CASE REPORT

Pseudohypoparathyroidism type 1B caused by methylation changes at the *GNAS* complex locusSabrina Poradosu,¹ Bert Bravenboer,¹ Rieko Takatani,² Harald Jüppner²¹Department of Endocrinology, Universitair Ziekenhuis Brussel, Brussels, Belgium²Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA**Correspondence to**
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SUMMARY

Pseudohypoparathyroidism type 1B (PHP1B) consists of a heterogeneous group of disorders characterised by resistance to parathyroid hormone (PTH). There are several different PHP1B subtypes that are all associated with methylation changes at *GNAS*. These epigenetic changes are caused by maternal deletions in *GNAS* or *STX16*, by paternal uniparental isodisomy of chromosome 20q (patUPD20q) or by undefined genetic mutations. The *GNAS* methylation changes are ultimately responsible for resistance to PTH signalling in the proximal renal tubules. However, there is no PTH resistance in the distal renal tubules nor in bone cells; consequently, patients with PHP1B have reduced urinary calcium excretion and can readily mobilise calcium (and phosphate) from the skeleton. We report a case of a sporadic PHP1B patient with broad *GNAS* methylation changes that were presumably caused by an unknown genetic mutation outside the *GNAS* locus. PTH resistance was preceded by several years by autoimmune negative hypothyroidism. Treatment consisted of calcium substitution and calcitriol.

BACKGROUND

Pseudohypoparathyroidism (PHP) is a heterogeneous group of disorders characterised by hypocalcaemia, hyperphosphataemia and elevated parathyroid hormone (PTH) levels due to resistance to this hormone. The two main subtypes are PHP type 1A (PHP1A) and type 1B (PHP1B). PHP1A is caused by loss-of-function mutations in those exons of *GNAS* that encode the α -subunit of the stimulatory G protein (G_{α}) downstream of the PTH receptor and numerous other G protein-coupled receptors. PHP1B can be caused by several different deletions within the *STX16/GNAS* complex, patUPD20q or as yet unknown mutations in other genes, leading to methylation changes at *GNAS* and, consequently, impaired G_{α} expression. Although the molecular and epigenetic differences between PHP subtypes are well established, it is not always possible to distinguish between them clinically or biochemically.^{1–2} For example, resistance to thyroid-stimulating hormone (TSH) was initially thought to occur only in PHP1A, but it is now well known that autoimmune negative hypothyroidism can occur also in patients with PHP1B, even before PTH resistance becomes clinically apparent.^{3–4} Furthermore, certain features of Albright's hereditary osteodystrophy (AHO, ie, brachydactyly, short stature, obesity and/or various degrees of intellectual impairment), which had been considered to be specific for PHP1A, can be observed in patients with PHP1B.^{5–8} In order to establish the diagnosis

of PHP1A or one of the different PHP1B variants, it is therefore necessary to perform genetic and epigenetic *GNAS* analyses.

This case illustrates the clinical, biochemical and genetic challenges in the diagnosis of this rare condition that should be considered in the diagnostic work up of patients with hypocalcaemia.

CASE PRESENTATION

A 24-year-old Belgian woman was sent to our outpatient clinic because of hypocalcaemia (1.68 mmol/L, normal range: 2.10–2.70 mmol/L) and an elevated intact PTH (289 ng/L, normal range: 15–65 ng/L). On presentation, the 25-hydroxy vitamin D level was low (6 ng/mL, normal range: 20–50 ng/mL), and phosphate and albumin levels were within normal limits (phosphate: 0.94 mmol/L, adult normal range: 0.8–1.5 mmol/L; albumin: 44 g/L, normal range: 35–50 g/L). Twenty-four hour urinary calcium excretion was normal (152 mg/24 h, normal range: 100–320 mg/24 h). The patient had been diagnosed with autoimmune negative hypothyroidism at the age of 20 years, when her TSH was measured because of unexplained weight gain. Her thyroid peroxidase antibodies were negative (<34 kIU/L); she had no history of previous thyroid disease and her thyroid gland was of normal size and consistency. None of her immediate family members had a history of thyroid disease or abnormal calcium regulation. However, the patient was known to have asthma-like symptoms for which she was treated with short-acting β -agonists as needed.

