GNAS-Related Loss-of-Function Disorders and the Role of Imprinting

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Introduction

The story of GNAS (guanine nucleotide-binding protein, α stimulating; MIM No. 610540) and parathormone (PTH) resistance began in 1942 when Fuller Albright [1] first described the absence of calcemic or phosphaturic response to parathyroid extracts in patients with ‘hypoparathyroidism’ and osteodystrophy. In the late 60s, immunometric PTH assays confirmed the association of clinically evident hypoparathyroidism and elevated circulating levels of PTH in patients with similar phenotypes [2]. Functional, then genetic, defects in Gsa, the α-stimulatory subunit of the G protein, have been identified in 1990 as the cause of the historical syndrome of Albright hereditary osteodystrophy (AHO) and PTH resistance, also termed ‘pseudohypoparathyroidism’ and osteodystrophy. In the late 60s, immunometric PTH assays confirmed the association of clinically evident hypoparathyroidism and elevated circulating levels of PTH in patients with similar phenotypes [2]. Functional, then genetic, defects in Gsa, the α-stimulatory subunit of the G protein, have been identified in 1990 as the cause of the historical syndrome of Albright hereditary osteodystrophy (AHO) and PTH resistance, also termed ‘pseudohypoparathyroidism’ and osteodystrophy. In 2000, the discovery of epigenetic changes at the GNAS locus controlling Gsa expression solved the mystery of isolated PTH resistance described as PHP type 1b (PHP1b) [4].

All the PHP type 1 subtypes (absence of cAMP and phosphaturic response to exogenous PTH infusion), although they overlap, have now been linked to a genetic or epigenetic defect in GNAS, whereas the cause of the PHP type 2 is still a matter of debate. Initially, PHP type 2 was
defined as an adequate rise in nephrogenic cAMP, albeit without phosphaturic response to exogenous PTH infusion, in the absence of AHO, and attributed to a defect in cAMP effectors [5]. This definition is challenged today by our and other groups’ recent discovery that mutations in the regulatory subunit of the protein kinase A (gene PRKAR1A) cause acrodysostosis, an extreme form of AHO with urinary cAMP response to exogenous PTH in the presence of hormone resistance, including PTH and thyroid-stimulating hormone (TSH) resistance [6–8]. Altogether, the former definition of PHP appears obsolete and should be progressively replaced by a classification of GNAS-related disorders with references to the mechanism of the diseases.

**GNAS Imprinting**

Gsa is one α-subunit of the heterotrimeric G proteins required for the signaling of the seven transmembrane domain receptors. Gsa is encoded by the imprinted GNAS locus. Imprinting refers to mechanisms that lead to the repression of gene expression from one parental allele. Genes subjected to parental imprinting contain imprinting control regions in differentially methylated regions (DMRs). In most loci, the parent-specific expressed transcripts are associated with a pattern of non- or low-methylated DNA, whereas the non-expressed transcripts are associated with a pattern of methylated DNA [9].
The imprinted human GNAS locus produces several transcripts comprising Gsa: A/B (also named 1A), extra-large (XL), the antisense transcript (AS) and the transcript coding neuroendocrine secretory protein 55 (NESP) [10]. Due to differential methylation of their promoters, most transcripts of this locus originate from one parental allele only. XL, A/B and AS are transcribed from the paternal allele; NESP is transcribed from the maternal allele only [11]. The promoter of Gsa is not differentially methylated, and therefore, Gsa expression arises from both alleles in most tissues (fig. 1). However, Gsa expression is restricted to the maternal allele in several tissues including the renal proximal tubule, the thyroid, the pituitary and the gonads [12–14]. In humans, two imprinting control regions of GNAS have been identified within or close to the GNAS locus. One is located within the STX16 gene and controls the establishment of imprinting at the A/B DMR only [15]; the other, encompassing AS exons 3 and 4, controls the establishment of imprinting over the entire GNAS locus [16–18].

