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Case Report

Pseudohypoparathyroidism-Ib due to novel heterozygous stop gain mutation in exon 8 of *STX16* gene: A case report

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ABSTRACT

We report a case of pseudohypoparathyroidism type 1b (PHP1b) manifesting in childhood with hypocalcemic seizures. Symptomatic hypocalcemia is a common emergency in the pediatric age group with vitamin D deficiency being a frequent underlying etiology and PHP is rare. Patients with PHP1b do not depict the Albright's hereditary osteodystrophy (AHO) phenotype typical of patients with PHP1a and pseudopseudohypoparathyroidism (PPHP). The resistance to parathyroid hormone (PTH) is documented mostly at renal tubular site of action in patients with PHP1b. Hypothyroidism is reported occasionally, signifying resistance to thyroid-stimulating hormone (TSH). Individuals with autosomal dominant and maternally inherited form of PHP harbor methylation defects at *GNAS* exon A/B, while sporadic and non-familial cases harbor methylation defects at other locus sites, including differentially methylated regions (GNAS-DMR). A novel heterozygous stop gain mutation c.C910T/p.Arg304X in exon 8 of the *STX16* gene (*Syntaxin 16*) was observed in our case. Resistance seems limited to the renal action of PTH alone as currently, TSH level is normal. Maternal *STX16* gene analysis results confirmed the modality of inheritance.

Keywords: Pseudohypoparathyroidism, hypocalcemia, Albright's hereditary osteodystrophy, methylation, *STX16* gene

INTRODUCTION

Pseudohypoparathyroidism (PHP) is classically associated with a triad of hypocalcemia, hyperphosphatemia, and high serum parathyroid hormone (PTH) levels. It is a diverse group of disorders with different clinical manifestations and underlying genetic etiologies but characterized by PTH resistance. As an entity, PHP is a rare disorder with a prevalence reported around 0.79 per 1,00,000 (Orphanet series). PHP is further classified into subtypes based on the inheritance pattern, genetic abnormalities, sites of PTH resistance, and presence of Albright's hereditary osteodystrophy (AHO) phenotype. The subtype PHP1 is caused by defects in the *GNAS* gene or in the 5' region of this gene locus. The genetic diagnosis and stratification of the imprinting pattern are imperative not only for molecular characterization but also for genetic counseling.

CASE REPORT

A 9-year-old male, born out of non-consanguineous union, presented with the first episode of a hypocalcemic seizure. He was born at full-term gestation with a birthweight of 2.85 Kg and

had an uneventful postnatal course. He was developmentally normal with no prior history of seizures. The general and systemic physical examination was normal with no clinical features of rickets or AHO such as round face, stocky habitus, brachydactyly, subcutaneous ossification, or dental anomalies. The anthropometric parameters were height 128.1 cm with BMI 16.8 kg/m^2.

On initial laboratory evaluation, the child had severe hypocalcemia with serum calcium 6 mg/dL (normal 8.5–10.2 mg/dL). Concurrent serum phosphorus was elevated, 6.8 mg/dL (normal 4.2–5.5 mg/dL), with normal serum albumin (3.6 g/dL), and normal serum alkaline phosphatase 229 IU/L (normal 111–425 IU/L) levels. Further evaluation of PTH-calcium axis showed, a high PTH of 924 pg/mL (normal 10–60 pg/mL) with normal 25-hydroxyvitamin D3 [25(OH)D] (67.1 ng/mL) levels. Disproportionately elevated PTH levels for hypocalcemia, elevated serum phosphorus, with a normal 25(OH)D, and serum creatinine levels suggested PHP. CT scan depicted calcifications in bilateral basal ganglia and frontoparietal gray-white matter junctions. Concomitantly, the correction of symptomatic hypocalcemia with intravenous calcium gluconate, followed by oral calcitriol and calcium supplements was initiated. The child responded to the treatment and was discharged at a serum calcium level of 8.1 mg/dL. Further investigations after the correction of hypocalcemia, including skeletal survey and electroencephalogram were normal.

Clinical exome analysis by next-generation sequencing yielded a novel stop gain mutation in *STX16* c.C190T/p. Arg304X, which was confirmed by Sanger sequencing. Maternal *STX16* gene analysis by Sanger sequencing confirmed the presence of the same variant, substantiating autosomal dominant maternal transmission. The index patient is the only progeny of the couple.

