

Calcification of Soft Tissues in a Family, Case Report and Review of Pseudohypoparathyroidism

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Abstract

Pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism are rare genetic disorders of calcium metabolism and resistance to the action of Parathormone. Although the genetic details of these conditions are not fully diagnosed, role of gene coding for stimulatory G protein locus was studied and imprinting in its inheritance was proved. We report three cases of a family member with calcification in soft tissues that show Pseudohypoparathyroidism in father and his sister and pseudo-pseudohypoparathyroidism in his son.

Keywords: Pseudohypoparathyroidism; Pseudopseudohypoparathyroidism; GTP-Binding Protein alpha Subunits; Bone Diseases

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Introduction

A rare genetic disorder of calcium metabolism is pseudohypoparathyroidism (PHP). Pseudo-pseudohypoparathyroidism (PPHP; OMIM #612463) is the more rare disorder of calcium metabolism [1]. These conditions could be seen separately or concomitant together in kindred [2]. They also variably accompanying with Albright hereditary osteodystrophy (AHO) that described by Albright et al. as the first hormone resistance syndrome [3-5]. They reported three cases with hypocalcemia and hyperphosphatemia with normal renal function tests. They had highly resistance to bovine parathyroid extract, introduced hypothesis of resistant to Parathormone (PTH) effect.

PHP refers to a heterogeneous group of resistance to the action of PTH and it is suspected by hypocalcemia despite high serum levels of PTH [6,7]. Symptoms of hypocalcemia such as tetany and seizure could be present or absent. Basal ganglia calcification and cataract might be concomitant finding in PHP. Usually, the PHP classified based on clinical features, biochemical signs, response to exogenous PTH and genetic evaluation [6]. Although recently a novel classification proposed by the EuroPHP network [8].

Case Presentation

In a routine visit of a 17 month old boy, asymptomatic multiple subcutaneous calcifications in trunk, limb and neck, which was sized maximum one centimeter in diameter were founded

(Figure 1). The lesions were painless and they didn't have history of trauma. The patient was born by cesarean section on 33 weeks of gestational age and his birth weight was 1650 grams. The preterm labor was because of premature rupture of membrane. He was admitted at birth due to respiratory distress and meconium aspiration syndrome. He had an acceptable hospitalization course and discharged after 10 days. Primary laboratory evaluations at 17 months age demonstrated normal total calcium (9.7 mg/dL), phosphorus (5.2 mg/dL), Alkaline phosphatase (733 U/L, in appropriate range for age), 25-OH Vitamin D (49 ng/ml, in appropriate range), PTH (24 pg/ml, in appropriate range), normal electrolyte and kidney function tests but a mild normochrome normocytic anemia (Hemoglobin 11 g/dL). Weight and height were under 1st percentile and head circumference was at 25th percentile, although, his socio-neurological development was mildly delayed in gross motor. The family history clarified that his father had extensive and large subcutaneous calcification especially on forearm, hand, abdominal wall, back and neck. After physical examination found that the father had short stature (1-3th percentile for age), shortening of forth metacarpal on both hands which were seen on clenched fist, loss of multiple teeth, stocky round face, brachydactyly, slight mental retardation and

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history of two times seizure in the past years. The patient's paternal aunt had similar objective finding similar to patient's father but she didn't agree to involve in our assessment. The parents was non-consanguineous and the mother had not the same problems and was normal in physical examination and anthropometric evaluation. The other 3.5 year old boy of this parent had not objective specific finding. In evaluation of 33 year old father, low total calcium level (6.9 mg/dL), high phosphorus (5.5 mg/dL), normal Alkaline phosphatase (277 U/L), very high PTH (650 pg/ml), normal kidney and thyroid function tests were found. The 25-OH-Vit D was in mid-normal range and ultrasound evaluation of kidneys do not demonstrate significant pathology. The brain CT scan showed calcification of basal ganglia and cerebellum nuclei (**Figure 2**).