**Disorders Related to GNAS Genetic and Epigenetic Defects**

**PHP1a (OMIM No. 103580)**

PHP1a is a rare autosomal dominant disease due to a defect in the expression or function of Gsa (table 1). Clinical features depend on the mono- or biallelic transcription of Gsa in tissues. In addition to resistance to hormones that signal through G-protein-coupled receptors (GPCRs), patients affected with PHP1a present with a collection of features including AHO and obesity. In our experience, PHP1a is diagnosed early (at about 6.5 years) during the investigation of a symptomatic hy-
pocalcemia, heterotopic ossifications, growth retardation, familial screening, hypothyroidism or developmental delay.

**Resistance to Hormones Binding to GPCRs**

Resistance to PTH manifests as the association of low serum calcium, elevated serum phosphate and elevated circulating PTH in the absence of 25-OH-vitamin D deficiency and renal insufficiency (fig. 2). Absent at birth, PTH resistance gradually develops during the first months or years of life [19]. Phosphate and PTH levels increase first, followed by a decrease in calcemia. In the renal proximal tubule, the defect in PTH signaling likely tapers the 1α-hydroxylation of 25-OH-vitamin D, hence the 1,25-(OH)₂-vitamin D production, and increases the tubular phosphate reabsorption. In the distal tubule, calcium reabsorption also depends on the PTH-driven cAMP production. However, most likely due to the biallelic expression of Gsa in the distal tubule, urinary calcium reabsorption is close to normal in patients with PHP1a (personal data) and may be involved in the long-term tolerance of PTH resistance. The disease is often revealed during events requiring increasing amounts of calcium such as vitamin D deficiency or pubertal growth spurt.

Resistance to TSH is found in most, if not all, patients and contributes to the diagnosis of PHP1a. It is characterized by an elevated TSH – 19.7 ± 3.3 mIU/l (mean ± SE) in our series and 4.9 mIU/l in the study of Balavoine et al. [20] – a low-normal free T4 with no goiter. Usually present at birth, TSH resistance may be revealed through neonatal screening programs. Patients affected with PHP1a also display resistance to TRH and calcitonin without symptoms [20, 21]. Cryptorchidism, often bilateral, is frequent in boys. A defect in INSL3 signaling, the hormone responsible for testicular descent, could be incriminated as its receptor couples to Gsa [22]. Girls present with delayed menarche (mean 14.0 ± 1.9 years, approximately 1.2 years later than counterparts) [23] and elevated follicle-stimulating hormone levels (150–200% of the upper normal range), yet normal luteinizing hormone levels [24, 25].

**Albright Hereditary Osteodystrophy**

Most patients affected with PHP1a present with bone dysplasia including brachymetacarpy (mainly 4th and 5th metacarpals), brachydactyly (fig. 3) and/or brachymetatarsy (present in all patients with variable degrees), narrowed lumbar shaft and undersized femoral necks. Absent at birth, bone dysplasia develops over time, especially
during puberty, and most likely results from the deficient PTH-related peptide signaling during endochondral bone formation. In fact, loss of function mutations of PTHLH (the gene encoding PTH-related peptide) are associated with similar bone shape abnormalities [26]. The bone density of the patients is roughly normal, yet patients seem prone to rheumatologic complications such as slipped femoral epiphysis or osteoarthritis [27, 28].

Adult short stature is common in patients: −2.5 ± 0.3 and −3.0 ± 0.9 (mean ± SD) height z-score in Long et al. [29] and our series, respectively. Most children with PHP1a have normal stature until they undergo rapid and premature closure of the epiphyses between 10 and 15 years of age (fig. 3). Short stature results from both deficient endochondral bone formation and growth hormone-releasing hormone resistance found in about 70% of the patients [30].

Heterotopic ossifications (osteoma cutis) are a specific feature of Gsa haploinsufficiency. Usually superficial (in the derma or subcutaneous fat), made of mature bone with central bone marrow elements, they may progress unforeseeably superficially or within deeper tissues [31].