In a follow-up of 3.5 years post-discharge, the patient remains seizure-free. At the last follow-up at 12.5 years of age, he is found to be growing well with an annual growth velocity of 6.1 cm/year and height of 157 cm (25–50th centile). He maintains normal weight and BMI (19.83 kg/m^2) on follow-up. He is symptom-free and maintains serum calcium at 7.9–8.2 mg/dL and phosphorus between 6.5–7.32 mg/dL with urine calcium to creatinine ratio of 0.02–0.05 on 0.75 μg of calcitriol (Rocaltrol®), 750 mg of oral calcium carbonate and 600 IU of cholecalciferol. Serial ultrasound examinations of kidneys done annually showed no nephrocalcinosis. Our follow-up strategy is continued monitoring and periodical titration of medicines to maintain serum calcium levels in the lower normal range with normal urine spot calcium/creatinine levels.

DISCUSSION

PHP is classically associated with a triad of hypocalcemia, hyperphosphatemia, and high serum PTH levels. PHP is further classified into subtypes based on the inheritance, genetic abnormalities, sites of PTH resistance, and presence of AHO phenotype. PHP1a is characterized by the presence of features of AHO and decreased end-organ responsiveness to hormones acting via receptors with Gs-coupled pathway such as PTH, thyroid-stimulating hormone (TSH), gonadotropins, and growth hormone-releasing hormone.[1] On the other hand, PHP type 1b (PHP1b) patients rarely depict features of AHO, and hormone resistance is limited to renal actions of PTH and occasionally TSH.

The PHP group of disorders are caused by mutations or deletions in the *GNAS* gene locus or upstream of the complex. The *GNAS* locus located at chromosome 20q13 region contains four differentially methylated regions (DMRs). *GNAS* locus products include Gsα along with four other transcripts—XLαs (extra-large Gsα), NESP55 and non-translated RNAs – A/B transcript and antisense transcript [Figure 1]. The transcription of all the products of *GNAS* gene is under the control of imprinting centers except Gsα. Both the alleles of Gsα are expressed in majority of tissues of the body except certain organs including proximal renal tubules, pituitary, gonads, and thyroid, where the maternal

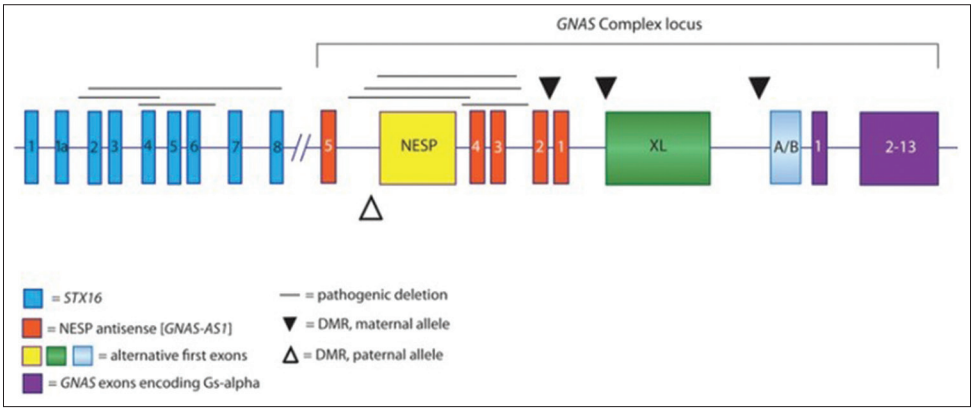

Figure 1: GNAS complex locus.

allele is expressed.[2] This tissue-specific allelic expression of Gsα is under influence of exon A/B.[3-5]

PHP1a is caused by heterozygous maternally derived mutations within the *GNAS* gene itself, whereas when inherited from the father, Gsα mutations cause pseudopseudohypoparathyroidism (PPHP) (AHO phenotype without calcium-PTH axis abnormality). PHP1b is characterized by PTH resistance with a lack of AHO phenotype. The genetic basis of this subtype can be linked to one of the following:

i. *GNAS* exon A/B (methylation defect) or
ii. Deletions in *STX16* gene upstream of *GNAS* locus.

