

Discordant Calcium and Parathyroid Hormone with Presumed Epileptic Seizures

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CASE DESCRIPTION

A 33-year-old woman presented for the first time at the age of 9 years with recurrent seizures and was subsequently treated for epilepsy for 2 years. At 11 years of age, it was noted that the patient had hypocalcemia with an increased parathyroid hormone (PTH)⁴ measured with use of an intact PTH assay. Serum calcium was 5.73 mg/dL [1.43 mmol/L; reference interval (RI) 8.82–10.42 mg/dL (2.2–2.6 mmol/L)]; serum phosphate was 10.42 mg/dL [3.36 mmol/L; RI 1.86–4.34 mg/dL (0.6–1.4 mmol/L)]; and PTH was 319.68 pg/mL [33.9 pmol/L; RI 8.49–68.84 pg/mL (0.9–7.3 mmol/L)]. Vitamin D (Total 25-OH; 25-OH D2 and D3) concentrations, magnesium concentrations, liver and renal function tests were all normal. No other endocrine abnormalities such as thyroid or gonadotropin resistance were detected. The patient also was of short stature and was overweight. However, she became overweight after the age of 13 years (body mass index at 13 years was 22.8 kg/m²), and the short stature was not significant, as she was at the lower end of the RI for height at 1.55 m. There was no cognitive impairment. Positive Chvostek sign was observed but not Trousseau sign.

The patient was placed on active vitamin D (alfacalcidol) and calcium supplements. The patient was followed up for 13 years; selected laboratory results are presented in Table 1. Although the patient was managed on calcium supplements and active vitamin D and followed up at a tertiary endocrine clinic, serum phosphate concentrations remained persistently high (Table 1). This was attributed to intermittent noncompliance and unavailability of active vitamin D. The patient had also been prescribed calcium carbonate as a phosphate binder.

QUESTIONS TO CONSIDER

1. What are the causes of hypocalcemia with an increased PTH concentration?
2. What is the explanation for the persistently increased PTH concentrations?
3. What is the role, if any, of molecular testing in a case like this?

DISCUSSION

The presence of an increased PTH with a calcium concentration below the RI along with the features of short stature and overweight should raise the suspicion of pseudohypoparathyroidism (PHP). Other possible causes include vitamin D deficiency and secondary hyperparathyroidism that is usually associated with chronic renal impairment. These conditions, however, were excluded, as vitamin D concentrations and renal functioning of the patient were within reference limits.

The patient was managed on vitamin D and calcium supplements, yet the PTH remained persistently increased. In patients with inadequate treatment, PTH may remain increased. This was also the case with this patient, as there were periods when treatment was not taken.

PTH concentrations also may be falsely increased owing to interference from heterophile antibodies, a common feature of earlier immunoassays. The introduction of the current intact PTH assay has markedly reduced this problem.

PSEUDOHYPOPARATHYROIDISM

In view of the patient's normal intelligence, lack of features of Albright hereditary osteodystrophy (AHO) other than considerations of overweight and short stature, and the hormonal resistance limited to PTH, PHP type 1b was suspected. The weight gain had occurred after the age of 13 years, and the height was thought to be at the lower end of the RI. The diagnosis of PHP type 1b was confirmed by use of a simple genetic test described previously in this journal by Weinhaeusel and colleagues (1) (Fig. 1). PHP is a rare condition characterized by a biochemical pattern of hypocalcemia, hyperphosphatemia, and in-

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⁴ Nonstandard abbreviations: PTH, parathyroid hormone; PHP, pseudohypoparathyroidism; RI, reference interval; AHO, Albright hereditary osteodystrophy; GNAS, GNAS complex locus; NESP55, neuroendocrine secretory protein-55.

Table 1. Trends of calcium, phosphate, and parathyroid hormone concentrations over 13 years of follow-up.^a

Dates	Calcium (mg/dL)	Phosphate (mg/dL)	Intact PTH (pg/mL)
11/18/2004	7.57 (8.82-10.22)	4.58 (2.70-4.50)	394 (12.3-65.1)
03/10/2005	7.45 (8.82-10.22)	5.61 (2.70-4.50)	358 (12.3-65.1)
06/30/2005	Ionized 3.29 (4.57-5.17)	6.48 (2.48-4.34)	330 (16.0-86.8)
11/17/2005	6.85 (8.22-10.26)	5.67 (2.48-4.34)	225 (16.0-86.8)
06/22/2006	7.82 (8.22-10.26)	4.90 (2.48-4.34)	303 (16.0-86.8)
11/30/2006	8.78 (8.22-10.26)	4.65 (2.48-4.34)	335 (16.0-86.8)
11/06/2008	7.94 (8.22-10.26)	4.74 (2.48-4.34)	300 (16.0-86.8)
09/02/2015	6.25 (8.62-10.02)	6.54 (2.42-4.40)	179 (12.3-87.7)

^a Values in the table are reported as concentrations (reference interval) expressed in traditional mass units. Conversion factors to standard international units: PTH pg/mL to pmol/L = 0.106X; calcium mg/dL to mmol/L = 0.250X; phosphate mg/dL to mmol/L = 0.323X.