Developmental delay and cognitive dysfunction have been reported repeatedly in textbooks, including the study by Fuller Albright [1] in 1942. About 70% of the patients affected with PHP1a exhibit a moderate to severe cognitive impairment (meaning that 30% do not have any impairment) [32].
Obesity

Obesity is a major feature of the disease, although patients are unevenly affected (mean body mass index 1.8 ± 0.3) [29]. Three contributing factors have been identified: resistance to epinephrine (a lipolysis-stimulating hormone acting through Gsa) [33], the potent anti-adipogenesis effect of Gsa established in vitro [34], and the loss of stimulation of energy expenditure by central melanocortins [35]. An additional layer of complexity arose through the discovery of the opposite effects of the GNAS transcripts on glucose metabolism and obesity [36].

Diagnostic Struggle

Obesity, AHO and mental retardation have been described in patients with 2q37 deletions and/or HDAC4 haploinsufficiency [37]. Brachydactyly and hormonal resistance are seen in patients with acrodysostosis and PRKAR1A mutations [6]. Hyperphosphatemia, AHO and TSH resistance allow the differential diagnosis with hyperparathyroidism due to vitamin D deficiency. To document the absence of urinary cAMP production and the increase in urinary phosphate excretion and to distinguish PHP type 1 from type 2, examination of the renal response to the infusion of exogenous PTH (former Ellsworth-Howard test replaced by the infusion of recombinant PTH 1–34) [6, 38] may be required. The erythrocyte bioassay to assess Gsa activity contributes to demonstrate the impact of novel mutations or to establish phenotype-genotype correlations [39].

Molecular Diagnosis of PHP1a

The diagnosis of PHP1a relies on the identification of a heterozygous loss-of-function mutation of the maternal coding sequence of GNAS (exons 1–13). All types of mutations can be found, such as deletions, insertions, amino acid substitutions or stop codons. Three hot spots are located in exons 6, 7 and 13; mutations may also lie in the alternatively spliced exon 3 [40]. The maternal origin of the mutated allele may be identified through parental transmission or haplotype studies [25]. Methylation changes at regulatory regions of GNAS have been found in a few patients with PTH resistance and mild features of AHO, exposing the overlap between the disease variants [41–43].

PHP Type 1c

Factually, PHP type 1c (PHP1c) describes patients with PTH resistance (fig. 2), AHO and normal Gsa in vitro activity. Thiele and colleagues [25, 39] have identified mutations in the C-terminal region of Gsa (p.R391X, p.E392K, p.E392X, p.L388R) in a subset of patients affected with PHP1c. These mutations prevent the coupling of Gsa to receptors, yet preserve cAMP generation through the activation of the adenylyl cyclase. Most in vitro assays of Gsa activity solely examine the ability of Gsa to activate the adenylyl cyclase, which explains the results found in PHP1c.

Pseudo PHP or Isolated AHO

Patients affected with pseudo PHP (pPHP) have first been identified within relatives of patients affected with PHP1a. They present with AHO, no hormone resistances and harbor the exact same mutation of Gsa than their siblings albeit on the paternal allele. Recent observations have shown that patients affected with pPHP are not obese [29] and do not have cognitive impairment [32]. Nonetheless, they are short and may have heterotopic ossifications, sometimes more severe than their PHP1a counterparts [44].

Progressive Osseous Heteroplasia (OMIM No. 166350)

Progressive osseous heteroplasia (POH) is a rare disorder of osteogenesis, developing during infancy, characterized by heterotopic bone in the derma and subcutaneous fat. Bone plaques eventually fuse and progress deeper into fascia, skeletal muscles, tendons and ligaments, leading to ankylosis and preventing natural limb growth. Inflammation may trigger or worsen the expansion of heterotopic bone. Kaplan and colleagues [45] noted features of AHO and subsequently identified GNAS as the disease-causing gene. Mutations in the coding sequence of Gsa found in patients with POH are also found in patients with PHP1a or pPHP, although they are exclusively located on the paternal allele, and principally, severely affect protein function. POH has been associated with intrauterine growth retardation, although some of the reported patients may be considered as pPHP rather than POH [44]. Altogether, we and others propose that pPHP and POH are both extremes of a common disease.

