

Clinical and Genetic Study of Pseudohypoparathyroidism Type 1b in Hong Kong Chinese

Ho-Ming Luk

Abstract

Pseudohypoparathyroidism type 1b (PHP-1b) is a rare congenital imprinting disease characterized by parathyroid hormone resistance, normal neurodevelopment and no feature of Albright hereditary osteodystrophy. It is caused by genetic and epigenetic defect of *GNAS* locus located at chromosome 20q13.32 region. We here have reported a case series of three cases of PHP-1b in Hong Kong Chinese. Two were sporadic and one was autosomal dominant (AD) subtype. All of them were identified by methylation specific-multiplex ligation dependent probe amplification method. The AD PHP-1b case had typical 3-kb *STX16* gene deletion. There was no paternal uniparental disomy chromosome 20 for those sporadic cases. Although PHP-1b had been well studied in western population, it is rarely reported in Chinese. This study is the first comprehensive study of PHP-1b for Hong Kong Chinese in the literature. With more awareness and better understanding of PHP-1b, it will result in judicious use of genetic testing and better medical management of this orphan disease.

Keywords: Pseudohypoparathyroidism type 1b; Chinese; Genetics

Introduction

Pseudohypoparathyroidism type 1b (PHP-1b) is a congenital imprinting disease that caused by genetic and epigenetic defect of *GNAS* locus located at chromosome 20q13.32 region. It is characterized by renal resistance to parathyroid hormone (PTH) and occasionally with mild resistance to thyroid stimulating hormone. Typically, PHP-1b patients do not have features of Albright hereditary osteodystrophy (AHO), though obesity, short stature and subtle bony abnormalities have been reported [1].

The *GNAS* complex contains at least four distinct differ-

entially methylated regions (DMRs). Through parent-of-origin effect with differential methylation on its different promoters, the *GNAS* locus would give rise to several transcripts, including alpha-subunit of the heterotrimeric stimulatory G protein α (*Gas*), the *Gas* extra-large variant (*XLas*), neuroendocrine protein 55 (*NESP55*), untranslated exon A/B (exon 1A) and antisense transcript (*AS*) [2]. The *NESP55* is maternal derived while the *GNAS XLas*, *AS* and *A/B* transcripts are exclusively paternal derived [2]. Consistent with this imprinted expression, the promoters of these genes are silenced through methylation. Therefore, *NESP55* should be methylated at paternal allele and the *GNAS XLas*, *AS* and *A/B* should be methylated at maternal allele in normal individual.

By different mechanism, PHP-1b disease is further divided into autosomal dominant (AD) and sporadic subtypes. In AD PHP-1b, the loss of imprinting at exon A/B DMR is due to microdeletion at the *STX16* gene that disrupts its *cis*-acting control element on *Gas* gene [2]. On the other hand, the sporadic PHP-1b has epigenetic abnormalities at multiple DMRs, but without copy number changes in *cis*- or *trans*-acting elements [3, 4].

In Hong Kong, sporadic PHP-1b has been reported in the literature [5]; however, there is no comprehensive clinical and molecular study of PHP-1b in tertiary wide basis. Here we have reported a series of three additional cases of PHP-1b in Hong Kong Chinese.

Case Reports

Case 1

A 12-year-old girl was referred from private hospital to regional pediatric department for suspected prolonged QTc syndrome and complex partial seizure. She was the first child of non-consanguineous Chinese couple, born at full term via elective lower segment cesarean section. The perinatal history was unremarkable and she enjoyed good past health before admission. Her developmental milestones were normal. She complained of recurrent episodes of facial automatism, eye staring, drooling of saliva and transient unresponsiveness that last for few minutes. All episodes were preceded by abnormal aura. There was no recent febrile illness. Physical examination showed no feature of AHO with body height of 142 cm (-1

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Clinical Genetic Service, Department of Health, 3/F Cheung Sha Wan Jockey Club Clinic, 2 Kwong Lee Road, Shamshuipo, Kowloon, Hong Kong SAR, China. Email: luksite@gmail.com

