

A Pseudohypoparathyroidism Type Ia Patient with Normocalcemia

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Abstract. Pseudohypoparathyroidism type Ia (PHP-Ia), one of 4 types of PHP, is a genetic disease characterized by clinical hypoparathyroidism caused by parathyroid hormone (PTH) resistance. In addition, patients with PHP-Ia show resistance to other hormones as well as Albright's hereditary osteodystrophy (AHO), a constellation of features including short stature, obesity, brachydactyly, ectopic ossifications, and/or mental retardation. Hypocalcemia is one of the hallmarks of PHP-Ia, but several PHP-Ia patients have been described to have normocalcemia. We encountered a 10-year-old girl with typical Albright's hereditary osteodystrophy with round face, short stature, brachydactyly, and obesity. Biochemical examination showed normocalcemia and increased PTH levels. Ellsworth-Howard test did not show any responses of urinary cAMP and phosphate. Based on these findings, she was diagnosed as having PHP-Ia with normocalcemia. Sequencing analysis of the *GNAS* gene identified a heterozygous missense mutation in exon 13 (R385H), which was previously reported in a PHP-Ia patient. The exact reason for her normocalcemia is not determined, but we must recognize heterogeneous biochemical findings even in PHP-Ia.

Key words: PHP-Ia, Albright's hereditary osteodystrophy, Missense mutation, Normocalcemia

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PSEUDOHYPOPARATHYROIDISM (PHP; MIM 103580) refers to end-organ resistance that primarily impairs the renal actions of PTH that mediates its action via Gs α -coupled receptor [1–3]. In addition, patients with PHP-Ia show resistance to other hormones as well as Albright's hereditary osteodystrophy (AHO), a constellation of features including short stature, obesity, brachydactyly, ectopic ossifications, and/or mental retardation.

PHP-Ia is caused by maternally inherited, heterozygous loss-of-function mutations in the *GNAS* gene encoding Gs α , whereas paternally inherited mutations

lead to AHO alone called pseudopseudohypoparathyroidism (PPHP) [1–4]. Patients with PHP-Ib usually show renal PTH resistance without AHO phenotypes [1–3]. Recent studies suggest that the loss of a maternal specific CpG methylation at exon A/B is likely to be one of the causes of PHP-Ib [3, 5].

Somatic and biochemical manifestations in PHP-Ia are sometimes heterogeneous even in the same family [1, 6]. Regarding serum calcium concentration, several PHP-Ia patients with normocalcemia have been described [6–13]

We encountered a 10-year-old PHP-Ia girl with normocalcemia. Sequencing analysis of the *GNAS* gene identified a missense mutation (R385H) in exon 13, which was previously reported.

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A report of case

A 10-year-old girl was referred to our department because of short stature. She was a full-term infant (41 weeks gestation) and was delivered after an uncomplicated pregnancy. She weighed 3,024 g with a length of 50.5 cm at birth. The neonatal period was uncomplicated. Her parents, elder sister and 2 younger brothers did not show any visible signs of AHO. Her father was 166 cm, and her mother was 150 cm. On physical examination, she was 123.1 cm (-2.3 SD for normal Japanese girl), and weighed 28.4 kg at referral. 22% obesity was observed on the Japanese Obesity Assessment Curve for school children. As shown in Fig. 1, height growth velocity had been gradually reduced from the age of 6. Her breast was Tanner stage II. She showed typical somatic and skeletal features of Albright's hereditary osteodystrophy with round face and brachymetaphalangism. Subcutaneous calcifications were not observed.

Skeletal maturation assessment by the plain radiographs revealed shortened metacarpals and metatarsals (Fig. 2). The RUS bone age was estimated to 10.9 years (BA/CA, 10.9/10.2) by the Japanese standard TW2 method from radiographs taken of her left carpal bone. Brain computed tomography did not show any abnormality.

Laboratory and endocrinological data are shown in Table 1. Her serum calcium level was normal (9.8 mg/dl, normal range, 8.0–11.0 mg/dl) and phosphate slightly increased (5.4 mg/dl, normal range; 3.6–4.6 mg/dl for this age). Her serum ALP was 565 IU/L (normal range, 104–338 IU/L). Her serum mid-portion PTH was very high (854 pg/ml; normal range 74–273, as measured by immunoradiometric assay, Mitsubishi Chemical). Her serum 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] was 61 pg/ml (normal range, 20–60 pg/ml). Urinary excretions of cAMP and phosphate did not increase after intravenous infusion of PTH, indicating PTH resistance in the kidney (Table 1). Her basal serum TSH, T3, T4, and free T4 levels were within normal ranges. Her TSH response after TRH stimulation and LH and FSH responses after GnRH stimulation were normal. GH provocative tests showed also normal responses of GH (Table 1).