PHP1b (OMIM No. 603233)

PHP1b is a rare disease due to defective Gsa signaling in selected tissues including the renal proximal tubule and the thyroid. Because of abnormal methylation at the
maternal A/B promoter of GNAS, Gsa expression is limited in those tissues resulting in hormonal resistance. In our experience, the mean age at diagnosis is 13, mostly due to symptoms of hypocalcemia; patients diagnosed after the age of 20 are not rare.

Resistance to Hormones Binding to GPCRs

PTH resistance is the main symptom of the disease (for a long time considered as the only one; fig. 2a, c) [10, 46]. As in PHP1a, PTH resistance develops over time [19, 47]. In contrast to PHP1a, in which haploinsufficiency affects all tissues, patients affected with PHP1b maintain a biallelic expression of Gsa in most tissues, in particular in bone. Consequently, their bones respond adequately to elevated PTH levels with an increased bone resorption and demineralization that may resemble rickets or Madelung deformity in children (fig. 4) [48] or severe primary hyperparathyroidism [49, 50]. TSH resistance is constant and usually mild (4.6 ± 1.0 mIU/l); in our series, TSH resistance ranged from 2.5 to 10.0 mIU/l [46]. In the report of Liu et al. [12], TSH ranged between 2.4 and 9.5 mIU/l, and in the report of Levine and colleagues [51], the TSH level was 4.5 mIU/l (reference range 0.5–4.5). Free T4 levels are within the normal range (fig. 2b). As in PHP1a, we found elevated calcitonin levels in 8 out of 10 patients investigated. Mantovani et al. [52] failed to identify either gonadotropin or growth hormone-releasing hormone resistance in those patients.

AHO and Obesity

Several features of AHO may be present, rarely all of them in a single patient [41–43]. Some patients present with a typical brachymetacarpal or slender heterotopic ossifications. In our series of patients, the mean body mass index, especially in girls, was significantly higher than that of the general population (1.0 ± 0.4; p = 0.019), and the final height was normal.

Molecular Diagnosis of PHP1b

Patients affected with PHP1b share a loss of methylation (LOM) at the maternal A/B DMR of GNAS [4]. In addition, some patients present with methylation chang-

![Fig. 4. Bone features of PHP1b. a, b Bone lesions resembling rickets with irregular and widened metaphysis in a 12-year-old boy with PHP1b and the recurrent 3-kb deletion in STX16. X-rays were performed at diagnosis because of bone pain. Transparent metaphyseal stripes (c) and increased bone matrix transparency (d, e).](image-url)
es at the GNAS locus, unequally affecting XL, AS and NESP DMRs [4, 15, 43, 53]. Several subtypes of PHP1b are recognized. About 15–20% of patients display a LOM restricted to the A/B DMR of GNAS, associated, in all reported cases except one, with a recurrent maternal 3-kb microdeletion within the STX16 gene [15, 43, 46]. In one family, the maternal deletion spanned 4.2 kb within the STX16 gene, and overlapped the latter by 1.2 kb [47]. These patients, often clustered in families, are diagnosed with autosomal dominant PHP1b. A very small number of families with autosomal dominant PHP1b show broad loss of imprinting at the GNAS locus and a deletion removing AS exons 3 and 4 [16, 18], or a deletion removing NESP and AS intron 4 [17]. In few sporadic cases, paternal uniparental disomy involving segments or whole chromosome 20 encompassing the GNAS locus is the cause of the absent maternal Gsa expression, hence PHP1b [54–56].

Most patients with PHP1b (80–85%) are sporadic and show broad loss of imprinting at the GNAS locus, including LOM at the A/B DMR and loss of imprinting affecting at least one additional DMR (gain of methylation at NESP, LOM at AS and/or XL) without microdeletions within the STX16 or AS genes or evidence for paternal uniparental disomy [43, 53]. They are described as having sporadic PHP1b.