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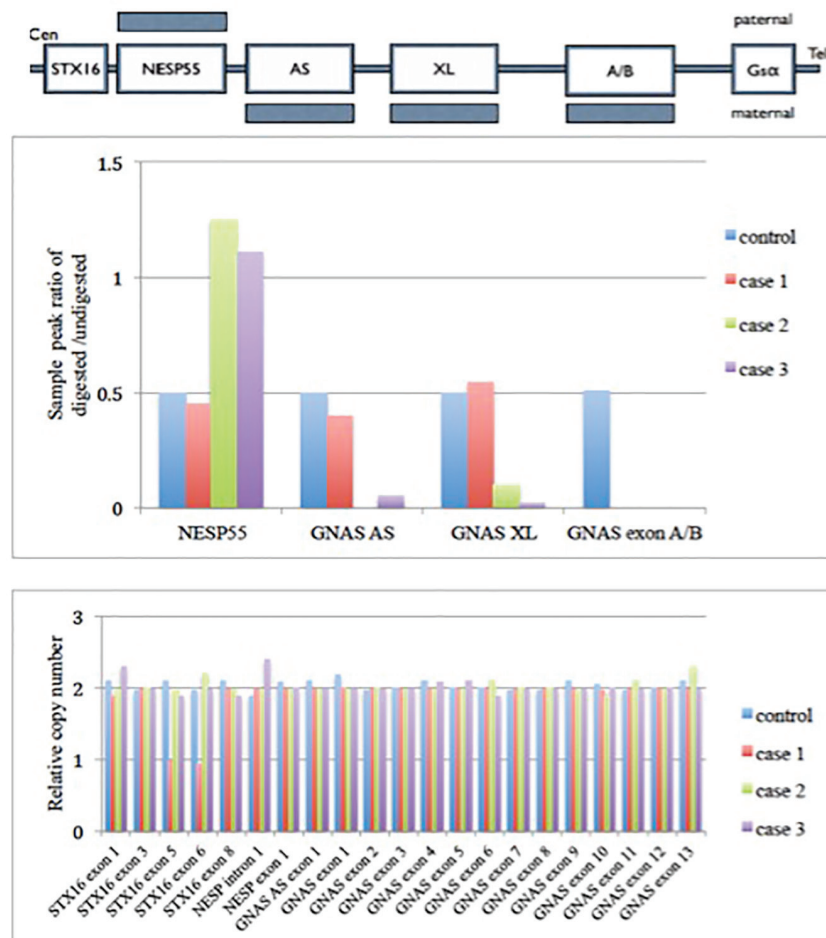


Figure 1. MS-MLPA (*GNAS* locus) results of these three cases. Upper panel shows a schematic representation of the *GNAS* locus region. Shaded bars represent the methylation state. Middle panel shows the methylation status of each differential methylation regions (DMRs) at the *GNAS* locus. The y axis is the ratio of digested to undigested signal change by restriction endonuclease enzyme over *GNAS* locus. In normal condition, the ratio should be 0.5. Value greater than 0.5 means hypermethylation and less than 0.5 means hypomethylation change over that region. There was hypomethylation at *GNAS* exon A/B in case 1 patient. There were hypermethylation at the *NESP55* and hypomethylation at *GNAS* AS, *GNAS* XL and *GNAS* exon A/B of the patient as compared with control in cases 2 and 3 patients. Lower panel shows the relative copy number change in different exons of *GNAS* locus. It shows there is deletion of exon 5 and 6 in *STX16* gene for case 1. There was no copy number change in *GNAS* locus among case 2 and 3. *Gas*: a subunit of the stimulatory G protein; *XLAs*: *Gas* extra-large variant; *NESP55*: neuroendocrine protein 55; A/B: untranslated exon A/B; AS: antisense transcript.

