Different Forms of Pseudohypoparathyroidism: Imprinted Disorders Caused by Different Coding and Non-coding Mutations in GNAS

Harald Jüppner
Massachusetts General Hospital and Harvard Medical School, Massachusetts, Boston, USA

Abstract. Pseudohypoparathyroidism (PHP) refers to disorders that are elicited by coding and non-coding mutations in GNAS, a complex gene locus encoding the α-subunit of stimulatory G protein (Gsα) and splice variants thereof. The complexity of the GNAS locus is reflected by several alternatively spliced sense and antisense transcripts, a parent-specific methylation pattern and consequently transcription from only one parental allele for all mRNAs, except for the mRNA encoding Gsα. PHP can be divided into two major groups that are caused either by heterozygous mutations in exons affecting Gsα (PHP type Ia: PHP-Ia) or by deletions of presumably regulatory regions affecting GNAS (PHP type Ib: PHP-Ib). The former group comprises besides PHP-Ia, pseudo-PHP (pPHP), and progressive osseous heteroplasia (POH). The phenotypes of these disorders are quite different and depend on the kind of mutation and whether it is inherited maternally or paternally. For example, PHP-Ia develops after maternal inheritance of a Gsα mutation, while paternal inheritance of the same mutation leads to pPHP or POH. Similarly, maternal inheritance of a deletion in STX16, the gene encoding syntaxin 16, upstream of GNAS leads to PHP-Ib, while inheritance of the same mutation from a male does not result in an obvious phenotype. In most familial cases of PHP-Ib, there is a loss of methylation affecting only exon A/B thus leading to active A/B transcription from both parental alleles, thereby suppressing Gsα transcription in the renal cortex and causing PTH-resistance.

Key words: pseudohypoparathyroidism (PHP), PHP type Ia (PHP-Ia), pseudo-PHP (pPHP), PHP type Ib (PHP-Ib), progressive osseous heteroplasia (POH), α-subunit of stimulatory G protein (Gsα), syntaxin 16 (STX16)
The term PHP was first reported by Dr. Fuller Albright (1942), when he worked at the Massachusetts General Hospital, Boston (5). His patients presented with hypocalcemia and hyperphosphatemia, and he showed that the disease was not caused by parathyroid hormone (PTH) deficiency, but instead by resistance towards PTH. Besides these endocrine abnormalities, he reported that affected individuals, now referred to as patients with PHP-Ia, have characteristic, but variable features such as round face, short stature, obesity, brachydactyly, heterotropic ossifications, and often mental retardation. These features are now termed Albright’s Hereditary Osteodystrophy (AHO).

Genetics of PHP-Ia

Several decades after Dr. Albright’s initial description, it became apparent that PHP-Ia is caused by heterozygous mutations in one of the 13 exons of GNAS encoding Gsα (6, 7). These mutations include nucleotide changes, deletions and insertions affecting the exons or introns, and splice junctions. It remains, however, unknown why only some of G protein-coupled receptors, such as the PTH/PTHrP receptor, are affected by these Gsα mutations while signaling through various other G protein-coupled receptors remains intact.

Over the last few years, it has become apparent that the GNAS locus is much more complex than initially thought (1, 2). For example, there are several different up-stream exons that are differentially methylated in a parent-specific manner and transcription occurs only from the non-methylated promoters. As a result, exons A/B (also termed exon 1A or exon 1’), XL (extra large stimulatory G protein, XLαs) and AS (antisense) are methylated only on the maternal allele, and the transcripts are derived only from the paternal allele. On the contrary, the NESP55 promoter is methylated on the paternal allele and active transcription occurs only from the maternal allele. With the exception of the AS transcript, all other alternative first exons splice onto exons 2 through 13.

