Genetics of pseudohypoparathyroidism

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Summary

Pseudohypoparathyroidism (PHP) is a group of disorders defined by the presence of renal parathyroid hormone (PTH) resistance, resulting in biochemically hypoparathyroidism in the presence of elevated serum PTH levels. Most PHP patients have diminished urinary cyclic AMP responses to administered PTH, indicating a defect in proximal PTH signaling. Heterozygous diminished urinary cyclic AMP responses to administered PTH is normally expressed primarily from the maternal allele. Familial diminished urinary cyclic AMP responses to administered PTH, is imprinted in a tissue-specific manner, and is poorly expressed from the paternal allele in various tissues, including renal proximal tubules. PHP type 1b patients have normal PTH responsiveness (3, 4) and some PHP patients present with hyperparathyroid bone disease due to the presence of a signaling defect involving the receptor, G protein, which couples hormone with cAMP production. cAMP mediates most of its actions by interacting with the cAMP-dependent protein kinase (protein kinase A), although other cAMP effectors have been more recently identified (10). cAMP mimics the effects of PTH on renal phosphate handling and vitamin D metabolism (11). At higher hormone concentrations the G protein Gs is activated, leading to stimulation of adenyl cyclase and generation of the second messenger cyclic AMP (cAMP). cAMP mediates most of its actions by interacting with the cAMP-dependent protein kinase (protein kinase A), although other cAMP effectors have been more recently identified (10). cAMP mediates the effects of PTH on renal phosphate handling and vitamin D metabolism (11). At higher hormone concentrations the PTH/PTHrP receptor is also able to activate the G protein Gs, which stimulates phospholipase C.

Most PHP patients (PHP type 1) have a markedly reduced urinary cAMP response to administered PTH (12, 13), indicating the presence of a signaling defect involving the receptor, Gs, or adenyl cyclase in renal proximal tubule cells. Rarely patients will have a normal cAMP response but a reduced phosphaturic response to PTH (PHP type 2), consistent with a defect downstream of cAMP generation (14). However the defect in PTH responsiveness in PHP type 2 patients is usually reversed after treatment with calcium or vitamin D, indicating that this is not a fixed genetic defect but rather a secondary and reversible effect of hypocalcemia and/or vitamin D deficiency (15). In most cases PHP type 1 results from reduced Gs activity due to genetic or epigenetic defects of the gene encoding it's specific α-subunit Gs. Like all heterotrimeric G proteins, Gs is composed of three subunits (α, β, and γ) which are all the products of separate genes (16). Gα interacts with both receptors and effectors, such as adenyl cyclase, and has a guanine nucleotide binding site. The β and γ subunits form tightly bound heterodimers. In the inactive state Gα has GDP bound to the guanine nucleotide binding site and is associated with βγ. The hormone bound receptor promotes GDP release, which leads to GTP binding and dissociation of Gα from βγ. The active GTP-

Introduction

Pseudohypoparathyroidism (PHP) is a group of disorders in which patients develop the signs and symptoms of hypoparathyroidism due to end organ resistance to parathyroid hormone (PTH) (1, 2). Patients with this disorder always present with elevated serum PTH levels, which is usually, although not always, associated with hypocalcemia and hyperphosphatemia. PTH resistance in PHP is primarily manifested in the renal proximal tubule. Because this tissue is unable to respond to PTH, patients have reduced renal phosphate clearance, leading to hyperphosphatemia. The combined effects of hyperphosphatemia and reduced PTH responsiveness leads to reduced synthesis of 1,25 dihydroxyvitamin D (1,25D) in proximal tubules. 1,25D deficiency leads to hypocalcemia through reduced gastrointestinal calcium absorption and PTH-stimulated calcium release in bone.

The PTH resistance in PHP appears to be limited to renal proximal tubules. Osteoblastic cells isolated from PHP patients have normal PTH responsiveness (3, 4) and some PHP patients present with hyperparathyroid bone disease due to the skeletal effects of chronically elevated PTH levels (5, 6). PTH action in the distal nephron (which promotes calcium reabsorption) also remains intact, and this is why PHP patients, unlike patients with primary hypoparathyroidism, are not prone to hypercalcemia upon treatment (1).
bound G\(_{\alpha}\) is capable of interacting with and stimulating adenyl cyclase. G\(_{\alpha}\) is inactivated by an intrinsic GTPase activity that hydrolyzes bound GTP to GDP. Mutations within specific residues (Arg\(^{201}\), Gln\(^{277}\)) critical for the GTPase reaction lead to constitutively active forms of G\(_{\alpha}\), and such somatic mutations are the genetic cause of various endocrine tumors, fibrous dysplasia of bone, and the McCune-Albright syndrome (2, 17-19). Recent studies suggest that G\(_{\alpha}\) may have other effectors besides adenyl cyclase, such as src tyrosine kinase (20).

