***GNAS* Locus and Pseudohypoparathyroidism**

Murat Bastepe^a Harald Jüppner^{a,b}

^aEndocrine Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, and

^bPediatric Nephrology, MassGeneral Hospital for Children, Massachusetts General Hospital, Harvard Medical School, Boston, Mass., USA

Key Words

Pseudohypoparathyroidism · Parathyroid hormone · Albright's hereditary osteodystrophy · *GNAS* locus

Abstract

Pseudohypoparathyroidism (PHP) is characterized by hypocalcemia and hyperphosphatemia due to resistance to parathyroid hormone (PTH). Patients with PHP-1a often present with additional hormonal resistance and show characteristic physical features that are collectively termed Albright's hereditary osteodystrophy (AHO). These features are also present in pseudopseudohypoparathyroidism (PPHP), but patients affected by this disorder do not show hormone resistance. PHP-1b patients, on the other hand, present predominantly with renal PTH resistance and lack any features of AHO. Most of these PHP forms are caused by defects in *GNAS* (20q13.3), an imprinted gene locus with multiple transcriptional units. PHP-1a and PPHP are caused by heterozygous inactivating mutations in those exons of *GNAS* encoding the α subunit of the stimulatory guanine nucleotide-binding protein (G_{α}), and the autosomal dominant form of PHP-1b (AD-PHP-1b) is caused by heterozygous mutations disrupting a long-range imprinting control element of *GNAS*. Expressed nearly in all cells, G_{α} plays essential roles in a multitude of physiological processes. Its ex-

pression in renal proximal tubules occurs predominantly from the maternal allele, and this tissue- and parent-specific imprinting of G_{α} is an important determinant of hormone resistance in kindreds with PHP-1a/PPHP and AD-PHP-1b.

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Deficiency of a hormone leads to decreased activity at its target organs, often leading to clinical manifestations that will require medical attention. Similar clinical abnormalities can also arise when a target organ becomes non-responsive to its hormone despite sufficient or even elevated levels of that hormone. Several different endocrine disorders result from such target-organ resistance, which are often caused by homozygous mutations that render hormone receptors inactive. Pseudohypoparathyroidism (PHP), on the other hand, exemplifies a different, quite unusual form of hormone resistance [1]. The molecular defect responsible for most types of PHP affects, rather than the hormone receptor, the ubiquitous signaling protein G_{α} (alpha subunit of the stimulatory guanine-nucleotide binding protein), which is downstream of many different G protein-coupled hormone receptors [2–4].

PHP refers to several distinct, but related, disorders in which resistance toward parathyroid hormone (PTH) is

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Dr. Murat Bastepe
Endocrine Unit
Massachusetts General Hospital
Boston, MA 02114 (USA)
Tel. +1 617 726 3966, Fax +1 617 726 7543, E-Mail bastepe@helix.mgh.harvard.edu

Table 1. Clinical and molecular features of the different PHP-I forms

	PTH resistance	Additional hormone resistance	Typical AHO features	<i>GNAS</i> defects
PHP-Ia	yes	yes	yes	Gsα mutations
PPHP	no	no	yes	Gsα mutations
POH	no	no	some cases	Gsα mutations
PHP-Ib	yes	some cases	no	<i>STX16</i> deletions affecting <i>GNAS</i> imprinting

the most prominent feature. However, resistance toward thyroid-stimulating hormone (TSH) and several other pituitary hormones that mediate their actions through G protein-coupled receptors is often encountered as well. As a result of PTH resistance, patients with PHP have diminished serum concentrations of 1,25-dihydroxy vitamin D3 [5, 6] and develop hypocalcemia and hyperphosphatemia. While these manifestations are also present in hypoparathyroidism [7], an elevation in serum PTH concentration indicates target-organ resistance to this hormone, which is a distinctive finding in PHP. Consistent with PTH resistance, administration of exogenous biologically active PTH fails to induce an appropriate increase in urinary phosphate and cAMP excretion [1, 8]. However, resistance seems to occur only in the proximal renal tubules, while the actions of PTH are unimpaired in other target tissues, such as bone [9, 10] and the thick ascending tubule [11]. Because of these latter unimpaired PTH functions, an elevated PTH concentration can maintain normocalcemia in PHP patients for prolonged periods of time. Nevertheless, episodes of hypocalcemia with associated clinical manifestations, such as muscle spasms or seizures, do occur in these patients and usually require treatment with oral calcium supplements and 1,25-dihydroxy vitamin D. Since an elevated PTH level is usually the most sensitive indicator of PTH resistance, asymptomatic patients with normal calcium and phosphate levels should also be treated in order to normalize PTH levels and, thereby, to prevent hyperparathyroid bone disease. In patients undergoing treatment, besides blood biochemistries, urinary calcium excretion needs to be monitored at regular intervals, i.e. yearly, to avoid persistent hypercalcemia and/or hypercalciuria.