According to clinical and para-clinical findings, such as hypocalcemia, hyperphosphatemia, elevated PTH, shortening of metacarpus, facial sign and subcutaneous calcification, the father's disease is a classic form of PHP type I (a or c) with AHO features but without obvious other evaluated hormone resistance. Differential diagnosis for subcutaneous ossification in our target child were: dystrophic soft tissue calcification, causing chondro-calcinosis, Fibrodysplasia ossificans progressive, post-traumatic osteoma cutis, metastatic calcification, idiopathic tumoural calcinosis, scleroderma, dermatomyositis, cysticercosis, myositis ossificans and soft tissue sarcoma. These wide range of differential diagnosis from trauma to malignancy were simply narrowed when his father's observed after a simple family history taking! Multiple subcutaneous calcifications in a child when his father shows a classic form of PHP, strike PPHP in the mind because of its genetic nature. As we discuss it in the next section, in a family with history of PHP when the father is the origin of gene, its imprinting led to PPHP in child. When a child has PPHP, and the father shows PHP features, the father's type of PHP is Ia. In the absence of genetic study and evaluation of

urine cAMP after synthetic PTH administration it is difficult to say our diagnosis is definite. According to European PHP network classification for PHP the father case has two major criteria and is a case of "inactivating PTH/PTHrP signaling disorder" (iPPSD) without genetic study.

Pathophysiology

Because of critical function of calcium ion in much vital enzymatic process and electrical activity of neuron, muscle conductivity and contractility and many other well diagnosed functions, calcium adjustment in body fluid and cells tightly regulate by multiple organ interactions [9]. Hypocalcemia evokes parathyroid glands to increase parathyroid hormone (PTH). Parathyroid chief cells can synthesize, process, store PTH and rapidly secrete stored hormone, in addition, these cells can replicate when chronically stimulated [10]. Minutely, PTH controls the ionized calcium level of intra and extra cellular fluid. PTH directly triggers calcium elevating responses on bone and renal cells and indirectly increase intestinal calcium absorption along with bone and kidney calcium fluxes into blood, induced by increased 1, 25 (OH)₂ Vit. D_{3as} another renal response to PTH. The major stimulator of PTH secretion is decreased ionized calcium. Moreover, Phosphate, magnesium, catecholamines, and other stimuli can partly affect PTH secretion [10-12]. Severe hypomagnesemia could reduce secretion of PTH

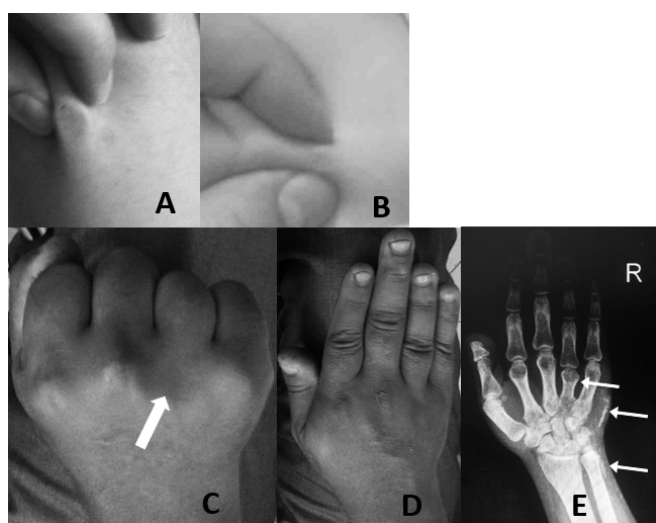


Figure 1 Subcutaneous ossification in child, A and B. Absence of fourth metacarpal head in father, C and D. Right hand radiogram shows short fourth metacarpus and multiple subcutaneous ossifications in father, E.

