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Pseudohypoparathyroidism type 1B associated with assisted reproductive technology

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Abstract

Evidence suggests an increased incidence of imprinting disorders in children conceived by assisted reproductive technologies (ART). Maternal loss-of-methylation at *GNAS* exon A/B, observed in pseudohypoparathyroidism type 1b (PHP1B), leads to decreased expression of the stimulatory Gsa. We present a patient conceived by ART, who presented at age 4 years with delayed neurocognitive development and persistently increased creatine kinase (CK). At 6 years an elevated PTH was detected with normal calcium and a low 25(OH) vitamin D level (25OHD). Physical exam showed a narrow forehead, nasal bridge hypoplasia and micropenis. After normalizing vitamin D, PTH remained elevated and PHP1B was therefore considered as the underlying diagnosis. An almost complete loss-of-methylation was observed at *GNAS* exons A/B

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and AS, but not at exon XL, which was associated with a gain-of-methylation at exon NESP. There was no evidence of a microdeletion within the *GNAS/STX16* region and analysis of several microsatellite markers for the *GNAS* region on Chr.20q revealed no evidence for paternal uniparental disomy (patUPD20q).

Keywords

assisted reproductive technology; creatine kinase; pseudohypoparathyroidism

Introduction

The term pseudohypoparathyroidism (PHP; MIM 103580) was first introduced in 1942 by Albright et al. [1] to describe patients with hypocalcemia and hyperphosphatemia, and resistance to the phosphaturic actions of parathyroid extracts. Besides PTH-resistance, these patients often develop resistance to other hormones that act via G protein-coupled receptors, such as thyroid stimulating hormone (TSH), gonadotropins and growth hormone-releasing hormone (GHRH) [2]. They furthermore show various additional abnormalities, referred to as Albright's hereditary osteodystrophy (AHO) that can include different features such as short metacarpals and – tarsals, round face, ectopic subcutaneous ossifications and various degrees of mental retardation. Once it became possible to measure PTH in the circulation, levels were shown to be elevated in similar patients, who are now referred as being affected by PHP type 1a (PHP1A) [2, 3]; this disorder is caused by inactivating mutations involving those exons of the maternal *GNAS* allele encoding the α -subunit of the heterotrimeric stimulatory G protein (G α) thus explaining the approximately 50% reduction in G-protein activity encountered in this disorder. In contrast, paternally-inherited mutations manifest with some, but not all AHO features in the absence of endocrine abnormalities; this condition is referred to as pseudopseudohypoparathyroidism (PPHP) [4].

PHP1B (MIM#603233) another form of PHP that was initially thought to be caused by mutations in the parathyroid hormone (PTH) receptor because most reported cases showed only evidence for PTH-resistance and usually no AHO features [5, 6]. Patients affected by this PHP variant show normal PTH secretion in response to hypocalcemia, but they fail to appropriately increase urinary cAMP and phosphate excretion by the proximal renal tubules in response to PTH [6]. Interestingly, there is no obvious PTH resistance in the distal renal tubules or in bone cells; consequently, patients affected by PHP1B have reduced urinary calcium excretion and often show increased bone resorption [5, 6]. Most PHP1B cases are sporadic and remain unresolved at the molecular level with the exception of a few cases resulting from paternal uniparental isodisomy of chromosome 20q (patUPD20q). A familial form of PHP1B with an autosomal dominant mode of inheritance (AD-PHP1B) had been reported in 1980 [7], but it was not until much later that multiple families with this disorder were analyzed, which provided linkage to the *GNAS* locus on chromosome 20q and revealed that the disease becomes apparent only if the disease-associated allele is inherited from a female [8, 9].

PHP1B patients are usually identified by hypocalcemia-associated neuromuscular irritability symptoms, because AHO features are not frequently present in PHP1B. Nevertheless, a substantial fraction of PHP1B patients remain asymptomatic.