Albright et al. [1] described patients affected by PHP for the first time in 1942. Different clinical variants of this disorder have since been reported, and today PHP is subdivided into two main types. In PHP-I, both nephrogenous cAMP generation and phosphate excretion following

exogenous PTH administration are blunted compared to those observed in normal subjects [1, 8]. In PHP-II, nephrogenous cAMP generation appears normal, but the urinary excretion of phosphate is impaired [12]. This review will mainly focus on PHP-I and its subtypes, because only a few cases of PHP-II have been reported, and the nature of the molecular defect responsible for this PHP variant remains elusive. However, it should be noted that the medical treatment of the abnormalities caused by hormone resistance is currently the same for patients with either PHP-I or PHP-II.

Loss-of-Function Mutations of Gsα, Multiple Hormone Resistance and AHO

Two main subtypes of PHP-I have been described: PHP-Ia and PHP-Ib (table 1). Patients with PHP-Ia have, in addition to PTH-resistance, Albright's hereditary osteodystrophy (AHO), a constellation of physical features, which may include obesity, short stature, heterotopic ossification, brachydactyly and/or mental retardation. PHP-Ia patients furthermore demonstrate additional hormonal abnormalities, including hypothyroidism and hypogonadism due to target-organ resistance to TSH and gonadotropins [2, 3]. Recent studies using growth hormone-releasing hormone (GHRH) infusion tests also suggest that growth hormone deficiency is a common hormonal abnormality among PHP-Ia patients, probably reflecting resistance at the GHRH receptor in pituitary somatotrophs [13, 14]. In contrast, the hypothalamus-pituitary-adrenal axis appears to be unaffected [13, 15, 16], as do the actions of numerous other hormones that mediate their actions through G protein-coupled receptors, including vasopressin [16, 17].

PHP-Ia is caused by heterozygous mutations located in *GNAS* exons 1–13, which encode Gsα (fig. 1). This ubiquitously expressed signaling protein stimulates adenylyl

Fig. 1. Heterozygous inactivating mutations affecting Gsα are found in patients with PHP-Ia, PPHP and POH. Exons 1–13 of *GNAS* and intronic spaces (white boxes and connecting lines) are shown, but recently identified exons located 5' (centromeric) to exon 1 are not included (see figure 3 for a scheme of the entire *GNAS* locus). Splicing pattern (dotted lines) indicates the two main splice variants of Gsα, the short and the long versions, which either include or exclude exon 3; these versions are thought to have similar biological activity.

cyclase when activated by an agonist-occupied G protein-coupled receptor, thereby generating the second-messenger cAMP in response to a wide range of agonists. In fact, a large variety of physiological processes rely on normal functioning of this signaling protein [18]. Homozygous disruption of Gsα is embryonically lethal in mice, and heterozygous disruption of Gsα leads to high mortality during early postnatal development, alterations in energy metabolism, and defects in various hormone actions [19, 20]. In patients with PHP-Ia, numerous different mutations have been identified in nearly all of the 13 exons that encode the Gsα protein (for a list of mutations, please see OMIM entry 139320 at <http://www.ncbi.nlm.nih.gov>). These mutations include missense and nonsense changes, as well as insertions and deletions that either alter pre-mRNA splicing or introduce frame-shifts causing early termination. Many of the Gsα mutants are not expressed due to instability of their mRNA or due to poor localization to the cell membrane. Other mutants fail to facilitate productive coupling to a variety of G protein-coupled receptors, thus leading to diminished or no cAMP generation. Easily accessible tissues from PHP-Ia patients, such as skin fibroblasts and erythrocytes, reveal an ~50% reduction in Gsα mRNA/protein, which is consistent with the heterozygous inactivating nature of these *GNAS* mutations. As a result, hormone-induced cAMP generation is reduced to ~50% in biochemical assays involving re-constitution of patient-derived cell membranes with membranes of cells that lack functional Gsα,