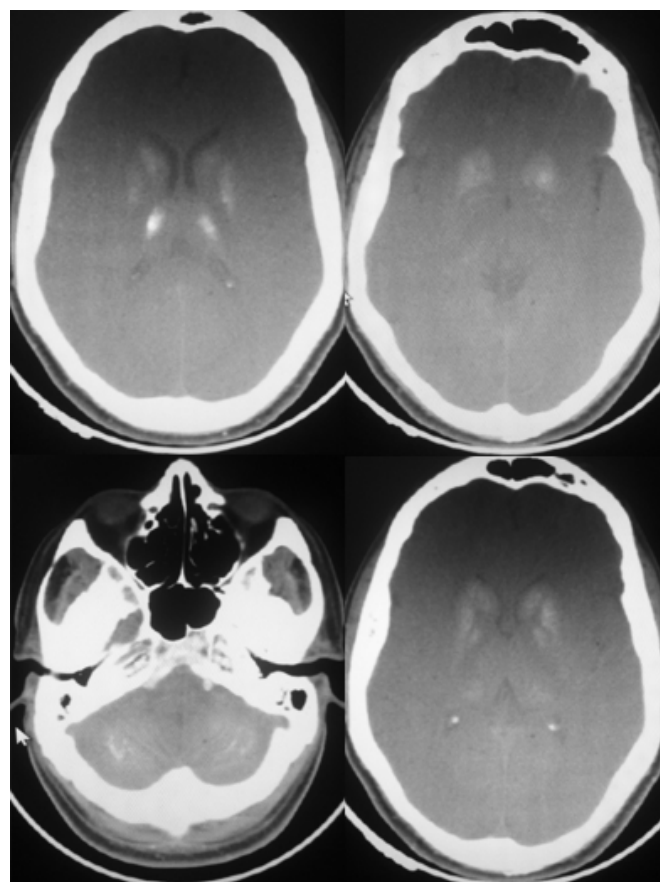


Figure 2 Thickening of the calvaria, bilateral calcification of basal ganglia and cerebellar nuclei was seen in father's brain CT scan.

and increase target organ resistance [13]. Brief main action of PTH on kidneys is stimulation of calcium reabsorption, Inhibition of phosphate transport and stimulates the synthesis of $1,25(\text{OH})_2\text{D}_3$ [14-16] hallmark of PHP is impairment of renal action of PTH. Although, PTH effect in the thick ascending tubule seems unimpaired, resistance to PTH in the proximal tubule occur [17]. Effect of PTH on bone is correlated to hemostatic state of calcium, type of bone and type or duration of PTH administration. Across the board, PTH increase the bone resorption [18,19].

Gene role

The role of genes in forming of parathyroid cells is partly diagnosed, as well as its roles in parathyroid function and target organ response. Combination of PTH and PTH related peptide (PTHrP) activate PTH receptor (PTH1R) that lead to activating the guanine-binding proteins (G protein). Heterotrimeric G proteins are composed of three subunits and are crucial mediators of signal transduction pathways of more than 1000 G protein coupled receptors [20]. The stimulatory GTP-Binding Protein alpha Subunits ($\text{Gs}\alpha$) triggers the activation of the adenylate cyclase (cAMP) leading to specific cellular functions of PTH. Studies have shown significant roles of *hoxa3*, *pax9*, *GATA3*, *gcm2* and *GCMB* genes in producing and function of parathyroid glands [21-25].

An alteration of the gene coding for Gsa (*GNAS*) locus causes PHP Ia and PHP Ib [26]. *GNAS* location is on the long arm of chromosome 20 (20q13.3); the very structurally similar murine homolog is on chromosome 2 [27]. The $\text{Gs}\alpha$ is the most abundant and best characterized gene product that encoded by exons 1–13 [28]. Although, inheriting the heterozygous gene encoding the subunit of heterotrimeric $\text{Gs}\alpha$, from the mother lead to PHP Ia, when the same gene inherited from the father lead to PPHP (Figure 1). The $\text{Gs}\alpha$ is an essential expressed gene in about all cells and has main roles in many of physiological processes (Figure 2). This essential role is because of its presentation in downstream of many different G protein-coupled hormone receptors [5,29]. $\text{Gs}\alpha$ has parent-specific imprinting that discussed later. *GNAS* gene has extensively influence on chondrocytes maturation and its damages could led to defect in growth palate including brachydactyly and Madelung deformity [30]. Recent studies have shown that *GNAS* mutations involved exons 2 through 13 reduces the birth weight of patients while patients had mutations

in exon1/intron 1 have had higher birth parameter, suggesting the role of *GNAS* in fetal development [31]. Although 70% of patients with PHP Ia and in their sibs affected with PPHP have *GNAS* mutations, in the remain cases the molecular evaluations fail to show definite mutation [5].