SD). Investigation on admission showed hypocalcemia with ionized calcium of 0.84 mmol/L (normal range (NR): 1.1 - 1.35 mmol/L) and hyperphosphatemia at 2.79 mmol/L (NR: 0.72 - 1.43 mmol/L). The renal function and magnesium level were normal. ECG showed prolonged QTc interval of 492 ms at heart rate of 80/min (N: < 440 ms). Intravenous calcium supplement was given to correct the symptomatic hypocalcemia. The QTc interval was normalized and complex partial seizure was stopped afterwards. Further investigations showed elevated PTH at 50.4 pmol/L (NR: 1.6 - 6.9 pmol/L) and moderate vitamin D deficiency with total 25OH vitamin D level of 28 nmol/L (NR: 50 - 220 nmol/L). Thyroid function was normal. Computerized tomography of brain showed no abnormal calcification. She was diagnosed as PHP and put on vitamin D and calcium supplement. She was then regularly followed up

in endocrine clinic. Latest calcium and urine calcium/creatinine ratio were normal. Regular ultrasound kidney showed no nephrocalcinosis. The family history was negative for endocrine or developmental problem.

Case 2

An 11-year-old boy was admitted to accident and emergency department for carpedal spasm. He was the first child of non-consanguineous Chinese couple, born at full term via normal vaginal delivery. The perinatal history was unremarkable. He had history of attention deficient hyperactivity disease and atypical absence seizure that were managed by private neurologist since 10 years old. On admission, the ionized calcium

Table 1. Summary of the Clinical, Biochemical and Genetic Abnormalities for All PHP-1b Patients in This Study

Case	Age of onset	Ca (NR: 2.12 - 2.64 mmol/L)	PO4 (NR: 1.05 - 1.80 mmol/L)	PTH (NR: 1.6 - 6.9 pmol/L)	AHO features	Methylation status of <i>GNAS</i> locus	Copy number change in <i>STX16</i> gene	Diagnosis
1	12	1.21	2.79	50.4	No	Loss of methylation at <i>GNAS</i> exon A/B	Deletion exon 5 and 6 of <i>STX16</i> gene	Autosomal dominant PHP-1b
2	10	1.27	3.23	30.3	No	Hypermethylation at <i>NESP55</i> and loss of methylation at <i>GNAS AS/GNAS XL/GNAS</i> exon A/B	Normal	Sporadic PHP-1b
3	67	1.46	1.93	22	Short stature, shortened fourth metacarpal bone	Hypermethylation at <i>NESP55</i> and loss of methylation at <i>GNAS AS/GNAS XL/GNAS</i> exon A/B	Normal	Sporadic PHP-1b

NR: normal range.

was 0.66 mmol/L (NR: 1.1 - 1.35 mmol/L) and phosphate level was 3.23 mmol/L (NR: 0.72 - 1.43 mmol/L). The renal function and magnesium level were normal. Physical examination showed no feature of AHO with body height of 145 cm (-0.5 SD). There was carpopedal spasm and positive Chvostek's sign, which have been aborted by correction of hypocalcemia. Further investigations showed elevated PTH at 30.3 pmol/L (NR: 1.6 - 6.9 pmol/L) and mild vitamin D deficiency with total 25OH vitamin D level of 39 nmol/L (NR: 50 - 220 nmol/L). Thyroid function was normal. Computerized tomography of brain showed symmetrical calcification of basal ganglia, and patchy calcification at subcortical U-fibers at bilateral frontal and right parietal region. X-ray hand showed no shortened fourth and fifth metacarpal bone. He was diagnosed as PHP. Vitamin D and calcium supplement was given regularly. The family history was non-contributory. He was then regularly followed up in pediatric unit. There was no recurrence of atypical absence seizure and carpopedal spasm afterwards.

