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CASE REPORT

Identification of a novel GNAS mutation for pseudohypoparathyroidism in a Chinese family

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Abstract Pseudohypoparathyroidism (PHP) is a heterogeneous group of diseases characterized by hormone resistance to receptors that stimulate adenylate cyclase. PHP-Ia patients show specific Gs-alpha protein deficiency, PTH/TSH/gonadotropin resistance, and a phenotype characterized by Albright hereditary osteodystrophy (AHO). Many heterozygous mutations in the GNAS gene encoding the Gs protein have been identified in PHP-Ia. We describe two boys with hypocalcemia and elevated serum levels of PTH in a Chinese family. The 13 exons of the GNAS gene were amplified using 15 pairs of GNAS-specific primers and analyzed by direct sequencing. We found a novel frame shift mutation in exon 11 of the GNAS gene identified in both of the two boys and their mother. This report provides another example of a Gs-alpha mutation leading to PHP.

Keywords Pseudohypoparathyroidism · Albright hereditary osteodystrophy · Gsα gene

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Introduction

Pseudohypoparathyroidism (PHP) embraces a heterogeneous group of disorders, whose common feature is resistance to parathyroid hormone (PTH), e.g., hypocalcemia, hyperphosphatemia, but with high plasma levels of PTH. It was the first human disease to be ascribed to deficient responsiveness to a hormone by otherwise normal target organs [1]. PHP is generally classified as types Ia, Ib, Ic, and II according to different mutations in the guanine nucleotide-binding protein α-stimulating polypeptide (GNAS) gene leading to each unique phenotype. Albright hereditary osteodystrophy (AHO) is often associated with PHP, characterized by several distinct clinical manifestations, including brachydactyly (i.e., variable shortening of the metacarpals, metatarsals, and phalanges), short stature, obesity, round face, and heterotopic calcification [2]. Some patients may have mental retardation.

PHP-Ia is caused by dominant mutations in GNAS gene that encodes for Gsα protein [3, 4]. GNAS gene is an imprinted gene, maternal inheritance of GNAS mutations produces offspring who have both AHO and multihormone resistance [termed pseudohypoparathyroidism type IA (PHP-Ia)] (a list of mutations is available at http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=139320&a=139320_AllelicVariant0001), while paternal inheritance of these mutations produces offspring who have only the AHO phenotype [termed pseudopseudohypoparathyroidism (PPHP)] [5, 6].

Here, we report a new heterozygous inactivating mutation in GNAS gene leading to PHP-Ia in a Chinese family.

Subjects and methods

Patients 1 and 2 are siblings, born in Oct, 1990 and Jan, 1994, respectively. They are boys. From ages 1 to 3 years,

the two patients showed signs of tetany in their hands and occasionally suffered epileptic seizures. They were noted to be of short stature in age cohorts. In 2003, both patients were referred to an endocrinologist. At that time, patient 1 was at age 13, his body height was 127 cm (−3.70SD), body weight was 32 kg (−1.41SD), while patient 2 (age 9) was 113 cm tall (−3.39SD), and his weight was 24.5 kg (−0.83SD). In addition to the short stature, both of them were also noted to have a rounded face (Fig. 1) and brachydactyly (Fig. 2) of the third, fourth, and fifth proximal phalanges bilaterally. The laboratory findings showed hypocalcemia and hyperphosphatemia. Hypocalcemia and hyperphostemia were confirmed again, and the increased serum PTH also was found and confirmed by two repeats (Table 1). Both of them were treated with 900–1800 mg elemental calcium and 180–375 IU Vit D3 supplementation daily. They were all primary school pupils without obvious metal retardation or impairment in sexual development. However, the thyroid and gonadal hormone levels and intelligence quotient were not tested in these two patients.