Recently, in a large series of 40 patients, Mantovani et al. show that more than one half of patients that previously clinically diagnosed as PHP Ia but have not mutations in $\text{Gs}\alpha$ -coding exons, substantially, could be classified as sporadic PHP-Ib after molecular study [32]. Nanclares et al. in 2006 investigate 4 unrelated patients who have mild AHO features, TSH and PTH resistance were thought to have PHP Ia [33] they suggest that some overlap may exist between PHP Ia and PHP Ib in molecular and clinical features. This means, some patients with molecular finding of PHP Ib may have AHO feature, unexpectedly. Mariot et al. report a female case of AHO and PTH resistance without *GNAS* Loss-of-Function mutation [34] this indicates heterogeneity in PHP Ib and these patients may have AHO feature. Unluturk et al. describe a case of clinically diagnosed PHP Ib with favorite genetic mutation but mild AHO feature including short fourth metacarpals, round face and slightly short stature [35] this mutation also was seen in her affected sister without AHO feature and her unaffected mother. In addition, the index case and her sister have increased fractional excretion of uric acid and decreased serum uric acid level that normalized after 3 months of treatment for hypocalcemia.

Classification of PHP

Resistance to PTH in idiopathic and inherited forms called Pseudohypoparathyroidism. Hypocalcemia and hyperphosphatemia is present in classic PHP and phosphate diuresis or elevation in calcium are not happen after administration of exogenous PTH [36,37]. Several forms described for PHP, The two main subtypes are type Ia and Ib, other types are Ic and type 2. Some patients in the family of PHP genetically have same mutation in *GNAS1* and phenotypically have AHO feature but have not resistant to PTH that called PPHP [38]. Usually, PPHP classified as a subtype of PHP because of its genetic correspondence, see (Table 1 and Figure 3). According to Orphanet reports, prevalence of PHP (including types Ia and Ib) and PPHP (including types Ia, Ib and PPHP), has been estimated at

Table 1: Differentiating the multiple subtypes of PHP [6,64].

PHP subtype	Serum calcium	Serum P	Serum PTH	Urine cAMP after synthetic PTH administered	Hormone resistance	AHO features	GNAS defect	Activity of Gsa
PHP Ia	↓	↑	↑	↓	Multiple: PTH, TSH, Gn, GHRH	Yes	Maternal inactivating mutation	↓
PHP Ib	↓	↑	↑	↓	PTH, TSH	No	Imprinting dysregulation	NI
PHP Ic	↓	↑	↑	↓	Multiple: PTH, TSH, Gn	Yes	Few inactivating mutation reported	NI
PHP II	↓	↑	↑	NI		No	-	NI
PPHP	NI	NI	NI	NI	No	Yes/no	Paternal inactivating mutations	↓

Abbreviations: PHP, pseudohypoparathyroidism; PPHP, pseudo-pseudohypoparathyroidism; P, phosphorous; PTH, parathyroid hormone; cAMP, cyclic adenosine monophosphate; AHO, Albright's hereditary osteodystrophy; Gsa , alpha subunit of the stimulatory G-protein; NI, Normal; Gn, Gonadotropins; TSH, Thyroid stimulating hormone; GHRH, Growth-hormone-releasing hormone.

	Gene function in proximal renal tubules (%)		Gene function in other organs (%)	
Normal	100		100	
PPHP	100	Resistance to PTH	50	AHO features
PHP <u>Ia</u>	0		50	
PHP <u>Ib</u>	0		100	

Figure 3 PTH resistance and AHO feature caused by GNAS gene dysfunction in renal tubules and other organs in PHP and PPHP.