Case 3

A 67-year-old lady was referred to endocrine outpatient clinic for persistent limb numbness. Baseline investigation showed hypocalcemia of 1.46 mmol/L (NR: 2.12 - 2.64 mmol/L) and hyperphosphatemia of 1.93 mmol/L (NR: 1.05 - 1.80 mmol/L). PTH was elevated at 22 pmol/L (NR: 1.6 - 6.9 pmol/L) and mild vitamin D deficiency with total 25OH vitamin D level of 27 nmol/L (NR: 50 - 220 nmol/L). The renal function, alkaline phosphatase, magnesium and thyroid function were normal. Physical examination showed she had body height of 148 cm (-1.5 SD) with mild bilateral shortening of fourth metacarpal bone. However, there was no other evidence of AHO. There was no evidence of other hormone resistance and she had normal intelligence. The family history was negative for endocrine or developmental problem. Based on the biochemical abnormalities, PHP was diagnosed. She was put on calcium together with vitamin D supplement and was regularly fol-

lowed up in endocrine clinic.

Molecular diagnostic algorithm

Based on hypocalcemia, hyperphosphatemia and elevated PTH, the diagnosis of PHP was substantiated. Without clinical features of AHO and normal intellectual development, the most likely diagnosis was PHP-1b. Therefore, the methylation status of *GNAS* DMRs together with the copy number change at *GNAS* DMR and *STX16* gene should firstly evaluated by methylation specific-multiplex ligation dependent probe amplification assay (MS-MLPA) by using SALSA MLPA ME031-A2 *GNAS* kit from MRC-Holland (Amsterdam, The Netherlands) under the manufacturer's instructions. Paternal uniparental disomy at chromosome 20 (upd(20)pat) was excluded in sporadic PHP-1b by using single nucleotide polymorphism (SNP) array (Agilent SurePrint G3 Human CGH + SNP Microarray 4x180K). Direct sequencing of *GNAS* gene was also performed in all these patients. Informed consent was obtained prior to the genetic studies.

Results

Total three patients with PHP-1b were reported in this case series. There were two sporadic PHP-1b and one autosomal dominant PHP-1b cases. The MS-MLPA study results are shown in Figure 1. The biochemical abnormalities and genetic alternations are summarized in Table 1. There was no *GNAS* mutation being detected by sequencing method in these patients. And no upd(20)pat was being detected in those sporadic cases (Fig. 2).

