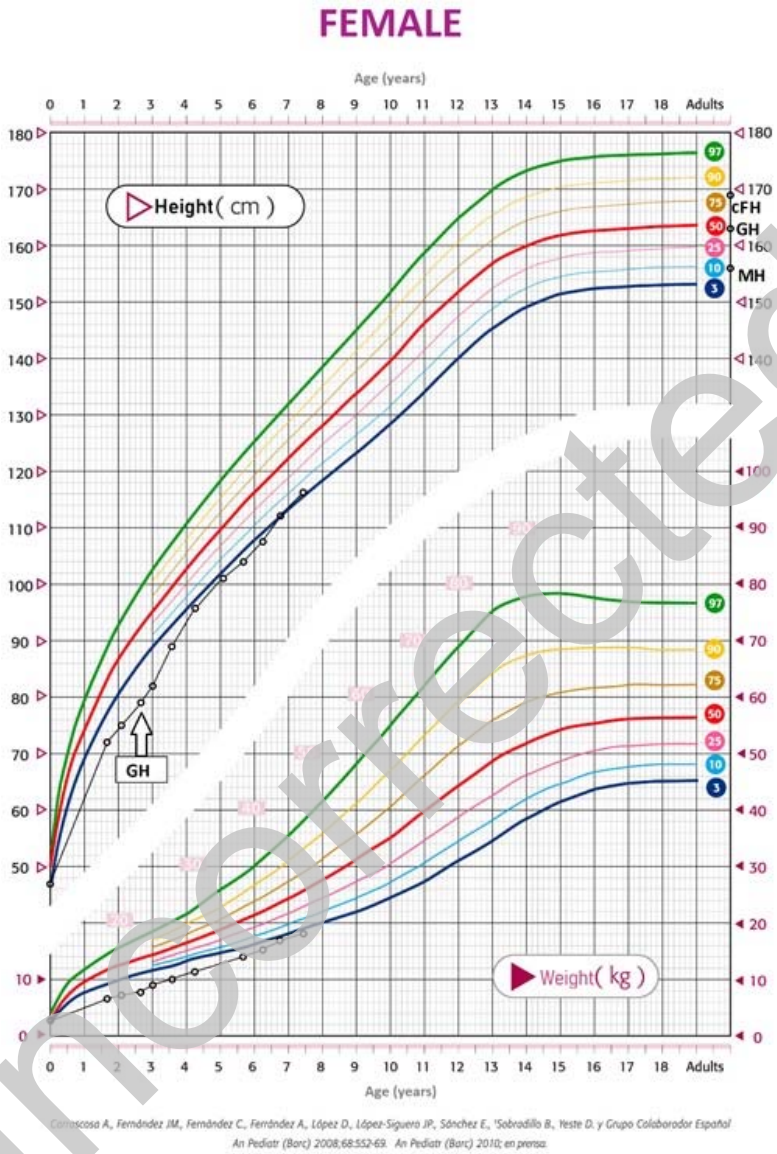

Figure 2.