



Pseudohypoparathyroidism type 1b due to paternal uniparental disomy of chromosome 20q: A case report

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Pseudohypoparathyroidism type 1b (PHP 1b) is the result of end organ resistance to parathyroid hormone (PTH) in the absence of any features of Albright's hereditary osteodystrophy. There are two subtypes of PHP 1b with different genetic mechanisms. One subtype is related to a maternally derived 3kb microdeletion involving *STX 16* gene, and is inherited in an autosomal dominant mode. Familial autosomal dominant inheritance of PHP 1b is relatively rare. The other subtype is associated with more extensive loss of imprinting at the *GNAS* locus that affects at least one additional differential methylated (hypermethylation at neuroendocrine secretory protein and hypomethylation at antisense transcript and or extra-large stimulatory G protein region) without microdeletion of the *STX 16* or *AS* gene. It can be sporadic due to an imprinting defect in the *GNAS* gene. In our case, an 8-year-old girl was referred for suspected PHP with no feature of Albright hereditary osteodystrophy. Blood test results revealed hypocalcemia and hyperphosphatemia. Elevated PTH was also checked. There was no family history of endocrine or developmental problem. Her intelligence was normal, but she had inferior sociability at that time. Based on above, we diagnosed a rare case of paternal uniparental disomy of the long arm of chromosome 20 as the cause of PHP 1b by microsatellite marker test of chromosome 20.

Key words: Pseudohypoparathyroidism, Parathyroid hormone, Uniparental disomy.

Introduction

Pseudohypoparathyroidism (PHP) is the result of end-organ resistance to parathyroid hormone (PTH). PHP is characterized by hypocalcemia, hyperphosphatemia, and increased PTH levels. PHP is unusual and its exact prevalence is unclear. Identified PHP types include as type 1a, type 1b, type 1c, and type 2. PHP type 1a presents with character of Albright hereditary osteodystrophy (AHO), such as short stature, obesity, round face, subcutaneous ossifications, brachydactyly, and mental retardation. In PHP type 1a, end-organ resistance is usually not limited to the PTH

actions, but also most commonly influence thyroid stimulating hormone and, rarely, gonadotrophins and growth hormone releasing hormone [1,2]. PHP 1b typically shows no features of AHO and is characterized by renal PTH resistance but preserved PTH responsiveness in bone and other tissues resulting in lack of AHO features. Generally, PHP 1b also presents no other endocrine abnormalities, although resistance to thyroid-stimulating hormone has been rarely reported. PHP type 1c is the rarest subtype. Furthermore, It is characterized by AHO and, resistance to PTH and other hormones in face of normal *Gsα* activity.

PHP type 2 does not present with any other hormone resis-

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Conflict of interest: The authors declare that they do not have any conflicts of interest.

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tance and is characterized by normal urinary cyclic adenosine monophosphate (cAMP) excretion and reduced phosphate excretion. Patients with type 2 have no evidence of AHO such as acrocystosis. The underlying pathophysiology of PHP is a genetic and epigenetic change in the adenylate cyclase-stimulating G alpha protein (*GNAS*) locus, which encodes the alpha subunit of the receptor G-protein, which changed messenger action [3]. In general, patients with PHP 1b lack mutations within *GNAS* exons encoding *Gs α* , but have methylation and imprinting defects that result in the absence of maternal *Gs α* expression. We report a sporadic PHP 1b due to paternal uniparental disomy (UPD) of chromosome 20 with clinical manifestations and familial genetic analysis.

Case

An 8-year, 10-month-old girl, was carried into the emergency room due to 1–2 minute episode of convulsion without aura. She had complained for weeks of a tingling sensation in both hands and of fatigue. Her mother had recently noted unconsciousness and lack of reply to verbal interactions. She was born at 41 weeks of gestation by normal spontaneous vaginal delivery. Birth weight was 4.18 kg (>95 percentile). Development was appropriate to her age. Past history was unremarkable except for infantile asthma. She showed some difficulties in peer relationships at the elementary school level. A psychological evaluation