**Diagnostic Struggle**

There is no major difference between the phenotypes of patients affected with familial or sporadic forms of PHP1b [46]. Therefore, diagnosis of PHP1b relies on (1) identification of LOM at the A/B DMR of GNAS, (2) characterization of the methylation pattern of the entire GNAS locus, and (3) the search for deletions within STX16 or GNAS and paternal uniparental disomy.

**Disease Inheritance**

The reader has already appraised the complexity, diversity and uncertainty of molecular processes affecting the GNAS-imprinted locus, hence triggering disease variants. Parental allelism, genomic localization, genomic or epigenomic lesions arbitrate disease transmission and the phenotype for upcoming babies. Schematically, PHP1a and PHP1b occur through maternal transmission, whereas pPHP and POH are paternally transmitted. Uniparental disomy should be erased through germinal transmission.

**Management of Patients with PHP1a and PHP1b**

**Hormones**

The objective of PTH resistance treatment is defined as follows: (1) maintain calcemia within the low-normal range (2.0–2.5 mM), (2) prevent hypercalcuria (in children, urinary calcium excretion <6 mg/kg/day or a ratio of urinary calcium/urinary creatinin <0.3 mM/mM), and (3) prevent bone resorption due to elevated PTH. For children, the key treatment is 1α-hydroxylated vitamin D (calcitriol or alfacalcidol), adjusted on growth velocity rather than weight (highest doses during periods of high-velocity growth like infancy or puberty). Unlike patients with hypoparathyroidism, treatment with alfacalcidol or calcitriol rarely leads to hypercalcuria. There is no specific recommendation for 25-OH-vitamin D therapy; however, both the residual activity of the Gsa protein for some patients and the observation that 25-OH-vitamin D facilitates calcium absorption in hypocalcemic patients [57] suggest that the 25-OH-vitamin D level within normal range may help disease management. Calcium supplements (250–1,000 mg according to age) are recommended during the year following the diagnosis of PTH resistance. Adults usually manage to maintain their calcemia and PTH with 25-OH-vitamin D and calcium supplements. Blood calcium, creatinine and PTH, urinary calcium (urine spot in young children, 24-hour urines after age 5), and renal ultrasound should be monitored at regular intervals to adjust treatment.

TSH resistance is usually treated in patients with PHP1a by oral thyroxin according to weight to reach normal free T4 level. Except during pregnancy, patients with PHP1b do not require treatment for their TSH resistance.

Off-label growth hormone has been used in patients with PHP1a and short stature with variable results. Unfortunately, clinical trials are lacking to prove the efficacy of the drug [58].

**Bone and Obesity**

The treatment of heterotopic ossifications is one of the most important challenges of this disease. Small and non-problematic ossifications should remain untouched, as they often recur after surgery. Use of non-steroidal anti-inflammatory drugs, thiosulfate or bisphosphonates has been reported; by analogy with ossifications following hip replacement, non-steroidal anti-inflammatory drugs should be considered in case of painful recurrence or surgery [59].

Dietary and lifestyle measures to prevent obesity and supportive care for cognitive functions are recommended.
as soon as the diagnosis of PHP1a is established. Cannabinoid receptor type 1 antagonists have been used occasionally for obesity treatment, but are now off the market [60].

**Prenatal Diagnosis, Pregnanacies and Births**

For patients affected with Gsa haploinsufficiency, pre-implantation diagnosis is feasible [61], albeit indebted to the severity of the gonadotropin resistance and to countries' ethical laws. At birth, mutations or epigenetic anomalies may be screened on cord blood [47].

During pregnancies, physicians should pay special attention to maintain TSH levels <2.5 IU/l, according to international guidelines [62], and correct hypocalcemia by all means. In case of uncontrolled maternal hypocalcemia, or vitamin D deficiency, newborns are at high risk of neonatal hyperparathyroidism and hypercalcemia. TSH resistance may be present at birth. Therefore, treatment with thyroxin should be started in newborns with elevated TSH (approximately 10 μg/kg/day) even before the result of the molecular biology analysis [63].

**References**