Fig. 2. Gender of the parent transmitting the *GNAS* defect determines the phenotype of the offspring in PHP-Ia/PPHP and AD-PHP-Ib kindreds. In certain tissues, including renal proximal tubules, Gsα expression from the paternal allele (gray rectangle) is silenced, and transcription takes place from the maternal allele (white rectangle) only. Therefore, when an inactivating Gsα mutation (black dot) is inherited from a female carrier, the expression level of Gsα in these tissues is nearly or completely abolished, and thus hormone resistance develops (PHP-Ia). In contrast, when the same inactivating Gsα mutation (black dot) is inherited from a male carrier, the expression level of Gsα remains unaltered, and thus the hormone response is normal (PPHP). Both after maternal and paternal transmission of the Gsα mutation, the offspring develops AHO, a finding that led to the hypothesis that the pathogenesis of AHO involves haploinsufficiency of Gsα in some of the tissues in which Gsα expression is biallelic. In AD-PHP-Ib kindreds, the mode of inheritance of PTH resistance is similar to that in PHP-Ia/PPHP kindreds. Maternal transmission of the deleted putative control element in *STX16* (black dot) leads to a loss of *GNAS* exon A/B imprinting and PTH resistance, while paternal transmission of the same defect leads to a normal hormone response.

such as turkey erythrocytes or the *cyc*⁻ clone of S49 lymphoma cells [2]. When non-hydrolysable GTP analogs are used in these assays instead of a receptor agonist, however, certain Gsα mutants that are defective in receptor coupling, but not in adenylyl cyclase stimulation, appear fully functional [2, 21]. Although this observation reflects primarily the type of stimulant used in the functional assay, it has led to the definition of another PHP-I subtype, termed PHP-Ic. However, as expected, there are no apparent differences between the clinical phenotypes of patients affected by PHP-Ia and those affected by PHP-Ic.

Two disorders, pseudopseudohypoparathyroidism (PPHP) and progressive osseous heteroplasia (POH), are closely related to PHP-Ia at the molecular level, despite having distinct clinical phenotypes. Patients with PPHP have the typical features of AHO but do not present with hormone resistance [22]. PPHP is also caused by hetero-

zygous inactivating mutations in *Gsa* coding exons of *GNAS*, and in fact, PHP-Ia and PPHP can be found in the same kindreds, but not in the same sibship. The phenotype in the offspring depends on the gender of the parent transmitting the molecular defect (fig. 2). Paternal transmission causes PPHP (AHO only), whereas maternal transmission of the same mutation causes PHP-Ia (AHO plus hormone resistance) [23, 24]. Based on several lines of recent evidence, this imprinted mode of inheritance for hormone resistance reflects the imprinted nature of *Gsa* expression in certain tissues (see below).

Patients with POH suffer from severe heterotopic ossification involving skeletal muscle and deep connective tissue, and often lack hormone resistance and typical AHO features [25]. Heterozygous mutations in the *GNAS* exons encoding *Gsa* have also been identified in this group of patients. In fact, some of the identified mutations are identical to those found in patients with either PHP-Ia or PPHP [25–27]. It has been reported that the POH phenotype develops only after paternal inheritance of these mutations [27]. Interestingly, maternal transmission of the same molecular defect in the next generation, which could be investigated thus far only in one large POH kindred, leads to AHO apparently without evidence of hormone resistance [27]. However, some POH cases have been reported to have a combination of hormone resistance and AHO after maternal transmission of the *Gsa* defect [26, 28], and it is therefore conceivable that some mothers with POH can have children affected by AHO and clinical and laboratory findings characteristic of PHP-Ia. This could indicate that POH represents the extreme end of the spectrum of AHO features. Furthermore, inheritance of *Gsa* mutations from a female appears to result in quite variable hormone resistance, i.e. the phenotype caused by maternal *Gsa* mutations can range from PHP-Ia, which occurs in the majority of the cases, to one that is indistinguishable from PPHP (AHO alone), which occurs only rarely. The consequences of *Gsa* mutations are therefore complex, and secondary factors, such as genetic background, are likely to influence the severity of the disease.

