

# Pseudohypoparathyroidism Type 1b: First Case Report in Chinese and Literature Review

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## Abstract

**Objective:** Pseudohypoparathyroidism type 1b is a rare genetic endocrine disease. We would like to increase awareness of this condition and highlight what clinical geneticists can contribute in the patient management. **Method:** Case report and literature review. **Case presentation:** A 10-year-old boy was referred for suspected pseudohypoparathyroidism with parathyroid hormone resistance on biochemical investigation. Physical examination showed no features of Albright hereditary osteodystrophy and normal intelligence. There was no family history of endocrine or developmental problem. Based on above, the diagnosis of pseudohypoparathyroidism type 1b was suspected. It was subsequently confirmed by epigenetic change over *GNAS* gene using methylation genetic study. **Conclusion:** Pseudohypoparathyroidism 1b is a rare endocrine disease caused by epigenetic change in *GNAS* gene. All patients with parathyroid hormone resistance should be referred to the clinical geneticist for proper assessment, genetic testing, and genetic counselling.

## Key words

Chinese; Epigenetic; Pseudohypoparathyroidism type 1b

## Introduction

Pseudohypoparathyroidism (PHP) is a clinically and genetically heterogeneous disease characterised by parathyroid hormone resistance (PTH). It is an uncommon disorder and can be further divided into several subtypes based on the presence of hormonal resistance pattern, molecular abnormalities and Albright hereditary osteodystrophy (AHO). Pseudohypoparathyroidism type 1b

(PHP-1b) (MIM#603233) is a rare subtype of PHP that is caused by loss of imprinting at the *GNAS* locus at chromosome 20q13.32 region. Methylation study targeting the *GNAS* gene will show specific pattern including hypermethylation at the *NESP55* and hypomethylation at the *NESPAS*, *GNAS XL* and *GNAS A/B* differential methylation regions. Affected individuals mainly have renal resistance to PTH with some patients also having mild resistance to thyroid stimulating hormone. Typically, PHP-1b patients do not have features of AHO, though obesity, short stature and subtle bony abnormalities have been reported.<sup>1</sup> Up to now, no more than 100 cases of PHP-1b were reported in the literature. Here we reported the first case in Chinese population that was confirmed by molecular testing.

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## Case Report

The proband was a 10-year-old boy. He was the first child of a non-consanguineous Chinese couple. His perinatal history was uneventful, and he enjoyed good past health

with normal development. There was no family history of endocrine problems or developmental delay. He initially presented with absence seizure at the age of 6. Investigations at that time showed hypocalcaemia, hyperphosphataemia and elevated PTH level. The initial biochemistry results were summarised in Table 1.

Based on the initial workup, the diagnosis of PHP was made. Further investigations after correction of hypocalcaemia, including brain imaging and electroencephalogram were normal. Skeletal survey showed no rachitic changes. He was put on vitamin D and calcium supplement and was regularly followed up in the endocrine clinic. Repeated ultrasound kidney showed no nephrocalcinosis. He was subsequently referred to Clinical Genetic Service for genetic investigation and counselling at the age of 10.

Physical examination at the genetic clinic showed normal growth parameters with both body weight and height at the 25th percentile. He had no cataract or craniofacial dysmorphism. There was no features of AHO like round face, brachydactyly and shortened metacarpal/metatarsal bones. Examination of parents was also normal. In view of the normal intelligence and lack of AHO features, PHP type 1b was suspected. Molecular testing using methylation specific-multiplex ligation dependent probe amplification (MS-MLPA) targeting the *GNAS* gene (SALSA MLPA kit ME031-A1 MRC-Holland) was performed. The results showed hypermethylation at the *NESP55* and hypomethylation at the *NESPAS*, *GNAS XL* and *GNAS A/B* differential methylation regions (Figures 1a and 1b) compared with normal controls. There was no copy number change of the *GNAS* and *STX16* genes. Parental genetic testing were normal. Paternal uniparental disomy of chromosome 20 was excluded by single nucleotide

polymorphism (SNP)-array study (Agilent SurePrint G3 Human CGH+SNP Microarray 4x180K) (Figure 1c). The overall picture substantiated the diagnosis of sporadic PHP-1b due to imprinting defect.