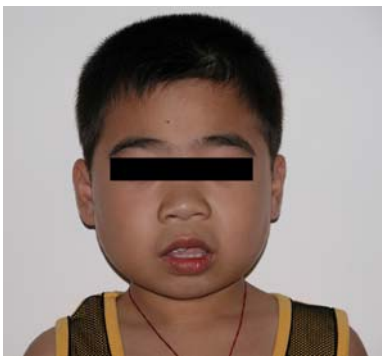

Fig. 1 Photo of the patient showing round face

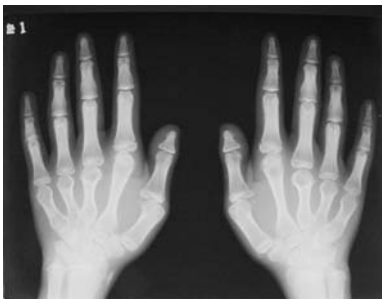

Fig. 2 Radiograph of the hands showing shortened metacarpals

In both patients, the CT scan displayed heterotopic calcification of their basal ganglia (Fig. 3). Based upon the endocrine and clinical manifestations, a presumptive diagnosis of PHP-Ia was made.

Since the two patients showed a complete AHO phenotype and typical laboratory findings, although the PTH infusion testing was impeded by the lack of commercially available PTH and Gsα protein activity was not measured, the diagnosis of PHP Ia was highly suspected.

The body height of the patients' father and mother were 162 cm (−1.56SD) and 147 cm (−2.27SD), respectively. Neither of them had rounded face or brachydactyly. Both of them were unavailable for biochemistry examination data because of their unwillingness to do so. However, their mother was highly suspected to be a case of PPHP. In order to confirm the diagnosis, the genetic analysis of GNAS was performed in two patients and their parents, together with another 12 available biological members of this family (Fig. 4).

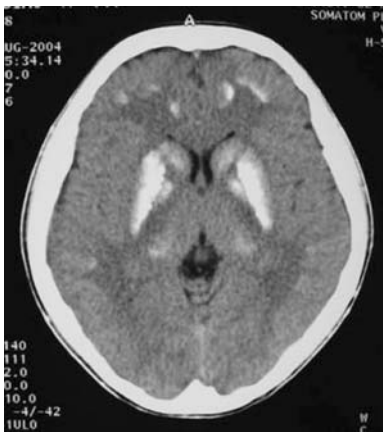

Fig. 3 CT scan of heterotopic calcification of the basal ganglia

Table 1 Clinical, biochemical, and molecular data in two patients with GNAS mutations

Case	Sex	Age (years)	Diagnosis	Clinical signs	Imaging	Ca (mmol/l); normal range: 2.1–2.6	P (mmol/l); normal range: 0.97–1.62	PTH (pmol/l); normal range: 0.7–5.6	GNAS mutation
1	M	14	PHP-Ia	Br, RF, SS	HC, SM	1.26	3.03	19.8	Exon 11 Codon 291
2	M	10	PHP-Ia	Br, RF, SS	HC, SM	1.49	2.47	19.6	Exon 11 Codon 291

Legend (arabic numbers indicate the two patients)

M male, *Br* brachydactyly, *HC* heterotopic calcification, *RF* round face, *SS* short stature, *SM* short metacarpals

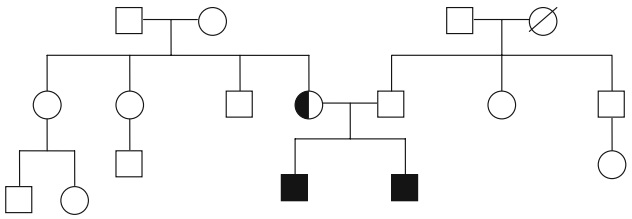

Fig. 4 Family pedigree

The study subjects provided written informed consent for genetic analysis in this study.

The clinical and biochemical findings are summarized in Table 1.

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes by protease-K digestion and phenol–chloroform extraction [7] in the two patients, their parents, the 12 other available biological family members (Fig. 4), and 60 unrelated controls.

The 13 exons of the GNAS gene were amplified using 15 pairs of GNAS-specific primers designed by us using Primer 3.0 software. Two and four sets of primers were designed for exons 13 and 1, respectively (Table 2). Amplified exons include each bordering intron region.

