

From pseudohypoparathyroidism to Albright's hereditary osteodystrophy: A spectrum of clinical phenotypes due to inactivating mutations in the *GNAS* locus

Giedre Grigelioniene^{1,2}

¹Astrid Lindgrens Children Hospital and Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden

Physiology of the parathyroid hormone (PTH)

PTH is secreted from parathyroid glands and its main function is to maintain calcium and phosphate homeostasis. The target organs of PTH are kidney and bone, where action of the hormone is mediated through the type I PTH/parathyroid hormone-related peptide receptor (PTHrP1R). This receptor primarily signals through stimulatory heterotrimeric G protein. The activation of the receptor by PTH results in the release of the α subunit (G_{α}) from the heterotrimer, leading to stimulation of adenylyl cyclase and generation of the cAMP [1]. cAMP is a second messenger in the cell and acts mainly by stimulation of protein kinase A. Secretion of PTH is tightly regulated and increases in response to low serum calcium.

PTH has several action mechanisms: 1) PTH stimulates synthesis of 25-hydroxyvitamin D 1- α hydroxylase in renal proximal tubule, leading to an increased serum levels of 1,25 dihydroxyvitamin D₃, the active vitamin D metabolite, which

enhances absorption of calcium and phosphate in the intestine; 2) In the distal renal tubule PTH enhances reabsorption of calcium from the glomerular filtrate; 3) PTH increases the urinary excretion of phosphate by inhibition of phosphate re-uptake from the glomerular filtrate; 4) PTH stimulates bone turnover and mobilizes calcium and phosphate from the bone matrix.

PTH signals through a G-protein coupled receptor

G-protein coupled receptors are widely used for transmembrane signal transduction of many hormones, neurotransmitters and other substances, including follicle-stimulating hormone, thyroidea-stimulating hormone, parathyroidea hormone related protein, pheromones, calcitonin etc. This receptor superfamily shares the same structure: seven membrane spanning helices connected by intra- and extracellular loops [2]. Upon the ligand binding, the signal is transmitted to the G-protein which consists of three subunits, α , β and γ . When the ligand activates the receptor, it induces exchange of guanidine diphosphate (GDP), which is bound to the α subunit of the heterotrimeric protein, for guanidine triphosphate (GTP). This

²: Correspondence to:
Giedre Grigelioniene, MD, PhD
Clinical Genetics, L5:02
Karolinska University Hospital Solna
17176 Stockholm
Sweden
Tel: +46 5177 0455
Fax: +46 8 327734
E-mail: Giedre.Grigelioniene@ki.se

leads to the dissociation of the α subunit from the β and γ dimer. The activated α subunit then in its turn stimulates adenyl cyclase, which leads to the cAMP production. There is a great diversity in each type of G-protein subunits. For example, there are at least 16 different α subunits known in mammals, some of them expressed in most of the cells (as $G_{s\alpha}$), whereas others are cell-type specific e.g. $G_{i1}\alpha$ (transducin, which is specific for rod cells in retina).

Studies of homozygous $G_{s\alpha}$ knockout mice indicate that $G_{s\alpha}$ signalling is essential for survival [3]. Interestingly, a child with a severely reduced $G_{s\alpha}$ activity has been recently reported, suffering from morbid obesity, severe PTH resistance and prothrombotic state [4]. In this case, the $G_{s\alpha}$ activity in platelets was only 10–20% [4], suggesting that at least some remaining activity of $G_{s\alpha}$ is necessary for human survival.

Inactivating mutations in $G_{s\alpha}$ encoding locus in humans cause a broad spectrum of phenotypes. The variability of these clinical features might be explained by dominant nature of the mutations and imprinting effects in the *GNAS* locus.

***GNAS* locus on chromosome 20q13 codes for several gene products and has a complex regulation**

Several alternative transcription start sites in the locus give rise to multiple gene products, which also have distinct imprinting patterns in different tissues and have different functions. Many transcripts from the *GNAS* locus have been described [5], but in this review only $G_{s\alpha}$, $XL\alpha_s$, a large variant of $G_{s\alpha}$, transcript A/B and NESP55 will be mentioned. $XL\alpha_s$ and $G_{s\alpha}$ are identical over the entire C terminal portion encoded by exons 2–13, but they have distinct N termini (Figure 1). Studies on animal models have demonstrated that $XL\alpha_s$ promotes growth, lipid accumulation, decreases metabolic rate and elevates serum glucose, whereas normal function of $G_{s\alpha}$ is to decrease fat mass, lipid accumulation, elevate metabolic rate and decrease serum glucose and insulin levels [6–9]. $G_{s\alpha}$ is expressed in most tissues, whereas $XL\alpha_s$ is found in neuroendocrine tissues and is expressed from the paternal allele [10, 11].

