

# A novel mutation in pseudohypoparathyroidism type 1a in a Chinese woman and her son with hypocalcaemia

Vicki HK Tam \*, Sammy PL Chen, Chloe M Mak, LM Fung, CY Lee, Albert YW Chan

## ABSTRACT

Pseudohypoparathyroidism is a rare genetic disorder characterised by end-organ resistance to parathyroid hormone due to a defect of the guanine nucleotide-binding protein alpha that simulates activity of the polypeptide 1 (*GNAS*) gene. Patients with type 1a pseudohypoparathyroidism display different features of Albright's hereditary osteodystrophy as well as multi-hormone resistance. We describe a Chinese woman and her son, who presented with different symptoms of pseudohypoparathyroidism and clinically manifested different degree of Albright's hereditary osteodystrophy. Genetic study detected a mutation [NM\_000516.4(*GNAS*):c682C>T (p.Arg228Cys)] in the *GNAS* gene.

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<sup>1</sup> VHK Tam \*, FHKCP, FHKAM (Medicine)

<sup>2</sup> SPL Chen, MRCP (UK), FHKAM (Pathology)

<sup>2</sup> CM Mak, MD, FHKAM (Pathology)

<sup>1</sup> LM Fung, FRCP (Edin), FHKAM (Medicine)

<sup>3</sup> CY Lee, FRCP (Edin), FHKAM (Paediatrics)

<sup>2</sup> AYW Chan, MD, FHKAM (Pathology)

<sup>1</sup> Department of Medicine and Geriatrics, Caritas Medical Centre, Shamshuipo, Hong Kong

<sup>2</sup> Kowloon West Cluster Laboratory Genetic Service, Chemical Pathology Laboratory, Department of Pathology, Princess Margaret Hospital, Laichikok, Hong Kong

<sup>3</sup> Department of Paediatrics and Adolescent Medicine, Caritas Medical Centre, Shamshuipo, Hong Kong

\* Corresponding author: vickitam@gmail.com

## Introduction

Pseudohypoparathyroidism (PHP) is characterised by hypocalcaemia and hyperphosphataemia due to resistance to parathyroid hormone (PTH). This was the first hormone resistance syndrome described in 1942 by Fuller Albright and his colleagues.<sup>1</sup>

There are three forms of PHP, namely: PHP-1, PHP-2, and pseudopseudohypoparathyroidism (PPHP). Evidently, PHP-1 differs from PHP-2 in that patients with the former show a blunted urinary cyclic AMP (cAMP) response to exogenous administration of PTH, whereas those with PHP-2 have normal urinary cAMP excretion but a blunted phosphaturic response. Moreover, PHP-1 is further classified into three different subtypes (1a, 1b, and 1c) based on the presence or absence of Albright's hereditary osteodystrophy (AHO), which typically includes short stature, obesity, brachydactyly, ectopic ossification, and mental retardation. Both PHP-1a and PHP-1c display features of AHO, but PHP-1b does not. Furthermore, PHP-1a is distinguished from PHP-1c in that it contains the inactivating mutation in the gene encoding  $G\alpha$  (*GNAS*).

Patients with both PHP-1a and PPHP carry heterozygous inactivating *GNAS* mutations. Apart from having AHO, patients with PHP-1a show resistance to hormones that act via G protein-coupled receptors. Patients with PPHP show only the features of AHO.

## Case report

A 44-year-old woman, with no significant past medical illness, presented to the Department of Orthopaedics and Traumatology of Caritas Medical Centre in 2006 because of progressive weakness and numbness in both lower limbs. These symptoms had been present for years and she was only recently unable to walk. Magnetic resonance imaging of the cervical spine revealed osteophytosis and thickening of posterior longitudinal ligament, resulting in narrowing of the spinal canal at multiple levels with compression on the cervical cord. She was diagnosed as having cervical spondylosis, and laminoplasty was performed in September 2007. Postoperatively, she was noted to have hypocalcaemia with a total serum calcium level of 1.81 mmol/L (reference range [RR], 2.10–2.60 mmol/L), and a serum phosphate level of 1.08 mmol/L (RR, 0.8–1.5 mmol/L). The serum PTH level was 258 pg/mL (RR, 11–54 pg/mL); her adjusted calcium level was 2.12 mmol/L.