PCR reaction mixtures (20 μl) contained 2 μl × Buffer, 0.5 μl dNTP, 0.5 μl Taq DNA-polymerase, 1 μl genomic DNA (50 ng/μl), 1 μl of each primer, and 14 μl ddH_2O. A Peltier Thermal Cycler 225 was used to perform primary denaturation for 3 min at 95°C followed by 40 cycles of denaturation for 45 s at 94°C, annealing for 45 s at 56–60°C, and extension for 1 min at 72°C. The final extension was 10 min at 72°C.

PCR fragments amplified from genomic DNA were analyzed by electrophoresis using 1% agarose gel containing ethidium bromide (EB) and the result was visualized using UV light. PCR products were purified by shrimp alkaline phosphatase (SAP). The purified PCR products were sequenced from two directions. The sequencing reactions were performed on a Peltier Thermal Cycler 225 using a BigDye Deoxy Terminator Cycle Sequencing Kit, then analyzed using an ABI PRISM 3700 Sequencer.

Results

A novel frame shift mutation was identified in both patients and their mother. Direct sequencing of exons 1–13 of the GNAS gene of the patients using genomic DNA revealed a heterozygous mutation in exon 11 at codon 291. This new mutation was a heterozygous 1-bp deletion, which results in the change in subsequent amino acids coding sequence. This frame shift mutation was not found in the father, nor other family members or any of the controls (Fig. 5). Such a mutation in exon 11 of GNAS gene has not been described previously in PHP Ia. In order to certify the results of this mutation, we used Swiss model 8.05 software to predict tertiary structure of mutational protein according to the amino acids sequence in GNAS gene from the patients (Fig. 6).

Discussion

In this report, we present two boys with typical features of AHO and resistance to PTH. Direct sequencing of 13 exons of GNAS gene in their family members demonstrated a

Table 2 Primers used to amplify GNAS, exon 1–13

Exon	Upper primer	Lower primer
1a	5′-TCAAACAAGGTTCCCTCCTG-3′	5′-CCGGGAAAGGTACACCCTAT-3′
1b	5′-CCATCCCTTCTTCTTGCTCA-3′	5′-GAGGGACAGCTCAAGGTCTG-3′
1c	5′-CTCCTCTGGCTCTCCTGCT-3′	5′-GGTCCCTTGGATCTTTGTCC-3′
1d	5′-AGACCGAGCCTGAAGACGAT-3′	5′-CTGGCATCCTGGGAAGTTT-3′
2	5′-TCAAAAGAAAAATTAAAACCAGCA-3′	5′-TTGTTGCACCAAAGATCCAA-3′
3	5′-GCAAAGGTGTGGGATTCTTC-3′′	5′-CCCCAGCTGGATCTCATTCT-3′
4	5′-GCTGCATGCAACTTCTGGTA-3′	5′-CACAAGTCGGGGTGTAGCTT-3′
5	5′-TTTTCTTTTCAATCCCACTGC-3′	5′-TCCATCTTGAACAAAGCCCTA-3′
6	5′-CAGCAGCTAATTGCGTGAAC-3′	5′-TGTCGCTGCACTAAAAGAAAAA-3′
7–8	5′-TCCTGTTTGCCCTAACCTTCT-3′	5′-GGACTGGGGTGAATGTCAAG-3′
8–9	5′-TGGCTTTGGTGAGATCCATT-3′	5′-CAAACCTGTTGTTCCAGATGC-3′
10–11	5′-TGGCTTTGCTCTCTTTGGTT-3′	5′-GCCTGACCGTTTGAAAAAGA-3′
12	5′-CTGCTCGCTGAGAAAGTCCT-3′	5′-ACCATGGAACTGGGTAGCTG-3′
13a	5′-CCGGGCCAAGTACTTCATT-3′	5′-TAAATTTGGGGGTTCCCTTC-3′
13b	5′-CTGCTACCCTCATTTCACCTG-3′	5′-AATGACGGGAATTTGCTCAG-3′