Discussion

This is the first comprehensive clinical and genetic case series

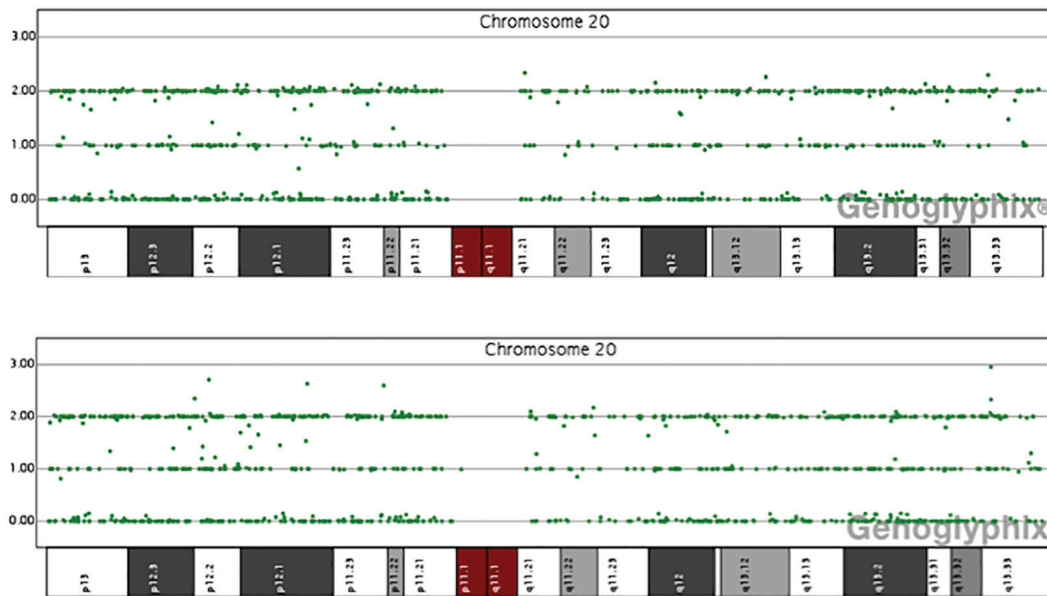


Figure 2. Data of single nucleotide polymorphism (SNP) array of chromosome 20 for patients 2 and 3. The y axis is the number of uncut alleles and x axis represents different region of chromosome 20. In normal condition, there are three lines of signals. In uniparental disomy, the central line signal will lose. Here it shows no evidence of paternal uniparental disomy of chromosome 20 in all our sporadic PHP-1b patients.

of PHP-1b patients in Hong Kong Chinese. By using the MS-MLPA method, total three cases of PHP-1b were confirmed molecularly, with the first case of Chinese AD PHP-1b being identified that has not been reported in the literature.

In this AD PHP-1b case, there was 3-kb deletion in the *STX16* gene being detected. This deletion encompassed exon 4 to 6 of *STX16* gene [6] and was the most frequent genetic mechanism for AD PHP-1b [7-9]. The frequent recurrence was due to the homologous recombination between the repeated sequences in intron 3 and 7 which flanked the deleted region. Apart from 3-kb deletion, maternally inherited microdeletion in other regions within *STX16*, *NESP55* and *GNAS AS* genes were also reported in AD PHP-1b patients [10-12]. Apart from deletion, maternal duplication of *GNAS* locus has also been reported to result in PHP-1b phenotype recently [13].

On family cascade screening in this AD PHP-1b family, her mother and younger sister also had the same 3-kb deletion in the *STX16* gene. However, methylation study showed there was hypomethylation of *GNAS* exon A/B in her younger sister but normal methylation status in her mother (Fig. 3). Biochemical screening showed the younger sister had asymptomatic hypocalcemia with elevated PTH, while mother had normal calcium and PTH level. As the maternal grandfather was not available for genetic testing, we postulated that the *STX16* gene deletion in her mother should either paternally inherited or happened *de novo* in the paternal allele that should not have clinical effect on her mother. But once she transmitted this deletion to her daughter, it would exhibit the parent-of-origin imprinting effect and develop PHP-1b disease. This is important for genetic counseling. As the recurrence risk of PHP-1b in maternal carrier was 50%, it has significant implication on her reproductive choice and

option in the future. Early identification of those asymptomatic PHP-1b carriers like younger sister of proband is also important as regular anticipatory surveillance and early treatment could reduce the morbidity and even mortality associated with hypocalcemia.

Concerning the genetic testing of PHP-1b, restriction fragment length polymorphism analysis using methylation sensitive enzyme together with Southern blot and bisulfite treated methylation specific PCR were commonly used in the past [14]. However, with the advancement in diagnostic method, MS-MLPA is currently the first line investigation for all suspected PHP-1b patients [6, 15], as it could simultaneously detect the aberrant methylation status of CpG islands and copy number change at the *GNAS* imprinting locus. Although it is very robust, MS-MLPA method is not without limitation. In MS-MLPA method, only the methylation status of HhaI site in selected CpG islands is being studied. It may not represent the status of entire CpG islands. Moreover, mutation or polymorphism over the probe ligation site will also affect the probe signal intensity [15] that would lead to erroneous results.