Parental Origin-Specific Expression of *Gsa* in Select Tissues

Mice with targeted disruption of *Gnas* exon 2 [19] have provided explanations for some of the findings observed in patients with *Gsa* mutations. Consistent with the essential roles of *Gsa* in many physiological processes,

homozygous disruption of *Gnas* exon 2 (*Gnas*^{E2-/E2-}) causes embryonic lethality during early post-implantation stages. Also, about three quarters of mice heterozygous for disruption of paternal *Gnas* exon 2 (*Gnas*^{E2+/pat-}) die within 24 h after birth, and an even higher percentage of mice with maternal disruption (*Gnas*^{E2+/mat-}) develop various neurological abnormalities within the first few weeks of life and die shortly thereafter. This high mortality is different from findings in humans with inactivating *Gsa* mutations (unless these mutations can be lethal during early human embryonic development), but it is similar to the findings observed in mice with either paternal or maternal uniparental disomy of the syntenic region on mouse chromosome 2 [29]. Both *Gnas*^{E2+/mat-} and *Gnas*^{E2+/pat-} mice that survive beyond weaning are significantly smaller, but are fertile and appear to have normal life spans. *Gnas*^{E2+/mat-} mice develop resistance to proximal tubular actions of PTH, while *Gnas*^{E2+/pat-} mice show no evidence of defective calcium metabolism. In keeping with these results, *Gnas*^{E2+/mat-} mice almost completely lack *Gsa* expression in the renal cortex, while *Gnas*^{E2+/pat-} and wild-type mice appear to have indistinguishable levels of *Gsa* expression in this portion of the kidney. On the other hand, hormone signaling in the renal medulla seems intact in both *Gnas*^{E2+/mat-} and *Gnas*^{E2+/pat-} mice, although *Gsa* expression levels are decreased to about 50% of wild-type levels [19]. These findings confirm that *Gsa* expression is predominantly maternal in some, but not all tissues, including the renal proximal tubule, thus explaining the maternal-specific inheritance of PTH resistance in PHP-Ia/PPHP kindreds and, to a certain extent, the limited spectrum of hormone resistance in PHP-Ia patients. Interestingly, *Gsa* expression in human fetal kidney cortex was shown to be biallelic using RT-PCR [30]. This finding could suggest that *Gsa* imprinting establishes later in life, or that it takes place only in a small subset of renal cortical cells.

Predominant maternal expression of *Gsa* has been documented in some human tissues, including thyroid gland [31–33], gonads [31], and pituitary [34]. On the other hand, expression of *Gsa* in the adrenal gland has been demonstrated to be biallelic [31]. These findings are consistent with the extent and imprinted inheritance of hormone resistance in PHP-Ia. Thus, hormone resistance, which occurs in patients carrying maternal *Gsa* mutations, develops only in those tissues where expression of *Gsa* is predominantly from the maternal allele. Physiological processes in other tissues are not detectably impaired, because an ~50% reduction in *Gsa* level and activity appears to be sufficient to maintain normal signaling

Fig. 3. The complex *GNAS* locus gives rise to multiple transcripts. Boxes and connecting lines indicate exons and introns, respectively. The five main transcriptional units derived from this locus and the utilized exons are indicated as rectangles below the gene depiction. *Gsa* transcripts are biallelically expressed except in a small number of tissues, including renal proximal tubules, thyroid, gonads and pituitary. XLas, A/B and antisense (AS) transcripts are paternally expressed, and NESP55 transcripts are maternally expressed. Promoters of these latter four transcripts are either maternally (m) or paternally (p) methylated, as indicated by + (methylated CpGs) and – (unmethylated CpGs).

events in most cells. Based on this model, AHO is predicted to develop as a result of defective cell signaling in certain cells or tissues where the remaining ~50% *Gsa* activity does not suffice to maintain normal cellular functions, thus resulting in haploinsufficiency. The *Gnas* exon 2 knockout model, however, does not explain the molecular pathogenesis underlying AHO, since apart from being smaller than their littermates, mice heterozygous for *Gnas* exon 2 disruption lack overt abnormalities that could be consistent with an AHO-like phenotype, such as the shortening of some bones due to growth plate defects. It is thus possible that additional mechanisms compensate for *Gsa* deficiency in the *Gnas* exon 2 knockout mouse model, and that these yet unknown mechanisms prevent such defects from developing entirely or from becoming severe enough to be detectable by routine analyses. In fact, only through the use of mice chimeric for wild-type and *Gnas*^{E2+/-} chondrocytes has it been possible to obtain evidence for haploinsufficiency of *Gsa* signaling in the growth plate cartilage [35].

