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Failure of tooth eruption and brachydactyly in pseudohypoparathyroidism are not related to plasma parathyroid hormone-related protein levels

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ABSTRACT

Background: Pseudohypoparathyroidism (PHP) is a genetic disorder characterized by resistance to the peripheral action of PTH due to maternally inherited heterozygous inactivating mutations in the coding sequence of $G_s\alpha$ or intronic regions of *GNAS* leading to aberrant splice variants (PHP1A), or methylation defects at *GNAS* (PHP1B). Brachydactyly is a clinical feature associated with both PHP1A and PHP1B, although it is more frequent in PHP1A patients. Loss-of-function mutations in *PTHLH*, the gene coding for parathyroid hormone related protein (PTHrP) were previously described in some patients with brachydactyly. Primary failure of tooth eruption (PFE) is related to some syndromes involving skeletal development, but it is also known as a nonsyndromic autosomal dominant condition. Previous studies showed that familial nonsyndromic PFE is caused by heterozygous mutations in the gene encoding the G protein-coupled receptor (PTH1R) for PTH and PTHrP. Thus, we hypothesized that PTHrP resistance could result in failure of tooth eruption (FTE) and/or brachydactyly in PHP.
Subjects and methods: Nineteen patients with a molecular diagnosis of PHP underwent dental panoramic radiography (DPR), hand radiography and had their PTHrP levels measured. Patients with alterations at DPR were submitted to clinical dental evaluation.
Results: Nine patients had FTE and 7 patients had brachydactyly; 4 patients presented both features and none of them presented high PTHrP levels. Fourteen patients had PTHrP levels within the normal range and only one patient had slightly elevated PTHrP levels. Additionally, three novel *GNAS* mutations were described.
Conclusion: We described the dental abnormalities in a large series of PHP patients that were followed in a single tertiary center. No relationship between plasma PTHrP levels and failure of tooth eruption, dental manifestations of PHP or brachydactyly was found. It is important that doctors pay attention to dental manifestations of the disease in order to refer patients to a proper care with dentists.

1. Introduction

Pseudohypoparathyroidism (PHP) is a rare heterogeneous genetic disorder characterized by resistance to the peripheral action of PTH due to maternally inherited heterozygous inactivating mutations in the coding sequence of $G_s\alpha$ or intronic regions of *GNAS* leading to aberrant splice variants (PHP1A), or methylation defects at *GNAS* (PHP1B), which results in hypocalcemia and hyperphosphatemia with elevated PTH levels. The common methylation defect found in PHP1B is loss of methylation in exon A/B, although other regions can also be affected (the differentially methylated regions: NESP55, AS and XL). PHP1B can be sporadic or familial with an autosomal dominant mode of inheritance (AD-PHP1B). Most cases of AD-PHP1B are due to heterozygous deletions of the *STX16* gene [1].

Brachydactyly is a clinical feature classically associated with PHP1A, although patients with PHP1B may also have brachydactyly [2]. The brachydactyly found in PHP involves a characteristic shortening of III, IV, and V metacarpals and I distal phalanx [1], which is quite similar to brachydactyly type E (BDE). BDE involves variable shortening of the metacarpals with essentially normal phalangeal length [3].

In some patients with BDE and short stature from five unrelated families, Klopocki *et al.* detected loss-of-function mutations in *PTHLH*, the gene coding for parathyroid hormone related protein (PTHrP). These authors noticed that two out of five families had dental problems,

Abbreviations: FTE, failure of tooth eruption; DPR, dental panoramic radiography; BDE, brachydactyly type E..

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which suggests the involvement of PTHrP in tooth development and/or eruption [4]. *In vivo*, Philbrick *et al.* showed that PTHrP-knockout mice die at birth with a chondrodystrophic phenotype; on the other hand, replacement of PTHrP expression in the chondrocytes of PTHrP-knockout mice resulted in the correction of the lethal skeletal abnormalities and generated animals that were small in stature and displayed a number of developmental defects, including cranial chondrodystrophy and failure of tooth eruption (FTE). Restoration of PTHrP expression in the enamel epithelium corrected the defect in bone resorption and restored the normal program of tooth eruption [5].

The clinical spectrum of tooth eruption disorders comprises syndromic and non-syndromic conditions ranging from delayed to total failure of eruption [6]. Primary failure of tooth eruption (PFE) is rare nonsyndromic autosomal dominant disorder, and previous studies showed that familial nonsyndromic PFE is caused by heterozygous mutations in the gene encoding the G protein-coupled receptor (PTH1R) for PTH and PTHrP [7–9].

PTH1R is a membrane receptor coupled to $G_s\alpha$ that binds PTH and PTHrP with similar affinity, and both ligands equally stimulate adenylate cyclase [10]. In both PHP1 A and PHP1B, there is resistance to the peripheral action of PTH. As PTHrP shares the same receptor, as well as the affected coupled-$G_s\alpha$, we hypothesized that resistance to PTHrP might also be found in PHP; by extension, PTHrP resistance could result in FTE and/or brachydactyly in PHP. We therefore measured plasma PTHrP levels and investigated the relationship to FTE and brachydactyly in 19 patients with PHP.