Despite recent advancement in understanding the epigenetic defects and PHP-1b, its exact pathogenesis was unknown. The proposed mechanism for AD PHP-1b was *STX16* gene deletion which would disrupt the cis-regulatory element of imprinting gene at the *GNAS* locus [2]. However, the mechanism of epimutation in sporadic PHP-1b that led to abnormal clinical phenotype was still yet to be elucidated. Sporadic PHP-1b accounts for 80-85% of the PHP-1b cases [16]. In the literature, about 2-20% of PHP-1b cases were reported to be associated with upd(20)pat [17-19], thus upd(20)pat should be excluded in all cases of sporadic PHP-1b, either by microsatellites analysis or SNP array study. Since micros-

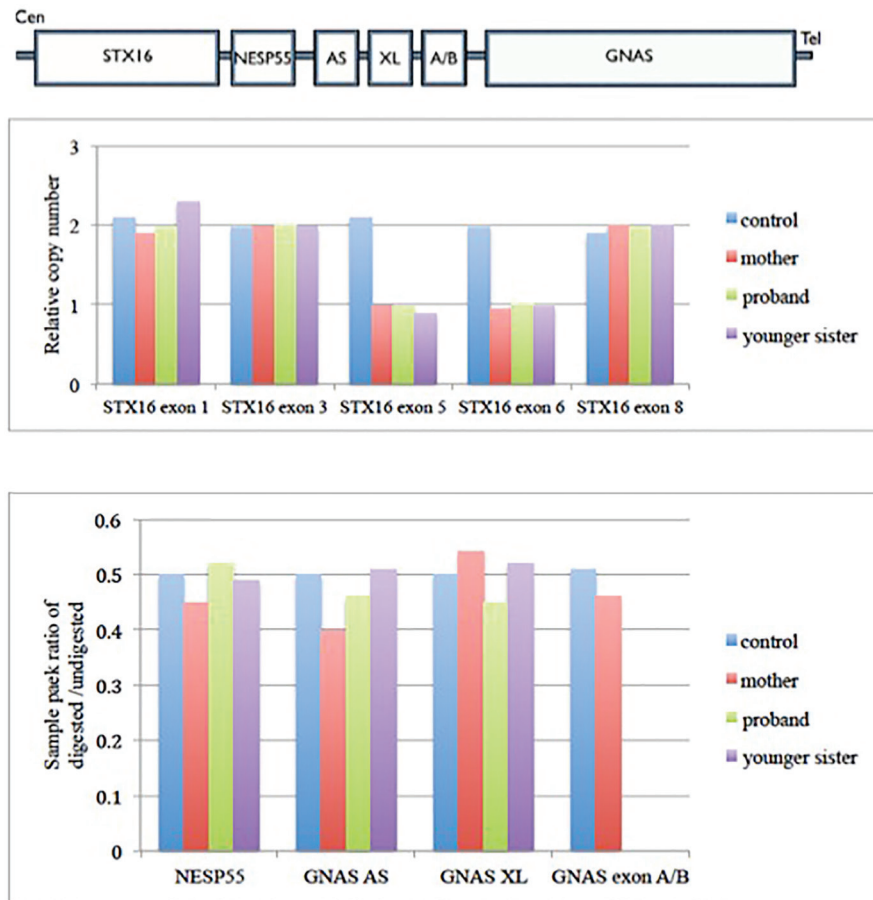


Figure 3. Family cascade screening for case 1 family. Upper panel shows the architecture of *GNAS* imprinting locus. Middle panel shows the relative copy number change in each exons of *STX16* gene in case 1 family. It shows there is deletion of exon 5 and 6 of *STX16* gene in proband, her mother and younger sister. Lower panel shows the methylation status of each differential methylation regions (DMRs) at the *GNAS* locus among this family. The y-axis is the ratio of digested to undigested signal change by restriction endonuclease enzyme over *GNAS* locus. In normal condition, the ratio should be 0.5. Value greater than 0.5 means hypermethylation and less than 0.5 means hypomethylation change over that region. There was hypomethylation at *GNAS* exon A/B in proband and her younger sister, but normal methylation status in their mother. *Gas*: a subunit of the stimulatory G protein; *XLas*: *Gas* extra-large variant; *NESP55*: neuroendocrine protein 55; A/B: untranslated exon A/B; AS: antisense transcript.

