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Resistance to GHRH but Not to PTH in a 15-Year-Old Boy With Pseudohypoparathyroidism 1A

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Pseudohypoparathyroidism 1A (PHP1A) consists of signs of Albright hereditary osteodystrophy (AHO) and multiple, variable hormonal resistances. Elevated PTH levels are the biochemical hallmark of the disease. Short stature in PHP1A may be caused by a form of accelerated chondrocyte differentiation leading to premature growth plate closure, possibly in combination with GH deficiency in some patients. Treatment of short stature with recombinant growth hormone (rhGH) in pediatric patients may improve final height if started during childhood. The 10 11/12-year-old boy with clinical signs of AHO presented for evaluation of short stature [height standard deviation score (SDS) −2.72]. Clinically his mother was affected by AHO as well. A heterozygous mutation c.505G>A (p.E169K) in exon 6 of the *GNAS* gene confirmed a diagnosis of PHP1A in the boy. However, hormonal assessment was unremarkable except for low serum IGF-1 (SDS −2.67). On follow-up, GH deficiency due to GHRH resistance was suspected and confirmed by clonidine and arginine stimulation tests. Treatment with rhGH (0.035 mg/kg) for 2 years resulted in catch-up growth (height SDS −1.52). At age 15 years the PTH levels and bone age of the patient remain within the normal range. In patients with PHP1A, short stature is caused by the effects of G_s-α deficiency on the growth plate. However, resistance to GHRH and the resulting GH deficiency might also contribute. Recombinant GH treatment increases growth in these patients. Diagnostic workup for GH deficiency as a factor contributing to short stature is recommended even in the absence of other hormonal resistances.

Freeform/Key Words: PHP1A, short stature, hormone resistence, growth hormone

Albright hereditary osteodystrophy (AHO) consists of clinical signs including short stature, round face, obesity, and characteristically shortened metacarpals. In 1988 the connection between AHO and the *GNAS* locus was identified [1]. Over time, mild cognitive impairment was also attributed to the condition [2].

GNAS (20q13.32) is a complex imprinted gene locus that encodes at least five gene products through alternative splicing and promoter activation [2]. One of the known gene products is G_s-α, the α-subunit of the stimulatory G protein that is crucial for signal

Abbreviations: AHO, Albright hereditary osteodystrophy; BMI, body mass index; PHP1A, pseudohypoparathyroidism 1A; PTH1R, PTH/PTH related peptide receptor; rhGH, recombinant growth hormone; SDS, standard deviation score.

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transduction through mediation of cAMP production after binding of several hormones to their receptors, including CRH, ACTH, GHRH, LH/CG, FSH, TSH, and PTH [2].

Loss-of-function of Gs-α or of genes encoding for proteins downstream of the signaling cascade [3] may cause a variety of signs and symptoms depending on parental origin, type (genomic or epigenetic), and location of the alteration. Hormone resistance with elevated PTH levels, hyperphosphatemia, and hypocalcemia manifests only with mutations located on the maternal allele [2].

Because of the lack of G_s-α derived from the paternal *GNAS* allele in the renal proximal tubule, thyroid, pituitary, and gonad, maternally derived genomic loss-of-function mutations cannot be compensated for and consequently may lead to resistance to PTH, TSH, GHRH, LH, and FSH. In most tissues with biallelic (paternal and maternal) *GNAS* expression a single heterozygous mutation will not interfere with sufficient gene function [2]. However, growth plate chondrocytes seem to require two functional copies of G_s-α for normal development. Accordingly, short stature and shortened metacarpals and metatarsals of AHO are possibly caused by haploinsufficiency of G_s-α in bone tissue independent of parental origin [4, 5].

In patients with pseudohypoparathyroidism 1A (PHP1A), the resistance to PTH is normally absent at birth and usually develops during infancy, following the time course of imprinting. However, delayed development of PTH resistance in a single case until the age of 22 years has been observed [3].

Resistance to GHRH is a common finding in PHP1A and may result in decreased IGF-1 levels and pathological response to some GH stimulation tests [6]. Short stature in children with PHP1A may develop over time and manifest late when epiphyseal closure is imminent.

Mantovani *et al.* [5] report GH deficiency in 17 of 22 children with PHP1A. In a cohort study by Germain-Lee *et al.* [7], all of nine patients with a GH deficiency had decreased IGF-1 levels, whereas all of four patients without GH deficiency had normal IGF-1 levels.

Patients presenting with short stature and GH deficiency usually respond with catch-up growth when treated with recombinant growth hormone (rhGH), similar to patients with isolated growth hormone deficiency [5]. However, the pubertal growth spurt seems to be limited because of premature closure of epiphyseal plates, resulting in unsatisfactory final height [8].