She reported suffering from skeletal pain, paraesthesia and episodic carpopedal spasms. Menstrual cycles were normal. Physical examination showed a short and obese stature (weight 102 kg, height 1.58 m, body mass index 40.9 kg/m²), positive Chvostek and Trousseau signs, and hyperreflexia of all four limbs. The patient's cognitive function was within normal limits. Her blood pressure was 120/80 mm Hg, further physical examination was normal. There were no subcutaneous ossifications, and neither brachydactyly nor other skeletal anomalies. As a result of a low 25-hydroxy vitamin D level, oral supplement with vitamin D was started together with calcium supplements, but serum calcium remained low and PTH remained elevated. Therefore, the diagnosis of pseudohypoparathyroidism was considered.

INVESTIGATIONS

The patient was hospitalised for intravenous administration of PTH(1–34) in order to assess

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PTH-stimulated urinary cyclic adenosine monophosphate and phosphate excretion, which showed no response.

Total body skeletal scintigraphy was performed because of bone pain, revealing no abnormalities despite prolonged PTH elevations, which could have increased bone resorption. However, a bone biopsy after tetracycline labelling revealed evidence only for osteomalacia, namely widened zones of unmineralised osteoid. Owing to the patient's neuromuscular signs and symptoms, we decided to perform an electromyogram, which showed signs of neither neuropathy nor myopathy. Besides the previously diagnosed hypothyroidism, no additional endocrine abnormalities were noted.

Analysis of *GNAS* exons 1–13, which encode the $G\alpha$, revealed no genetic mutation. Multiplex ligation-dependent probe amplification (MLPA)³ provided no evidence for *GNAS* or *STX16* deletions. However, methylation-specific MLPA³ showed a loss of methylation at *GNAS* exons A/B and AS, and a gain of methylation at *GNAS* exon neuroendocrine secretory protein (figure 1), thus confirming the diagnosis of PHP1B; similar to a few previously reported cases,⁹ a normal methylation pattern was observed at *GNAS* exon XL. Several microsatellite markers across the *GNAS* locus were analysed for the patient and her mother, to exclude patUPD20q. The patient showed heterozygosity for markers D20S86, 907-rep2, 543J19-TTA and D20S171, yet homozygosity for 261P9-CA and 806M20-CA; however, the patient and her mother showed no discordance for the latter two markers.

DIFFERENTIAL DIAGNOSIS

Hypocalcaemia can be caused by decreased synthesis of PTH, altered responsiveness to PTH, vitamin D deficiency or resistance to 1,25(OH)₂ vitamin D. Our patient demonstrated low serum calcium with very high PTH, even after normalisation of the vitamin D level. Absence of brachydactyly, presence of normal cognitive functions and lack of mutations in the *GNAS* exons encoding $G\alpha$ made PHP1A as the underlying disease

unlikely; in fact, multiple *GNAS* methylation changes established the diagnosis of PHP1B. We obtained no evidence for a deletion in *STX16* or *GNAS*, which can be the cause of autosomal dominant PHP1B forms. PatUPD20q, that is, duplication of the entire long arm of chromosome 20 or of a chromosomal segment comprising the *GNAS* locus, appeared unlikely since methylation at *GNAS* exon XL was normal. Consistent with this conclusion, analysis of several microsatellite markers located within the chromosome 20q13 region excluded a paternal duplication of a large region surrounding the *GNAS* locus. Our patient was therefore most likely affected by the sporadic form of the disease, which has not yet been defined at the molecular level.