was not done. At the time of admission, her height was 140 cm (>97 percentile), weight was 33 kg (75–90 percentile), and body mass index was 16.8 kg/m² (50–75 percentile). Blood pressure was normal (102/66 mmHg). She had a round face. Dental characteristics included slightly enlarged pulp chamber and microdontia, but no hypoplasia. Metacarpal features of AHO were absent. Blood test results revealed hypocalcemia (ionized calcium 2.0 mg/dL, normal range 4.5–5.4 mg/dL; total calcium 4.5 mg/dL, normal range 8.2–10.5 mg/dL), hyperphosphatemia (phosphate 7.4 mg/dL, normal range 2.3–4.5 mg/dL). Elevated PTH (415 pg/mL, normal 15–65 pg/mL) was also checked. 25 Hydroxyvitamin D3 level was 20.98 ng/mL (normal range 8.0–51.9 ng/mL) and, 1,25-dihydroxyvitamin D3 was 64.14 pg/mL (normal 15–65 pg/mL). Normal levels of thyroid hormone and other pituitary hormones were evident. Electrocardiogram revealed prolonged QT (corrected QT interval 0.45 sec). Electroencephalogram revealed moderately severe diffuse cerebral dysfunction, which was more severe in the left hemisphere. Computed tomography scan revealed calcification in both basal ganglia and the right frontal lobe (Fig. 1). The patient was initially treated with intravenous calcium. Oral calcium supplement and 2 μ g of 1, 25-hydroxylated vitamin D metabolite (calcitriol) were supplied when the total calcium level was 8.1 mg/dL. Her two brothers and parents did not have any phenotypic and biochemical proof of PHP. The sequence was normal. Methylation specific-multiplex ligation-dependent probe amplification (MS-MLPA; ME031 probemix; MRC-Holland, Amsterdam, The Netherlands) to detect gene dosage and methylation status in *GNAS* region showed an abnormal methylation pattern of paternal UPD (Fig. 2). To confirm patUPD20, 11 short tandem repeat markers dispersed across chromosome 20 were assessed (Fig. 3). The patient was diagnosed as paternal UPD in chromosome 20. Molecular genetic analysis for the paternal UPD in chromosome 20 in her brothers was normal.



Fig. 1. Calcifications in both basal ganglia were evident on brain computed tomography.

Discussion

PHP 1a is caused by heterozygous inactivating mutation of the *GNAS* gene that encodes the stimulatory G protein, which is necessary for the action of PTH and other hormones including thyroid stimulating hormone. The stimulatory *Gs α* that stimulates adenyl cyclase and generates cAMP is expressed by both maternal and paternal alleles of *GNAS* in most tissues. However, only the maternal allele of *GNAS* is expressed in the proximal renal tubule, thyroid, gonads, and pituitary [4].