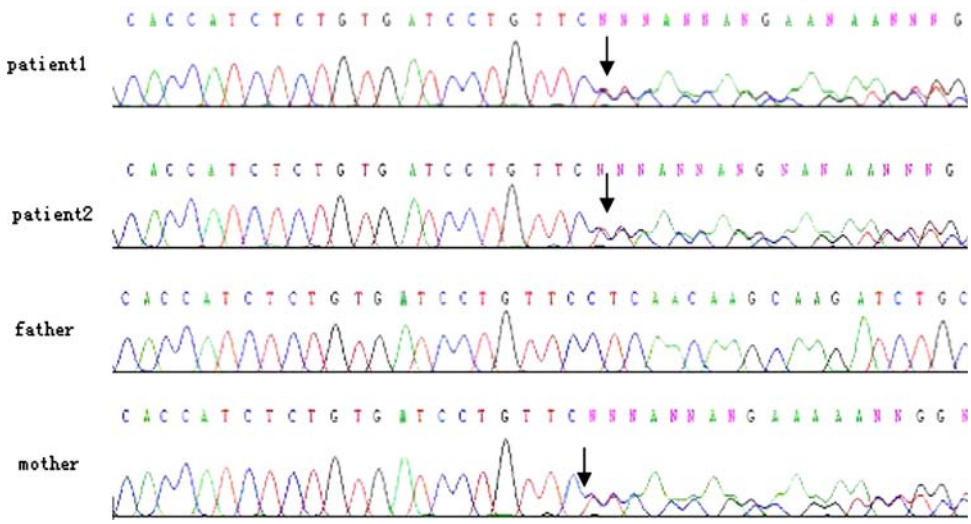

Fig. 5 The result of DNA sequencing analysis. The *arrow* shows a 1-bp frame shift deletion at codon 291

new heterozygous mutation in GNAS in the two boys and their mother. Genotyping in the boys' father revealed no sequence variation. Thus the diagnosis of PHP-Ia was confirmed in the two patients.

Different diseases are associated with mutations in GNAS: somatic activating mutations occur in several endocrine tumors and in McCune-Albright syndrome (a condition characterized by polyostotic fibrous dysplasia and endocrine dysfunction, such as hyperthyroidism and precocious puberty); PHP is caused by germline loss-of-function mutations.

In this study, we have identified a novel frameshift mutation in GNAS within affected members of two patients with features of AHO from their mother, which results in a premature stop codon within the mutant allele.

Many mutations leading to PHP-Ia and PPHP have been reported. But only the deletion in exon 7 can be considered a hot spot mutation [8–10] involving about 35% of all mutations described. Shapira et al. [11] reported two patients with Albright's hereditary osteodystrophy, multiple hormone resistance, and deficient Gsα activity. In their first patient, there was a heterozygous 1-bp deletion in exon 5 affecting proline 116, which introduced a stop codon 16 amino acids downstream. Also, this mutation has been shown to prevent the generation of a normal full-length Gsα protein, resulting in a partial deficiency (50%) of Gsα activity. In their second patient, an insertion of a C in exon 10 at codon 267 was detected. In an Italian families with PHP-Ia and features of AHO, Mantovani et al. [12] found another 2 frameshift mutations which were identified in exons 1 and 11, causing a premature stop codon in the mutant allele. However, no mutation was detected in the families in which PPHP was the only clinical manifestation. Luisa de Sanctis et al. [13] had performed GNAS mutational analysis in 43 patients with PTH resistance and/or AHO. Sequencing of the whole coding region of the GNAS gene, eight new mutations, a common deletion of 4-bp in exon 7, and two previously reported mutations had been identified in 18 subjects from 15 families.

PHP Ia shows an autosomal-dominant mode of inheritance. Retrospective analysis of published cases revealed that in the case of maternal transmission, children develop PHP Ia, whereas paternal transmission leads to offspring with PPHP [14], suggesting that GNAS might be an imprinted gene. Genomic imprinting is an epigenetic phenomenon affecting a small number of autosomal genes by which one allele (paternal or maternal) has partial or total loss of expression [15]. If the GNAS paternal allele is imprinted in hormone target tissues, then maternal transmission of a GNAS mutation would lead to markedly decreased Gsα expression (and hormone resistance) due to imprinting of the paternal allele and mutation of the maternal allele. Actually, the differential methylation is the most important candidate for imprinting mark [16]. For example, in renal proximal tubules only the maternal gene is expressed. Normally, this is sufficient to mediate PTH action. However, with mutations that impair or interfere with methylation of the maternal gene, then no GNAS is expressed in proximal tubule cells. In the previous study, Luisa de Sanctis et al. [13] found that of all of those who had PHP-Ia, the mutated alleles were inherited from the mothers, who in turn had AHO (PPHP), consistent with the proposed imprinting mechanism. Indeed, in our study, the two patients with PHP-Ia inherited the disease from their mother, in agreement with a possible role of paternal imprinting in the genesis of AHO.