atellites analysis is more labor intensive, SNP array might be the first line recommended genetic method to exclude upd(20)pat in sporadic PHP-1b patient. One additional benefit of SNP array is it can measure the size of uniparental disomy. There were reported epigenotype-phenotype correlation between the size of upd(20)pat segment and the clinical features [19, 20]. The longer the segment of upd(20)pat, the earlier the onset of symptoms and the more likely to have overgrowth features like macrosomia and macrocephaly [20]. However, such epigenotype-phenotype correlation could not be demonstrated in non-upd(20)pat related PHP-1b. On the other hand, the degree of methylation aberration was not useful in predicting the severity and type of disease manifestation [21].

With more cases of PHP being studied extensively by molecular technique, it is noted that the *GNAS* related disease is more genetically heterogeneous and complicated than previously thought. There are molecularly and clinically overlapping between the PHP-1a and PHP-1b diseases [1, 22]. Some

PHP-1a patients have epigenetic alternation in *GNAS* imprinting region and some PHP-1b patients have *GNAS* gene mutation being reported [23, 24]. Despite this, there was no difference in clinical features and genetic abnormalities between Chinese and non-Chinese PHP patient, therefore based on the current evidence and recommendations [5], we propose the molecular diagnostic algorithm for PHP as Figure 4.

In conclusion, in the presence of hypocalcemia, hyperphosphatemia, elevated PTH, but without intellectual disability or features of AHO, the diagnosis of PHP-1b should be considered. Epimutation study rather than sequencing of the coding region in the *GNAS* gene should be performed as the first line genetic investigation. Although there is no difference in clinical manifestations between AD PHP-1b and sporadic PHP-1b, their differentiation by genetic testing is of paramount importance for genetic counseling, reproductive choice option and family cascade screening. All cases of PHP-1b should be managed by multidisciplinary teams which included clinical

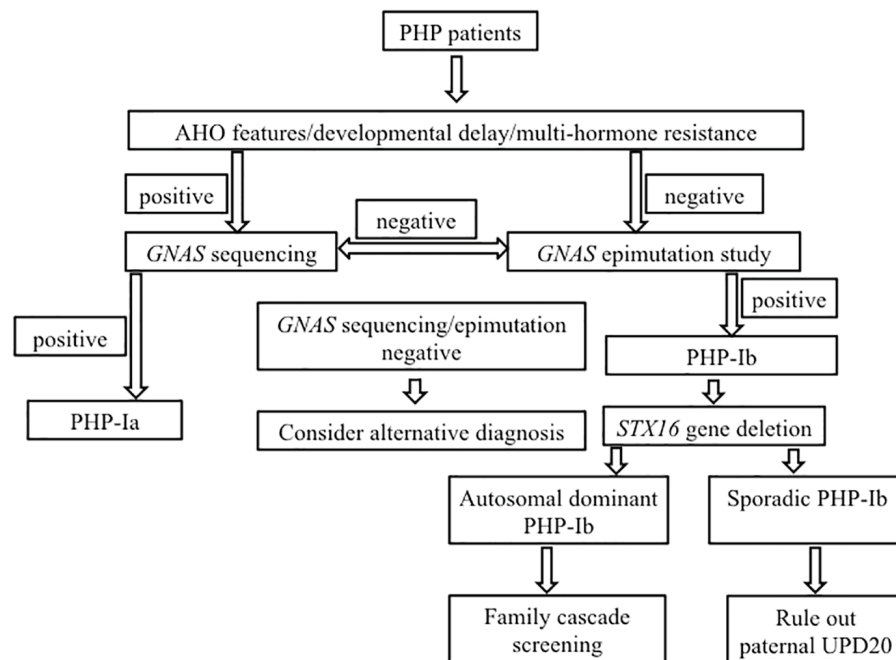


Figure 4. Diagnostic algorithm for molecular genetic testing for patient with PHP. AHO: Albright hereditary osteodystrophy; UPD: uniparental disomy.

geneticist, so as to provide the best quality of medical care and treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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