

## Original

# Two Cases of Pseudohypoparathyroidism Type Ia in Duozygotic Twins with Different Phenotypes

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**Abstract.** Pseudohypoparathyroidism (PHP) type Ia is characterized by hypocalcemia due to PTH resistance and by features of Albright's hereditary osteodystrophy, including short stature, obesity, subcutaneous calcification and brachydactyly. A wide variety of clinical and biochemical manifestations have been reported. We report two cases of PHP type Ia in duozygotic twins with different phenotypes. The proband was a 10-yr-old girl. She showed subcutaneous ossification, shortening of the metacarpal bone, short stature, obesity and round face. She had normocalcemia (8.9 mg/dl), high-normal phosphate (5.0 mg/dl) and increased levels of serum intact PTH (152 pg/ml) and TSH (9.17  $\mu$ IU/ml) levels. Her twin younger brother had atypical Albright's hereditary osteodystrophy with only mild obesity and subcutaneous calcifications, but he showed a low level of serum calcium (7.0 mg/dl) and high levels of serum phosphate (7.6 mg/dl), intact PTH (377 pg/ml) and TSH (6.9  $\mu$ IU/ml). We diagnosed them as having PHP type Ia on the basis of clinical and biochemical findings, Ellsworth-Howard test and family history. There is considerable variability in clinical and biochemical features of PHP type Ia even among affected duozygotic twins. The differences of intrauterine environment and growth history cannot account for the variable phenotypes of PHP type Ia. Even if a patient shows no AHO features, examination of all family members should be undertaken.

**Key words:** pseudohypoparathyroidism type Ia, Albright's hereditary osteodystrophy, duozygotic twins, phenotype

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## Introduction

Pseudohypoparathyroidism (PHP) type Ia is an autosomal dominant condition characterized

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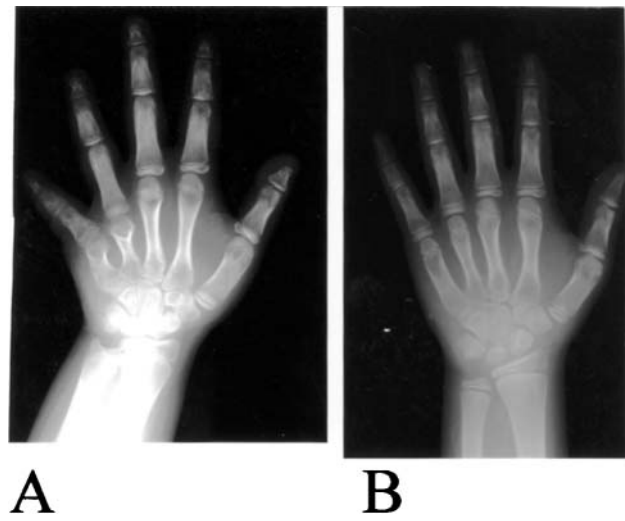
by target organ unresponsiveness to a number of hormones that share the same signaling mechanism, *i.e.*, a Gs protein-coupled receptor (1). Heterozygous loss of function mutations in the stimulatory Gs protein  $\alpha$ -subunit ( $Gs\alpha$ ) gene inherited from the mother lead to the development of PHP type Ia (2). This disease is associated with Albright's hereditary osteodystrophy (AHO) and resistance to PTH and some other hormones whose receptors are coupled to Gs protein (1). AHO includes short stature, obesity, a round face,

brachydactyly, subcutaneous ossification, and mild to moderate mental retardation (3). Considerable variability occurs in the clinical and biochemical manifestations even among affected members of a single family, and all of these features may not be present in every case (4). We report two cases of PHP type Ia in duozygotic twins with different phenotypes.