2. Patients and methods

2.1. Patients and molecular studies

The study protocol was approved by the Ethics Committee of the Clinics Hospital of University of Sao Paulo (CAPPesq – HCFMUSP) and informed consent was obtained from all patients or their parents.

Nineteen patients were selected on the basis of presence of PTH resistance (i.e., elevated PTH levels, hypocalcemia and/or hyperphosphatemia, with normal renal function) and molecular diagnosis of PHP, with ages between 8 and 48 years. Nine females and 10 males were selected for this study.

As these conditions are rare, genetic tests were only performed for the *GNAS* and *STX16* genes that are known to be related to PHP syndromes. We used the following techniques: PCR and direct sequencing of *GNAS*, and methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). The results were compared to the wild type *GNAS* sequence published at www.ensembl.org (ENST00000371085).

2.2. Biochemistry

Blood samples from the patients were drawn between 7 and 10 am after an overnight fast. Serum levels of calcium, ionized calcium and phosphate were measured using standard laboratory methods. PTH concentrations were measured by Immulite 2000 Intact PTH assay (DPC, Los Angeles, CA, USA) and PTHrP levels by immunoassay using a C-terminal PTHrP assay (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, USA).

2.3. Radiology and clinical dental evaluation

Brachydactyly was identified through clinical evaluation or X-ray. On X-ray examination, we used the metacarpal sign to identify the brachydactyly, which consists of drawing a line tangentially to the circumference of the heads (distal ends) of the fourth and fifth metacarpals. Normally, the extension of this line passes distal to the head of the third metacarpal, and the metacarpal sign is negative. If the line runs through the distal end of the third metacarpal, it indicates a specific relationship, and the metacarpal sign is positive [11].

All patients underwent dental panoramic radiography (DPR) to determine FTE. DPR could also detect short blunted roots, widened pulp chambers and/or calcified intrapulpal deposits and hypodontia. Additionally, patients with alterations at DPR were submitted to clinical dental evaluation to characterize enamel hypoplasia.

3. Results

Six patients harbored mutations in the $G_s\alpha$ coding region or intronic regions of *GNAS* (PHP1A) and 13 presented with methylation defects at *GNAS* (PHP1B). We identified deletion of exons 4–6 of the *STX16* in only three patients with PHP1B. We did not identify any other deletion of *STX16* or differentially methylated regions NESP or AS of the *GNAS*. Three novel *GNAS* mutations were described (Table 1).

Fourteen patients had PTHrP levels within the normal range; this group included three patients with normal DPR. Only patient 7 had slightly elevated PTHrP levels; she had brachydactyly but without dental alterations. Four patients had slightly low PTHrP levels. We also measured PTH and total calcium levels concomitantly with PTHrP (Table 1). All patients had normal renal function (data not shown).

Four patients had normal DPR for their age and thus did not undergo further dental evaluation. Fifteen patients were clinically evaluated. We found FTE in 9 patients and brachydactyly in 7 patients; 4 patients presented both features and none of them presented with high PTHrP levels (Table 1).

Additional dental manifestations detected were: short blunted roots (12/19), widened pulp chambers and/or calcified intrapulpal deposits (4/19), hypodontia (1/19) and enamel hypoplasia (12/15) as shown in Table 1. The most prominent dental manifestations of our cohort can be seen in patients 10 and 16 (Fig. 1).

FTE and additional dental findings were present in both PHP types, whereas brachydactyly was more prevalent in PHP1A.

4. Discussion

PTHrP is expressed in many normal tissues, such as neuroendocrine tissues, normal keratinocytes, endothelial cells, smooth muscle, lactating mammary tissue, tooth and bone; it acts as a paracrine regulator in these sites [12]. So far, only three conditions in which PTHrP is found in the circulation and acts in an endocrine manner have been reported. These are the syndrome of humoral hypercalcemia of malignancy (HHM), in which PTHrP is made by tumors and circulates to the bone to stimulate bone resorption; lactation, in which PTHrP is produced in the breast and released into the general circulation; and fetal life, where PTHrP controls maternal-to-fetal placental calcium transport [13]. Plasma PTHrP levels have not previously been measured in PHP. We believed that PHP might also be a condition in which systemic levels of PTHrP were elevated, and that this might contribute to FTE or to the dental manifestations of PHP and brachydactyly.

In the present study, we measured plasma PTHrP levels and investigated FTE and brachydactyly in 19 patients with PHP. We also described the dental abnormalities in this large series of PHP patients followed in a single tertiary center. Plasma PTHrP levels were not significantly elevated in our cohort.

The relationship between PTHrP and FTE and/or brachydactyly had never been studied in PHP. Contrary to our expectations, no association between plasma PTHrP levels and FTE or dental manifestations of PHP and brachydactyly was found. This lack of association might be explained by local resistance to PTHrP in enamel epithelium and chondrocytes, respectively. Furthermore, we did not detect any relationship between FTE and brachydactyly.

In the medical literature, there is a lack of information concerning PHP and dental findings. In addition to other dental abnormalities, FTE was found in both PHP1A and PHP1B in our cohort. All PHP1A patients had at least one dental alteration. In contrast, in the PHP1B group, we observed significant variability with regard to dental manifestations,