PHP 1b is generally not related with AHO. All lack methylation

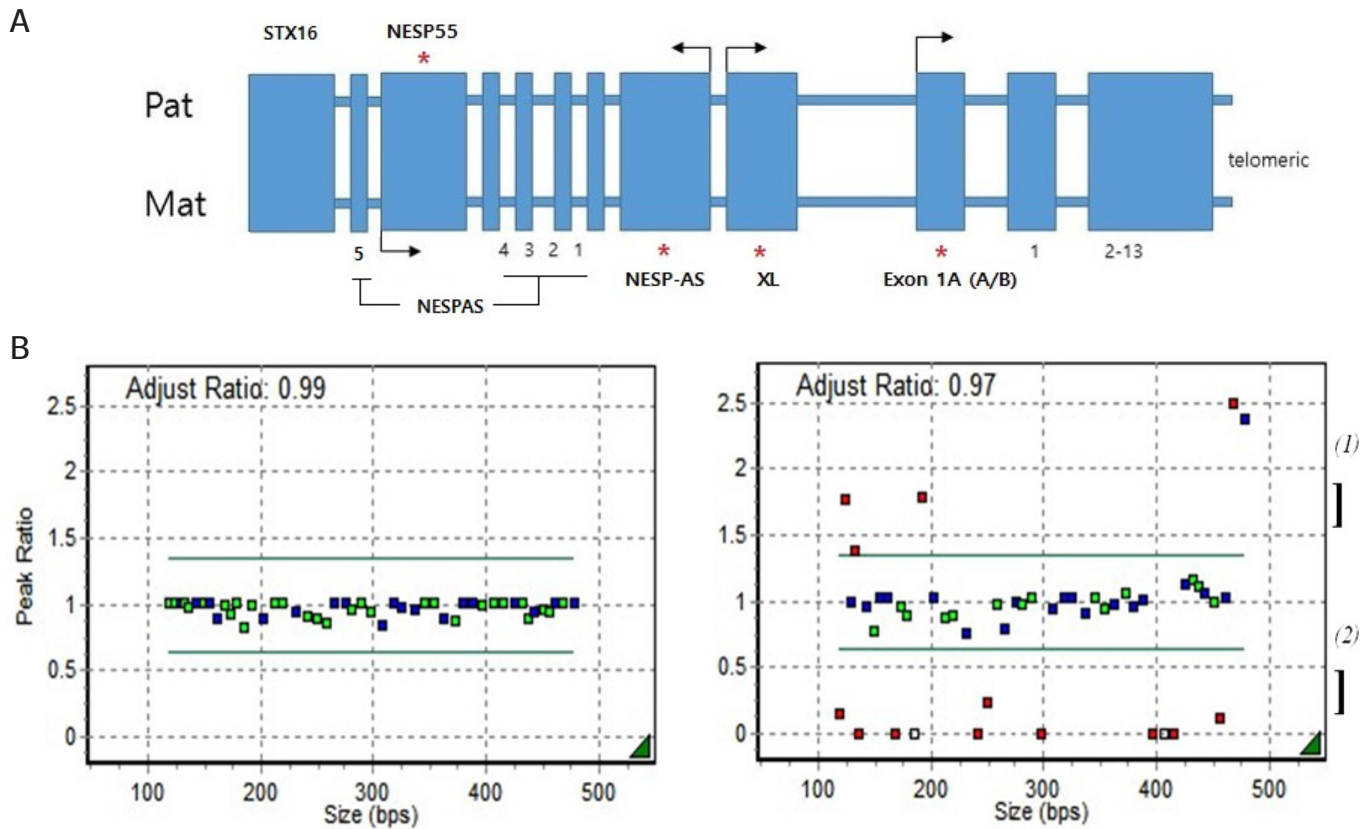


Fig. 2. (A) Methylation patterns of *GNAS* locus included methylation specific multiplex ligation dependent probe amplification analysis. (B) Results of methylation specific multiplex ligation dependent probe amplification (MS-MLPA) analyses. MS-MLPA was performed with ME031 probemix (MRC-Holland). The left panel depicts normal dosage of genes in *GNAS* locus, indicative of no large deletion or duplication mutation in *GNAS* locus. The right panel depicts abnormal methylation status, in which the neuroendocrine secretory protein (NESP) region has hypermethylation status indicated by restriction enzyme *HhaI* resistance (1), whereas NESP-AS (antisense), extra-large stimulatory G protein (XL), and *GNAS* 1A regions show hypomethylation status indicated by *HhaI* sensitivity (2). This loss of maternal methylation pattern and gain of paternal methylation pattern indicate presumed paternal uniparental disomy, at least in the *GNAS* locus. Abnormal hypermethylation spots (1) were for NESP, 123 bp, 132 bp, and 192 bp, respectively. Hypomethylation spots (2) were for NESP-AS (135 bp, 167 bp, and 249 bp), XL (119 bp, 299 bp, 415 bp, and 427 bp), and exon 1 alternative (A/B) of *GNAS* (240 bp and 397 bp). *The NESP region is paternally methylated region, and NESP-AS, XL, and exon 1 alternative (A/B) of *GNAS* are maternally methylated regions. Pat, paternal allele; Mat, maternal allele; NESP55, neuroendocrine secretory protein 55.

of the alternate exon 1A, an epigenetic defect that is introduced to suppress expression of the functional exon 1-containing *Gsα* transcript in renal tissues only. Thus, the loss of methylation of the maternal exon 1A allele gives rise to the silencing of the maternal in addition to paternal *Gsα* allele, causing PTH resistance specifically in renal proximal tubule cells [5,6]. Our patient presented at about 9 years of age with hypocalcemia with an elevated PTH level without AHO phenotype. Thyroid function test was normal, and no other endocrine abnormalities were evident. PHP 1b is caused by deletions in the differentially methylated region of the *GNAS* locus, situated chromosome 20q13.11 [7,8]. In other cases, it has been shown to be sporadic, caused by imprinting defect in the *GNAS* gene. Paternal uniparental isodisomy of the long arm of chromosome 20 may be the rare factor of PHP 1b, as in this case.

The identification of hereditary forms of PHP 1b is essential for clinical management and genetic consultation. A maternally inherited 3-kb deletion is present within the *STX16* gene, and is inherited in autosomal dominant PHP 1b, which is featured by isolated loss of methylation at the exon A/B differentially methylated region of *GNAS*. The mechanisms underlying broad *GNAS* methylation changes are still unknown and are obviously sporadic cases. There may be an epigenotype-phenotype correlation in paternal UPD 20q, depending on the length of the UPD segment [8,9]. The longer the segment of upd(20)pat, the earlier the start of symptoms and the more likely to have overgrowth marks like macrosomia and macrocephaly [9]. The degree of methylation aberration was not useful in predicting the severity and type of disease manifestation. Like in our case, paternal uniparental isodisomy of total chromosome 20 can be the rare

