Recessive versus imprinted disorder: consanguinity can impede establishing the diagnosis of autosomal dominant pseudohypoparathyroidism type Ib

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Abstract

Hypocalcemia and hyperphosphatemia with low/normal parathyroid hormone (PTH) levels can be observed in hypoparathyroidism (HP), a disorder that may follow an autosomal dominant (AD) or autosomal recessive (AR) mode of inheritance. Similar biochemical changes are also observed in pseudohypoparathyroidism (PHP) type Ia and Ib, but affected patients usually show elevated PTH levels indicative of hormonal resistance. Features of Albright’s hereditary osteodystrophy (AHO) are typically not observed in patients affected by familial forms of PHP-Ib, which are most frequently caused by maternally inherited, heterozygous microdeletions within STX16 and are associated with isolated loss of methylation at GNAS exon A/B.

We established the molecular defect in two children of consanguineous Turkish parents, who presented with hypocalcemia, hyperphosphatemia, and low 25-OH vitamin D levels, but initially normal or only mildly elevated PTH levels, i.e. findings that do not readily exclude HP. After normalizing serum magnesium levels, hypocalcemia and hyperphosphatemia persisted, and PTH levels increased, suggesting PTH resistance rather than PTH deficiency. Because of the absence of AHO and parental consanguinity, an AR form of PHP-Ib appeared plausible, which had previously been suggested for sporadic cases. However, loss of GNAS methylation was restricted to exon A/B, which led to the identification of the 3-kb STX16 microdeletion. The same mutation was also detected in the healthy mother, who did not show any GNAS methylation abnormality, indicating that her deletion resides on the paternal allele.

Our findings emphasize the importance of considering a parentally imprinted, AD disorder even if consanguinity suggests an AR mode of inheritance.

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Introduction

Hypocalcemia and hyperphosphatemia with inappropriately low/normal or only slightly elevated parathyroid hormone (PTH) levels are typically observed in patients affected by isolated hypoparathyroidism (HP), a rare disorder that can follow an autosomal recessive (AR) or an autosomal dominant (AD) mode of inheritance (1–6). HP can be caused by impaired synthesis or secretion of PTH as a result of mutations in the PTH gene itself (2, 3) due to activating mutations in the calcium-sensing receptor (4) or due to homozygous or heterozygous mutations in glial cells missing B (5, 6).

Abnormalities in serum calcium and phosphorous similar to those encountered in HP are also observed in patients with pseudohypoparathyroidism type Ia (PHP-Ia), a disorder that is caused by heterozygous, inactivating mutations in those GNAS exons encoding the α-subunit of the stimulatory G protein (Gₛα). Affected individuals show, in addition to PTH resistance, resistance toward few other hormones that mediate their actions through Gₛα-coupled receptors, and they usually exhibit features of Albright’s hereditary osteodystrophy (AHO) such as round face, short stature, obesity, brachydactyly, heterotopic ossifications, and mental retardation, i.e. clinical findings not present in HP (7, 8).

In contrast, patients affected by PHP type Ib (PHP-Ib) typically show PTH and, in some cases, TSH resistance without any features of AHO, although few reports have revealed subtle evidence for these developmental changes (9–11). At least two distinct types of epigenetic/genetic defects have been described in AD-PHP-Ib,
which lead to indistinguishable clinical and laboratory phenotypes. These familial forms of PHP-Ib are caused by maternally inherited, heterozygous deletions within or upstream of the GNAS locus, which are associated either with a loss of maternally GNAS methylation imprints or with a loss of exon A/B methylation alone (12–15); paternal inheritance of these deletions does not lead to laboratory or clinically obvious abnormalities. Similar to AD-PHP-Ib caused by deletions within GNAS, sporadic PHP-Ib cases display broad GNAS imprinting defects, but no molecular defect has yet been identified. Haplotype sharing with an unaffected sibling has been demonstrated in some sporadic PHP-Ib cases (16), suggesting either de novo mutations, small paternal uniparental iso- or heterodisomy within the chromosome 20q13.3 region, or an AR form of PHP-Ib that could be caused by homozygous or compound heterozygous mutations in a different gene.

Here, we describe clinical and laboratory findings of a patient, who presented with hypocalcemia, hypomagnesemia, and hyperphosphatemia, and inappropriately normal PTH levels, but no evidence for developmental abnormalities. After correction of serum magnesium levels, PTH levels increased suggesting PHP-Ib rather than HP, which was subsequently confirmed through epigenetic and genetic investigations.

Case report

An 11-year-old Turkish female (Fig. 1A; patient #3) presented to clinic with tetany, and laboratory testing revealed hypocalcemia, hypomagnesemia, and hyperphosphatemia (Fig. 1B: 7.0 mg/dl (normal: 8.4–10.4); 1.1 mg/dl (normal: 1.6–2.5); and 9.1 mg/dl (normal: 2.5–4.5) respectively). Alkaline phosphatase was 639 U/l (normal: 300–1100), and PTH was 44 pg/ml (normal: 9–55); the urinary calcium-to-creatinine ratio was 0.2). Her height was 147 cm (0.3 SDS above the mean of age-matched Turkish controls), her weight was 51 kg (1.4 SDS above the mean of age-matched Turkish controls), and her body mass index was 23.6 kg/m² (1.6 SDS above the mean of age-matched Turkish controls). She revealed hypocalcemia, hypomagnesemia, and hyperphosphatemia (5.3 ng/ml; normal: 6–46). Because PTH was initially normal despite hypocalcemia, it was remeasured after correction of the hypomagnesemia, i.e. 10 days after the first measurement, and at that time, it was shown to be mildly elevated at 86.9 pg/ml (normal: 12–72) when the serum levels of Ca, Pi, and Mg were 7.4, 7.8, and 1.8 mg/dl respectively. Based on the laboratory findings at presentation, a tentative diagnosis of HP in combination with vitamin D deficiency was made. Treatment with oral calcium carbonate and calcitriol was therefore initiated. However, the doses of both medications were probably too low since PTH levels increased further (107–203 pg/ml) and hyperphosphatemia persisted (5.3 and 5.7 mg/dl; Fig. 1B), indicating PTH resistance rather than HP. It was therefore suspected that she might be affected by PHP, e.g. PHP-Ib because of the absence of AHO. Thyroxine (T₄) and TSH were within normal limits.