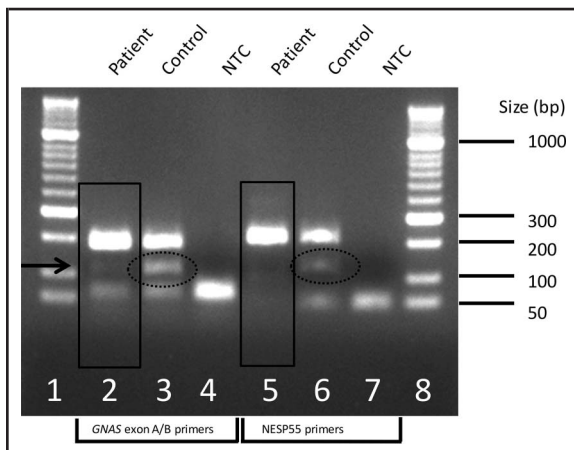


Fig. 1. Methylation testing of the patient's DNA compared with a control DNA.

Lane 1 & 8: Hyperladder 50 bp (Bioline); lane 2 & 5: Deaminated patient DNA amplified with use of *GNAS* exon A/B primers (lane 2) and NESP55 primers (lane 5); lane 3 & 6: Deaminated control DNA amplified with use of *GNAS* exon A/B primers (lane 3) and NESP55 primers (lane 6); lane 4 & 7: no-template controls (NTC). *GNAS* exon A/B multiplex PCR contained primers able to amplify both the methylated (113 bp) and unmethylated (180 bp) version of the *GNAS* exon A/B. The unmethylated allele product could be amplified from only the patient's sample, not the control sample. NESP55 multiplex PCR contained primers able to amplify both the methylated (219 bp) and unmethylated (129 bp) versions of NESP55. The methylated allele product could be amplified from only the patient's sample, not the control sample, which is indicative of a 4.7-kb NESP differentially methylated region (DMR) deletion, as these patients do not have the unmethylated allele. The result clearly identifies this as PHP-1b.

creased PTH concentrations (2). It refers to a group of disorders characterized by the physiologic inability to respond appropriately to PTH. The biochemical pattern of hypocalcemia and hyperphosphatemia is typical for hypoparathyroidism, but in PHP, PTH is increased. Thus, the term PHP refers to PTH resistance. PHP exists in several subtypes based on PTH resistance and the presence or absence of features of AHO, which include short stature, rounded face, central obesity, subcutaneous ossification, and mild mental retardation (2). The subtypes are subdivided on the basis of the biochemistry and the phenotypic presentation of the mutations.

PTH is the principal hormone that regulates calcium and phosphate homeostasis. It is secreted from the parathyroid gland in response to hypocalcemia. In patients with hypoparathyroidism, PTH is either absent or too low to bring about the desired response. Thus the fundamental difference between hypoparathyroidism and pseudohypoparathyroidism is that in PHP, PTH concentrations are more than adequate, but there is a lack of receptor response to PTH. PTH acts directly on the bone and kidneys. With regard to bone, PTH has receptors on osteoblasts leading to stimulation of osteoclasts that are responsible for bone resorption, thus leading to increased concentrations of calcium.

The physiological role of PTH is mainly resorptive, which leads to mobilization of both calcium and phosphate from the bone into the circulation. The clinical manifestations of long-term increased PTH have been documented to range from a decrease in bone density to debilitating conditions such as osteosclerosis (3). However, bone density scans and radiological imaging did not show any abnormality.

In our patient, the biochemical pattern revealed decreased calcium and increases in both phosphate and PTH. The coexistence of hypocalcemia, increased PTH associated with hyperphosphatemia, made the diagnosis of PHP a possibility. The biochemical pattern was con-

sistent with PHP subtype 1b, which was subsequently confirmed by analyzing the DNA sample recently obtained from the patient.

Unlike PHP-1a, which is characterized by presence of AHO features (2), generally patients with PHP-1b are defined by renal PTH resistance limited to the renal proximal tubule and the absence of AHO features (1). However, Zeniya and colleagues (4) recently described a patient who was diagnosed with sporadic PHP-1b with clinical features of AHO. PHP-1b results from changes in methylation that occur at the *GNAS* complex locus (*GNAS*) (2). PHP-1b can either follow an autosomal dominant pattern or a sporadic pattern (2, 5). The autosomal dominant form most commonly occurs as result of microdeletions in *STX16*, the gene encoding syntaxin 16, while the sporadic form has predilection to *GNAS* microdeletions involving exon NESP55 (6, 7). However, there is loss of methylation in the maternal exon A/B in both forms. In our patient, loss of methylation was detected at the exon A/B with a deletion observed at NESP55, as these patients do not have the NESP55 unmethylated allele (1) (Fig. 1). Although the genetic defects are distinct between the 2 PHP-1b groups, the biochemical pattern is similar, which leads to similar management (8).