Based on these clinical and biochemical findings, she was diagnosed as having PHP-1a with normocalcemia. Subsequently treatment with 1,25-dihydroxyvitamin D3 [$1,25(\text{OH})_2\text{D}_3$] (0.02 $\mu\text{g/kg/day}$) was initiated.

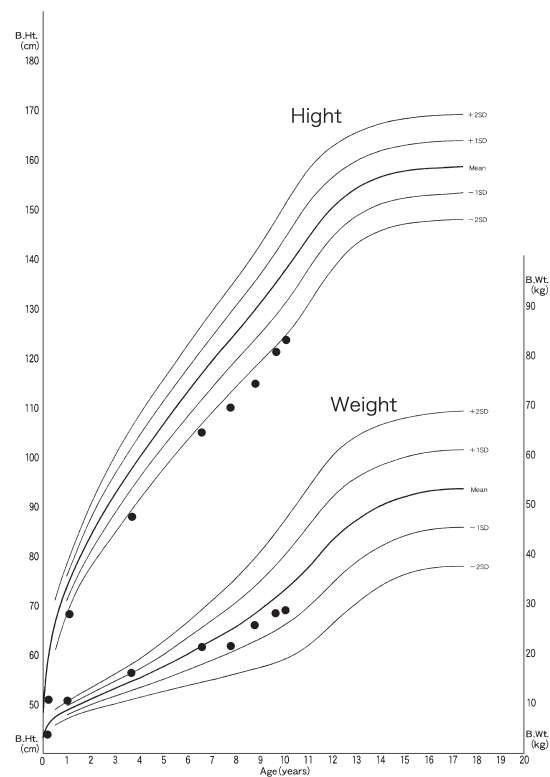


Fig. 1. Growth chart of the patient. Values were plotted on cross sectional growth chart for Japanese girls (0–18 years), 2000.

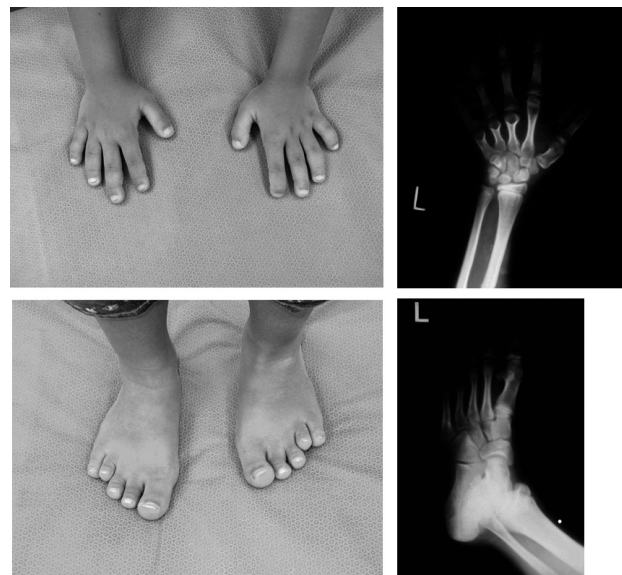


Fig. 2. Metacarpal shortening and metatarsal shortening in the patient.

Shortened third, fourth and fifth metacarpals were detected on both right and left hands and also on both right and left feet. Roentgenogram showed shortening of third, fourth and fifth metacarpals on the left hand and left foot.

Table 1. Summary of laboratory data for the patient with PHP 1a

		Reference range
Serum Ca (mg/dl)	9.8	8.0–11.0
Serum P (mg/dl)	5.4	3.6–4.6 for this age
Mid-portion PTH (pg/ml)	854	74–273
CRE (mg/dl)	0.4	0.5–1.0
Serum T3 (ng/ml)	138	70–167
Serum T4 (mg/dl)	6.6	4.8–10.5
Serum free T4 (ng/dl)	1.1	0.8–1.7
TRH stimulation		
TSH (μ U/ml)	4.8 → 36.2	
IGF-1 (ng/ml)	397.7	170–962
GH (ng/ml) ^b		
Arginine stimulation	5.4 → 9.5	
Clonidine stimulation	8.2 → 13.5	
L-dopa stimulation	0.7 → 23.9	
GnRH stimulation		
LH (mIU/ml)	0.8 → 28.4	
FSH (mIU/ml)	8.0 → 23.9	
Ellsworth-Howard test ^a		
cAMP response		
U4-U3 (mmol/h/m ²)	0.71	1>
U4/U3	2.17	10>
Phosphate response		
(U4 + U5) – (U2 + U3)	–6.3	35>
(mg/2 h)		

^a PTH (1–34)-infusion test (Ellsworth-Howard test) was performed using bolus injection of the active synthetic PTH-(1–34), at a dose of 100 U/m².