The gene encoding G\(_{\alpha}\) is known as GNAS and is located at 20q13 (21). The G\(_{\alpha}\) coding region is spread over 13 exons. Alternative splicing of exon 3 results in the generation of long and short forms of G\(_{\alpha}\) protein that are all capable of mediating signaling between receptor and adenyl cyclase. GNAS is now recognized to be a complex imprinted gene which generates multiple gene products through the use of alternative promoters and first exons which splice onto a common set of downstream exons (exons 2-13, see Fig. 1) (16). The most upstream promoter generates transcripts for the chromogranin-like protein NESP55, which is structurally and functionally unrelated to G\(_{\alpha}\). The next promoter generates transcripts for XL\(_{\alpha}\), a neuroendocrine-speciﬁc G\(_{\alpha}\) isoform that has an extra-long amino-terminal extension encoded within its speciﬁc ﬁrst exon. NESP55 and XL\(_{\alpha}\) are oppositely imprinted: NESP55 is expressed only from the maternal allele and its promoter region is DNA methylated on the paternal allele and its promoter is methylated on the maternal allele (22, 23). The XL\(_{\alpha}\) promoter region also generates paternal-speciﬁc antisense transcripts that are likely to be important for paternal NESP55 imprinting (24). The next promoter region (the exon 1A region), which is located 35 kilobases downstream of the XL\(_{\alpha}\) promoter, is methylated on the maternal allele and generates unmethylated transcripts of unknown function from the paternal allele (25, 26). Just downstream of exon 1A is the G\(_{\alpha}\) promoter, which is unmethylated even though, as discussed below, G\(_{\alpha}\) is imprinted in a tissue-specific manner (27).

PHP type 1b patients have renal PTH resistance in the absence of AHO, which results from a GNAS imprinting defect. Rarely patients with the PHP type 1a phenotype have no evidence for a G\(_{\alpha}\) defect, and this has been referred to as PHP type 1c. In this review we will focus on the genetic and epigenetic defects associated with PHP types 1a and 1b, respectively.

**Pseudohypparathyroidism type 1a (Albright hereditary osteodystrophy)**

AHO is a congenital syndrome in which patients present with short stature, brachydactyly, obesity, subcutaneous ossifications, facial features such as hypertelorism or depressed nasal bridge, and mental defects or developmental delay (Fig. 2). The severity of the phenotype varies signiﬁcantly, and 50% or less present with neurobehavioral problems (27, 28). Even within the same kindred, some affected patients also develop resistance to PTH, thyrotropin and gonadotropins (PHP type 1a) while other affected patients develop AHO but have no evidence for hormone resistance (a condition also known as pseudopseudohypoparathyroidism [PPHP]).

All AHO patients (both PHP type 1a and PHP type 1b patients) have a ~50% reduction in G\(_{\alpha}\) bioactivity in membranes from various tissues, such as blood cells and ﬁbroblasts (29, 30), and this is due in most cases to a similar reduction in G\(_{\alpha}\) mRNA and/or protein expression (31-33). As these ﬁndings would predict, most AHO (both PHP type 1a and PHP type 1b) patients have heterogeneous inactivating mutations within or surrounding the G\(_{\alpha}\) coding region exons (34-36). These mutations are spread throughout the G\(_{\alpha}\) coding region exons with the exception of exon 3—probably because transcripts with this exon spliced out still produce a functional G\(_{\alpha}\) protein. There is no obvious differences in phenotype between patients with exon 3 mutations (which only affect G\(_{\alpha}\) expression) from mutations in the other downstream exons which are common to all transcripts, suggesting that the phenotype results speciﬁcally from G\(_{\alpha}\) deﬁciency. One 4 base pair deletion in exon 7 has been identiﬁed in >22 families, which probably results from a pseudophakic bypass of DNA polymerase and slipped-strand mispairing during replication (37). Several other mutations have also been reported in more than one kindred, but most mutations have been identiﬁed in one kindred each.

