Clinical and Molecular Characterization of Patients with Pseudohypoparathyroidism

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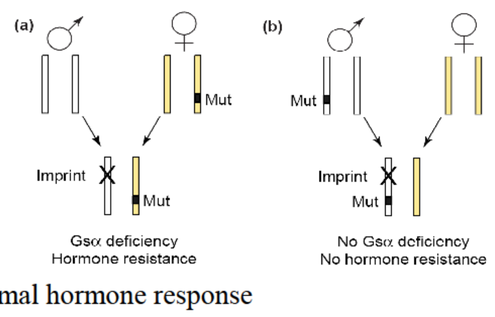
Background

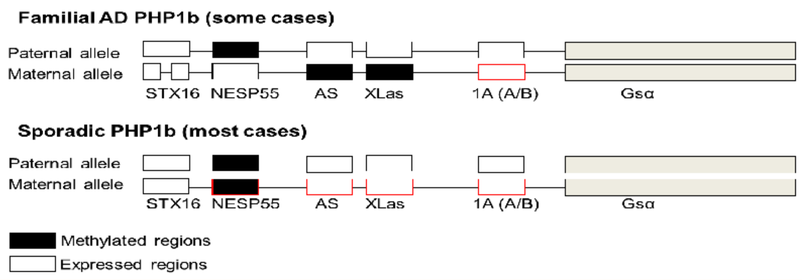
Pseudohypoparathyroidism (PHP)

- ✓ A heterogeneous group of disorders characterized by hypocalcemia, hyperphosphatemia, and Albright hereditary osteodystrophy (AHO)
- ✓ Results from abnormality of the *GNAS* locus

Classification and clinical features of PHP

- ✓ **PHP1a (multiple hormone resistance with AHO)**
  Maternally-inherited inactivating *GNAS* mutation with tissue-specific imprinting
  → Multiple hormone resistance with AHO
- ✓ **Pseudopseudohypoparathyroidism (PPHP) (AHO only, no hormone resistance)**
  Paternally-inherited *GNAS* mutation → AHO with normal hormone response
- ✓ **PHP1b (multiple hormone resistance only, no AHO)**
  Methylation defect in maternal allele (**paternal UPD**): Gsα loses expression in renal tubule leading to PTH resistance without affecting the expression in other tissues





Objectives

- This study was performed to investigate clinical features, outcomes, molecular characteristics of patients with pseudohypoparathyroidism (PHP) and pseudopseudohypoparathyroidism (PPHP).

Subjects and Methods

- **31 patients (15 males and 16 females) from 26 unrelated families**
- ✓ Clinical features of pseudohypoparathyroidism: hypothyroidism, hypocalcemia, increased PTH, and Albright hereditary osteodystrophy
- ✓ Clinical data such as presenting symptoms, clinical courses, and endocrinologic findings were analyzed retrospectively.
- **Molecular analysis of the *GNAS* gene**
- ✓ Mutation analysis of GNAS:
  - PCR amplification of all coding 13 exons and exon-intron boundaries using specific primers
  - Direct sequencing of the PCR products using an ABI3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
- ✓ Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA)

Results

Molecular analysis of *GNAS*

Table 1. Molecular analysis of *GNAS* in patients with PHP and PPHP

Subtype	Number	*GNAS* mutation		*GNAS* methylation defect
		Base exchange	Amino acid change	
PHP1a	3	c.348_349ins(C)	p.V117Rfs*23	
	3	c.85C>T	p.Q29*	
	1	c.348_349ins(C)	p.V117fs*23	
	1	c.1160A>C	p.H387P	
	1	c.802G>T	p.E268*	-
	1	c.313-2A>T	Splice site mutation	
	1	c.82A>T	p.K28*	
	1	c.312+5G>A	Splice site mutation	
	1	c.565_568del	p.D189_Y190delinsMfsX13	
	1	c.659+1G>A	Splice site mutation	
PHP1b	12			Paternal UPD
	2	-		*STX16* del, loss of methylation of *GNAS* exon 1A
PPHP	2	c.565_568del	p.D189_Y190delinsMfs*14	
	1	c.565_570del	p.Y190Cfs*19	

- ✓ Maternally-inherited *GNAS* mutations were identified in 14 patients with PHP1a, and paternally-transmitted mutations in 3 patients with PPHP.
- ✓ Paternal uniparental disomy was identified in 12 patients with PHP1b. Two patients of PHP1b demonstrated loss of methylation on exon 1A of *GNAS* on maternal allele with a deletion of *STX16*.

Clinical and Endocrinological Characteristics at Diagnosis

Table 2. Baseline characteristics of patients with PHP and PPHP.