## Discussion

With the coexistence of hypocalcaemia, hyperphosphataemia, elevated PTH levels without vitamin D and renal abnormalities, the diagnosis of PHP was easily made. However, in difficult cases, the diagnosis could only be confirmed by PTH infusion test or subcutaneous challenge before the molecular era. With more understanding of the underlying genetic and epigenetic mechanisms of PHP together with the highly sensitive and specific *GNAS* assay, *GNAS* molecular testing should be considered the first line investigation for all suspected PHP cases. The detection rates for typical PHP-1a and PHP-1b by *GNAS* mutation/epimutation screening are approximately 70% and 80-90%, respectively.<sup>2</sup> Apart from confirmation of the clinical diagnosis that serves to guide patient management, knowledge of the underlying genetic or epigenetic defect also facilitates genetic counselling in terms of risk assessment and prenatal diagnosis.

In contrast to PHP-1a characterised by presence of AHO features, developmental delay and/or other hormones resistance which is caused by heterozygous maternally derived mutations of the *GNAS* gene, PHP-1b is caused by loss of imprinting at the *GNAS* complex locus. The *GNAS* locus is located at chromosome 20q13.32 region and consists of at least 4 differential methylated regions (DMRs) (Figure 1a). By parent-specific methylation of different promoters, different transcripts are produced from the *GNAS* locus, namely *Gsα* ( $\alpha$  subunit of the stimulatory G protein), *XLαs* (*Gαs* extra-large variant), *NESP55* (neuroendocrine protein 55), *A/B* (untranslated exon A/B) and *AS* (antisense transcript).<sup>3-6</sup> The *NESP55* is maternally expressed while *XLαs*, *A/B* and *AS* are paternally expressed.<sup>3,6</sup> All PHP-1b patients have loss of methylation at the *A/B* DMR. There are 2 subtypes of PHP-1b with different genetic mechanisms. One subtype, accounting for 10-15% of PHP-1b cases, is associated with a maternally derived 3 kb microdeletion involving the *STX16* gene, and is inherited in autosomal dominant fashion.<sup>7</sup> The other subtype is associated with more extensive loss of imprinting at the *GNAS* locus that affects at least one additional DMR (hypermethylation at *NESP* and hypomethylation at *AS* and/or *XL* region) without microdeletion of the *STX16* or *AS*