Multiple Imprinted Transcripts at the *GNAS* Locus

Over the past several years it has become apparent that additional, previously unrecognized *Gsa* splice variants are derived from *GNAS* and its mouse homolog *Gnas*

(fig. 3). These include XLas, a large variant of *Gsa* expressed primarily in neuroendocrine tissues and the nervous system. XLas differs from *Gsa* by sequences derived from a novel first exon that splices onto exons 2–13 [36–38]. XLas and *Gsa* are thus identical over the entire C-terminal portion but they have distinct N-termini. Another protein product of *GNAS* is NESP55, a chromogranin-like neuroendocrine secretory protein. As in the case of XLas, a novel first exon splices onto exons 2–13, but because of a termination codon within this novel exon, NESP55 does not share an amino acid sequence with *Gsa* [39, 40]. Transcripts encoding XLas and those encoding NESP55 are exclusively derived from the paternal or the maternal *GNAS* allele, respectively [36, 40]. *GNAS* also gives rise to the A/B transcript, which uses exon A/B as the alternate first exon (also referred to as exon 1A or 1') [41–43], and an antisense transcript, which consists of distinct exons that do not overlap with any of the other exons [44, 45]. Both A/B and antisense transcripts, which are thought to be non-translated, show ubiquitous expression and they are derived, in nearly all investigated tissues, from the paternal allele only [42, 44, 45]. Consistent with their parent-specific expression, the promoter regions of all these additional *GNAS* transcripts are within differentially methylated regions (DMRs), and in each case the non-methylated promoter drives the expression [36, 40, 42, 44, 45]. In contrast, the promoter of *Gsa* lacks methylation and is biallelically active in most

tissues [37, 46]. Thus, the *GNAS* locus is more complex than previously recognized. The fact that multiple *Gsa* splice variants share exon 2 could explain, at least partly, why the *Gnas*-exon 2 knockout mouse model does not entirely phenocopy PHP-Ia and PPHP patients. Comparative studies with other mouse models, such as mice with disruption of *Gnas* exon 1 [47–49] and mice with disruption of exon XL [50], will likely reveal additional mechanisms underlying hormonal resistance and the pathogenesis of AHO in PHP-Ia/PPHP.

Imprinting Defects of *GNAS* and PTH Resistance

The second subtype of PHP-I, PHP-Ib, is characterized by PTH resistance in the absence of AHO features [2]. Until recently, hormone resistance in PHP-Ib was thought to be limited to the renal actions of PTH, but recent reports have shown that other hormone actions, including the actions of TSH, can also be impaired [33, 51, 52]. Most PHP-Ib cases are sporadic, but some cases are familial and show an autosomal dominant mode of inheritance (AD-PHP-Ib). As is the case for PHP-Ia, the severity of PTH resistance varies significantly from patient to patient, even within the same family [4, 53]. Also, like hormone resistance in PHP-Ia, PTH resistance in PHP-Ib develops only after maternal transmission of the genetic defect (fig. 2) [52, 53]. Despite these similarities between the two forms of PHP, mutations within the *GNAS* exons encoding *Gsa* do not seem to be present in most patients with PHP-Ib [54, 55]. However, the disease gene in AD-PHP-Ib maps to a telomeric region on chromosome 20q containing the *GNAS* locus [52, 53]. Furthermore, most AD-PHP-Ib cases demonstrate a loss of methylation at the exon A/B DMR of *GNAS*; this epigenetic defect is concomitant with biallelic expression of A/B transcripts [52]. Similarly, most sporadic PHP-Ib cases show *GNAS* methylation changes at multiple DMRs, consistently including the exon A/B DMR [56]. In fact, one of these sporadic PHP-Ib cases has been shown to have paternal uniparental isodisomy of the entire chromosome 20q [51]. Taken together, these findings suggest that the mutations responsible for different forms of PHP-Ib affect control elements regulating differential methylation of *GNAS* and allele-specific gene expression.