In contrast to the sporadic variant of PHP1b, which is associated with broad *GNAS* imprinting abnormalities involving multiple DMRs, the autosomal dominant variant of PHP1b (AD-PHP1b) is most commonly associated with maternally derived microdeletions in the *STX16* gene.[4,5]

The exon A/B, which controls allele expression of Gsα, is in turn regulated by a long-range imprinting control region—a part of linked centromeric gene, *STX16*.[3] *STX16* mRNA is expressed in all tissues and has biallelic expression. The plausible explanation of implication of loss of function mutation in one allele of *STX16* as etiology of AD-PHP1b is thus explained by it being key regulator of Gsα. However, it is postulated that the regions between these microdeletions probably harbor a cis-acting imprint control region that is instrumental for determining methylation patterns at *GNAS* exon A/B, thus determining Gsα tissue-specific imprinting and final expression and explaining the etiopathogenesis.[6] A 3-kb microdeletion within *STX16* of *GNAS* is commonly reported as a cause of AD-PHP1b in unrelated families and sporadic cases.[7] A heterozygous 4.4-kb microdeletion involving exons 2-4 in *STX16* was later identified as novel etiology in 2005.[5] More recently, Elli *et al*. reported a larger 24.6 kb deletion involving exons 3–8.[8] These different size microdeletions described above are found to be overlapping and thus confirm the role of *STX16* regulating Gsα.

We report a novel heterozygous stop gain mutation c.C910T/p.Arg304X in exon 8 of the *STX16* gene [Figure 2]. Based on the literature review *STX16* gene variant has not been reported in any research publication or case study or disease databases (ClinVar/Human Gene Mutation Database/Leiden Open Variation Database). The presentation thus follows rules which the imprinted diseases follow—transmission from female carrier causes PHP1b, whereas transmission from male carrier does not lead to disease. Of the PHP1B cases, 15–20% are familial, with an autosomal dominant mode of inheritance (AD-PHP1B) through the maternal lineage.[8]

Another peculiar feature of AD-PHP1b is that PTH resistance does not manifest immediately after birth. The published data on PHP1b has variable age of presentation ranging from infancy to adulthood. Our patient was asymptomatic till

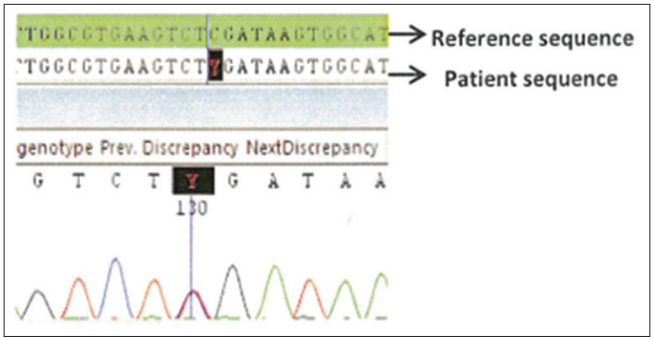

Figure 2: Novel heterozygous stop gain mutation in *STX16* in our patient.

9 years of age. The time lag in clinical presentation which was observed in our index patient has also been reported in other case reports as well. The delay is also worth noting in patients with PHP1a. The two postulations supporting the delayed age of presentation include the following:

i. One hypothesis proposes that both alleles of Gsα are expressed in renal tissue during early life, shown by RT-PCR from mouse and human fetal renal tissues[9]
ii. Other hypothesis suggests that actions of PTH are carried out through XLαs in early life.[6]

PTH action through the receptor complex, therefore, seems intact in the kidneys during the infancy and early childhood period.

The targeted investigation to establish genetic etiology in suspected PHP1b patients is methylation specific-multiplex ligation-dependent probe amplification (MS-MLPA) to identify deletions/duplications affecting *GNAS* and the methylation status of *GNAS* DMRs. The clinical manifestations of familial and sporadic forms are similar and thus, genetic testing is the only modality to differentiate between the two. The implications of genetic confirmation cannot be undermined. Thus, if a female patient is diagnosed with PHP and mutation is found in the GNAS locus (comprising of *GNAS, NESP55, NESPas,* and *STX* genes), the percentage of future progeny affected can be predicted, that is two out of four. Similarly, with the identification of same abnormality in male patients, like in our index case, the risk is two out of four for the progeny to develop PPHP.

CONCLUSION

We describe here an association between epigenotype (loss of methylation of exon A/B) and genotype (deletion in *STX16 gene*). We also identified a novel deletion in the *STX16* locus as a cause of AD-PHP1b. The understanding of the molecular mechanisms involved in the pathogenesis of PHP1b is evolving and we are yet to understand the subgroup of PHP1b which is caused by methylation defects of exon A/B. This suggests the existence of other and still unidentified genetic

rearrangements to explain the pathogenesis. Physicians need to be aware of this entity of PHP1b in view of its modality of inheritance, and distinct clinical, biochemical and phenotypic difference from PHP1a.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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