### Case Report

#### Case 1

The proband, a 10-yr-old girl, was referred to our department with subcutaneous ossification and brachydactyly. She was born as the first born of twins at term with routine discharge from the hospital. There was no evidence of consanguinity. Her birth weight was 2646 g and body length was 46 cm. A small hard tumor was noticed on her left ankle at the age of 6 mo, but no definite diagnosis was made at that time. She began gaining weight excessively from early infancy, and she was observed from 6 yr old as simple obesity by a local pediatrician. She did not show hypocalcemia in the local hospital. At the age of 10 yr, a tumor of 20 mm in diameter was noticed on her back, and a biopsy was performed by a dermatologist. Osteoma cutis was diagnosed with the histological findings. She was referred to our department by the dermatologist because she had brachydactyly as well as osteoma cutis. Her height and weight at that time were 130.6 cm (-1.27 SD) and 38.3 kg, respectively. Percent of relative body weight was +40.6%. Her bone age was 12 yr and 4 mo, and her height SD score for bone age was -3.28 SD. She had typical AHO with a round face, short neck, brachymetaphalangism (Fig. 1) and obesity. No mental retardation was found. Nail hypoplasia of the feet and hands was noted. Dental root defects of 8 teeth were observed. She had breast budding (Tanner stage II) and no pubic hair. Examination of the eyes, ears, nose, throat, chest, heart, and abdomen disclosed no other abnormalities. Both Chvostek's sign and Trousseau's sign were absent.



**Fig. 1** Panel A (case 1): Radiograph of the left hand showing shortening of the 4th and 5th metacarpals. Panel B (case 2): No brachymetaphalangism was found.

The results of a neurological examination were within normal limits. Head computerized tomography (CT) showed multiple subcutaneous calcifications without intracranial calcification. Biochemical and hormonal profiles are shown in Table 1. She had normocalcemia, high-normal phosphate and increased serum PTH and TSH levels. An Ellsworth-Howard test showed the absence of the phosphaturic response and the absence of the expected rise in urinary and plasma cyclic AMP (data not shown). We diagnosed her as having a PHP type Ia on the basis of clinical and laboratory findings, and the Ellsworth-Howard test. Family members underwent clinical and hormonal examinations as well as hand radiography. The pedigree is shown in Fig. 2.

#### Case 2

The patient was the younger twin brother of the proband. His birth weight was 2394 g and body length was 43.5 cm. He began gaining weight excessively from early infancy and received lifestyle advice from a doctor. At the age of 10 yr, he was referred to our department because of

**Table 1** Biochemical and hormonal characteristics of the family members

	Case 1	Case 2	mother	father	normal range
Calcium (mg/dl)	8.9	7.0	9.5	8.9	8.7–10.0
Phosphate (mg/dl)	5	7.6	2.7	3.5	2.5–4.6
Intact PTH (pg/ml)	152	377	26	44	10–60
TSH ( $\mu$ IU/ml)	9.17	6.9	1.96	–	0.6–4.1
Free T4 (ng/dl)	1.0	0.8	1.3	–	0.9–1.6
25-OHD (ng/ml)	21	28			10–29
1,25-(OH)2D3 (pg/ml)	43.1	41.5			13–79
IGF-1 (ng/ml)	340	330			Boys: 87–405 <sup>a</sup> Girls: 60–514 <sup>a</sup>
Bone-specific ALP (U/l)	102	113			Boys: 97.3 $\pm$ 25.0 (5) <sup>b</sup> Girls 107.7 $\pm$ 29.3(5) <sup>b</sup>
Osteocalcin (ng/ml)	22	39			Boys: 12.5 $\pm$ 3.8 (5) <sup>b</sup> Girls 15.4 $\pm$ 9.0 (5) <sup>b</sup>
Urinary NTX (nmol BCE/mmol Cre)	335.5	683.8			Boys: 222.3 $\pm$ 23.7 (6) <sup>b</sup> Girls: 353.5 $\pm$ 23.2 (6) <sup>b</sup>
Urinary DPD (nmol/mmol Cre)	27.1	24.4			Boys: 45.7–67.4 (7) <sup>c</sup> Girls 45.5–71.2 (7) <sup>c</sup>
BMD (Z score, L2 - L4 DEXA)	-0.22	-0.14			

25-OHD; 25-hydroxyvitamin D, 1,25-(OH)2D3; 1,25-dihydroxyvitamin D, NTX; N-telopeptides of type I collagen. DPD; deoxypyridinines, BMD; bone mineral density, DEXA; dual energy X-ray absorptiometry. a Normal values for boys and girls aged 9 ~ 10 yr. b Normal values for boys and girls with Tanner stage II. Data are given as mean  $\pm$  SD. c Normal values for boys and girls aged 10 yr. Data are given as interquartile range.