The promoter for exon 1 is not methylated, and transcripts encoding Gsα are therefore derived from both parental alleles in most of tissues. However, in the proximal renal tubules Gsα is only expressed from the maternal allele, but the underlying mechanisms leading to parent-specific expression in this and few other tissues are still unknown. Due to expression that is restricted to the maternal allele, heterozygous inactivating Gsα mutations lead to a complete loss of this important signaling protein in the proximal renal tubules, resulting in PTH-resistance in this tissue and thereby explaining why these GNAS mutations need to be inherited maternally in order to cause PHP-Ia. Pseudo-PHP, characterized by AHO without PTH-resistance, occurs only if GNAS mutations affecting exons 1 through 13 are inherited paternally (Fig. 1).

In order to analyze the roles of the different GNAS exons, Dr. Weinstein’s group at the NIH and the groups in the UK headed by Drs. Kelsey and Peters have generated mice with ablation of different exons (8, 9). For example, removal of exon XL showed that the XLαs protein is critically involved in post-natal growth in mice. Furthermore, deletion of exon 1a (equivalent of the human exon A/B) showed that exon 1a-derived transcripts are important for reducing Gsα expression, indicating that there is competition between 1a and Gsα transcription. Disruption of GNAS exon 2 induces maternally inherited mild PTH-resistance by affecting the transcripts encoding Gsα and XLαs; no evidence for PTH-resistance was seen, as expected, when this deletion was paternally inherited (10). Surprisingly, there were no obvious phenotypic changes, which resemble the AHO phenotype in humans.
Different Forms of Pseudohypoparathyroidism

POH, a Disorder also Caused by GNAS Mutations

Recently, Dr. Shore and her colleagues reported another disorder, progressive osseous heteroplasia (POH) that is caused by mutations in the 13 exons encoding Gsα (3). Affected individuals show new bone formation usually affecting deep connective tissues and skeletal muscles, which can lead to disabling abnormalities (Fig. 2). It appears likely that POH is an extreme variant of pPHP. This conclusion is particularly based on findings in one pedigree with this disorder, where the father, who carries a Gsα mutation, is healthy, but has 5 daughters who are all affected by POH; because of this and other findings, it seemed likely that POH occurs only when the Gsα mutation is inherited paternally.

When the same mutation was transmitted by the females, their children developed AHO without obvious hormonal resistance. These children showed no evidence for hypocalcemia or hyperphosphatemia, but no data were reported regarding the serum levels of TSH and PTH. It thus appears plausible that the maternally inherited Gsα mutation leads in this family to relatively mild hormonal resistance. The question remains, however, whether the penetrance of hormonal resistance is incomplete due to incomplete silencing of Gsα expression from the paternal allele, or whether the XLαs can compensate, at least to some extent, for the lack of Gsα expression in the proximal renal tubules.

For the purpose of clarifying some of these questions, Dr. Bastepe in our laboratory prepared cell lines in which exon 2 of mouse GNAS is disrupted (11). Due to exon 2 disruption, these cell lines (Gsα−/−) lack Gsα and XLαs expression, and

Fig. 1 Gsα deficiency in PHP-Ia/pPHP. Due to parent-specific methylation of the alternative exons in the region up-stream of the Gsα exons, transcripts are generated either from the paternal or the maternal allele. Although exon 1 is not methylated, expression of Gsα occurs only from the maternal allele in the proximal renal tubules; the mechanism for this is not known. When Gsα mutations are inherited maternally, affected individuals develop hormonal resistance and AHO (PHP-Ia). On the other hand, if the mutations are inherited paternally, AHO develops without hormone resistance (pseudo-PHP).

Fig. 2 Progressive osseous heteroplasia (POH). POH is caused by mutations in one of the exons 13 encoding Gsα. The patients have new bone formations affecting their deep connective tissues and the muscles, leading to severe disabling disorders. It is likely that POH is a variant of pPHP (from Shore et al., 2002, with permission). ©2002 Massachusetts Medical Society.
consequently have no detectable formation of cAMP when challenged with different agonists. When the Gsα<sup>−/−</sup> cell lines were transfected with a single plasmid encoding either the PTH/PTHrP receptor, XLαs, or Gsα, no increase was in cAMP observed in response to the PTH(1-34). However, when the cells were co-transfected with two plasmids encoding the PTH/PTHrP receptor and XLαs, or the PTH/PTHrP receptor and Gsα, PTH-dependent cAMP formation was observed; remarkably, the PTH-dependent increase in cAMP formation was virtually equivalent for XLαs and Gsα (Fig. 3).