TREATMENT

The patient was treated with oral calcium substitution and calcitriol uneventfully since the diagnosis was made 10 years prior; note that either calcitriol or alfacalcidol can be used for treatment of hypocalcaemia. Despite stable normocalcaemia, normal vitamin D levels and slightly elevated PTH values, the patient still suffers from intermittent skeletal and muscle pain, which has, however, become less frequent and less intense. No evidence for nephrocalcinosis has developed on the current treatment. TSH levels have fluctuated slightly and treatment with oral levothyroxine has been adjusted accordingly.

OUTCOME AND FOLLOW-UP

Follow-up of our patient takes place at our outpatient clinic every 6 months, and consists of a thorough clinical examination and biochemical determination of serum calcium, phosphate and PTH levels, assessment of kidney function and a 24 h urine collection to assess urinary calcium excretion. Treatment has been well tolerated and the course of her disease remains uneventful. During follow-up, 25-hydroxy vitamin D levels have varied between 19 and 51 ng/mL. Urinary calcium and phosphate excretion have remained within normal levels.

Figure 1 Genetic analysis of the *GNAS-STX16* locus. (A) Schematic depiction of the region extending from the syntaxin 16 (*STX16*) gene to the *GNAS* complex locus located about 250 kb further telomeric. The maternal (Mat) and the paternal (Pat) allele are shown. Boxes represent different *STX16* and *GNAS* exons. Several alternatively spliced transcripts are derived from *GNAS*: $G\alpha$ transcript, exons 1–13; A/B transcript, exon A/B spliced onto exons 2–13; XL α transcript, exon XL spliced onto exons 2–13; NESP55 transcript: exon NESP spliced onto exons 2–13. Note that the NESP exon comprises an in-frame termination codon; the portion of the NESP55 messenger RNA (mRNA) derived from exons 2–13 is therefore located in the 3'-non-coding region. A non-coding antisense mRNA is derived from antisense exons 1–5 (numbers in italics). Note that $G\alpha$ is expressed from both parental alleles; **, locations of differentially methylated regions; vertical arrows, locations of different microsatellite markers. (B) Analysis of the *STX16/GNAS* region by multiplex ligation-dependent probe amplification provided no evidence for an allelic loss at the different *GNAS* and *STX16* exons. (C) Methylation-specific multiplex ligation-dependent probe amplification assay using probes that are specific for exons NESP, AS, XL and A/B. Our patient's DNA revealed loss of methylation at *GNAS* exons AS and A/B, and a gain of methylation at *GNAS* exon NESP55, but no changes at *GNAS* exon XL. In contrast, analysis of DNA from the healthy mother and other healthy controls showed ~50% methylation at all four differentially methylated regions.

DISCUSSION

PHP1A is caused by mutations affecting the maternal *GNAS* exons 1–13, while PHP1B is caused either by maternal deletions in *GNAS* or *STX16*, by *patUPD20q* or by as yet undefined genetic mutations; the PHP1B variants are all associated with *GNAS* methylation changes that reduce *Gsα* expression from this parental allele.^{1 2} Loss of the maternal *GNAS* methylation imprints in association with the normally reduced or absent *Gsα* expression from the paternal allele results in a severe reduction or complete lack of the signalling protein in the proximal renal tubules, thus causing PTH resistance, which explains the elevated PTH levels. Resistance towards PTH in this portion of the kidney impairs downregulation of *NPT2a* and *NPT2c* expression, thus leading to enhanced phosphate reabsorption. Furthermore, reduced vitamin D 1-hydroxylation most likely contributes to reduced intestinal calcium absorption and to impaired negative feedback regulation of PTH production. However, PTH continues to work in other target tissues such as the distal renal tubules thereby allowing PTH-dependent calcium reabsorption, which limits the risk of developing hypercalciuria and nephrocalcinosis. Likewise, bone remains PTH responsive making it possible to maintain normocalcaemia for prolonged periods of time through PTH-dependent bone resorption.