familial investigations. His height and weight were 134.1 cm ( $-0.69$  SD) and 37.7 kg, respectively. Percent of relative body weight was +25.3%. His bone age was 12 yr and 3 mo, and his height SD score for bone age was  $-2.0$  SD. His testes volume was 4~5 ml (Tanner stage II). Brachydactyly and mental retardation were not found. Dental enamel hypoplasia and root defects were not observed. Both Chvostek's sign and Trousseau's sign were absent. Head CT showed multiple subcutaneous calcifications with intracranial calcification. A subcutaneous calcification was also found by hand radiography. He had atypical AHO with only mild obesity and subcutaneous calcifications. Biochemical and hormonal profiles are shown in Table 1. He had hypocalcemia, hyperphosphatemia, and increased serum PTH and TSH levels. An Ellsworth-Howard test showed the absence of the

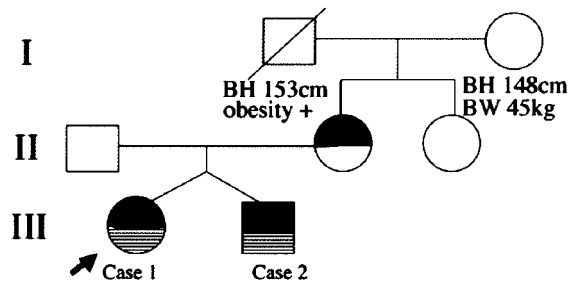
phosphaturic response and the absence of the expected rise in urinary and plasma cyclic AMP (data not shown). We also diagnosed him as having a PHP type Ia on the basis of clinical and laboratory findings, the Ellsworth-Howard test and family history.

### Propositus' mother and father

Clinical and biochemical features of the mother and father are shown in Fig. 2 and Table 1. The mother had short stature, round face and mild obesity. No brachydactyly, mental retardation or subcutaneous calcification was found. All biochemical findings were within normal ranges. The father did not have any clinical or biochemical features.

### Endocrinological tests

Cases 1 and 2 underwent the following



	Age (yr)	Height SD score	Phenotypic features					
			Ob	RF	SC	Br	DD	MR
Case1	10	-1.27SD (* -3.28SD)	+	+	+	+	+	-
Case2	10	-0.69SD (* -2.0SD)	+	+/-	+	-	-	-
mother	38	-2.0SD	+	+	-	-	-	-
father	42	+0.82SD	-	-	-	-	-	-

\*; height SD score for bone age BH; body height  
RF; round face, Ob; obesity, SC; subcutaneous calcification  
Br; brachydactyly, DD; dental defect, MR; mental retardation

**Fig. 2** Pedigree of the affected family. The AHO phenotype is indicated by Black boxes. Hatched symbols show the presence of PTH resistance.

endocrinological tests (Table 2). Human CRH, TRH, LHRH and GHRH were administered simultaneously, and GH stimulation tests, *i.e.*, insulin and clonidine stimulation tests were performed on separate days after an overnight fast. Case 1 had increased peak levels of ACTH and TSH, delayed FSH peak, and low response of GH, whereas Case 2 had increased peak levels of ACTH and LH, and delayed FSH and LH peaks.

### Molecular studies (Case 1)

Total genomic DNA was isolated from peripheral blood leukocytes of case 1, and the 13 coding exons and intron-exon boundaries of *GNAS1* were analyzed as previously described (8). DNA analysis was performed after obtaining informed consent. No mutation was identified in coding exons and intron-exon boundaries of *GNAS1*.

### Discussion

These two cases are particularly interesting because they demonstrate a variety of clinical and hormonal features. Case 1 had typical features of

**Table 2** Endocrinological Provocation Tests

CRH, TRH, LHRH, GHRH stimulation test (CRH 1.5  $\mu\text{g}/\text{kg}$ , TRH 0.5  $\text{mg}/\text{m}^2$ , LHRH 0.1  $\text{mg}/\text{m}^2$ , GHRH 1  $\mu\text{g}/\text{kg}$  i.v.)

	Case 1		Case 2		normal range peak
	basal	peak	basal	peak	
ACTH (pg/ml)	25.7	187.0	70.1	370	17.2~135.3
Cortisol ( $\mu\text{g}/\text{dl}$ )	3.2	16.2	7.9	16.4	13.1~35.6
TSH ( $\mu\text{IU}/\text{ml}$ )	9.17	43.5	6.9	29.3	10~35
PRL (ng/ml)	2.9	9	2.8	8.8	more than twice of basal value
LH (mIU/ml)	<0.6	13.6	2	30.7 (120 min)	male 18.2~38.0, female 8.5~15.5
FSH (mIU/ml)	6.5	25 (120 min)	4.4	10.4 (120 min)	male 5.8~22.3, female 8.3~20.0
GH (ng/ml)	1.4	15.8	1.8	16.9 (120 min)	>15

Insulin stimulation test (regular insulin 0.1 U/kg i.v.)

