Pseudohypoparathyroidism type 1B in a patient conceived by in vitro fertilization: another imprinting disorder reported with assisted reproductive technology

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Abstract

Pseudohypoparathyroidism type 1B (PHP1B) is characterized by renal tubular resistance to parathyroid hormone (PTH) leading to hyperphosphatemia, hypocalcemia, elevated PTH, and hyperparathyroid bone changes. PHP1B is an imprinting disorder that results from loss of methylation at the maternal *GNAS* gene, which suppresses transcription of the alpha subunit of the stimulatory G protein of the PTH receptor. Emerging evidence supports an association between assisted reproductive technologies (ART) and imprinting disorders; however, there is currently little evidence linking PHP1B and ART. We present a twin boy conceived by ART to parents with no history of subfertility who presented at age 12 with bilateral slipped capital femoral epiphysis and bilateral genu valgum deformity. Clinical and laboratory investigation revealed markedly elevated PTH, low ionized calcium, elevated phosphorus, TSH resistance, and skeletal evidence of hyperparathyroidism, leading to the diagnosis of PHP1B. A partial loss of methylation at the *GNAS* exon A/B locus was observed. The patient's dizygotic twin sibling was asymptomatic and had normal laboratory evaluation. This is the second reported case of a child with PHP1B conceived by ART, further supporting the possibility that ART may lead to an increased risk for imprinting defects.

Keywords Pseudohypoparathyroidism type 1B · Imprinting disorder · IVF · Slipped capital femoral epiphysis

Introduction

Pseudohypoparathyroidism (PHP) is a heterogeneous group of disorders characterized by end organ resistance to parathyroid hormone (PTH) leading to hypocalcemia and hyperphosphatemia. The most common subtype, PHP1A (OMIM 103580), is caused by inactivating mutations in the exons of the maternal *GNAS* allele that encodes the alpha subunit of the stimulatory guanine nucleotide-binding protein ($G_s\alpha$) [1]. In addition to PTH resistance, patients with PHP1A often develop resistance to other hormones that act via G

protein-coupled receptors including thyroid-stimulating hormone, gonadotropins, and growth hormone-releasing hormone [2]. PHP1A is further associated with a constellation of other abnormalities referred to as Albright hereditary osteodystrophy (AHO) which includes short stature, developmental delay, round facies, depressed nasal bridge, brachydactyly, and dental abnormalities [1]. Pseudopseudohypoparathyroidism (OMIM 612463), another condition in the PHP family, is caused by paternal inheritance of the *GNAS* mutation rather than maternal inheritance as seen in PHP1A [3]. These patients develop most of the findings of AHO but in the absence of PTH resistance, as only the *GNAS* maternal allele is expressed in the renal tubules [4, 5].

The PHP1B (OMIM 603233) subtype of PHP is characterized by isolated renal resistance to PTH, leading to hyperphosphatemia, hypocalcemia, and elevated PTH. These patients usually lack the distinct anatomic features of AHO and typically have no other endocrine abnormalities, although TSH resistance has been reported [6]. This disorder is not caused by mutations within *GNAS* exons that directly encode $G_s\alpha$. It instead results from methylation defects at

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differentially methylated regions (DMRs) of the *GNAS* maternal allele causing decreased expression of $G_{\alpha s}$. As only the maternal allele is expressed in the kidney, renal PTH resistance manifests. Since the paternal allele is intact and expressed in bone, skeletal effects of hyperparathyroidism are observed, including osteitis fibrosa cystica and ectopic ossifications. The majority of cases of PHP1B are sporadic, but autosomal dominant transmission has also been reported [7]. Hormonal resistance seen in PHP1B develops if the disease-associated allele is maternally inherited, while paternal inheritance is not associated with any endocrine abnormalities [8, 9]. Paternal uniparental disomy of chromosome 20q (where *GNAS* is found) may explain a subset of sporadic cases [9].

In the past decades, an increased risk of imprinting disorders has been described among infants born from assistive reproductive technology (ART). Evidence of a potential association with ART is strongest for Beckwith–Wiedemann syndrome (BWS) and Angelman syndrome (AS) [10, 11]. However, a number of reports have also identified infants born through ART with other imprinting disorders including retinoblastoma, Silver–Russell syndrome, and Prader–Willi syndrome (PWS) [12].

To our knowledge, only one case of PHP1B following ART has been reported to date [13]. We report a twin boy conceived by ART with PHP1B and partial loss of methylation at the *GNAS* exon A/B locus, presenting with bilateral slipped capital femoral epiphysis (SCFE) and bilateral genu valgum deformity.