**Table 1** Initial Biochemistry workup. Normal ranges were shown in brackets

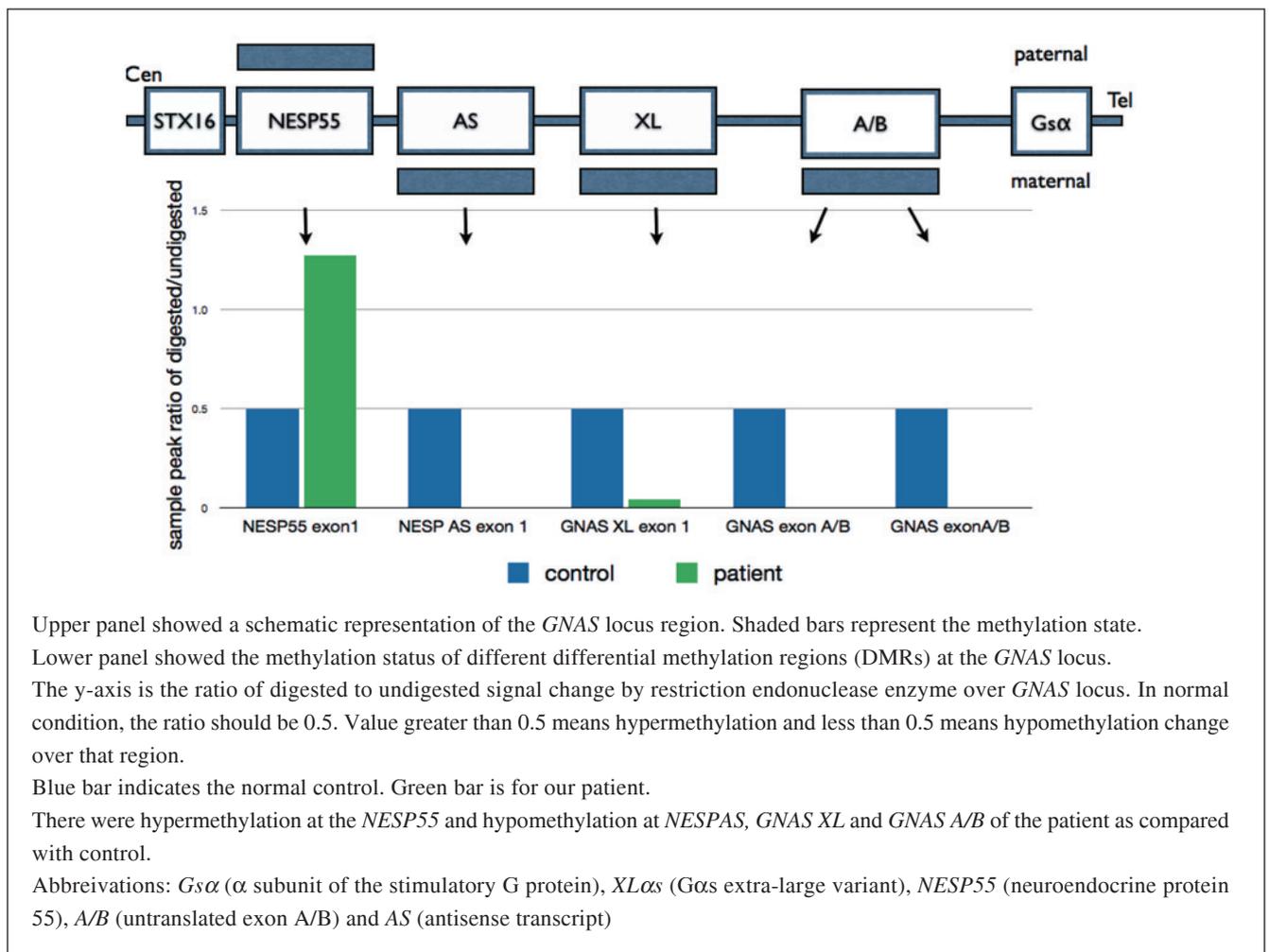
	At presentation
Corrected Ca, mmol/l (2.07-2.37)	1.60
PO <sub>4</sub> , mmol/l (0.95-1.72)	2.93
ALP, U/L (82-296)	167
PTH, pmol/l (1.30-9.30)	44.9
Mg, mmol/l (0.72-0.97)	0.80
Plasma urea and creatinine	Normal
Urine Ca/Creatinine ratio, mmol/mmol (0.06-0.74)	<0.01
Vitamin D level	Not done
Thyroid function	Normal

gene. It is sporadic and accounts for 80-85% of the cases.<sup>7</sup> In order to study the epigenetic defects in PHP-1b, methylation study with techniques like methylation specific-multiplex ligation dependent probe amplification (MS-MLPA), methylation-specific polymerase chain reaction or pyrosequencing can be used. Despite recent progress in the understanding of the epigenetic defects associated with PHP-1b, the exact pathogenesis is yet to be elucidated.