NESP55 is also expressed in the central nervous system and is chromogranin-like secretory protein, expressed in neuroendocrine cells [12]. $G_{s\alpha}$ is involved in signalling of many hormones, including PTH, thyroidea stimulating hormone and gonadotropins, as well as autocrine/paracrine factors [1], thus mutations in this locus may result in combined hormone resistance with broad phenotype variability.

Investigation of adult human tissues has shown that $G_{s\alpha}$ is predominantly expressed from maternal allele in thyroidea, ovaries, proximal renal tubule and pituitary gland [3, 13, 14], whereas biallelic expression has been shown in bone, adipose tissue and adrenals [15]. It is not completely clear when the imprinting of different parental alleles takes place in humans. In rodents, the maternally methylated regions are established at gametogenesis, whereas the paternal methylation takes place after fertilisation [16]. The clinical observation that renal PTH resistance develops after infancy [17, 18] suggests that either tissue-specific inactivation of the paternal allele in renal proximal tubule takes place during the first years of life, or the expression of $XL\alpha_s$ from the paternal allele initially compensates for the deficiency of $G_{s\alpha}$.

Pseudohypoparathyroidism (PHP)

PHP is a heterogeneous group of disorders occurring due to insensitivity of target organs to PTH. This end-organ resistance mainly affects the renal proximal tubule, as the actions of PTH in bone and distal tubule appear to be unimpaired [19, 20]. Clinically, PTH resistance results in hypocalcemia, hyperphosphatemia and increased levels of serum PTH. In addition, some patients with PHP show skeletal dysplasia phenotype called Albright's hereditary osteodystrophy, which includes short stature, round faces, obesity, ectopic ossification, brachydactyly, short metacarpal and/or metatarsal bones [21, 22]. PHP can be divided into two distinct types: patients with type I fail to excrete both phosphate and cAMP in the urine in response to administration of biologically active PTH, while patients with type II show failure of only phosphate excretion in response to the same stimuli. PTH type II will not be discussed

in this review as the genetic defect is not known and only few cases have been described in the literature.

PHP type I is caused by heterozygous mutations in the *GNAS* locus.

PHP is characterized by a broad phenotype variability, which depends not only on the dominant

nature of the disease causing mutation, but also on complex imprinting mechanisms (i.e., parental origin of the mutation). The *GNAS* locus codes for several gene products which are differentially expressed from paternal or maternal allele in different tissues (Figure 1). The alpha subunit of the G-protein coupled receptor, $G\alpha$, is biallelically expressed in most tissues, except renal proximal tubule, thyroid and hypophysis. In those tissues $G\alpha$ is mainly transcribed from the maternal allele [3, 13, 14]. The tissue-specific expression of