^b GH was measured by immunoradiometric assay using Daiichi radioisotope[®]. This assay was done before recombinant kit for GH measurement was available. Each GH value was corrected by the method of calculation recommended by Growth Foundation in Japan.

By this treatment, serum mid-portion PTH levels have gradually decreased to 200–300 pg/ml. Her urinary calcium excretion has remained below 0.2 (calcium (mg/dl)/creatinine (mg/dl)).

We obtained the approval of the study by the institutional ethical committee of Kansai Medical University and Matsubara Municipal Hospital and the parents gave their written informed consent for DNA analysis. The 13 exons and exon-intron boundaries of the *Gsα* gene were amplified by polymerase chain reaction (PCR) using oligonucleotide primers as described in a previous report [14]. Direct sequencing from both 5' and 3' ends of the amplified genomic DNA fragments revealed the substitution of histidine for arginine at position 385 (R385H) in exon 13 of the *GNAS* gene

(Fig. 3), which was previously reported in a patient with PHP-1a [9].

Discussion

Our patient had typical AHO phenotypes and renal PTH resistance, but showed normocalcemia. Sequence analysis of the patient identified R385H mutation, which was previously described [9]. Previous *in vitro* study demonstrated that this mutant *Gsα* is unable to interact with hormone receptor and results in uncoupling of adenylyl cyclase from cell surface receptor. Therefore, R358H mutation is the cause of our patient's condition.

As mentioned, several patients with PHP-1a have been reported to have normocalcemia [7–13]. A patient with R385H was also described to have normocalcemia [9]. One may infer that R385H is milder than other mutations of the *GNAS*. However, it is generally thought that there is no clear genotype-phenotype correlation in PHP-1a [1, 3]. Thiele *et al.* [13] have reported a mutation of exon 3 in a PHP-1a patient with normocalcemia. Usually in *Gsα*, there are two long (*Gsα*-L) and two short (*Gsα*-S) splice variants, created by alternative splicing. Their *in vitro* study demonstrated that the protein expression of *Gsα*-L reduced, but the expression of *Gsα*-S was not altered. Thus, one possibility for normocalcemia may be that *Gsα*-S could only partly compensate for impaired *Gsα*-L function.

Another explanation for her normocalcemia is plenty of dietary intake of calcium and vitamin D. Normal or increased serum concentrations of 1,25(OH)₂D were reported in some patients with PHP-1a [11, 15]. From the interview of her parents, there was no particular eating habit of excess calcium and vitamin D, but her serum 1,25(OH)₂D was within normal range. This may contribute to maintain normal serum calcium levels.

Thirdly, several studies demonstrated PTH action on bone at least in some patients with PHP-1a by radiographic and histological findings, and decreased bone mineral density [7, 8, 16, 17]. Furthermore, *in vitro*, Ish-Shalom *et al.* [10] showed that bone cells from patients with PHP-1a had normal response to PTH administration. Thus, although we did not determine PTH action on bone, normocalcemia may be partly explained by normal skeletal responsiveness to PTH in our patient.

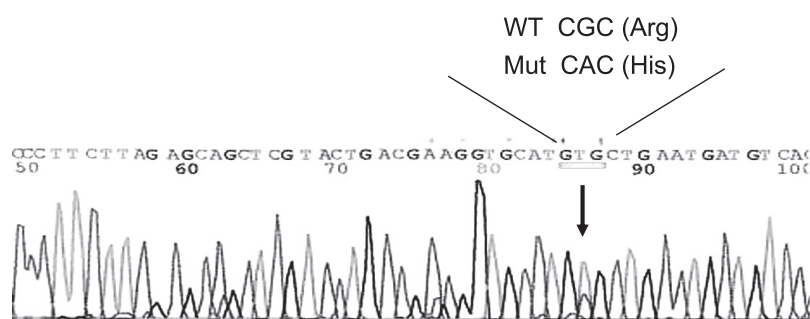


Fig. 3. Direct sequencing analysis of the exon 13 in the *GNAS* gene. Antisense sequence was shown. Mutated and normal nucleotides (C and T) were present (arrow). This caused a missense mutation (R385H).

It is usual practice to adjust therapy with vitamin D compounds to maintain normocalcemia. As described above, some patients with PHP-Ia have normal skeletal normal responsiveness to PTH. Therefore, serum PTH should be suppressed to normal levels in order to protect against bone loss, even though they have normocalcemia [10, 11]. This rationale our therapy of 1,25(OH)₂D₃ in this patient.

In summary, we encountered a PHP-Ia patient with normocalcemia caused by R385H mutation in the

GNAS gene.

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