A heterozygous 3-kb deletion has been identified in the affected individuals and unaffected carriers of 14 unrelated AD-PHP-Ib kindreds and two apparently sporadic PHP-Ib cases, but not in healthy controls [57]. Consistent

with the mode of inheritance in AD-PHP-Ib, the disease is associated with maternal inheritance of this deletion. Interestingly, the identified deletion is located approximately 220 kb centromeric of exon A/B and disrupts another gene, *STX16*, which encodes a member of the SNARE family of proteins; these are involved in intracellular trafficking [58, 59] (fig. 4). There is no evidence to suggest that *STX16* is an imprinted gene, and those individuals of AD-PHP-Ib kindreds who inherit the deletion from their fathers lack hormone resistance. Therefore, the involvement of *STX16* and/or haploinsufficiency of its protein product, syntaxin-16, in the pathogenesis of PHP-Ib appear unlikely (however, a critical role for this protein in the establishment of methylation during female germ cell development remains as a possibility). Instead, given that all the affected individuals with this deletion show loss of methylation at exon A/B and no epigenetic defects at other *GNAS* DMRs, and given that all but one AD-PHP-Ib kindred (see below) with the exon A/B methylation defect alone carry the same deletion, it is more likely that the 3-kb deletion disrupts a *cis*-acting imprinting control element essential for the establishment and/or maintenance of the methylation imprint at exon A/B (fig. 4).

Recently, another deletion has been discovered in an AD-PHP-Ib kindred in whom the 3-kb deletion was not detected and in whom the epigenetic defect was shown to be limited to exon A/B. This new deletion also affects *STX16* and overlaps with the previously identified 3-kb deletion by about 1286 bp [60], thus strengthening the hypothesis that the *STX16* region comprises a putative regulatory element necessary for *GNAS* exon A/B methylation. Nucleotide sequences of the deleted *STX16* regions do not share significant homology with nucleotide sequences of regions deleted in other disorders of imprinting, such as Beckwith-Wiedemann syndrome [61] and Prader-Willi/Angelman syndromes [62]. However, there appears to be significant evolutionary conservation in this region when compared to the regions of synteny in mouse and rat, although the highly conserved areas are mostly limited to exons encoding the syntaxin-16 protein [57]. Hence, the molecular mechanisms underlying the establishment and/or maintenance of the methylation imprint at *GNAS* exon A/B and the involvement of *STX16* in this process remain to be investigated. Mouse models of PHP-Ib carrying deletions at the mouse *Stx16* locus will help unravel some of these mechanisms, and are thus likely to further improve our understanding of gene regulation at the *GNAS* locus, and possibly, at other imprinted genomic loci.

Fig. 4. Identical heterozygous 3-kb deletions in AD-PHP-Ib kindreds presumably disrupts a putative regulatory element that controls the establishment and/or the maintenance of exon A/B methylation. The identified deletion, ~220 kb upstream of *GNAS* exon A/B, is located within *STX16*, the gene encoding syntaxin-16, and is flanked by two almost identical direct repeats of 391 bp (triangles). Rather than disruption of *STX16*, which does not appear to be imprinted, it is likely that disruption of a putative imprinting control element positioned

within this gene is responsible for the loss of exon A/B methylation in AD-PHP-Ib patients. This epigenetic change and/or the associated derepression of maternal A/B transcript expression is postulated to silence maternal *Gsα* expression in proximal renal tubules. Because paternal *Gsα* expression in this tissue is primarily silenced under normal conditions, this maternal silencing results in a marked reduction in *Gsα* levels, leading to PTH resistance. N = NESP55; AS = antisense.

Deletions within *STX16* are not present in most sporadic PHP-Ib cases [57]. Remarkably, all of the analyzed sporadic cases appear to have, in addition to the exon A/B methylation defect, complete or incomplete methylation defects at all other *GNAS* DMRs, including gain of methylation at exon NESP55, and loss of methylation at exon XL and the promoter of the antisense transcript. These cases may represent a genetically distinct form of PHP-Ib that is caused by a mutation that disrupts another regulatory element necessary for the imprinting of the entire *GNAS* locus. Alternatively, it is conceivable that some of these cases represent a recessive trait, and that they are caused by homozygous inactivating mutations in a gene encoding a putative trans-acting factor. Further investigations will be required to identify the underlying genetic defect(s) in this form(s) of PHP-Ib.

