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Case Report Open Access

Pseudohypoparathyroidism Type Ia-Clinical Case with a Novel Mutation of *GNAS*1 Gene

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Abstract

Pseudohypoparathyroidism (PHP) is a rare autosomal dominant disorder resulting from loss of function mutations in the GNAS gene. Several forms of PHP are noted. PHP type 1a occurs most commonly and is characterized by physical features termed Albright's Hereditary Osteodystrophy (AHO), a constellation of physical features which may include short stature, obesity, round facies, heterotopic ossification, brachydactyly and mental retardation, and increased levels of parathyroid hormone (PTH) due to the end organ hormone resistance to its action. Here we report a new GNAS mutation in a 3.5 years old African American female patient with a history of round facies, developmental delays, obesity and seizure disorder; she was admitted for apneic episode and noted to have prolonged QTc interval on cardiac monitor. A lab evaluation showed severe hypocalcaemia, hyperphosphatemia, high PTH with normal magnesium and alkaline phosphatase levels. She also had slightly elevated Thyroid Stimulating Hormone (TSH) levels indicative of type 1 a PHP where resistance to multiple Gs protein-coupled hormones (e.g. PTH, TSH, Luteinizing Hormone (LH), Follicular Stimulating Hormone (FSH), and Growth Hormone Releasing Hormone (GHRH)) is present. Full genomic DNA sequencing of the exons and adjacent intronic regions of the GNAS gene revealed a novel heterozygous mutation in intron 7, c.585+1G>A, in both the patient and her mother.

Keywords: Pseudohypoparathyroidism; Pseudopseudohypoparathyroidism; Novel mutation in *GNAS* gene

Abbreviations: FT: Full Term; AGA: Appropriate for Gestational Age; PCR: Polymerase Chain Reaction; PHP: Pseudohypoparathyroidism; PPHP: Pseudopseudohypoparathyroidism

Background

Parathyroid Hormone (PTH) exerts it effects on specialized target cells that express specific plasma membrane G protein–coupled receptors called type 1 PTH receptor (PTH 1-r). PTH 1-r is most abundantly present in bone and kidney.PTH 1-r receptor couples with G-proteins that consist of α, β and γ subunits [1]. The activation of adenlylate cyclase is mediated by the Gs- protein at the guanine nucleotide- binding site on the α-subunit. This results in stimulation of phospholipase C, which activates protein kinase A and protein kinase C signaling pathways and leads to a rapid generation of the second messengers cAMP, [2,3] inositol 1,4,5-trisphosphate and diacyl glycerol, and cytosolic calcium [4,5].

The *GNAS* gene encodes the alpha subunit of $G_s\alpha$ that couples PTH receptors to stimulate adenylyl cyclase [6]. Pseudohypoparathyroidism (PHP) is associated with both genetic and epigenetic mutations in the *GNAS* gene [7-9]. Several variants of PHP have been described and all are rare. The molecular defects in the *GNAS* gene encoding the alpha subunit of $G_s\alpha$ result in 3 different forms of the disease: PHP type 1a, PHP type 1b, and pseudopseudohypoparathyroidism (PPHP) [10]. So far PHP type 1a is the best understood among these different forms of PHP. PHP type 1a, also known as Albright hereditary osteodystrophy (AHO), is characterized by a constellation of physical features which may include short stature, obesity, round facies, heterotopic ossification, brachydactyly and mental retardation and biochemical abnormalities such as hypocalcaemia, hyperphosphatemia and increased levels of parathyroid hormone (PTH). Interestingly, maternally inherited *GNAS* mutations cause hormone resistance in addition to the AHO phenotype [11-13]. In contrast, paternally transmitted *GNAS* mutations are associated with AHO only without any hormone resistance, which is why it is referred to as PPHP.

Case

Here we report a case of 3.5 years old African American female, born full term (FT), appropriate for gestational age (AGA) to non-consanguineous parents. She has a history of developmental delays and seizure disorder. Her initial admission was for a hypopnea/ apnea episode and during that monitoring she was noticed to have prolonged QTc interval on the cardiac monitor.

Initial laboratory work up showed a very low serum ionized calcium level of 2.6 mg/dL (4.5-5.3) and high phosphate level of 8.1 mg/dl (4.0-7.0) with normal magnesium and alkaline phosphatase levels. She was given intravenous calcium infusion to correct her serum calcium levels initially and was later switched to oral calcium and calcitriol (Table 1). Her BMI was 22 kg/m^2 (>97 percentile), with short hands (Figure 1), round facies and some developmental delays. She was diagnosed with seizure disorder in the past but no laboratory evaluation showing her calcium levels at the time of diagnosis was available to review. She was started on anti-seizure medications and remained seizure free since starting treatment. In the meantime her PTH levels were elevated and her vitamin D levels were significantly reduced (Table 1). She received stoss therapy in addition to her calcium supplements to restore her vitamin D levels. During her follow up visit in 3 months her calcium, phosphate and vitamin D

