Identification of a novel mutation in a patient with pseudohypoparathyroidism type Ia

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Abstract

Pseudohypoparathyroidism type Ia (PHP Ia) is a disorder characterized by multiform hormone resistance including parathyroid hormone (PTH) and Albright hereditary osteodystrophy (AHO). It is caused by heterozygous inactivating mutations within Gs alpha-encoding GNAS exons. A 9-year-old boy had typical clinical and laboratory abnormalities including hypocalcemia, hyperphosphatemia, PTH resistance, multihormone resistance and AHO (round face, short stature, obesity, brachydactyly and osteoma cutis) which were suggestive of PHP Ia. He had a history of repeated convulsive episodes which started from the age of 2 months. Cranial computed tomography scan showed bilateral calcifications in basal ganglia and his intelligence scale indicated mild mental retardation. Family history revealed that maternal relatives of the patient, grandmother and two siblings of his mother, had features suggestive of AHO. Sequencing of the GNAS gene of the patient identified a heterozygous nonsense mutation within exon 11 (c.637 C>T). The C>T transversion results in an amino acid substitution from Gln to stop codon at codon 213 (p.Gln213*). To our knowledge, this is a novel mutation in GNAS.

Key words: pseudohypoparathyroidism (PHP), pseudohypoparathyroidism type Ia (PHP Ia), GNAS gene, Albright hereditary osteodystrophy (AHO)
**Introduction**

Pseudohypoparathyroidism (PHP) is a disorder of end-organ resistance primarily affecting the actions of parathyroid hormone (PTH)\(^1\). It is characterized by hypocalcemia and hyperphosphatemia in association with increased secretion of parathyroid hormone due to decreased target tissue responsiveness to PTH. Patients with PHP Ia are not only resistant to PTH, but also to other hormones that bind to receptors coupled to stimulatory G protein (Gs alpha)\(^2\). The GNAS gene is located on chromosome 20q13.11 and consists of 13 exons and 12 introns\(^3\). PHP-Ia is caused by heterozygous mutations affecting one of the 13 GNAS exons encoding Gs alpha\(^4\).

We report here a 9-yr-old boy with PHP Ia with a nonsense mutation of c.637 C>T in the GNAS gene, which is novel.
Case report

A 9-year-old boy was admitted to our hospital with painful subcutaneous soft tissue masses with calcifications on the right toes and posterior chest wall. He had a history of repeated convulsive episodes from the age of 2 months. Physical examination showed short stature (120 cm, 3rd percentile), round face (Fig.1), brachydactyly with short metacarpals (Fig.2) and subcutaneous ossifications on the 2nd toe tip and 1st web space of the right foot and the left lower posterior chest wall (Fig.3). His weight was 30 kg (50th percentile), thus having a normal body mass index (20.83 kg/m², 85~90th percentile).

He was the first child of non-consanguinous Korean parents. He was born at full term by cesarean section with a birth weight of 3.3 kg. Family history revealed that his maternal grandmother had features suggestive of AHO, such as short stature, round face, obesity and brachydactyly. His mother had normal appearance apart from short stature, but two of her siblings had short stature, round face and obesity. We could not make further investigation because the patient lost contact with his mother and maternal family after his parents got divorced.

Laboratory tests revealed hypocalcemia (4.2 mg/dL, normal range 8.8-10.8 mg/dL), hyperphosphatemia (8.9 mg/dL, normal range 3.7-5.6 mg/dL) and elevated serum PTH (146.5 pg/mL, normal range 9-65 pg/mL). He also showed elevated plasma TSH (6.978 uIU/mL, normal range 0.5-4.8 uIU/mL), FSH (5.6 uIU/mL, normal range 0.26-3.0 uIU/mL) and LH (1.2 uIU/mL, normal range 0.02-0.3 uIU/mL), which suggest multiple hormone resistance. Urinary phosphorous and cAMP response after infusion of synthetic human PTH (teriparatide acetate) was attenuated (Ellsworth-Howard test) (Table 1). Cranial computed tomography scan demonstrated bilateral calcifications in the basal ganglia (Fig.4). His intelligence scale (KEDI-WISC) indicated mild mental retardation (Intelligence quotient 67).
He was diagnosed as having PHP type Ia based on physical and laboratory findings; hypocalcemia, hyperphosphatemia, PTH resistance, presence of AHO, multiple hormone resistance and mental retardation. He was started on phosphate-binding substance (aluminum hydroxide 80 mg/kg/day), vitamin D (alfacalcidol 1 mcg/day) and calcium carbonate (50 mg/kg/day). Three months later, his serum calcium was elevated from 4.2 to 6.0 mg/dL, and phosphorus was decreased from 8.9 to 8.6 mg/dL. The patient's clinical symptoms of pain, seizure and hyperactivity were reduced.

To support the diagnosis of PHP type Ia, we performed DNA analysis of the GNAS gene. Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification kit following the manufacturer’s instructions (Promega, Madison, WI). All coding exons and their flanking introns of the GNAS gene were amplified using primer sets designed by the authors. Polymerase chain reaction (PCR) was performed with a thermal cycler (model 9700, Applied Biosystems, Foster City, CA, USA) as follows: 32 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C of 30 seconds. After treatment of amplicon (5 uL) with 10 U shrimp alkaline phosphatase and 2 U exonuclease I (USB Corp., Cleveland, OH, USA), direct sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) on theABI Prism 3100 x 1 genetic analyzer (Applied Biosystems).