Case presentation

A 12-year-old boy presented with a 1-year history of periodic hip, knee, and ankle pain. The hip pain was persistent, greater in the right hip than the left, and had worsened over time. The patient walked with a limp due to the pain. There was no history of trauma and no history of bone fractures. The patient denied tremors or muscle cramps. He had a balanced diet with adequate vitamin D intake and took no medications.

The patient was one of dizygotic twins conceived by in vitro fertilization (IVF). The mother was 25 years of age and the father was 38 years of age at the time of conception. Father has two healthy children from a previous marriage, conceived naturally. Mother has had five previous pregnancies all with the patient's father and all of which were ectopic. Mother's hysterosalpingogram showed no abnormalities, and the cause of her ectopic pregnancies is unknown. The couple elected to use IVF to ensure an intrauterine pregnancy. Neither parent has any history of subfertility.

The patient was born appropriate for gestational age by Cesarean section at 35 weeks gestation due to breech presentation of twin brother. The patient had an unremarkable neonatal course. At presentation, he had normal intelligence and

no features of AHO. Height and weight were at the 75th and 50th percentiles, respectively, and pubertal development was Tanner IV. Physical exam revealed restricted range of motion of both hips associated with pain with internal rotation worse on the right than the left and bilateral genu valgum deformity.

X-ray studies demonstrated bilateral slipped capital femoral epiphysis (Fig. 1a), a 7.8-mm lucency in the distal fibular metadiaphysis of the right ankle, (Fig. 2), and bilateral genu valgum deformity (Fig. 3a). Labs showed mildly low ionized calcium, mildly elevated phosphorus, elevated alkaline phosphatase, and markedly elevated PTH (1113 pg/mL; normal range 15–65). Free T4 was normal, TSH was elevated (4.86 mIU/mL; normal range 0.27–4.20) and antithyroglobulin and antiperoxidase antibodies were negative. Full laboratory values are detailed in Table 1. Given these lab values, skeletal evidence of hyperparathyroidism, and lack of clinical features of AHO, PHP1B was strongly suspected. After informed consent was obtained, methylation analysis of the *GNAS* exon A/B was performed by The Johns Hopkins DNA Diagnostic Laboratory which demonstrated partial loss of methylation at 27% (normal $\geq 41\%$), which confirmed the diagnosis of PHP1B.

The patient was started on calcitriol 0.25 mg orally twice daily and oral calcium carbonate to give approximately 30 mg of elemental calcium/kg/day. Calcitriol was increased to 0.5 mg in the morning and 0.25 mg at night after 8 days. The patient underwent bilateral percutaneous screw fixation of slipped capital femoral epiphyses after 8 days of treatment. Laboratory values normalized after 1 month of treatment. Mild elevation of TSH persisted, and the patient was also treated with levothyroxine due to apparent TSH resistance. The patient continues on calcium, calcitriol, and levothyroxine supplementation. Doses were adjusted to maintain calcium, phosphorous, PTH, urine calcium-to-creatinine ratio, free T4, and TSH levels in the normal range. The bone pain resolved. X-ray findings obtained 8 months after surgery showed well-healed femoral physis with no further evidence of slipping of the capital epiphysis (Fig. 1b) and resolution of genu valgum (Fig. 3b). The patient returned to full activity by 12 months after the start of treatment. Dizygotic twin brother and mother were asymptomatic and had normal laboratory evaluation.

Discussion

Herein, we report a male conceived by IVF who was diagnosed with PHP1B following presentation with bilateral slipped capital femoral epiphysis and bilateral genu valgum deformity. Genetic testing showing partial loss of methylation at *GNAS* exon A/B which, coupled with the patient's clinical presentation and laboratory findings, confirms the diagnosis of PHP1B.

Fig. 1 **a** Frog leg view showing bilateral slipped capital femoral epiphysis (black arrows). **b** Frog leg view 8 months after undergoing bilateral percutaneous screw fixation and calcium and calcitriol

supplementation demonstrating well-healed femoral physes with no further evidence of slipping of the capital epiphysis

Notably, laboratory investigations were prompted after the patient presented with bilateral slipped capital femoral epiphysis. PHP1B is a highly unusual cause of SCFE, and only five previously reported cases of SCFE associated with PHP1B could be identified [14–18]. Although this patient presented at the typical age for SCFE, he lacked other common risk factors such as obesity, significant thyroid disease, history of steroid treatment, or kidney disease. The possibility of PHP1B should be considered in patients presenting with SCFE, especially in the absence of other risk factors. Both in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have been associated with increased relative risk of imprinting disorders, including Beckwith-Wiedemann syndrome, Angelman syndrome, and Silver-Russell syndrome [10–12]. Strong evidence from animal models supports the biological plausibility of a causal link between imprinting errors and ART. In one study on mice by de Waal et al., IVF placenta displayed hypomethylation of imprinting control regions on select imprinted genes and displayed a global reduction in

DNA methylation levels [19]. Epigenetic perturbations associated with ART have also been demonstrated in sheep and cattle [20–22].