The patient was brought up by her step-mother since she was young. She got divorced and had no siblings and lost contact with her biological parents and their families. She also had a history of oligomenorrhoea since menarche with only one to two menstrual periods per year. On physical examination, her body weight was 87.2 kg and height 1.61 m, with a body mass index of 33.6 kg/m<sup>2</sup>. She was obese with a moon face, but there was no definite

brachydactyly. The clinical diagnosis was PHP.

Other investigations revealed that she had subclinical hypothyroidism with a serum thyroid-stimulating hormone (TSH) level of 7.64 mIU/L (RR, 0.50-4.70 mIU/L) and serum free T<sub>4</sub> level of 10.7 pmol/L (RR, 9.1-23.8 pmol/L). The anti-thyroglobulin antibody titre was < 1/100 but the anti-microsomal antibody titre was 1/24 600. Her serum follicle-stimulating hormone level was 20 U/L, serum luteinising hormone level was 14.8 U/L, and serum oestradiol level was <73 pmol/L. The short synacthen test (using 250 µg tetracosactrin) showed a baseline serum cortisol level of 77 nmol/L and peak level of 500 nmol/L. She was started on calcitriol and calcium supplement as well as thyroxine replacement.

She only had one child, a 16-year-old son who also had "calcium problem". He was followed up by the Department of Paediatrics and Adolescent Medicine of our hospital. In 2003, he had presented with a generalised tonic-clonic convulsion at the age of 12 years. At that time, his serum calcium level was 1.46 mmol/L, phosphate level of 1.98 mmol/L, and a PTH level of 70 pg/mL. The thyroid function test was normal. He was obese and tall with a body weight of 60 kg (at 97th percentile in the growth chart) and height of 166.3 cm (>97th percentile). He suffered from mild mental retardation and studied in special school. There was mild shortening of the fourth and fifth metacarpals (Fig). He was diagnosed as having PHP with AHO features, and received calcitriol and calcium supplement. Over the years, he had gone through a normal puberty. The brachydactyly had become more prominent.

We performed a mutation analysis on the *GNAS* gene of both the mother and her son. Genomic DNA of both patients was extracted from peripheral blood leukocytes using the QIAamp



FIG. X-ray hands of patient's son showing brachydactyly

## 在一對患有假性副甲狀腺低下症母子的 嶄新基因發現

譚可璣、陳栢林、麥苗、馮麗明、李靜賢、陳恩和

假性副甲狀腺低下症是一種罕見的遺傳病。患者的G $\alpha$ 蛋白基因(*GNAS*)異常,無法對副甲狀腺素的指令產生反應。依疾病分型患者可有遺傳性骨發育不全症及其他與G $\alpha$ 蛋白有關的激素功能不足的症狀。本文報告一名女性及其兒子均患有假性副甲狀腺低下症,他們被發現時有不同的病徵及不同程度的遺傳性骨發育不全症。分子基因檢查發現他們的*GNAS*基因均有突變[NM\_000516.4(*GNAS*):c.682C>T (p.Arg228Cys)]。

Blood Kit (Qiagen, Hilden, Germany). The coding exons and the flanking regions of the *SPG4* gene were amplified using a polymerase chain reaction and sequenced. The numbering of nucleotides was based on GenBank accession number NM\_000516.4 with 394 amino acids. Protocol is available on request.

A heterozygous missense mutation, NM\_000516.4(*GNAS*):c.682C>T (p.Arg228Cys), in the *GNAS* gene was identified in both the proband and her son. This was a novel mutation, with involvement of a structurally non-conservative substitution of the evolutionary conserved amino acid change predicted to affect protein function by Sorting Intolerant From Tolerant analysis, Polyphen-2 and MutationTaster analyses. Screening in 300 normal chromosomes did not suggest this as a polymorphic site. The diagnosis of the mother was PHP-1a with mild AHO, and of her son was PHP-1a with AHO.