1/295,000 in a Japanese study and 1/150,000 in Italy, respectively. Female were twice more affected by PHP than men [39]. In PHP I subtypes the exogenous PTH fails to increase serum calcium level or increase the urinary phosphate and cAMP secretion, due to impairment in PTH receptor coupling [26,38]. But, in PHP type II the same test as above fails to increase serum calcium level or increase the urinary phosphate secretion whereas the cAMP urinary secretion is normal, these means the defect is distal to cAMP generation point [37,40]. In PHP Ia (OMIM #103580) and PHP Ic (OMIM #612462) the feature of AHO including short stature, rounded face, foreshortened metacarpals and metatarsals, obesity [41], stocky habitus, obesity, developmental delay, dental hypoplasia, soft tissue calcification/ossification and subcutaneous calcifications are present, but in PHP type Ib (OMIM #603233) there are not [42-46]. Hypocalcemic patients may have sign and symptoms of decreased ionized calcium such as tetany or seizure [47]. Signs and symptoms that are suggest deficiencies of thyrotropin, the gonadotropins, glucagon, antidiuretic hormone, adrenocorticotropin, and growth hormone-releasing hormone may present in PHP Ia, because these peptide hormones use the alpha subunit of the Gsa protein for production of cAMP [48]. Although, men with PHP Ia may show features of hypogonadism and infertility, these conditions is less obvious in men than women. Multi-hormone resistance and presence of AHO both seen in PHP Ia and PHP Ic, but partial deficiency of Gsα activity, about 50%, is only seen in PHP Ia, see (Figure 3) [49]. Although, Mental retardation has been suggested to be an additional clinical characteristics of AHO since its first reports, but the severity and frequency of this sign are not well accepted [50,51]. Albright et al. 10 years after the first report, describe patients showing the features of AHO without any evidence of PTH resistance were also described previously [52] this syndrome called PPHP and could be found as sporadic or in known kindred of PHP. The PPHP frequently seen in family with history of PHP Ia with inheritance called genetic imprinting that in this case means, when the impaired Gsα gene inherit from father, the child exhibits PPHP, and when inherit from mother, child shows PHP manifestations and have the same percentage of Gsα activity deficiency in cell membranes [7,38,53-55].

Patients suffering from PTH resistance and hypocalcemia/osteomalacia without AHO features are classified in PHP 2 group. Genetic aspect of PHP 2 is not well known until now

[56]. Progressive osseous heteroplasia (POH, OMIM #166350), inherited like PPHP from the father gene. POH characterized by heterotopic ossifications not only in subcutaneous tissue but expanding into deep muscles and connective tissues. They have not hormone resistance but have heterozygous mutation in GNAS [57]. Acrodysostosis is another AHO featured of this family. Some of them had cognitive impairment, severe AHO feature, PTH and TSH resistant and hypertension. Heterozygous mutation was found in some cases in PRKAR1A, PDE4D or PDE3A coding sequences [58-60]. Deletion of the 2q37.3 chromosomal region including HDAC4 could led to 2q37.3 deletion syndrome presenting with AHO feature and cognitive impairment/psychosis without PTH resistance [61]. The Blomstrand chondrodysplasia (OMIM #215045) is a lethal form of dwarfism, associated with biallelic inactivating mutation of the PTH1R gene [62]. A milder form of biallelic inactivating mutation of the PTH1R gene is Eiken epiphyseal dysplasia, presented with short stature and elevated PTH in some cases [63]. Recently, European PHP network proposed a novel classification for PHP [8] they proposed the term “inactivating PTH/PTHrP signaling disorder” (iPPSD) instead of pseudohypoparathyroidism. The iPPSD classification recommended the following categories: iPPSD: clinical/biochemical diagnosis based on the major/minor criteria (Table 2), in the absence of genetic investigation; this category is recommended for individuals with a pure clinical suspicion of iPPSD and lack of complete (epi) genetic testing.

iPPSD1: loss-of-function mutation in PTH1R.

iPPSD2: loss-of-function mutation in GNAS Gsa sequence.

iPPSD3: methylation change(s) at one or more GNAS DMRs, associated with or without a genetic (deletion) or cytogenetic (UPD) defect.

iPPSD4: PRKAR1A mutation.

iPPSD5: PDE4D mutation.

iPPSD6: PDE3A mutation.

iPPSDx: lack of genetic/epigenetic defect identified following molecular investigation of known genes described above.

iPPSDn+1: the identification of a novel gene/molecular defect will lead to a disease named iPPSD7, then 8 and so on.