1. Case Report

A 10 11/12-year-old boy presented to the pediatric endocrine outpatient clinic for evaluation of short stature [height SDS −2.72; body mass index (BMI) SDS 1.15]. On clinical examination, signs of AHO with a round face and short hands were noted. He attended a school for children with learning disabilities. Medical history revealed mild developmental delay and neurosurgical therapy of craniosynostosis in infancy but was otherwise unremarkable. The X-ray of the left hand revealed short and broad metacarpals (Fig. 1). On hormonal assessment, decreased IGF-I (SDS −2.67) and 25-OH vitamin D (5.1 ng/mL, normal range 20 to 46 ng/mL), but normal PTH levels were detected. (Table 1). No subcutaneous ossifications were noted.

G_s-α activity in erythrocyte membranes was decreased to 63.8% (normal range 85% to 115%). The G_s-α activity was quantified by measuring the cAMP concentration by a radioimmunoassay according to a method described earlier [9]. Genetic assessment of the *GNAS* locus revealed a pathogenic heterozygous mutation c.505G>A (p.E169K) in exon 6, which has been previously described in another patient with PHP1A [10]. Methylation status of the GNAS locus investigated by methylation-specific multiplex ligation-dependent probe amplification (probe set ME031, MRC-Holland) was normal.

The patient's mother is also affected by AHO [brachymetacarpia, height 151.9 cm (−2.5 SD), weight 86 kg (2.4 SD), BMI 37.3 kg/m^2 (1.8 SD)], without evidence of hormone resistance

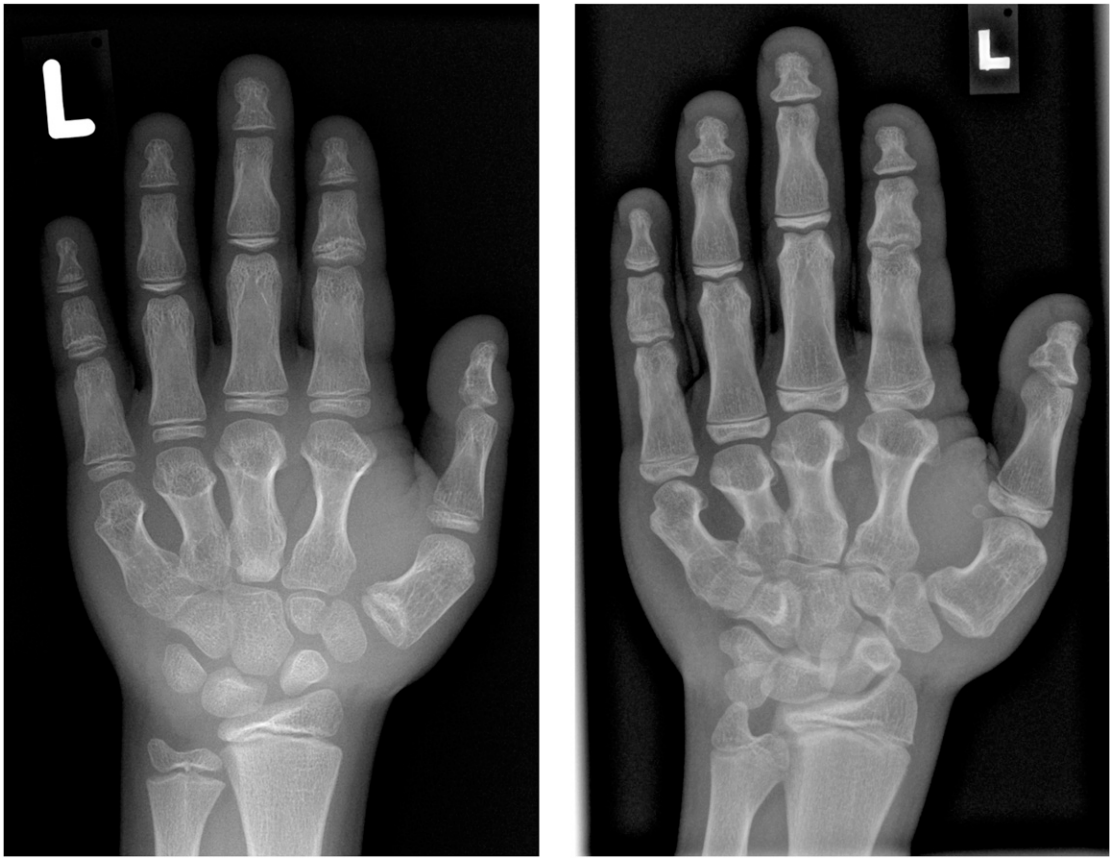

Figure 1. X-ray of the left hand with brachydactyly of a patient with PHP1A. Left: 10 11/12 y (bone age according to Greulich and Pyle, 11 6/12 y). Right: 15 1/12 y (bone age according to Greulich and Pyle, 15 6/12 y). The standards of Greulich and Pyle may not be adequate to assess bone age in patients with PHP1A.