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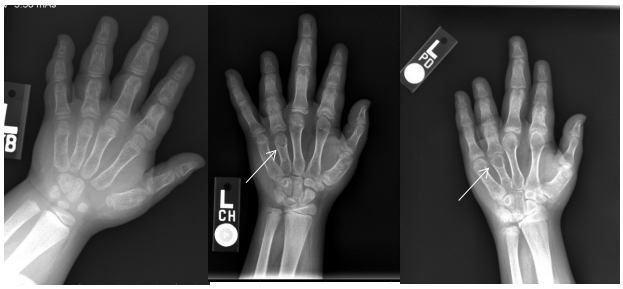
Laboratory evaluation	Admission	On therapy
Ionized calcium (4.5-5.3)	2.6 mg/dL	4.64 mg/dL
Total Calcium (8.5-10.6)	4.6 mg/dL	8.7 mg/dL
Phosphate (4.0-7.0)	8.1 mg/dL	6.7 mg/dL
PTH (15-65)	542 pg/mL	383 pg/mL
Alkaline phosphatase (140-400)	342 IU/L	240 IU/L
25, hydroxy vitamin D (20-100)	6 ng/mL	48 ng/mL
1,25 dihydroxy vitamin D (27-71)	80 pg/mL	32 pg/mL
TSH (0.50-4.50)	6.76 uIU/mL	5.64 uIU/mL
Free T4 (0.70-2.00)	1.01 ng/dL	0.70 ng/dL

Table 1: Laboratory evaluation showing calcium levels at the time of diagnosis in seizure disorder patient.



A (3.5yrs) B (8yrs) C (10yrs)

Figure 1: X-ray of the left hand at different ages showing the most pronounced defect in the recent films (Figures 1B and 1C). The osseous structure show diffuse demineralization and shortened appearance of the 4th metacarpal (see arrow) and 2nd and 4th proximal phalanges.

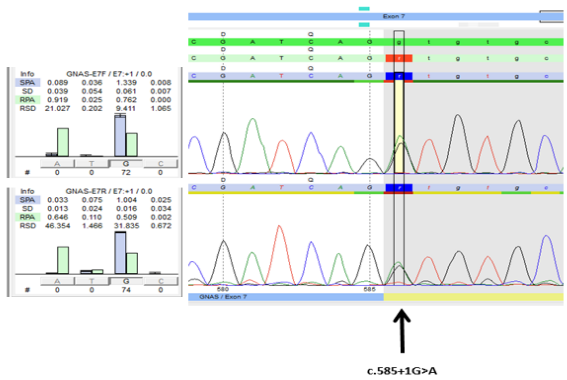


Figure 2: The Proband's Sanger Sequencing Result.
Bidirectional Sanger sequence was analyzed using Sequence Pilot (JSI Medical Systems). Each nucleotide has a result peak area (RPA) calculated based on the areas of the 20 nucleotides before and after it. The RPA is then compared to the statistical peak area (SPA), which is the average of all of the RPAs from previously analyzed samples. Forward sequence is on the top, reverse sequence is on the bottom. To the right are the electropherograms showing the peaks from the capillary electrophoresis. To the left are charts showing the RPA and SPA results for the c.585+1 position. The blue bar represents the previously analyzed samples (72 in the forward, 74 in the reverse) having wild type sequence at that position and the green bar represents the result at this position for the proband. The ratio of RPA/SPA in forward and reverse directions for c.585+1 are 57% and 51%, respectively, for the wild type G nucleotide. In both directions there is an A nucleotide that is not usually present and that has RPAs greater than the wild type nucleotide. Overall, sequencing results are consistent with the mutation c.585+1G>A. The same results were obtained in the maternal DNA sample.

levels had normalized but her PTH levels continues to stay high (Table 1). Because of her clinical and biochemical presentation we suspected pseudohypoparathyroidism and additional workup confirmed our diagnosis.

Because the proband's mother had short stature (142 cm), brachydactyly of her fingers, but normal hormone-related labs, we suspected PPHP.

Genomic DNA was extracted from whole blood; the exons and immediately adjacent intronic regions of *GNAS* were amplified by Polymerase Chain Reaction (PCR), Sanger sequenced bidirectionally and then analyzed using the software Sequence Pilot (JSI Medical Systems). Sequencing identified the *GNAS* mutation c.585+1G>A (Figure 2) in intron 7. This is not a previously reported disease associated mutation, but occurs in a highly conserved splice donor site that is essential for normal splicing. Similar *GNAS* loss of function mutations (c.432+1G>A and c.839+1G>C) have been reported in the +1 splice donor site of the surrounding introns 5 and 10 [14]. Furthermore, bioinformatic analysis predicts that this single nucleotide transition obliterates (wild type=6.46, mutant=-1.72) the strength of the wild type splice site [15]. Thus, it is extremely likely that c.585+1G>A is a deleterious loss of function mutation. DNA testing of the proband's mother revealed that she is also heterozygous for c.585+1G>A, thus confirming the putative clinical diagnosis of PHPP.

Discussion

Our patient had elevated PTH that did not normalize after correcting her hypocalcaemia and vitamin D deficiency. She also had persistent elevation of TSH. This observation is consistent with end organ resistance, in this case renal and thyroid, to more than one hormone sharing the same intracellular signaling pathway. She had developmental delays, obesity, round facies and short metacarpal bones (Figures 1A, 1B and 1C), all part of the AHO phenotype. The *GNAS* mutation c.585+1G>A is consistent with a loss of function, the molecular mechanism associated with PHP 1a. The proband's mother also carries *GNAS* c.585+1G>A and has features of AHO phenotype without any hormone resistance, suggesting that she inherited the mutation from her father.

Conclusion

We report a family where the proband is affected with PHP type 1a and her mother is affected with PPHP. The *GNAS* mutation c.585+1G>A is novel and adds to the spectrum of mutations associated with these disorders.

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