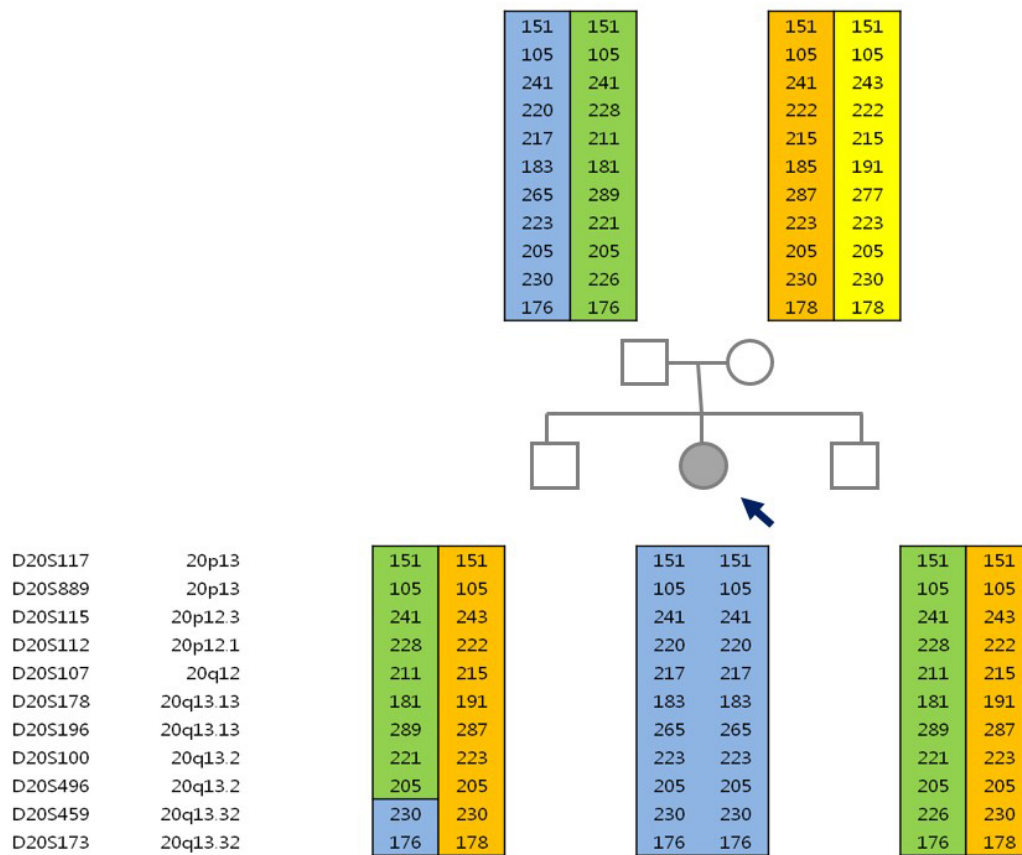


Fig. 3. Short tandem repeat marker test of chromosome 20. To identify pat uniparental disomy of the patient and her siblings, 11 markers dispersed across chromosome 20 were examined. The patient (arrow) had paternal marker sets in both alleles within a chromosome 20 region. The elder and younger brothers displayed normal segregations.

cause of PHP 1b.

Other transcripts produced by the locus are imprinted. For example, XL and A/B are paternally expressed, and NESP55 is maternally expressed [9]. These transcripts are produced by alternative first exons located upstream of exon 1 that separately splice onto exons 2-13. A noncoding antisense transcript Nespas is also exclusively paternally expressed. The promoters of the imprinted *GNAS* transcripts show differential methylation, with the imprinted allele being methylated and the active allele not being methylated. Most patients with sporadic PHP 1b have *GNAS* imprinting abnormalities involving multiple differentially methylated regions, but the molecular mechanisms underlying these *GNAS* imprinting defects remains unclear.

In our case, pat UPD of chromosome 20q12-13.32 which includes the *GNAS* locus was confirmed through the short tandem repeat marker test of chromosome 20. Several patients with PHP 1b due to pat UPD20 have been described with features including relatively high birth weight, early onset obesity, relatively tall stature, and macrocephaly [9]. In our case, elevated birth weight and tall stature were noted, but obesity and mac-

rocephaly were not evident. There was no family history of endocrine or developmental problem. Her intelligence was normal, but she had inferior sociability. We report a sporadic PHP 1b because of paternal UPD of chromosome 20 involving the long arm of chromosome 20 with clinical presentation and familial genetic analysis.

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