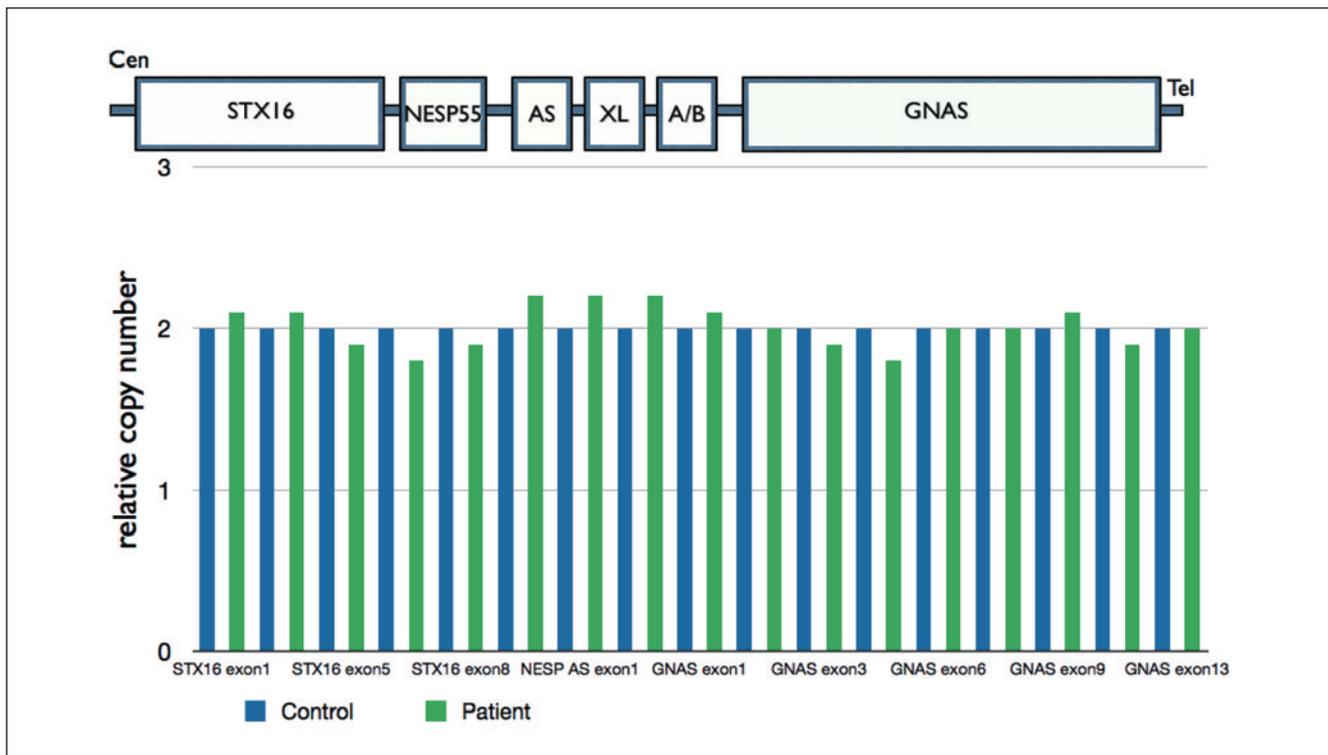
The clinical manifestations of autosomal dominant and sporadic forms of PHP-1b are similar. Therefore, distinguishing between them by appropriate molecular testing is important for genetic counselling. In the literature, about 2 to 20% of PHP-1b cases were reported to be associated with paternal uniparental disomy (UPD) chromosome 20, thus UPD20 should be excluded in all case of PHP-1b, either by microsatellites analysis or SNP-

array study. If UPD20 is excluded, the recurrence risk to subsequent siblings and offspring is negligible, while for the autosomal dominant form the recurrence risk is 50%.