It has also been suggested that the association between imprinting errors and ART may be secondary to causes of infertility or subfertility—such as oligospermia and advanced maternal age—rather than the manipulations involved in ART. The sperm of infertile males have been shown to have a higher incidence of imprinting errors [23–26]. In fact, a Dutch study comparing the prevalence of AS, BWS, and PWS among children conceived by ART to those conceived by subfertile couples without the aid of ART suggested the increased prevalence could be explained by subfertility alone [27]. The higher incidence of imprinted disorders in animal studies involving ART, however, strongly suggests the ART process itself contributes to the epigenetic disruptions, as these animals were not infertile or subfertile. It may be that both

Fig. 2 Medial oblique view of right ankle showing 7.8-mm lucency in distal fibular metadiaphysis (yellow arrow)

Fig. 3 **a** Bilateral genu valgum deformity noted before treatment. **b** Improvement of genu valgum 8 months after calcium and calcitriol supplementation

Table 1 Biochemical evaluation and treatment of the patient

Laboratory results	Normal values	Days after initiation of treatment				
		0	4	8	28	60
PTH (pg/mL)	10–65	1113	700	361	62	31.3
ALP (IU/L)	< 300	418			290	225
Phosphorous (mg/dL)	3.2–5.7	6.2	5.5		4.7	
Total calcium (mg/dL)	8.4–10.2	8.8	9.1		10.2	9.9
Ionized calcium (mg/dL)	4.8–5.2	4.6	4.8	4.7	5.1	
Magnesium (mg/dL)	1.5–2.5	1.9				
25(OH) D3 (ng/mL)	30–100	28				
1,25(OH) D3 (pg/mL)	24–86	60				
TSH (mIU/mL)	0.27–4.2	4.86			5.9	1.79
Free T4 (ng/mL)	0.8–2.0	1.1				1.6
Treatment						
Calcitriol (mg, AM/PM)		0.25/- 0.25	0.25/- 0.25	0.5/- 0.25	0.5/- 0.25	0.5/- 0.25
Calcium carbonate (mg/kg/day)		28	34	34	34	34
Levothyroxine (mcg)					75	75

PTH, parathyroid hormone; *ALP*, alkaline phosphatase; *25(OH) D3*, 25-hydroxyvitamin D; *1,25(OH)D3*, calcitriol; *TSH*, thyroid-stimulating hormone; *Free T4*, free thyroxine

subfertility and ART play a role in the increased risk of imprinting disorders seen in this population [28].

To our knowledge, this is only the second reported case of PHP1B in a patient conceived by ART. This is a sporadic case, as there is no family history of PHP1B and twin brother and mother are asymptomatic and had normal laboratory evaluation. The previously reported patient was also a sporadic case with incomplete loss of methylation at *GNAS* exon A/B [13]. That both of these cases are sporadic lends support to the hypothesis that ART may have played a role in the emergence of a methylation defect. Of note, the parents of the patient reported here had no history of subfertility or infertility, defined as year-long period of unwanted non-conception. Furthermore, neither advanced maternal nor paternal age was present in this case. In the previously reported case, the child was conceived after nine unsuccessful attempts with ART, to a mother that was 39 years old. This is significant because of evidence suggesting that imprinting defects seen in the setting of ART may actually be secondary to the causes of subfertility that necessitate ART or to advanced parental age. The absence of subfertility or advanced parental age in the present case further suggests that in this case ART may have contributed to a methylation defect.

Sporadic case reports linking imprinting disorders to the use of ART continue to appear in the literature. This report provides additional evidence to support such a link, adding PHP1B to the list of imprinting disorders that may be associated with ART. Nonetheless, further studies are required to

better define this association. Specifically, a clearer understanding of the mechanism by which ART might induce epimutations is needed. Prospective studies are also needed to further examine the incidence of epimutational changes in ART pregnancies and test the potential long-term effects of the technology. The number of children with imprinting disorders born by ART remains small and a vast majority of children conceived by ART are healthy. Further studies will be key in allowing patients and clinicians to understand the risks of ART and make informed decisions.

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Compliance with ethical standards

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