In summarizing these findings, PHP-Ia or pPHP are caused by mutations affecting one of the 13 exons encoding the Gsα; when these mutations are inherited maternally individuals develop AHO in combination with PTH-resistance, namely PHP-Ia. When the same mutations are inherited paternally, the affected individuals develop AHO without PTH-resistance, namely pseudo-PHP. In POH families, paternal inheritance of mutations in one of the exons encoding Gsα elicits POH. Maternal inheritance, on the other hand, leads to AHO without apparent PTH-resistance, but this aspect has not yet been thoroughly studied in kindreds affected by this disorder. In fact, it is likely that pseudo-PHP and POH are extremes of a disease spectrum. Furthermore, it appears likely that the degree of PTH-resistance is quite variable and that Gsα can lead either to PHP-Ia or to AHO with only mild, possibly subclinical hormonal resistance.

**Genetics of PHP-Ib**

Pseudohypoparathyroidism type Ib (PHP-Ib) is characterized by hypocalcemia, hyperphosphatemia, and high PTH levels, and in some case, the serum level of the thyroid-stimulating hormone (TSH) is also elevated, but none of these patients have AHO (1, 2). When tested, Gsα activity of the peripheral blood cells was shown to be normal, and most cases have no mutation affecting the GNAS exons encoding Gsα. There are sporadic and familial forms of the disease, including an autosomal dominant form (AD-PHP-Ib).

Results from the sequence analyses of genomic DNA and cDNA as well as genetic linkage studies had previously excluded mutations in the PTH-receptor, i.e. the PTH/PTHrP receptor (1, 2). We therefore used genetic linkage studies to define the underlying genetic cause of AD-PHP-Ib using genomic DNA from a large five-generation kindred living in the USA (12). The male proband, who was diagnosed with pseudohypoparathyroidism in the 1940s, had two sisters, who are also affected by this disorder; one of these sisters has an affected son. The proband had 5 children, who are all healthy and they all have no evidence for PTH-resistance. However, two of his daughters have affected children and grandchildren. The maximal theoretical lod score for this kindred was calculated to be more than 4.5. By analyzing this pedigree and several others, it became apparent that AD-PHP-Ib is also an imprinted disorder, i.e. the genetic defect leads to PHP-Ib only if the mutation is inherited maternally; when inherited
Different Forms of Pseudohypoparathyroidism

We initially determined that the genetic locus resides on chromosome 20q13.3, in a chromosomal region that comprises about 2.5 million base pairs and contains the GNAS locus at its telomeric end. After reducing the critical interval, two different deletions were identified about 220 kb up-stream of the differentially methylated exon A/B.

Subsequently, Dr. Weinstein’s group showed that most patients affected by PHP-Ib have a loss of methylation affecting only the exon A/B, which was later confirmed through our own studies (13, 14). Our fine mapping studies revealed that the mutation leading to AD-PHP-Ib resides centromeric of NESP55, within a region comprising about 280 kb (Fig. 4). We subsequently identified an allelic loss in the region that contains STX16, the gene encoding syntaxin 16 (4). Inspection of this region revealed two almost perfect 391 base pair repeats, which suggested that a deletion had most probably occurred between these two repeats. Southern blot analysis confirmed that all the affected and unaffected obligate carriers showed a smaller band of 8.9 kb band derived from the mutant allele in addition to the band derived from the wild-type allele of 11.9 kb indicating that there was a 3-kb deletion between those 2 repeats (Fig. 5). The deletion was also identified by means of the polymerase chain reaction (PCR) using forward and reverse primers just outside of these repeat regions.