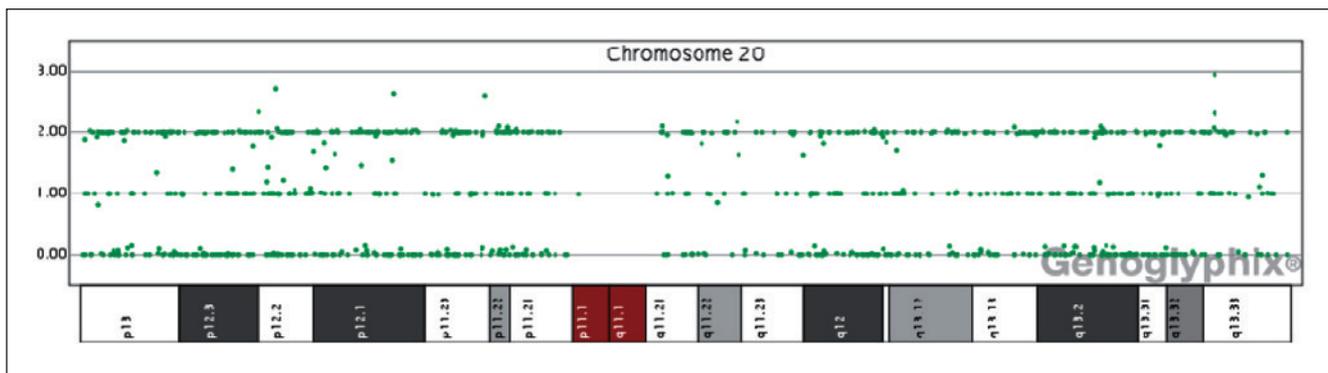
In summary, we have reported the first case of sporadic PHP-1b due to imprinting defect in Chinese. In the presence of biochemical features of PHP like hypocalcaemia, hyperphosphataemia, elevated PTH, normal vitamin D and renal function, but lack of intellectual disability or skeletal features of AHO, the diagnosis of PHP-1b should be the considered. Epigenetic/epimutation study rather than sequencing of the coding region of *GNAS* gene should be performed as the initial genetic investigation (Figure 1d). All cases of PHP should be managed by specialists from multiple disciplines including clinical geneticist, so as to provide the best medical management and genetic counselling.



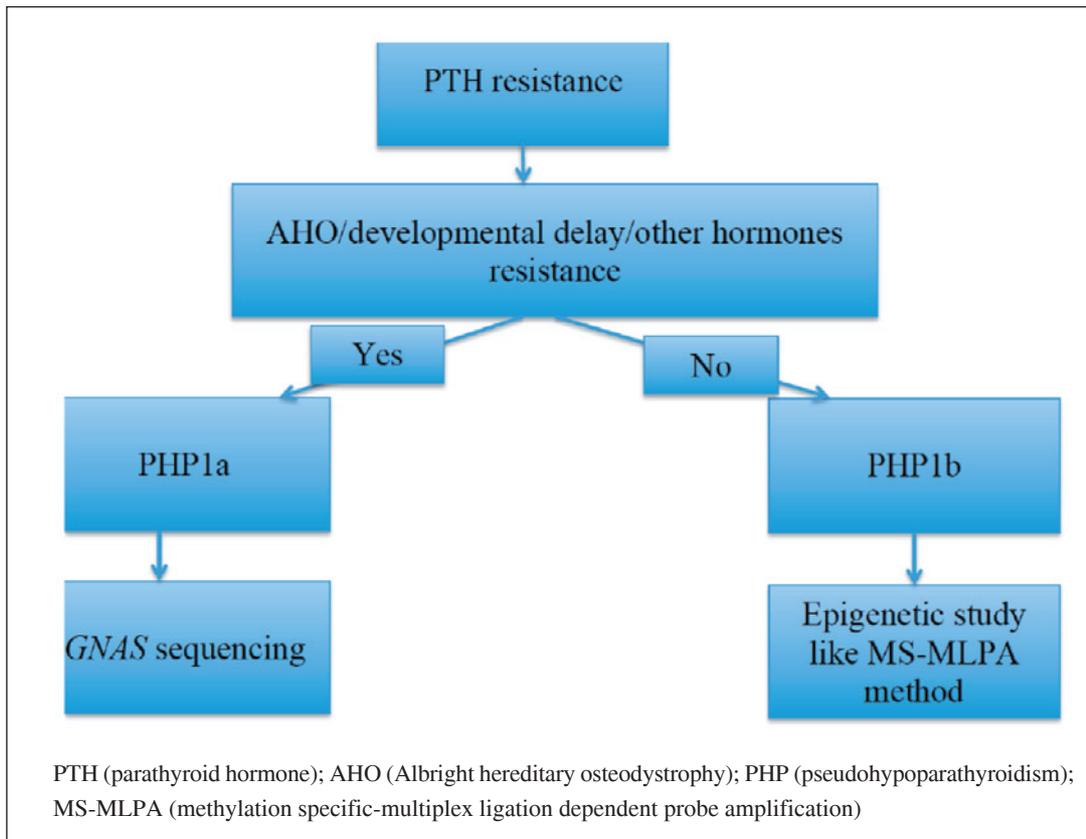
**Figure 1a** MS-MLPA (*GNAS*) result of this patient.