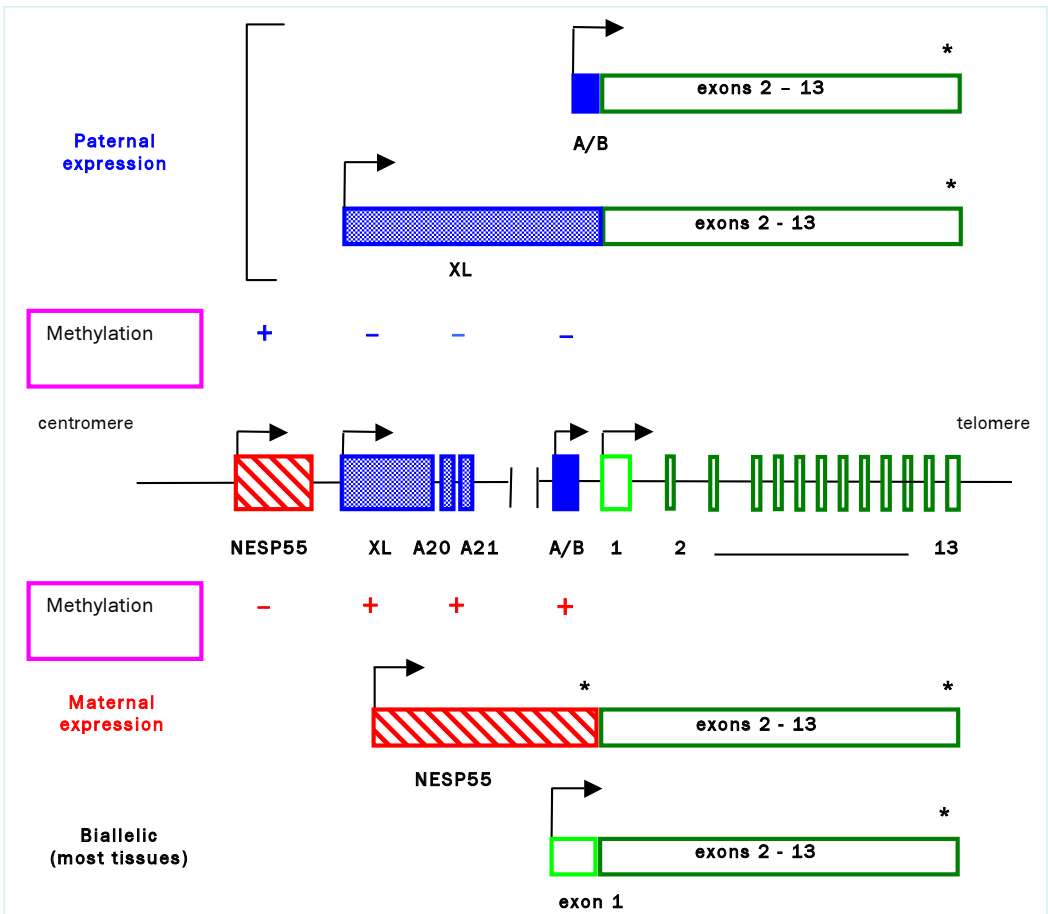


Figure 1

Simplified picture of different transcripts from the *GNAS* locus and imprinting pattern. Several transcripts are known in this locus, but only 4 alternative transcription start sites and products are shown. These include $G\alpha$, $XL\alpha$ s, a large variant of $G\alpha$, transcript A/B and NESP55. $XL\alpha$ s and $G\alpha$ have different N termini, but are identical at the C-terminal part encoded by exons 2–13. $G\alpha$ expression is biallelic in most tissues, with some exceptions including renal proximal tubuli, thyroid, pituitary and gonads. Different promoters of the *GNAS* locus are differentially methylated (shown by +) and unmethylated (indicated by -), in the paternal and maternal alleles. Boxes indicate exons, lines — introns. Associated Professor Murat Bastepe is thanked for advice during the preparation of this figure.

paternal versus maternal alleles explains the clinical variability of the phenotypes in PHP type I.

Different phenotypes in PHP depend on the parental origin of mutation.

Type I PHP can be divided into four different forms: 1) type Ia (PHPIa), 2) type Ib (PHPIb), 3) pseudopseudohypoparathyroidism (PPHP), and 4) progressive osseous heteroplasia (POH). Another form of PHP, type Ic, has also been described, but it remains unclear, whether this is a distinct entity or it is a subvariant of PHPIa.

PTHla is characterized by phenotype of Albright's hereditary osteodystrophy, resistance to different hormones signalling through G_{α} -coupled pathways, and/or varying degree of mental retardation and obesity. The hormone resistance includes PTH insensitivity in the proximal renal tubule, leading to hypocalcemia and hyperphosphatemia and increased levels of PTH. Moreover, many patients with PHPIa have also partial insensitivity to thyroidea stimulating hormone, gonadotropines and growth hormone releasing hormone (reviewed in [23-25]). Some individuals with PHPIa suffer from calcificates in the skin or deeper tissues [26]. The phenotype of Albright's hereditary osteodystrophy includes disproportionate short stature with shortening of the limbs, short digits, metacarpals IV-V and metatarsals (Figure 2).

PHPIa is caused by heterozygous inactivating mutations in exons 1–13 of the G_{α} gene, inherited maternally. The phenotype develops because of the decreased expression of the G_{α} in bone, and an absence of the G_{α} in the proximal renal tubule. The mutations in G_{α} causing PHPIa are of different types, from constitutional deletions of 20q to nonsense and missense mutations in the coding region, and are summarized in OMIM entry +139320. The same mutations inherited paternally do not cause renal insensitivity to PTH, and result either in Albright's hereditary osteodystrophy and/or in calcifications of the skin or deeper tissues, called *osteoma cutis* and progressive osseous heteroplasia respectively.

PHPIb is defined by PTH insensitivity without Albright's hereditary osteodystrophy. As in PHPIa,

the severity of PTH resistance in PHPIb varies among the patients [28]. In PHPIb, the hormone resistance is not only limited to PTH, a mild resistance to thyroidea stimulating hormone has also been described [29, 30]. Individuals with PHPIb do not show Albright's hereditary osteodystrophy phenotype, but in some cases might have ossifications in the skin or connective tissues [26].