## Discussion

It appears that PHP-1a is an autosomal dominant disease in which full clinical and metabolic abnormalities may not be present initially, but become apparent later. Patients with PHP-1a showed a heterozygous inactivating germline mutation in *GNAS*, the gene encoding the  $\alpha$ -subunit of the stimulatory GTP binding protein (G $\alpha$ ). This could lead to a reduced G $\alpha$  protein level and cellular activity and thus the clinical resistance phenotype.<sup>2</sup>

The *GNAS* gene maps to 20q13 and contains 13 exons.<sup>3</sup> The mutation can be localised in the entire coding region of the gene. All exons can be affected by loss-of-function alterations with the exception of exon 3, where no mutations have been detected to date.<sup>4</sup> The hot-spot mutations accounting for about 20% of all mutations so far described have been identified on exon 7.<sup>5</sup> As for the types of mutations, small insertions, deletions and amino-acid substitutions predominate. Our patient showed a novel heterozygous missense mutation of exon 9 in the *GNAS* gene, which is considered to be

functionally deleterious.

Maternal inheritance of this *GNAS* mutation leads to PHP-1a (ie AHO plus hormone resistance), while paternal inheritance of the same mutation leads to PPHP (ie AHO only).<sup>6</sup> This imprinted mode of inheritance for hormone resistance can be explained by the predominantly maternal expression of *Gsα* in certain tissues, including renal proximal tubules.<sup>7</sup> In our case, the son inherited the same molecular defect from his mother, resulting in PHP-1a. It could be postulated that the proband should also inherit the condition from her mother, but this remains to be substantiated as there is not much helpful information available.

Our patient (the mother) also had subclinical hypothyroidism; the anti-microsomal antibody was positive. She had evidence of hypogonadism (oligomenorrhoea and low oestradiol levels). By contrast, her son had normal thyroid function, and he had a normal puberty. Co-existing endocrinological abnormalities may ensue, as individuals with PHP-1a also demonstrate resistance to other hormones such as TSH, gonadotropins, and growth hormone-releasing hormone. Fernandez-Rebollo et al<sup>8</sup> showed that most patients with PHP-1a have TSH resistance, which is usually mild, and manifests during childhood or adolescence. Goitre and anti-thyroid antibodies are usually absent.<sup>9</sup> Clinical evidence of hypogonadism is common in PHP-1a, particularly in females, and manifests as delayed sexual maturation, amenorrhoea, oligomenorrhoea, and/or infertility. Affected individuals usually have slight hypoeestrogenism, but no definite evidence of increased basal or gonadotropin-releasing hormone-stimulated levels of circulating gonadotropins.<sup>10</sup> Mantovani and Spada<sup>11</sup> demonstrated that growth hormone deficiency is also common in patients with PHP-1a. However, the relevance of growth hormone deficiency on final height and obesity in these patients is not certain, because PPHP patients (who do not have hormonal resistance) also have short statures together with obesity. That study also evaluated the adrenocortical and corticotropin responsiveness in patients with PHP-1a; all of whom showed a normal response to 1 µg adrenocorticotropin and to corticotropin-releasing factor. Normal pituitary-adrenal function in these patients suggested that the presence of *Gsα* imprinting within the pituitary gland is cell-type specific.<sup>12</sup>

Spinal cord compression is a rare neurological complication of PHP or PPHP, which is due to ossification of the posterior longitudinal ligament that may compress the spinal cord.<sup>13</sup> Presentations include spastic paraparesis, tetraparesis, and urinary incontinence.<sup>14</sup> Many such patients endure long-term disability despite neurosurgical intervention. Our patient had a long history of neurological

symptoms but presented late. After laminoplasty, she still had residual weakness. Hence, spinal cord compression should also be considered in patients with PHP or PPHP who present with neurological symptoms.

We have demonstrated a novel mutation in the *GNAS* gene in a small Chinese family with PHP-1a. One family member presented with spinal cord compression, which is a rare complication in PHP caused by ectopic ossification. Moreover, associated endocrinopathies, especially hypothyroidism and hypogonadism, are common in PHP-1a.

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