Regulation of Imprinted Gene Expression from *GNAS*

As discussed above, *GNAS* contains at least four distinct DMRs. Two of those DMRs, the maternally methylated region encompassing the promoter of the antisense transcript and the maternally methylated region encompassing exon A/B, represent distinct methylation imprint marks. Both DMRs, unlike the DMR at exon NESP55 and the DMR at exon XL, are established in the germ line [42, 63]. Based on the findings in AD-PHP-Ib patients, the establishment and/or maintenance of the exon A/B imprint mark requires a long-range control element located within *STX16*. Furthermore, because failure to establish the exon A/B imprint does not affect the integrity of other DMRs, including the imprint mark at the promoter of the antisense transcript, it appears that distinct mechanisms govern the epigenetic regulation affecting two methylation imprint marks within the *GNAS* locus. Additional investigations are required to identify individual control elements involved in the imprinting of different *GNAS* domains.

The promoter for *Gsα* transcription is not located within a DMR and, consistent with that finding, expression of *Gsα* takes place in most tissues from both parental alleles [37, 46]. However, *Gsα* promoter activity is evidently silenced from the paternal allele in a few tissues. Certain findings in PHP-Ib patients may provide clues regarding the molecular events governing this allele-specific *Gsα* silencing in certain tissues. Methylation of the exon A/B DMR and/or promoter activity driving A/B expression appears to play important roles in allele-specific *Gsα* expression in renal proximal tubules and, possibly, in other tissues in which *Gsα* shows imprinted expression. A possible mechanism underlying allele-specific *Gsα* silencing entails a promoter competition model in which either the promoter of A/B transcripts or the promoter of *Gsα* transcripts can be active on the same allele. This hypothesis is based on the correlation between the maternal silencing of *Gsα* expression and the maternal derepression of A/B expression that presumably takes place in the renal proximal tubules of PHP-Ib patients. However, given that the A/B transcript is broadly expressed [44, 45, 64], such a mechanism appears unlikely. Alternatively, it is plausible that silencing of the *Gsα* promoter on the paternal allele involves, under normal conditions, the methylation-sensitive binding of a putative tissue-specific trans-acting factor, such as a boundary element or a transcriptional repressor. Binding of this trans-acting factor to the exon A/B DMR would be expected to limit the effect of a putative enhancer element on the *Gsα* promoter, or to directly suppress the activity of that promoter. Evidence

for this latter hypothesis has been obtained from mice with targeted deletion of the exon A/B DMR [65, 66]. Upon paternal inheritance of the deleted allele, these mice show derepression of paternal *Gsα* transcription and thus an elevated *Gsα* level in brown adipose tissue where *Gsα* is normally silenced on the paternal allele [65]. These mice appear to have elevated *Gsα* levels also in the renal proximal tubules, since they show increased basal signaling through the renal PTH/PTHrP receptor as evidenced by ~50% reduction in serum PTH levels compared to wild-type animals. Future studies will likely distinguish between different types of trans-acting factors responsible for paternal *Gsα* silencing.

Conclusion

Analysis of PHP patients over the past several years has provided remarkable new insights into the understanding of the complex *GNAS* locus that gives rise to *Gsα* and several other splice variants. Although a number of genetic defects responsible for different PHP forms have been identified within the *GNAS* locus, molecular mechanisms underlying hormone resistance and imprinting defects remain incompletely understood. It will be important to identify patients in whom PHP is caused by novel *GNAS* defects, as careful laboratory investigations of those defects will likely further our knowledge of this complex gene and this unique disorder.

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Note Added in Proof

Recently, deletions of the *GNAS* locus have been identified in two unrelated kindreds with AD-PHP-Ib in whom the affected individuals show methylation abnormalities at multiple *GNAS* DMRs, including one at exon A/B. In each case, the deletion removes the NESP55 DMR, which comprises exon NESP55, and exons 3 and 4 of the antisense transcript. On maternal transmission, the deletion disrupts all the maternal imprints of *GNAS* and causes PTH resistance. It thus appears that NESP55 DMR harbors a *cis*-acting element required for the proper imprinting of the entire maternal *GNAS* allele.

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