Patients with PHPIb have maternally inherited mutations in the *GNAS* locus; however, those mutations are not in the coding exons of the locus, but in so-called differentially methylated region, DMR. Those mutations are methylation



Figure 2
Brachydactyly and short metacarpals III–V in PHPIa. Clinical picture (A) and X-ray (B) of the hands of an adult woman with PTHla. Published with permission.

defects, consistently including loss of imprinting in maternal exon A/B [31]. Previous studies have indicated that exon A/B DMR controls the expression of *Gsα* [32]. Paternal mode of expression of A/B transcripts from both alleles (demethylation of A/B DMR) would lead to decreased *Gsα* expression in the tissues where only maternal *Gsα* is transcribed. Consequently, the imprinting defect of A/B DMR results in decreased maternal *Gsα* expression in renal proximal tubule and thyroid, and thus hormone resistance in these tissues.

PHPIb has been described in both familial and sporadic forms. Most of the sporadic forms of PHPIa show loss of imprinting in exon A/B, whereas familial cases are caused by deletions located upstream of the *GNAS* locus and always involving the *STX16* gene. Deletions at two distinct sites have been described: microdeletions at the *STX16* locus, which is ~220 kb centromeric of *GNAS*, and larger deletions including NESP55 DMR [33, 34]. Both types of mutations lead to an abnormal methylation of exon A/B, suggesting an existence of cis-acting imprinting control elements of the *GNAS* locus [35]. Furthermore, the NESP55 deletion causes broad imprinting defects involving all the maternal imprinting pattern of the *GNAS* gene. It has been demonstrated that the loss of imprinting in maternal exon A/B of the *GNAS* locus leads to biallelic expression of A/B transcripts and decreased *Gsα* expression in renal proximal tubules [36]. In the sporadic PHPIb

cases, the methylation of exon A/B is also affected, however, the underlying genetic defect in those cases has not been clarified yet. The regulatory elements responsible for a proper methylation of DMR region of the *GNAS* locus as well as other imprinted loci might be involved in the pathogenesis of sporadic PTHIb. This is suggested by finding of hypomethylation in multiple maternally methylated loci, including *GNAS* locus, in cases of Beckwith-Wiedemann syndrome, where the causative mutations are not found yet [37].

Pseudopseudohypoparathyroidism (PPHP) (Albright's hereditary osteodystrophy): PPHP is defined by a skeletal dysplasia phenotype described above, but not hormone resistance in the end organs [21]. Phenotypes of PHPIa and PPHP have been described in the pedigrees depending on whether the genetic condition is inherited maternally or paternally [38, 39]. Paternally inherited mutations in the coding exons of the *Gsα* result in the skeletal dysplasia phenotype and/or subcutaneous calcifications, but not in PTH resistance in the proximal renal tubule, as the normal maternal allele is expressed in this tissue.

Progressive osseous heteroplasia and osteoma cutis (POH and OC): POH is a rare condition characterized by heterotopic ossification in subcutaneous and deep connective tissue, muscles and fascia in the absence of PTH resistance and Albright's hereditary osteodystrophy phenotype, whereas *osteoma cutis* is defined

Table 1

Phenotypes and inheritance of *GNAS* mutations in PHP type I.

Phenotype	Parental origin of the mutation	<i>GNAS</i> defect
PHPIa (Albright's hereditary osteodystrophy + PTH resistance)	Maternal	<i>Gsα</i> mutations in the coding region
PHPIb (PTH resistance only)	Maternal	Imprinting defect of the <i>GNAS</i> locus
PPHP (Albright's hereditary osteodystrophy)	Paternal	<i>Gsα</i> mutations in the coding region
Progressive osseous heteroplasia & <i>osteoma cutis</i>	Paternal	<i>Gsα</i> mutations in the coding region

by superficial calcifications [40]. POH has to be differentiated from another condition with ossification of the soft tissues, *fibrodysplasia ossificans progressiva* (FOP). In the individuals with FOP, ossifications occur after trauma and are always preceded by an inflammatory reaction in the tissues. Furthermore, FOP is characterized by specific dysmorphic features [40, 41].

POH mutations in $Gs\alpha$ gene are inherited paternally and some of them are identical with those in PHPIa and PPHP [26]. It is not clear why some of the patients would develop POH only, while others have PPHP phenotype, but most probably the explanation might be found in the phenomena of phenotype variability and variable penetrance of autosomal dominant disorders. Individuals with POH in combination with AHO features as well as PTH resistance have also been reported (so called syndromic POH), indicating that POH is at the very extreme end of a *GNAS*-related phenotypes [26]. Osteoma cutis is a mild form of POH, where only superficial calcifications are found, and this condition is not associated with PTH resistance.

Conclusion

PHP type I is a hormone resistance syndrome caused by inactivating mutations or imprinting defects in the *GNAS* locus. One of the multiple transcripts from this locus is $Gs\alpha$, a protein involved in transmembrane signalling. The complex regulation of the locus and its imprinting pattern gives the explanation to the phenotypic variations of the PHP type I. The phenotypes and the inheritance pattern in PHP type I are summarized in table 1.

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