**Figure 1 - General organization of the GNAS locus.** The maternal (Mat, above) and paternal (Pat, below) alleles of GNAS are depicted with regions of methylation shown above (METH) and splicing patterns shown below. Transcriptionally active promoters are indicated by horizontal arrows in the direction of transcription. The alternative first exons for NESP55 (NESP), XL\(_{\alpha}\), paternal untranslated mRNA transcripts (exon 1A), and G\(_{\alpha}\) (exon 1) are shown splicing onto a common downstream exon (exon 2). The ﬁrst exon for paternal antisense transcripts are also shown (NESPAS). Common downstream exons 3-13 as well as the downstream exons of the antisense transcript (NESPAS) are not shown. G\(_{\alpha}\) is paternally imprinted in some tissues, indicated by the dashed arrow. The diagram is not drawn to scale.

**Figure 2 - Features of AHO.** A. Photograph of AHO patient showing obesity, rounded face, and short stature. B. Hand radiograph showing brachydactyly of the 4th metacarpal bone.
Most inactivating mutations are frameshift or splice junction mutations that prevent mRNA expression or missense mutations which lead to unstable proteins and reduced protein expression. With few exceptions there is no obvious genotype-phenotype correlation. However some missense mutations lead to specific defects in G\(\alpha _s\) biochemical function. Various mutations altering carboxy-terminal residues prevent G\(\alpha _s\) protein receptor interactions (38, 39). Mutations in Glu\(^{259}\) or Arg\(^{231}\) within the conformational switch regions lead to a receptor activation defect due to an inability to stabilize the active conformation (40, 41). A mutation at residue Arg\(^{231}\) leads to both increased GDP binding in the basal state and an increased rate of GTP hydrolysis in the active state (42, 43). The Ala\(^{366}\) to serine mutation leads to an interesting phenotype in which males develop the co-occurrence of PHP type 1a and gonadotropin-independent precocious puberty (44). This mutation promotes GDP release in the absence of hormone receptor stimulation.

At core body temperature the resulting protein is thermostable due to the lack of bound guanine nucleotide, resulting in PHP type 1a. At the slightly lower temperature of the testis, the protein is stable but is constitutively activated as GDP release is the rate-limiting step for G protein activation.

The fact that identical G\(\alpha _s\) mutations are present in both PHP type 1a and PPHP patients within the same kindred confirms that AHO is an autosomal dominant disorder that probably is an underlying cause of the AHO syndrome. Tissue-specific G\(\alpha _s\) expression in most tissues, and this haploinsufficiency is most likely the type 1a and PPHP, respectively) produce G\(\alpha _s\) maternally and paternally (heterozygous G\(\alpha _s\) imprints in most tissues, including the renal medulla and most likely bone. This is consistent with the fact that both maternal and paternal inheritance (the active maternal allele in proximal tubules, and therefore mutation of the active maternal allele leads to G\(\alpha _s\) deficiency in this tissue and renal PTH resistance). In contrast, paternal mutations have little effect on G\(\alpha _s\) expression or PTH resistance while mutation on the paternal allele has little effect on G\(\alpha _s\) expression or PTH sensitivity. This is confirmed by immunoblot results of renal cortical membranes isolated from wild type mice (WT) and mice with disruption of the G\(\alpha _s\) maternal allele (m/+). (Figure adapted from Weinstein, et al. Endocr Rev. 2001; 22:675-705).

Accompanying Features | Gene Defect |
---|---|
**PHP type 1a** | AHO, multihormone resistance |
| Maternal G\(\alpha _s\) null mutations |
**PHP type 1b** | Borderline TSH resistance in some cases |
| GNAs imprinting defect with maternal STX16 deletion (familial) |
| Paternal uniparental disomy of 20q |
| Maternal deletion of G\(\alpha _s\) isoleucine 382 |
**PHP type 1c** | AHO, multihormone resistance |
| No G\(\alpha _s\) defect; Defect unknown |
**PHP type 2** | Often calcium, vitamin D deficiency |
| None known; probable secondary physiological defect |

Figure 3 - Tissue-specific G\(\alpha _s\) imprinting and the effect of heterozygous null mutations. In renal proximal tubules (above) G\(\alpha _s\) is imprinted on the paternal allele (denoted with X). Mutation (Mut) on the active maternal allele (gray rectangle, left panel) leads to almost total G\(\alpha _s\) deficiency and PTH resistance while mutation on the paternal allele (right panel) has little effect on G\(\alpha _s\) expression or PTH sensitivity. This is confirmed by immunoblot results of renal inner medulla membranes from the same mice (46). (Figure adapted from Weinstein, et al. Endocr Rev. 2001; 22:675-705).