(PTH 40.9 pg/mL, normal range 14 to 72 pg/mL; TSH 3.65 μU/mL, normal range 0.27 to 4.2 μU/mL). She has not been tested for GH deficiency.

She was born preterm at about 32 weeks in Turkey. No information about her birth weight and length was available. There were no apparent signs of cognitive deficits; she had attended and completed a level 2 school. She carries the same *GNAS* mutation as her son. In the clinically unaffected father, sequencing of *GNAS* revealed a wild type sequence. Methylation pattern of *GNAS* was normal in both parents.

On follow-up visits an annual growth rate of 3 cm/y (height velocity SDS −2.04), and consistently low IGF-1 levels (SDS −2.54) prompted testing for GH deficiency. After clonidine and arginine stimulation, a GH peak of 6.13 ng/mL (normal value >12.5 ng/mL) and 7.07 ng/mL (normal value >7.25 ng/mL), respectively, confirmed GH deficiency. Treatment with rhGH was initiated at age 12 10/12 years and resulted in catch-up growth with a maximum height velocity of 13.5 cm/y (SDS +2.82) accompanied by the onset of puberty (Fig. 2) despite radiographic evidence of premature closure of the metacarpal epiphyseal plates (Fig. 1).

At age 13 6/12 years, an elevated serum phosphate (1.74 mmol/L; normal range 0.87 to 1.58 mmol/L) was noted, and a slightly elevated PTH (65.7 pg/mL; normal value <65 pg/mL) was noted 1 year later. 25-OH vitamin D and serum calcium levels remained within the normal range. At the most recent visit at age 15 1/12 years, both serum phosphate and PTH were within the normal range again (Table 1).

A TRH test at age 12 years suggested a mild resistance to TRH (TRH test, baseline TSH 5.6 mU/L; TSH peak, 33.4 mU/L; TSH after 120 minutes, 11.3 mU/L). However, so far TSH

Table 1. Clinical (Testicular Volume, Height SDS, Growth Velocity SDS, and BMI SDS) and Biochemical Information of the Patient

Age (y)	PTH (pg/mL)	25-OH Vitamin D (ng/mL)	PO_3^{4-} (mmol/L)	TRP (%)	TmP/GFR (mg/dL)	Ca^{2+} (mmol/L)	TSAP (U/L)
11.0	37.6	**5.1**	1.23			2.45	202
11.6	14.8	30.4					
12.2	21.2	35.9	1.10	**88.7**	**2.6**	**2.53**	191
12.6	16.7	37.6	1.07	**90.3**	**2.8**	2.50	206
12.8			Start of GH treatment (0.035 mg/kg/d)				
13.1	17.8	32.0	1.32			2.38	348
13.6	39.5	34.4	**1.74**			2.39	**392**
14.1	60.1	34.9	**1.74**			2.48	**488**
14.3	23.9	32.6	1.20			2.46	**402**
14.6	**65.7**	31.2	**1.65**			2.39	386
15.1	31.5	30.6	1.10	**98.2**	3.9	2.47	376
15.6	52.2	35.5	1.39			2.30	244

(*Continued*)

and fT4 values remain within the normal range without supplementation therapy [11/2018, TSH 1.04 mU/L (normal range 0.5 to 4.33); free T4, 14.5 pmol/L (normal range 10.57 to 22.62)].

An increase in serum testosterone was first detected at age 11 7/12, and clinical signs of puberty (pubarche and testicular volume >3 mL) were noted at age 13 0/12, shortly after the onset of GH therapy. Pubertal development has progressed continuously since then. The patient reports erections but no ejaculations. Gonadotropin levels have been within the normal range.

2. Discussion

The patient presents with an unusual course of PHP1A with a specific and peculiar bone phenotype with severe brachydactyly, craniosynostosis, and short stature, a manifestation of GH deficiency due to GHRH resistance, but without clear manifestation of resistance to PTH up to the age of 15 years. This course differs from the case of the other patient in whom the same mutation was previously detected: a 3 11/12-year-old girl who presented with obesity, brachymetacarpia, and PTH resistance (hyperparathyroidism and hypocalcemia) [10].