There can be considerable overlap between the molecular and clinical features of PHP1A and PHP1B. For example, several patients, initially thought to be affected by PHP1A because of their skeletal AHO features, revealed no loss-of-function mutation affecting *GNAS* exons 1–13, yet were found to have *GNAS* methylation abnormalities, thus establishing the diagnosis of PHP1B.^{5–8} Similarly, TSH resistance is not restricted to PHP1A, but has been observed also in different PHP1B variants. In fact, as shown for other PHP1B cases,^{3 4} an elevation in TSH levels, in our case, preceded the development of PTH resistance by several years. This indicates that silencing of paternal *Gsα* expression can be particularly prominent in the thyroid. It

Learning points

- ▶ Pseudohypoparathyroidism (PHP) refers to a heterogeneous group of rare disorders characterised by resistance to parathyroid hormone (PTH).
- ▶ PHP type 1B (PHP1B) is characterised by hypocalcaemia, hyperphosphataemia and elevated levels of PTH due to proximal tubular resistance to the action of PTH at the postreceptor level.
- ▶ Resistance to thyroid-stimulating hormone can precede the onset of PTH resistance by many years.
- ▶ Establishing the diagnosis of PHP1B requires genetic and epigenetic analyses of *GNAS* locus.

remains to be determined whether our patient's obesity and short stature are manifestations of AHO that could be related to abnormal *Gsα* expression in pituitary, brown fat tissue and/or portions of the central nervous system, that is, tissues in which this signalling protein is derived mainly from the maternal allele.

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REFERENCES

- 1 Mantovani G, Elli FM, Spada A. *GNAS* epigenetic defects and pseudohypoparathyroidism: time for a new classification? *Horm Metab Res* 2012;44:716–23.
- 2 Bastepe M. The *GNAS* locus and pseudohypoparathyroidism. *Adv Exp Med Biol* 2008;626:27–40.
- 3 Molinaro A, Tiosano D, Takatani R, *et al.* TSH elevations as the first laboratory evidence for pseudohypoparathyroidism type 1b. *J Bone Miner Res* 2015;30:906–12.
- 4 Romanet P, Osei L, Netchine I, *et al.* Case report of *GNAS* epigenetic defect revealed by a congenital hypothyroidism. *Pediatrics* 2015;135:e1079–83.
- 5 Perez de Nancres G, Fernandez-Rebollo E, Santin I, *et al.* Epigenetic defects of *GNAS* in patients with pseudohypoparathyroidism and mild features of Albright's hereditary osteodystrophy. *J Clin Endocrinol Metab* 2007;92:2370–3.
- 6 Mariot V, Maupetit-Méhouas S, Sinding C, *et al.* A maternal epimutation of *GNAS* leads to Albright osteodystrophy and parathyroid hormone resistance. *J Clin Endocrinol Metab* 2008;93:661–5.
- 7 Mantovani G, de Sanctis L, Barbieri AM, *et al.* Pseudohypoparathyroidism and *GNAS* epigenetic defects: clinical evaluation of Albright hereditary osteodystrophy and molecular analysis in 40 patients. *J Clin Endocrinol Metab* 2010;95:651–8.
- 8 Sharma A, Phillips J, Jüppner H. Hypoplastic metatarsals—beyond cosmesis. *New Engl J Med* 2015;373:2189–90.
- 9 Maupetit-Méhouas S, Mariot V, Reynès C, *et al.* Quantification of the methylation at the *GNAS* locus identifies subtypes of sporadic pseudohypoparathyroidism type 1b. *J Med Genet* 2011;48:55–63.

Patient's perspective

I had serious problems with cramps and muscle pains in the past. I am very happy that my treating physician made a final diagnosis explaining my bothersome symptoms. I am now taking vitamin D and calcium, and my symptoms have improved but have not disappeared completely. The outcome of the genetic testing done in Boston has been very helpful to better accept my chronic disease.

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