Imprinting is also the likely explanation for why PHP type 1a patients do not have skeletal PTH resistance (3) and do not lose the ability of PTH to stimulate calcium reabsorption in the distal nephron (7). Likewise it can explain why PHP type 1a patients do not show clinical resistance to other hormones also
known to work through $\alpha_s$ (e.g. adrenocorticotropic, vasopresin), as $\alpha_s$ is not imprintd in their respective target tissues.

Because of the non-specific nature of many of the features of AHO and the presence of other genetic defects that can mimic AHO [e.g. acro-osteolysis (56), AHO-like syndrome mapped to distal chromosome 2 (57)], the diagnosis of AHO should not be made in the absence of a clear family history of PHP type 1a, multihormone resistance, or documented genetic or biochemistry $\alpha_s$ defect. While typically AHO is associated with relatively benign scutaneous ossifications, a few patients with hetrozygous $\alpha_s$ mutations develop large plate-like ossifications that invade into deeper tissues such as muscle and cause severe deformity and reduced mobility (referred to as progressive osseous heteroplasia) (58). The mutations in these patients are often identical to those found in more typical AHO patients, so it does not appear that this more severe form of ectopic ossification results from more severe $\alpha_s$ mutations.

**Pseudohypoparathyroidism type 1b**

PHP type 1b patients have renal PTH resistance that is similar to that in PHP type 1a patients, but lack the features of the AHO phenotype. As in PHP type 1a, the urinary cAMP response to administered PTH is markedly reduced in these patients, indicating a proximal signaling defect (53). Several lines of evidence have ruled out PTH/PTHrP receptor defects as the cause of PHP type 1b (59, 60). In contrast to PHP type 1a, PHP type 1b is associated with normal $\alpha_s$ expression and bioactivity in erythrocyte membranes (53, 61), ruling out typical loss-of-function mutations within or surrounding the GNAS gene. PHP type 1b patients have renal PTH resistance that is similar to that observed in proximal tubules and lower levels of circulating PTH (consistent with multihormone resistance, or documented genetic or biochemistry $\alpha_s$ deficiency). While typically AHO is associated with relatively benign scutaneous ossifications, a few patients with heterozygous $\alpha_s$ mutations develop large plate-like ossifications that invade into deeper tissues such as muscle and cause severe deformity and reduced mobility (referred to as progressive osseous heteroplasia) (58). The mutations in these patients are often identical to those found in more typical AHO patients, so it does not appear that this more severe form of ectopic ossification results from more severe $\alpha_s$ mutations.

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higher PTH sensitivity), presumably due to the absence of the co-isocitrate negative regulatory element on the paternal allele. In contrast, the maternal deletion had no effect.

One family has been reported in which PHP type 1b resulted from a inherited Gαs missense mutation (deletion of isoleucine 382) within the carboxyl terminal region of Gαs (39). Biochemical analysis of the mutant protein showed it to be unable to be activated by the PTH/PTHrP receptor, although it could be activated by other Gαs receptors. Because of this selective receptor coupling defect the affected patients developed PTH resistance without developing AHO or other hormonal defects. Consistent with the paternal imprinting of Gαs, the mutation only resulted in PTH resistance when it was inherited maternally. Because Gαs is also partially imprinted in thyroid cells (48-50), one might expect that PHP type 1b patients may also have evidence for TSH resistance. Although it was originally reported that TSH resistance is not a feature of PHP type 1b, it has been more recently shown that almost 50% of PHP type 1b patients confirmed to have the exon 1A methylation defect have evidence for borderline or mild TSH resistance as well (50, 66).

Conclusions

PHP is a genetically heterogeneous group of disorders which is defined by the presence of renal PTH resistance. Because Gαs is normally expressed primarily from the maternal allele in renal proximal tubules, Gαs deficiency and PTH resistance may result from either loss-of function genetic mutations (PHP type 1a) or epigenetic changes (PHP type 1b) in the GNAS maternal allele which lead to loss of Gαs expression or expression of a bioinactive protein. Mutations on the maternal allele in PHP type 1a also lead to mild to moderate hormone resistance in other organs where Gαs is normally highly expressed and is probably involved in the underlying cause of the AHO phenotype. Patients with these mutations paternally only develop the AHO phenotype. Central PHP type 1b is usually associated with a deletion mutation in a closely linked gene that presumably leads to a specific Gαs imprinting defect by unknown mechanisms. Almost all PHP type 1b patients have lost the maternal-specific imprinting of the exon 1A GNAS gene. The epigenetic change presumably leads to Gαs deficiency in tissues, such as proximal tubules, where Gαs is normally expressed primarily from the maternal allele.

References


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