**Figure 1b** The relative copy number change in different exons of *GNAS* locus. It show there is no deletion or duplication in different exons of *GNAS* locus for this patient.



**Figure 1c** Data of single nucleotide polymorphism (SNP) array of chromosome 20. Y-axis is the number of uncut alleles and X-axis represents different region of chromosome 20. In normal condition, there are three lines of signals. In uniparental disomy, the central line signal will lose. Here it showed no evidence of paternal uniparental disomy of chromosome 20 of our patient.



**Figure 1d** Suggested genetic investigation for parathyroid resistance.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

- De Nanclares GP, Fernandez-Rebollo E, Santin I, et al. Epigenetic defects of GNAS in patients with pseudohypoparathyroidism and mild features of Albright's hereditary osteodystrophy. *J Clin Endocrinol Metab* 2007;92:2370-3.
- Mantovani G, Linglart A, Garin I, Silve C, Elli FM, de Nanclares GP. Clinical utility gene card for: Pseudohypoparathyroidism. *Eur J Hum Genet* 2013;21. doi:10.1038/ejhg.2012.211
- Hayward BE, Bonthron DT. An imprinted antisense transcript at the human GNAS1 locus. *Hum Mol Genet* 2009;835-41.
- Liu J, Yu S, Litman D, Chen W, Weinstein LS. Identification of a methylation imprint mark within the mouse Gnas locus. *Mol Cell Biol* 2000;20:5808-17.
- Coombes C, Arnaud P, Gordon E, et al. Epigenetic properties and identification of an imprint mark in the NESP-GNASXL domain GNAS imprinted locus. *Mol Cell Biol* 2003;23:5475-88.
- Bastepe M. The GNAS Locus: Quintessential Complex Gene Encoding G $\alpha$ 1, XL $\alpha$ s, and other Imprinted Transcripts. *Curr Genomics* 2007;8:398-414.
- Maupetit-Méhouas, Azzi S, Steunou S, et al. Simultaneous hyper- and hypomethylation at imprinted loci in a subset of patients with GNAS epimutations underlies a complex and different mechanism of multilocus methylation defect in pseudohypoparathyroidism type 1b. *Hum Mutat* 2013;34:1172-80.
- Bastepe M, Lane AH, Juppner H. Paternal uniparental isodisomy of chromosome 20q—and the resulting changes in GNAS methylation—as a plausible cause of pseudohypoparathyroidism. *Am J Hum Genet* 2001;68:1283-9.
- Bastepe M, Altug-Teber O, Agarwal C, Oberfield SE, Bonin M, Juppner H. Paternal uniparental isodisomy of the entire chromosome 20 as a molecular cause of pseudohypoparathyroidism type 1b (PHP-1b). *Bone* 2010;48:659-62.
- Fernandez-Rebollo E, Lecumberri B, Garin I, et al. New mechanisms involved in paternal 20q disomy associated with pseudohypoparathyroidism. *Eur J Endocrinol* 2010;163:953-62.