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CASE REPORT

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, brachydactylity, 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,

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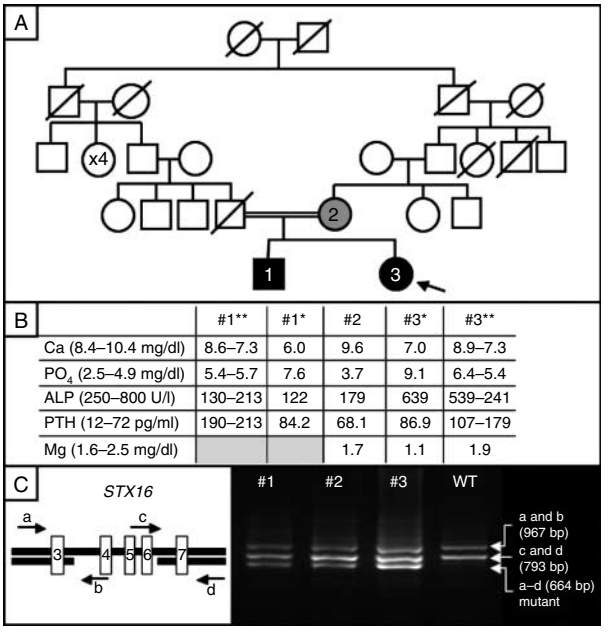
which lead to indistinguishable clinical and laboratory phenotypes. These familial forms of PHP-Ib are caused by maternally inherited, heterozygous deletions within or upstream of the *GNAS* locus, which are associated either with a loss of all maternal *GNAS* methylation imprints or with a loss of exon 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 low at 0.01–0.05 (normal: <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/m^2 (1.6 SDS above the mean of age-matched Turkish controls). She revealed no evidence for AHO. The physical examination was within normal limits, and the past medical history was unremarkable; birth weight had been 3000 g, and she had shown normal growth and development. At the time of presentation, her pubertal development was consistent with Tanner stage III.

Because of the low serum calcium and magnesium levels, she was first treated with i.v. calcium gluconate (500 mg/kg per day) and $MgSO_4$ (100 mg/kg per day) for 3 days. She furthermore received oral vitamin D (300 000 IU) because of a low 25-OH vitamin D level (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



	#1**	#1*	#2	#3*	#3**
Ca (8.4–10.4 mg/dl)	8.6–7.3	6.0	9.6	7.0	8.9–7.3
PO_4 (2.5–4.9 mg/dl)	5.4–5.7	7.6	3.7	9.1	6.4–5.4
ALP (250–800 U/l)	130–213	122	179	639	539–241
PTH (12–72 pg/ml)	190–213	84.2	68.1	86.9	107–179
Mg (1.6–2.5 mg/dl)			1.7	1.1	1.9

Figure 1 (A) Pedigree of the AD-PHP-Ib family showing a mode of inheritance that is consistent with an autosomal recessive disorder. (B) Laboratory findings. *, at presentation; **, range after normalization of serum magnesium level in the index case. For conversion of metric unit to SI unit; multiple by 0.25 for Ca, 0.3229 for phosphorus and 0.18 for Mg to mmol/l, 0.102 for PTH to pmol/l. (C) Analysis of the *STX-16* region by multiplex PCR using primers a, b, c, and d (arrows) leading to the identification of the previously described heterozygous 3-kb microdeletion comprising exons 4–6. The shortest PCR product, which was present in the two affected family members, #1 and #3, and in unaffected carrier #2, is derived from the mutant allele and amplified by primers a and d. The 967-bp and 793-bp products representing the wild type allele were amplified by primers a/b and c/d, respectively, representing the mutant product, is amplified by primers a and b, while the 793-bp product is amplified by primers c and d represents the wild-type allele.

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 (T_4) 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). T_4 and TSH were

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.

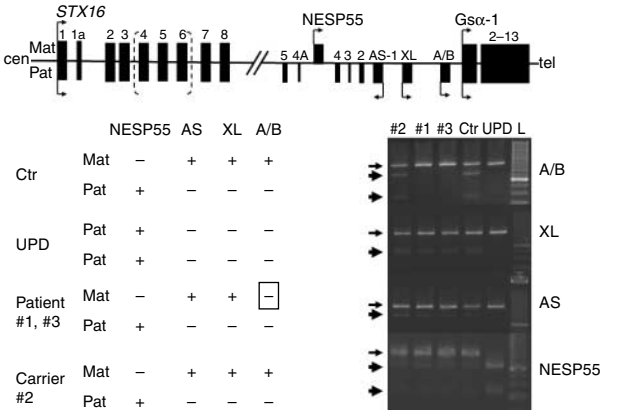


Figure 2 Schematic representation of the human *STX16* gene and the *GNAS* locus. Exons are shown as black boxes; arrows indicate the transcriptional direction (sense, antisense) and allelic origin (mat, maternal: above the line; pat, paternal: below the line). Hatched bracket shows deleted region within *STX16*. Lower left panel: methylation changes observed in the differentially methylated regions (DMR) for several individuals; '+', methylated DMR; '−', non-methylated DMR; Ctr, healthy individual; UPD, patient with paternal uniparental isodisomy of chromosome 20q; patients #1 and #3 with 3-kb *STX16* deletion; carrier #2, healthy mother of #1 and #3. Lower right panel: bisulfite-treated genomic DNA was amplified by PCR and then digested with the endonuclease *Fau*I for exon A/B, *Bst*UI for exons XL and AS, with *Aci*I for exon NESP55 to assess the methylation status. Only the products derived from the methylated allele were digested (thick arrows); absence of digestion thus indicates that genomic DNA was unmethylated before bisulfite treatment (thin arrows).

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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