However, delayed development of PTH resistance in PHP has recently been described by Usardi *et al.* [11] and in a new consensus statement [3]. In these reports the oldest patients developing PTH resistance were 3 and 22 years old, respectively.

Furthermore, this case highlights the importance of thorough endocrine evaluation of short stature to differentiate effects of GNAS mutation on the growth plate and on the pituitary. Although GH deficiency has been diagnosed via conventional stimulation tests in the past [7, 12], the diagnosis of GH deficiency in PHP1A may be difficult because stimulation tests using arginine infusion or insulin-induced hypoglycemia bypass the hypothalamic GHRH activation by directly acting on the pituitary via somatostatin inhibition [13]. In the case of GH deficiency due to GHRH resistance, these tests may lead to false normal results and may explain the near normal results of the arginine stimulation test in the presented case.

Unlike other patients with PHP1A, this patient exhibits a pubertal growth spurt without accelerated bone age [8]. The reason for this finding is unclear. Based on the most recent height measurement and bone age, the patient's adult height prediction at age 15

Table 1. Clinical (Testicular Volume, Height SDS, Growth Velocity SDS, and BMI SDS) and Biochemical Information of the Patient (Continued)

LH (U/L)	FSH (U/L)	Serum Testosterone (nmol/L)	Testicular Volume (mL)	IGF-1 SDS	IGFBP-3 SDS	Height Velocity SDS	Height SDS	BMI SDS
0.1	0.6	<0.35	2	**−2.67**	−1.12		**−2.72**	1.15
0.5		1.13	3	**−2.54**	−0.50	**−2.04**	**−2.75**	0.65
0.6	2.0	0.95	3	**−2.30**	−0.59	**−2.58**	**−2.91**	0.57
0.9	1.9	3.61		−1.65	−0.30	−1.84	**−2.81**	0.21
			Start of GH treatment (0.035 mg/kg/d)					
0.6	1.7	1.65	6	−0.29	0.01	−0.34	**−2.67**	0.50
0.3	2.2	0.92	6	0.90	0.72	0.97	**−2.44**	0.42
0.5	2.5	1.19	10	1.43	1.29	**2.82**	−1.93	0.85
1.6	2.7	3.82		1.67	1.16	**2.04**	**-2.05**	1.13
1.0	3.4	0.94	12	1.37	1.28	**2.21**	−1.86	1.01
1.0	3.5	17.34	15	**2.22**	1.12	1.16	−1.58	1.10
0.8	5.2	1.92	17.5	1.79	1.21		−1.55	1.09

Biochemical parameters: In plasma, PTH, normal value <65 pg/mL. In serum, 25-OH vitamin D, normal range = 20.0 to 46.0 ng/mL. PO_3^{4-} (phosphate), normal range 0.87 to 1.58 mmol/L. Ca^{2+} (calcium), normal range 2.15 to 2.52 mmol/L. TSAP (total serum alkaline phosphatase), age-appropriate normal range, 74 to 390 U/L. TRP (renal tubular reabsorption of phosphate), normal range 83% to 97%. TmP/GFR (ratio of renal tubular maximum reabsorption of phosphate to glomerular filtration rate), normal range 3.4 to 7.5 mg/dL. Values out of the age-appropriate normal range are shown in bold.
Abbreviation: IGFBP-3, serum insulin-like growth factor-binding protein 3.

6/12 (corresponding bone age 15 10/12) is 167.7 cm (±1.3 cm) (−1.9 SDS, Kromeyer-Hausschild).

Chondrocyte differentiation is modified through the PTH/PTH related peptide receptor (PTH1R) [4]. PTH1R not only activates G_s and thereby the adenylyl cyclase pathway but also $G_{q/11}$, another heterotrimeric G protein subunit, which activates the phospholipase C pathway [4].

In vivo experiments in mouse models showed that loss of function of G_s-α causes chondrocyte hypertrophy in murine growth plates, whereas loss of function of G_q results in delayed hypertrophy in these cells [4].

If alterations in the timing of hypertrophy of murine growth plate chondrocytes may be translated to alterations of bone age in human adolescents, activation of PTH1R may be a critical regulator of bone maturation.

Consequently, PTH excess in the presence of G_s dysfunction may cause an imbalance in favor of increased G_q-mediated hypertrophy of chondrocytes in the absence of G_s-mediated inhibition of hypertrophy of chondrocytes, possibly contributing to bone age acceleration and reduced final height in PHP1A.

In line with this hypothesis, the patient presented in this report displays a normal pubertal growth spurt without acceleration of bone age, unlike many patients from previous reports [3, 5].