AD-PHP1B is caused by maternal deletions within *STX16* or *GNAS* that cause loss-of-methylation at one or several differentially methylated regions within this complex locus, which reduce Gs α expression [6]. Because Gs α expression from the paternal *GNAS* is reduced or absent in several tissues, including proximal renal tubules, the pituitary gland, gonads and the thyroid gland, maternal *GNAS* mutations lead to little or no Gs α in these tissues, thus explaining the different clinical and biochemical manifestations [10]. Recently, TSH resistance has also been documented in a large subset of patients, whereas they maintain a normal responsiveness to GHRH [11].

Although still controversial, there is some evidence suggesting an increased incidence of imprinting disorders in children conceived by assisted reproductive technologies (ART), specifically Angelman syndrome and Beckwith–Wiedemann syndrome [12]. However, no associations between ART and PHP1B have been described to date.

Here we report a Chilean patient conceived by ART with sporadic PHP1B due to an incomplete loss-of-methylation at *GNAS* exons A/B and AS, but not XL, and a gain-of-methylation at exon NESP, who presented with unique osteomuscular symptoms.

Case presentation

The proband, a 4 year-old boy, was referred by a pediatric neurologist after learning difficulties were observed after admission to preschool. The patient was born in April of 2007, as the first child of non-consanguineous Chilean parents, after nine previous unsuccessful attempts with ART. In vitro fertilization (IVF) was performed with intracytoplasmic sperm injection (ICSI); the mother was 39 years old. Pregnancy was complicated by mild gestational diabetes, managed with diet and a depressive disorder treated with sertraline and clonazepam during last trimester. Delivery was by cesarean section at 37 weeks because of vasa previa; despite lack of bleeding during pregnancy. Ultrasounds during pregnancy did not show abnormalities. At birth cranial deformity with flattened frontal bone and microcephaly, the birth weight was 2510 g (−1.39 SD), the birth length 47 cm (−0.94 SD) and a head circumference of 32 cm (−1.83 SD), Apgar scores were 9 at 1 min and at 5 min. Neonatal screening tests were normal. He was discharged feeding normally, being breast fed until 4 months of age. His developmental milestones were delayed; he walked independently at the age of 24 months, sphincter control at age 3 years. At age 4, when he was first evaluated by neurology, a mild global delay, clumsiness and attention deficit disorder were described. Cognitive evaluation using Wechsler test showed: global IQ 77 mean (low, 4th percentile; IC 95%, 69–79), verbal IQ 85 (low-normal, 16th percentile; IC 95%, 79–93), working memory 59 (mild deficit, 0.3th percentile; IC, 95%, 55–70), processing speed: 88 (low normal, 21st percentile; IC 95%, 80–98), perceptual reasoning 77 (low, 6th percentile; IC, 95%, 71–86). Evaluation using the abbreviated NEPSY–II divided into two content domains: 1. Attention and executive functioning and 2.

Memory, showed a decreased function for the first test (6.5) and a low normal for the second domain (7) (normal range: 7–13; median: 10).

When first examined by neurology, he had normal antropometric measurements (see Figures 1–3: growth chart). Osteotendineous reflexes were diffusely increased, without pedal clonus or extensor plantar reflex. Following an extensive investigation, including metabolic studies, renal and liver function, neuroimaging and electroencephalogram, only persistently increased serum creatine kinase (CK) levels were identified. Echocardiogram and ECG showed no abnormalities. He was started on supportive therapy for his learning, difficulties with attention and clumsiness; methylphenidate was started at age of six. Searching for asymptomatic etiology of high CK, elevated PTH was detected (199 pg/mL, normal range: 10–65 pg/mL); he was therefore referred to endocrinology at 6 years of age for further investigations. Normal calcium and alkaline phosphatase, normal high phosphorus, low 25(OH) Vitamin D (18.3 ng/mL) and slightly elevated TSH level, with normal free T4 and negative thyroid antibodies. Physical exam was unremarkable except for a narrow forehead (sutures closed), nasal bridge hypoplasia, both testis were descended, but he revealed a micropenis (penile length 3.2 cm, more than –2 SDS) (see Figures 4 and 5). The latter was treated with intramuscular testosterone with excellent clinical response (penile size increased to 5.5 cm (–0.56 SDS). His height was +0.63 SDS, his BMI +1.48 SDS. Vitamin D supplementation increased 25(OH)D to 25.9 ng/mL, but PTH remained elevated. A diagnosis of PHP1B was therefore considered and he was started on calcitriol treatment and levothyroxine (Table 1).