Sequencing of the amplified GNAS genomic DNA fragments revealed a heterozygous nonsense mutation within exon 11 (c.637 C>T) (Fig.5). The C>T transversion results in an amino acid substitution from Gln to stop codon at codon 213 (p.Gln213*). To our knowledge, this is a novel mutation in GNAS. We also performed DNA sequencing of the GNAS gene of his father, but no mutation was detected.
Discussion

PHP is a heterogeneous disease characterized by PTH resistance and classified as types Ia, Ib, Ic and II, according to its different pathogenesis and phenotype\(^5\). In contrast to the situation in hypoparathyroidism, in PHP the parathyroid glands are normal or hyperplastic and they can synthesize and secrete PTH \(^6\).

Heterozygous inactivating mutations within Gs alpha-encoding GNAS exons are found in patients with PHP-Ia, who also show resistance to other hormones and a constellation of physical features called AHO. Patients who exhibit AHO features without evidence for hormone resistance, who are said to have pseudopseudohypoparathyroidism (PPHP), also carry heterozygous inactivating Gs alpha mutations\(^1\). There is some evidence to suggest that the Gs alpha mutation is paternally transmitted in patients with PPHP and maternally transmitted in patients with PHP-Ia. The gene may be imprinted in a tissue-specific manner\(^6\).

In our case, the patient presented the characteristics of AHO and hormone resistance. DNA sequencing of the patient and his father revealed that only the patient had mutations. The presence of slight AHO in the mother’s side of the patient may suggest that the mutation came from his mother, but we were unable to confirm this because the DNA of the patient’s mother or maternal family was not available at the time of the study. Although we could not obtain molecular data from the maternal family of the patient, considering variation of phenotypes due to genomic imprinting, we were able to explain that the patient had the genotype and phenotype of PHP Ia. In the maternal family of the patient, his mother had milder features suggestive of AHO, whereas her mother and two siblings had characteristics that were more strongly suggestive of AHO. We can suspect the possibility of a hypofunctioning GNAS mutation like mosaicism, but it was impossible to validate.
Mutational analysis of GNAS gene is a useful method for identifying genetic abnormalities as well as making diagnosis and differentiation of various subtypes of PHP\textsuperscript{7).} There are previous reports of Korean patients with PHP Ia and PPHP who were confirmed by genetic analysis. Park et al.\textsuperscript{8)} reported a nonsense mutation of c.94A>T (p.K32X) and a frame shift mutation of c.344_345insT (p.V117RfsX23) in patients with PHP Ia, of which the former was novel. In a study of patients with PHP Ia and PPHP, Jin et al.\textsuperscript{7)} identified two novel mutations; c.569_570del mutation (p.Tyr190CysfsX19) and a splicing mutation (c.659 + 1G>A). GNAS has been termed one of the most complex gene loci in the human genome\textsuperscript{3).} Although many mutations have been identified in GNAS, c.637 C>T nonsense mutation is deemed novel. This identification stresses that GNAS gene analysis is important for the diagnosis of PHP. Further investigation is required to determine the various genotypes of PHP.
References


Table 1: The results of Ellsworth-Howard test

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<tr>
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<th>Urine phosphorous (mg/hr)</th>
<th>Urine c-AMP (nmol/mg cr)</th>
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<tbody>
<tr>
<td>U1</td>
<td></td>
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<tr>
<td>U2</td>
<td>11.3</td>
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<tr>
<td>U3</td>
<td>29.0</td>
<td>1.6</td>
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<tr>
<td>U4</td>
<td>20.2</td>
<td>0.3</td>
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<td>U5</td>
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The urine sample obtained 2 hours before administration of human PTH (Fosteo, teriparatide) was designated U1, and samples collected thereafter at 1-hour interval were designated U2, U3, U4, and U5. PTH was injected at the time between U3 and U4.

Phosphaturic response : (urinary phosphate U4 + urinary phosphate U5) – (urinary phosphate U2 + urinary phosphate U3)

cAMP response : Urinary cAMP U4 – urinary cAMP U3

In this case, phosphaturic response (0.25 fold) was blunted (normal range : 10 fold) and urinary cAMP excretion was not increased.
Figure 1. General appearance of the patient shows short stature and round face.
Figure 2. (A) The patient’s hands and (B) an X-ray of right hand show brachydactyly with short metacarpals.
Figure 3: (A) The patient’s posterior chest wall with subcutaneous ossifications and (B) a whole body bone scan show irregular increased activity in the area of calcification. (C) An X-ray of the patient’s right foot shows calcification of subcutaneous tissue of 1\textsuperscript{st} web space (long arrow) and 2\textsuperscript{nd} toe tip (short arrow).
Figure 4: Cranial computed tomography scan shows multiple calcifications in cerebral subcortex and basal ganglia.
Figure 5: Sequencing analysis of exon 11 shows c.637 C>T nonsense mutation (red box).