While in our patient thyroid function was found to be normal, there have been recent reports of mild thyroid-stimulating hormone resistance in PHP-1b patients (9). In the kidneys, PTH stimulates 1- α -hydroxylase leading to formation of active vitamin D (1,25 dihydroxy vitamin D). PTH increases calcium reabsorption in the kidneys and inhibits phosphate reabsorption, promoting renal excretion of phosphate (phosphaturic effect). Active vitamin D stimulates absorption of calcium and phosphorus in the intestine and release from the storage pools from the bone.

The diagnosis of PHP can be easily made based on the coexistence of hypocalcemia, hyperphosphatemia and increased PTH provided that renal abnormalities and vitamin D deficiency have been excluded (2). Although the biochemical pattern provides guidance in terms of patient management, genetic determination of the underlying mutation is still essential (10).

CONCLUSION

Methylation defects in differentially methylated regions of *GNAS* are a feature of sporadic PHP-1b. The presentation of PHP may not always be obvious, and thus a high index of suspicion is required. These patients and their families can benefit from continued management at an endocrinology clinic. The genetic test for a methylation defect described by Weinhaeusel et al. (1) is simple and robust enough to be performed in most laboratories with basic molecular biology facilities and will facilitate rapid diagnosis in suspected cases.

POINTS TO REMEMBER

- Low calcium with an increased PTH and short stature should raise the suspicion of pseudohypoparathyroidism.
- Vitamin D deficiency and secondary hyperparathyroidism from renal disease may also cause low calcium and an increased PTH.
- Consider assay interference if calcium and PTH results are discordant.
- PHP-1b arises from methylation changes at the *GNAS* locus and be easily diagnosed with the methylation assay.

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Commentary

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Pseudohypoparathyroidism (PHP)² defines a heterogeneous group of rare and related disorders of end-organ resistance primarily impacting the actions of parathyroid hormone. In a patient with PHP type 1a or PHP type 1b, the typical biochemical pattern observed is hypocalcemia, hyperphosphatemia, and hyperparathyroidism, which may be present with or without (type 1b) physical abnormalities termed Albright hereditary osteodystrophy (AHO). On the other hand, the presence of AHO features in the absence of any endocrine abnormalities is indicative of pseudo-PHP (PPHP). This clinical case study illustrates the challenge of accurately diagnosing PHP and the role the laboratory plays in ruling out other causes of these biochemical abnormalities.

Chale-Matsau et al. report a case of a patient who presented with persistent hypocalcemia, hyperphosphatemia, and hyperparathyroidism over 13 years of follow-up. This patient's biochemical pattern was highly indicative of PHP, and it is important that the results are scrutinized for accuracy, especially in the absence of ge-

netic testing. In a patient with hypocalcemia, measurement of albumin or ionized calcium is important to distinguish true hypocalcemia (low ionized calcium) from factitious hypocalcemia (low albumin and total calcium but not ionized calcium). While in this case there was no mention of albumin, measurement of ionized calcium on 6/30/2005 was below the reference interval and confirmed true hypocalcemia. Other clinical causes of hyperparathyroidism such as vitamin D deficiency and chronic renal impairment must also be ruled out. These can be investigated by measurement of levels of serum 25-hydroxyvitamin D, serum creatinine, and/or cystatin C, which were normal in this patient.

In light of these true biochemical abnormalities and the lack of AHO features, this patient's presentation was consistent with PHP type 1b. Patients may be clinically managed even in the absence of confirmatory genetic testing. However, genetic testing is recommended and simple to perform when diagnosis remains uncertain.

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² Nonstandard abbreviations: PHP, pseudohypoparathyroidism; AHO, Albright hereditary osteodystrophy; PPHP, pseudo-PHP.

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Commentary

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As a rare disorder, the diagnosis of pseudohypoparathyroidism (PHP)² is challenging. This case illustrates, how-

ever, that an understanding of parathyroid action and of the genetic mechanisms underlying PHP can effectively guide its diagnosis.

The spectrum of severe hypocalcemia, hyperparathyroidism, and hyperphosphatemia raises suspicion for PHP. An increased parathyroid hormone (PTH) concentration distinguishes PHP from hypoparathyroidism. Severe vitamin D deficiency and renal dysfunction can resemble PHP, but measurement of glomerular filtration rate and 25-OH vitamin D should aid this distinction. If

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² Nonstandard abbreviations: PHP, pseudohypoparathyroidism; PTH, parathyroid hormone; PPR, PTH/PTH-related peptide receptor.