He remains asymptomatic and presents biochemical improvement: 25(OH)D 25.9 ng/mL, calcium 9.5 mg/dL, phosphorus 4.8 mg/dL, PTH 105 pg/mL, and CK 133 UI/L. Calcitriol dose titration has been done according to PTH levels.

Dual-energy X-ray absorptiometry (DEXA) revealed at age 8 years a normal areal and volumetric bone mineral density at the lumbar spine (L2–L4) (+1.4 SDS) and at the right and left hip (–1.1 SDS both).

Molecular analyses

This study was approved by the Institutional Review Board Committee at the Massachusetts General Hospital.

After obtaining written informed consent, genomic DNA was extracted from leukocytes of the patient and his parents. An incomplete loss-of-methylation was observed at *GNAS* exons A/B and AS (antisense transcript), but not at exon XL, and a gain-of-methylation at exon NESP (Figure 6).

Discussion

Herein, we report a male patient with biochemical findings typical for PHP1B in the absence of AHO features. Remarkably, laboratory investigations were prompted by laboratory studies pursued because of his neurological abnormalities that revealed asymptomatic total CK elevation.

Even though, the association between hypocalcemia and neuromuscular symptoms is fully recognized, there are few cases in the literature describing the association of low calcium levels, myopathy, and CK elevation [13, 14]. Moreover, to our knowledge the link between mild isolated neuromuscular symptoms as an initial manifestation of PHP1B was previously described only once as the presenting isolated symptom in a 12-year-old Japanese male, who presented with general fatigue, abnormal gait, and myalgia. Physical examinations in that patient revealed muscular weakness and atrophies in the lower legs, a shortening of the bilateral Achilles' tendons and absence of deep tendon reflexes. Bone metabolism profile was compatible, CK was elevated, and genetic testing confirmed the diagnosis of PHP1B [15]. In contrast, our patient did not have neuromotor symptoms and no hypocalcemia, and the only laboratory abnormality regarding the osteomuscular system was a raised CK.

A possible physiopathological explanation was proposed by Somjen et al. in 1985, who observed a two-fold increase in CK activity when stimulating bone-cell cultures grown in low $[Ca^{2+}]$ (0.125 mM), enriched in osteoblast-like cells, in vitro with PTH and prostaglandin E2 (PGE2), while calcitonin had no effect. Additionally, when these bone cells were grown in low calcium condition, the increase in CK activity after PTH stimulation was enhanced [16]. In PHP1B, there is no resistance to PTH in bone; thus cAMP-dependent CK elevations may explain the findings in our patient.

Cognitive impairment is well-established for PHP1A patients [17], but systematic investigations in PHP1B have not yet been conducted. Nonetheless, given that PHP1A and PHP1B are both caused by mutations within the *GNAS* locus, it would be conceivable to find significant overlap for both PHP1 variants [18].

Furthermore, our proband is currently under treatment for attention deficit hyperactivity disorder and a weak association between ART and drug-treated attention deficit hyperactivity disorder has been reported, but the association is no longer significant with adjustment for subfertility [19].

Genetic analysis in our patient showed an almost complete loss of methylation at *GNAS* exons A/B and AS, and a gain of methylation at exon NESP, but no evidence for a *STX16*/*GNAS* deletion.