3. Summary

PTH resistance might develop much later in individual patients with PHP1A than anticipated. Patients with clinical signs of AHO/PHP, short stature, and insufficient height velocity should be tested for GH deficiency, preferably with stimulation tests acting through stimulation of hypothalamic GHRH secretion (clonidine test, L-dopa-test). Because premature closure of epiphyseal plates in patients with PHP is common, timely diagnostic workup and close monitoring of therapy for short stature is recommended.

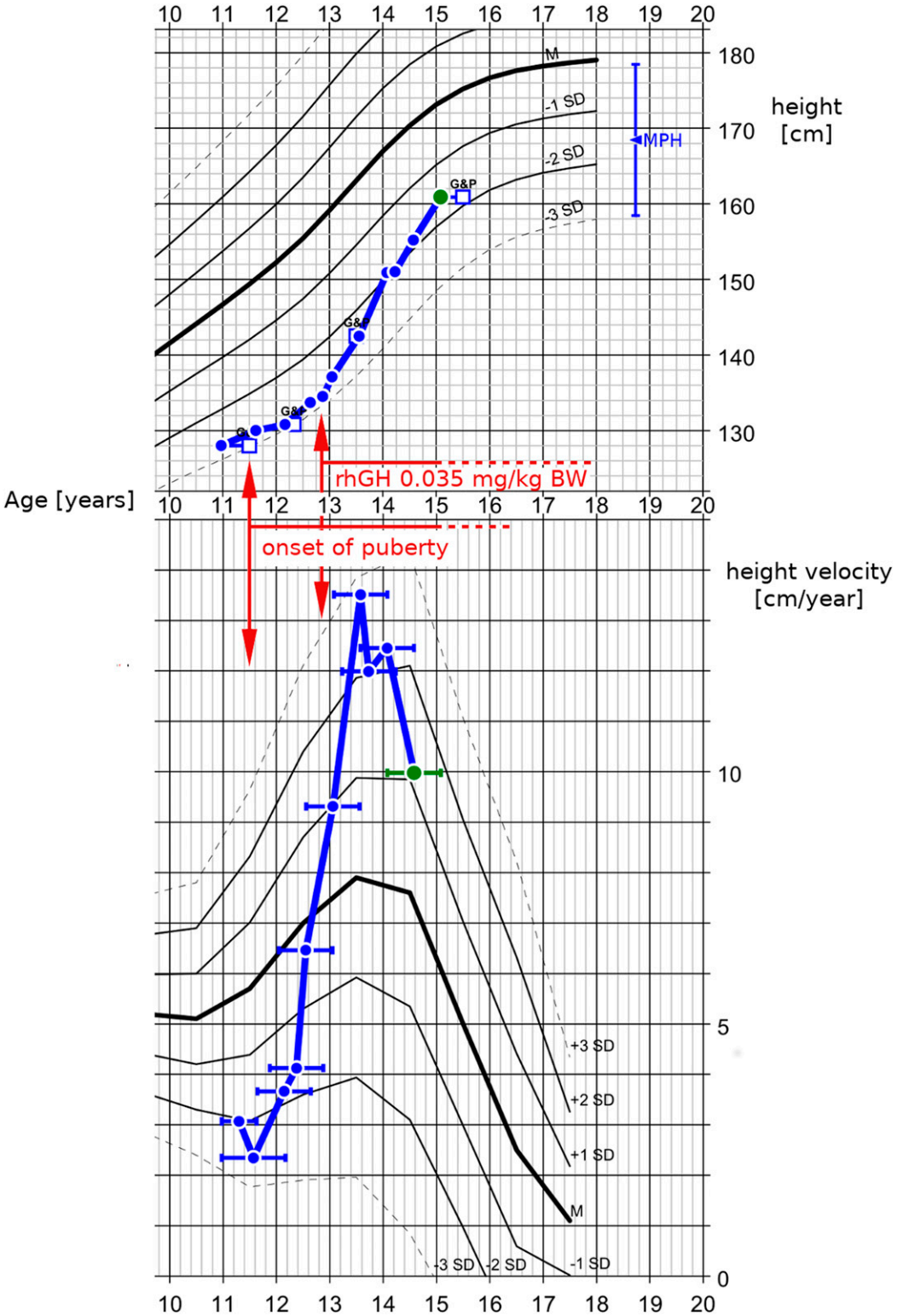

Figure 2. Height (upper chart) and height velocity (lower chart) from age 11 to 15 of the patient with PHP1A. Dots represent measured height (in cm), open squares indicate height corrected for bone age (according to Greulich and Pyle). The biochemical onset of puberty and the start of rhGH treatment are indicated in red.

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