In one fairly large family with loss of the A/B methylation alone, however, we could not obtain any evidence for the 3-kb deletion, although the lod score in this family was more than 2.0, thus suggesting linkage to this region. We therefore amplified STX16 between exons 1 and 6 by reverse-transcriptase PCR and determined that exon 1 spliced directly onto exon 5, indicating that exons 2 through 4 had been deleted in this particular family (15). This was also confirmed by means of the Southern blot analysis, and we could eventually conclude that the affected individuals in this family had a 4.4-kb deletion, which was slightly more centromeric than the 3-kb deletion,
but overlapped by about 1200 base pairs with this former deletion. These findings were confirmed by a multiplex PCR approach; a novel band was observed in affected individuals and obligate carriers, but not in healthy or unrelated individuals.

Based on our current findings, we believe that there is a critical region about 220 kb upstream of the GNAS locus that contains an imprinting control element, which is required for establishment or maintenance of normal methylation at the A/B locus. When this critical region is disrupted, the loss of the methylation leads to enhanced A/B transcription from the maternal allele and this in turn silences Gsα transcription, but presumably only in the proximal renal tubules and perhaps few other tissues.

So far, the same 3-kb deletion has been identified in 23 different families in our laboratory, but when we include other families reported in Japan and other countries, the total number of the AD-PHP-Ib families with the 3-kb deletion probably reaches 40. In our case, 22 out of 23 families had the 3-kb deletion, and the remaining one family had the 4.4-kb deletion; all these families showed a loss of methylation at exon A/B, but not at the other GNAS exons that are normally methylated in a parent-specific manner.

On the other hand, out of 19 sporadic PHP-Ib individuals that we studied, none had the deletion within the STX16 region. One of these patients turned out to have a paternal uniparental isodisomy of chromosome 20q (patUPD20q). Furthermore, all of them had a loss of the maternal imprints of the entire GNAS locus. However, there are other familial forms of this disease that do not have this type of methylation defect, and some of them have variable penetrance (16). Also, there are few other families that have no obvious deletion, and these have no loss of methylation at any of the GNAS loci. It is therefore quite possible that there are several additional forms of PHP-Ib, which are likely to have different genetic causes.

**Other Findings in PHP-Ib**

Finally, we would like to discuss some recent findings in individuals affected by PHP-Ib. For example, a girl, who was born in July of 2001, was shown to have a loss of methylation at exon A/B in DNA obtained from cord blood at delivery (15). Later on, we determined that this child had inherited the disease-associated haplotype and that she carries the 4.4-kb deletion. So far, she has been followed for about 3 yrs (Fig. 6). Her serum calcium stayed within the normal range throughout this observation period. As predicted based on the genetic studies, her serum phosphate remained at the upper end of the normal range and the serum PTH level started to increase dramatically at about 2 yrs of age. This indicated that PTH-resistance had developed over time.

In a recently reported Greek kindred with AD-PHP-Ib, only one male was clinically symptomatic (17). He suffered seizures when he was 25 yr old and was shown to have hypocalcemia and elevated PTH levels. Analysis of his genomic DNA revealed the 3-kb deletion just like his mother, his grandmother and some of his other relatives. Four
affected individuals in the group had decreased serum uric acid levels in comparison with the unaffected carriers and healthy individuals. Those individuals with hypouricemia revealed increased fractional excretion of uric acid. Although we do not know yet what these uric acid findings mean, it is interesting to note that patients with primary hyperparathyroidism have elevated serum uric acid concentrations, which normalize after parathyroid surgery.

Thus, in summary, there is an identical 3-kb deletion in most of the families with AD-PHP-Ib and only in one family did we find a 4.4-kb deletion; these deletions both lead to a loss of methylation affecting exon A/B, while all other GNAS exons are normally methylated. Both deletions are likely to disrupt an imprinting control element of GNAS that is necessary for methylation of the exon A/B. If the deletion is inherited maternally, individuals are affected; and if it is inherited paternally, the deletions lead to no apparent phenotype. Onset of PTH-resistance seems to be at about 2 yr of age. Some of the affected individuals showed hypouricemia due to increased renal uric acid excretion. The genetic defect in other PHP-Ib cases with distinct epigenetic characteristics remains unknown.

References