The older brother of the patient (Fig. 1A; patient #1) presented at the age of 18 years with pain in his arms and legs that had started about 5 years ago. Laboratory evaluation revealed hypocalcemia and hyperphosphatemia (6.0 and 7.6 mg/dl respectively), with mildly elevated PTH (84.2 pg/ml; Fig. 1B). T₄ and TSH were elevated.

![Figure 1](image-url)
within normal limits; there was no evidence for AHO. Because of the findings in his younger sister, the index case #3, therapy with oral calcium carbonate and 1,25(OH)2 vitamin D was initiated, which resulted initially in a further increase in PTH levels (190–213 pg/ml).

Lymphocyte DNA was extracted from both patients and the mother using standard methods after obtaining informed consents (12); the study was approved by Massachusetts General Hospital Institutional Review Board. The father had been healthy until he died in a traffic accident, and laboratory and genetic analyses could therefore not be performed. GNAS methylation analysis was carried out as described (12) using bisulfite-modified genomic DNA sequence analysis, which established normal methylation at three of the four differentially methylated GNAS regions, NESP55, AS, and XL. However, only exon A/B revealed a loss of methylation in both patients. GNAS methylation analysis of the mother showed no abnormality (Fig. 2). Because of consanguinity of the healthy parents, a recessive form of PHP-Ib had been initially considered. However, because of the loss of A/B methylation alone, we first searched for one of the two known microdeletions within the gene encoding syntaxin 16 (STX16), using multiplex PCR analysis as described (11). These studies revealed the previously reported 3-kb deletion within STX16, which was also identified in the unaffected mother (Fig. 1C), thereby establishing that both patients are affected by AD-PHP-Ib.

**Discussion**

In this report, we describe laboratory, epigenetic, and genetic findings in a patient, who presented with hypocalcemia, hypomagnesemia, and hyperphosphatemia. Because the parents of the patient are related and because serum PTH levels were initially normal, an AR form of HP was considered. After correction of serum magnesium levels, however, PTH concentrations increased while calcium levels remained low, making a form of PHP without AHO more likely. Because the patient’s brother showed similar biochemical abnormalities to the index case and because of the parental consanguinity, an AR form of PHP-Ib appeared plausible. However, subsequent epigenetic and molecular characterization revealed only a loss of GNAS exon A/B methylation, and the previously described heterozygous 3-kb STX16 deletion was identified in the affected siblings and their mother, thereby establishing AD-PHP-Ib. Both patients (#1 and #3) carry the mutation on the maternal allele, and both show an associated loss of exon A/B methylation and PTH resistance. In contrast, their healthy mother presumably carries the mutation on the paternal allele and, thus, does not show any loss of exon A/B methylation.

Only ~100 genes in the human genome undergo parent-of-origin-specific methylation, thereby limiting expression specifically to a single parental allele (17, 18). However, only less than ten imprinted genes have thus far been implicated in human diseases, including Prader–Willi syndrome, Angelman syndrome, Beckwith–Wiedeman syndrome, Silver–Russell syndrome, and transient neonatal diabetes (17, 19), as well as disorders that are caused by mutations within the GNAS locus. These include PHP-Ia, in which affected individuals carry maternally inherited, inactivating mutations located in those GNAS exons that encode Gsα (20–23). When inherited paternally, the same mutations lead to pseudo-PHP or progressive osseous heteroplasia, i.e. related disorders characterized by the presence of AHO features without hormonal resistance (20–23). AD-PHP-Ib can be caused by one of two different, maternally inherited microdeletions within STX16, a gene about 220-kb upstream of the GNAS locus; these 3- and 4.4-kb deletions, which are overlapping, are usually not associated with AHO-like abnormalities. PTH resistance, which can be quite variable, is observed only with maternally inherited STX16 deletions (12, 13, 16, 24, 25).

Because the consanguineous parents are healthy, and did not show any laboratory abnormalities, the siblings described herein were initially thought to be affected by
an AR disorder. However, identification of the 3-kb STX16 deletion and knowledge about the paternally imprinted mode of inheritance for AD-PHP-Ib led to the conclusion that consanguinity had been misleading. Since the rates of consanguineous marriages in the Turkish population are reported to be as high as 20–25%, our findings raise the possibility that additional cases of AD-PHP-Ib will be identified in Turkey or other countries with high frequency of marriages between closely related relatives (26).

In conclusion, we identified the 3-kb STX16 deletion, the most frequent cause of AD-PHP-Ib (12), in two siblings in whom an AR form of PHP-Ib was initially suspected because of the parental consanguinity. Our findings indicate that children from related parents are not necessarily affected by an AR disorder, and that a parentally imprinted disorder should be considered. For patients affected by PHP-Ib, whose parents are related, it is thus important to first exclude the known genetic defects before searching for a novel genetic locus, even though parental consanguinity suggests the possibility of an AR disorder.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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