	PHP1a (n=10)	PHP1b (n=11)	PPHP (n=2)	*P* value (1a *vs.* 1b)
Age at diagnosis (years)	10.25±8.86	10.22±4.36	5.88±7.48	0.5
Height-SDS	-0.84±1.93	-0.75±1.34	-2.75±1.48	0.45
Weight-SDS	0.46±1.74	-0.79±1.48	-2.1±2.74	0.23
BMI (kg/m^2)	21.95	19.07	16.4	0.06
AHO	9/10 (90%)	-	1/2 (50%)	
Mental retardation	3/10 (30%)	4/11 (36.4%)	1/2 (50%)	
Basal ganglia calcification	3/10 (30%)	5/11 (45.5%)	0/2	
Calcium (mg/dL)	6.61±1.62	6.34±1.49	9.85±1.34	0.35
Phosphorus (mg/dL)	8.34±2.49	7.36±1.71	5.45±0.07	0.16
ALP (IU/L)	393.2±246.6	272±128.9	522.5±135.1	0.08
Intact PTH (pg/mL)	383.6±324	375.5±193.4	276.5±369.3	0.47
25-OH-vitamin D (ng/mL)	28.4±19.36	24.05±13.22	93.56±71.33	0.29
1,25-$(OH)_2$ vitamin D (ng/mL)	67.68±15.3	43.88±18.92	82.35±19.59	0.01
TSH (μU/mL)	6.6±4.77	5.8±4.4	6.51±6.0	0.35
Free T_4 (ng/mL)	1.14±0.45	1.13±0.2	1.03±0.3	0.47

* Reference range; calcium 8.6 – 10.2mg/dL, Phosphorus 2.5 – 4.5 mg/dL, Alkaline phosphatase (ALP) 40-120 IU/L , intact parathyroid hormone (PTH) 9 – 74 pg/mL, 25-OH-vitamin D 30 – 60 ng/mL, 1,25-$(OH)_2$-vitamin D 18 – 70 ng/mL, Thyroid-stimulating hormone (TSH) 0.4 – 5.0 μU/mL, and free T4 0.8 – 1.9 ng/mL.

Treatment Outcomes

- L-thyroxine therapy in 3 patients with PHP1a and 3 with PHP1b at age 2.0 ± 1.8 years
- Calcium or vitamin D supplementation in 6 patients with PHP at age 9.2 ± 5.6 years

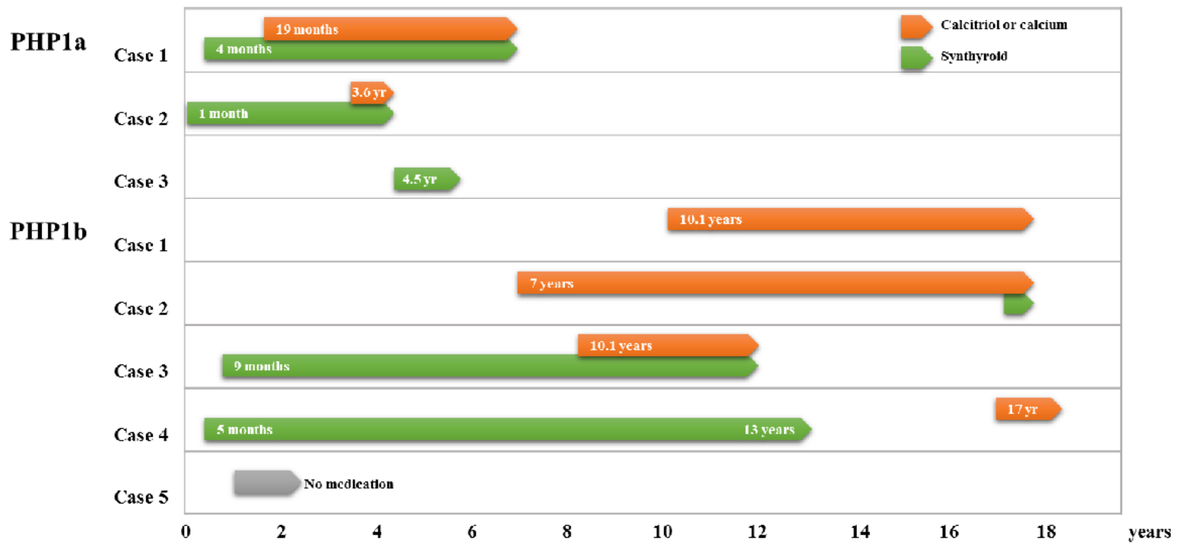


Fig. 1. Clinical course of PHP. Five patients manifested subclinical hypothyroidism earlier than the onset of hypocalcemia. Two subjects with PHP1b (cases 2 and 3) showed bimodal distribution of hypothyroidism and hypocalcemia.

Conclusions

- **Bimodal distribution of clinical features**
- ✓ **Hypothyroidism** in early infancy
  - Greater sensitivity to haploinsufficiency in thyroid than parathyroid cells.
- ✓ **Hypocalcemia** during children and adolescents:
  - Gsα expression in human fetal kidney cortex was shown to be biallelic using RT-PCR → could suggest that Gsα imprinting establishes later in life
  - Elevated PTH concentration can maintain normocalcemia for prolonged periods of time
  - Increased demands of calcium during pubertal growth
- **Growth patterns, pubertal progression, obesity, thyroid functions, serum PTH, calcium, and phosphorus levels → assessed on a regular basis** in order to introduce appropriate treatment in these patients

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DISCLOSURE STATEMENT

The authors have no financial relationships to disclosure or conflicts of interest to resolve.

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