Levine et al. [17] demonstrated that a defect in the stimulatory guanine nucleotide-binding regulatory protein

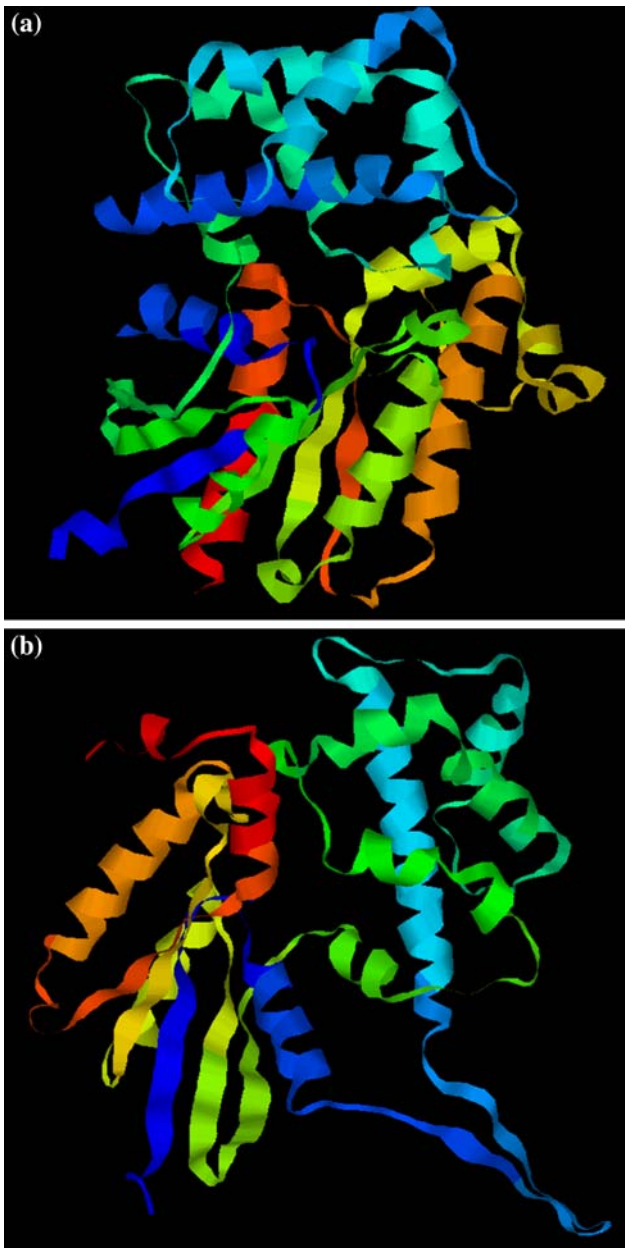

Fig. 6 The tertiary structure of Gsa protein. **a** Tertiary structure of mutational protein derived from the amino acids sequence in normal Gsa protein. **b** Tertiary structure of mutational protein derived from the amino acids sequence in Gsa protein from our patients

of adenylate cyclase was responsible for resistance to multiple hormones in some cases of PHP-Ia. Furthermore, Carlson and Brickman [18] described a blunted plasma cAMP response to the infusion of the beta-adrenergic agonist isoproterenol. The limitations of our study are lack of other hormone levels, PTH stimulatory test, and Gsα protein activity in patients and their parents.

In conclusion, a novel mutation within the Gsα gene (GNAS) leading to PHP-Ia was identified in a Chinese family. Our results further expand the molecular pathophysiology of PHP and underscore its genetic heterogeneity. Our results stress the importance of a complete investigation of GNAS gene in patients of AHO and PHP Ia, especially when testing for Gsα activity is not feasible.

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