	Case1 peak	Case2 peak	normal range peak
GH (ng/ml)	5.3	6.2	>10

clonidine stimulation test (clonidine 0.1  $\text{mg}/\text{m}^2$  p.o.)

	Case1 peak	Case 2 peak	normal range peak
GH (ng/ml)	7.1	12.6	>10

AHO except for mental retardation but showed normocalcemia and high-normal phosphate. On the other hand, Case 2 had atypical AHO with only subcutaneous ossification and mild obesity but showed hypocalcemia and hyperphosphatemia. The mother had an AHO phenotype, but calcium metabolism and serum PTH levels were normal, and it was suggestive of pseudo-PHP. The maternal grandfather had short stature (body height 153 cm) and obesity, however he had died before the referral of the present cases, and the details are uncertain. The maternal grandmother showed neither short stature nor obesity. If the maternal grandfather had AHO, it is supposed that the mother is pseudo-PHP. Analysis of the  $Gs\alpha$  activity or the *GNAS1* gene is required for definitive diagnosis. The *GNAS1* mutation was not identified in Case 1 by our approach. In another study, the mutation detection rate was 72% in PHP patients with reduced activity of  $Gs\alpha$  (9). The genetic defect may be located outside the coding region in the promoter region of *GNAS1* or in another regulatory region leading to  $Gs\alpha$  deficiency.

Considerable variability occurs in the clinical expression of AHO even among affected members of a single family, and all of the features may not be present in every case (4). On rare occasions, it may be impossible to detect any features of AHO in an individual with  $Gs\alpha$  deficiency (10). Our cases are dizygotic twins, whose intrauterine environment and growth history resemble each other very much. However, the phenotypes of both were completely different. The reason for the phenotypic difference is unknown, but we suggest that it is not caused by the environment. Tissue- or cell-specific imprinting of  $Gs\alpha$  has been suggested as an explanation of phenotypic variation (11, 12).

The PTH/PTHrP receptor not only mediates PTH-dependent regulation of calcium and phosphate homeostasis but also plays an important role in chondrocyte proliferation and differentiation and thus in bone growth and elongation (13).  $Gs\alpha$  mutations in the proliferative

layer of growth plate chondrocytes result in an insufficient PTHrP-dependent inhibition of chondrocyte maturation. Mantovani *et al.* reported that the clinical findings of osteodystrophy and obesity in PHP type Ia patients despite the presence of one normal  $Gs\alpha$  allele might be due to the presence of haploinsufficiency of this gene in bone and adipose tissue (12). Case 1 showed typical shortening of both 4th and 5th metacarpals and moderate obesity, whereas Case 2 had no brachydactyly and only mild obesity. The reason for this difference is not clear, though the degree of haploinsufficiency of *GNAS1* might explain the difference.

PHP type Ia is associated with resistance to multiple hormones, including PTH, TSH, gonadotropins and glucagons, whose effects are mediated by Gs-coupled pathways (14). Mantovani *et al.* reported the presence of imprinting of  $Gs\alpha$  paternal allele in selective tissues, such as the thyroid, pituitary and gonad, which, besides the kidney, are affected in PHP type Ia (15). On the other hand, both the paternal and maternal alleles were equally expressed in the adrenal gland (15). Patients with PHP type Ia usually show a normal hypothalamic-pituitary-adrenal axis. Both of our cases showed high basal TSH levels and delayed exaggerated response to LHRH, besides hyperresponsiveness of ACTH to the CRH test. These findings might indicate partial adrenal resistance. We suppose that the presence of 50% activity of the Gs protein in their adrenal gland is not sufficient in our cases. Careful observation of adrenal insufficiency might be needed in the future.

There is considerable variability in clinical and biochemical features of PHP type Ia even among affected dizygotic twins. The differences of intrauterine environment and growth history cannot account for the variable phenotype of PHP type Ia. Even if a patient shows no AHO features, examination of all family members should be undertaken.

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