Table 2: Definition of major and minor criteria for iPPSD and differential diagnoses [8].

		Assessment	Differential diagnosis	
Major criteria	1. PTH resistance	Ionized calcium, total calcium	Normocalcemic	
		Phosphate	hyperparathyroidism	
		Magnesium	Renal failure	
		PTH	Vitamin D deficiency or	
		Vitamin D (25OHD)	any kind of secondary	
		Creatinine	hyperparathyroidism	
		Urinary calcium		
		Urinary phosphate		
		PTH infusion test in challenging cases		
		2. Ectopic ossification	Detailed physical exam	Fibrodysplasia ossificans progressiva (FOP, OMIM #135100), post-traumatic osteoma cutis
			X-rays	
3. Brachydactyly type E (comprises the IV)	Clinical inspection (fist), hand and feet X-rays		Turner syndrome, tricho-rhino-phalangeal syndrome (TRPS), TRPS I, (OMIM #190350), TRPS-II (OMIM #150230) and TRPS-III, (OMIM #190351)	
	Minor criteria	1. TSH resistance	TSH, T4I, antibodies, imaging†	Mutations in the TSH receptor
		2. Other hormonal resistances	IGF-1 (GH stimulation test if necessary), calcitonin, LH, FSH, GnRH test	
		3. Motor and cognitive retardation or impairment	Computed tomography scan and/or MRI of the brain, psychopathological rating scales adjusted for age	
		4. Intrauterine and postnatal growth retardation	IUGR: gestational age, birth weight, birth length, head circumference, comparison to reference charts; post-natal growth: growth charts, X-ray of the left hand for determination of the bone age	
	5. Obesity/overweight	Weight SDS, BMI percentile, BMI z-score		
	6. Flat nasal bridge and/or maxillary hypoplasia and/or round face	Clinical inspection		
		iPPSD clinical diagnosis	(a) Presence of one major criteria, either number 1 or 2;	
			(b) Presence of major criteria number 3 and at least 2 minor criteria‡	

		Assessment	Differential diagnosis
†US in adults with hypothyroidism and no evidence for autoimmunity; thyroid imaging through thyroid scintigraphy and US in neonates diagnosed through screening for congenital hypothyroidism;			
‡Minor criteria are nonspecific (obesity/cognitive impairment); for instance, the association of BDE + obesity or BDE + cognitive impairment would not be relevant for our classification. By raising the number of minor criteria from 1 to 2, we will reduce the risk of overdiagnosing patients with iPPSD.			
From: Thiele S, et al. Pseudohypoparathyroidism to inactivating PTH/PTHrP signalling disorder (iPPSD), a novel classification proposed by the EuroPHP network. <i>European Journal of Endocrinology</i> . 2016;175(6):P1-P17.			

They believe that this new nomenclature will facilitate approach to the diagnosis of iPPSD and it would allow for the classification of patients in a more homogenous way and lead to better future observational and research studies in the field.

Conclusion

Although clinical presentation of PHP and PPHP was known and multiple organ involvement was explained in detail, the genetic and molecular pattern of this condition was not clearly distinct

and investigations are in progress. Despite the fact that according to clinical feature and simple laboratory tests, our index case has PPHP and his father and fathers' sister are cases of PHP Ia, but in lack of genetic study the autosomal dominant PHP Ib could not be rollout in patients' aunt [35].

Typical AHO feature, hypocalcemia, hyperphosphatemia and high PTH of father in addition to PPHP phenotype in his child, that is compatible with paternal imprinting, affirm the diagnosis of PHP Ia for father.

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