As was mentioned, our patient was conceived by ART. Worldwide use of assisted reproductive technology accounts for an estimated 1%–3% of all births [20]. There is growing evidence of an increased risk of imprinting disorders in ART children [21]. In addition to ART itself, different studies suggest that subfertility may implicate a greater risk of preexisting methylation defects and consequently imprinting disorders in the offspring of these couples [22–24]. In fact, Whitelaw et al. [25] evaluated epigenetic defects in the offspring of assisted conception either by IVF or ICSI vs. spontaneous pregnancies. They assessed DNA methylation in the paternally expressed gene 3 (*PEG3*), insulin-like growth factor II (*IGF2*), SNRPN (small nuclear ribonucleoprotein polypeptide N), long interspersed nuclear element 1 (*LINE1*), and the insulin gene (*INS*) in a retrospective cohort study of children born between 2002 and 2008. They observed a higher level SNRPN methylation in the offspring was linked to fertility treatment in the parents and this effect was apparently

specific to children conceived using ICSI. Interestingly, duplications or deletions of the SNRPN gene, which encodes the RNA-associated SmN protein, are strongly associated with neurodevelopmental disabilities [26]. Additionally, a higher level of methylation in this gene was also associated with a longer duration of infertility in the parents [25].

Our case was conceived by ICSI. Based on the available information, it is plausible that a link exists between the methylation defects that cause his PHP1B and the assisted conception.

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Established facts

- Increased incidence of imprinting disorders in children conceived by assisted reproductive technologies (ART)
- Pseudohypoparathyroidism is caused by imprinting abnormalities.

Novel Insights

- First report of a possible association between a methylation defects that causes PHP1B and assisted conception
- Increased creatine kinase level was associated with an increase in PTH concentration.

MRN 22373872-9	Last name Riquelme	First name Martin	Birth date 09-04-2007	Gender Male
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Figure 1.
Patient's weight chart.

MRN	Last name	First name	Birth date	Gender
22373872-9	Riquelme	Martin	09-04-2007	Male

Figure 2.
Patient's height chart.

MRN 22373872-9	Last name Riquelme	First name Martin	Birth date 09-04-2007	Gender Male
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Figure 3.
Patient's head circumference chart.

Figure 4.
Patient at the age of 6 years.
Note the narrow forehead (sutures closed), nasal bridge hypoplasia.

Figure 5.
Patient at the age of 9 years and 9 months.

Figure 6.

GNAS locus and transcripts.

GNAS is a complex imprinted locus that generates multiple transcripts using alternative first exons, which share common downstream exons (exons 2–13). Due to epigenetic regulation, transcripts originate from one parental allele, with the exception of *Gsa*, which is subject to tissue-specific imprinting. A schematic representation of paternally-derived mRNAs (top panel) and maternally-derived mRNAs (bottom panel). Patient's methylation defect is shown, with incomplete loss-of-methylation was observed at *GNAS* exons A/B and AS (antisense transcript), and a gain-of-methylation at exon NESP.

Table 1

Biochemical findings in our patient at different ages.

	Age	4 years 1 month	6 years	6 years 4 months	7 years 10 months	8 years 4 months	9 years	9 years 5 months
	Normal value							
CK	<190	U/L	263		269	218	122	163
PTH	10–65	pg/mL		199	183	206	105	176
TSH	0.7–5.7	μIU/mL		4.56	5.63	4.8	2.43	3.94
Free T4	1–2.1	ng/dL		0.86	0.99	1.14	1.43	1.24
25(OH) D3		ng/mL		18.3	25.9			1.47
AP	42–362	U/L	241		313	232	184	224
Calcium	8.5–10.5	mg/dL	9.3	9.3	9.5	9.3	9.5	9.7
Ionized calcium	1.2–1.38	mmol/L		1.06	0.92			
Phosphorus	3.7–5.6	mg/dL	5.3	5.5	5.4	4.7	4.8	4.6
Urinary calcium/Creatinine ratio						7		0.19

CK, creatin kinase; PTH, parathormone; TSH, thyroid stimulating hormone; Free T4, free thyroxine; 25(OH) D3, calcitriol; AP, alkaline phosphatase.