Potential Effects of Alendronate on Fibroblast Growth Factor 23 Levels and Effective Control of Hypercalciuria in an Adult with Jansen’s Metaphyseal Chondrodysplasia

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Context: Jansen’s metaphyseal chondrodysplasia (JMC) is a rare autosomal dominant disorder caused by activating mutations in the PTH 1 receptor (PTH1R; PTH/PTHrP receptor), leading to chronic hypercalcemia and hypercalciuria. Hypophosphatemia is also a hallmark of JMC, and recently, increased fibroblast growth factor 23 (FGF23) levels have been reported in this syndrome. Hypercalcemia has been associated with increased cardiovascular risk; however, cardiovascular disease has not been extensively investigated in JMC patients.

Objective: The aim of the study was to describe the long-term follow-up of a JMC patient with regard to the management of hypercalciuria, the evaluation of FGF23 levels under bisphosphonate treatment, and the investigation of cardiovascular repercussion of chronic hypercalcemia.

Results: The diagnosis of JCM was confirmed by molecular analysis (p.H223R mutation in PTH1R). The patient was followed from 5 to 27 yr of age. Asymptomatic nephrolithiasis was diagnosed at 18 yr of age, prompting pharmacological management of hypercalciuria. Treatment with alendronate reduced hypercalciuria; however, normocalciuria was only obtained with the association of thiazide diuretic. Serum FGF23 levels, measured under alendronate treatment, were repeatedly within the normal range. Subclinical cardiovascular disease was investigated when the patient was 26 yr old, after 19 yr of sustained mild hypercalcemia; carotid and vertebral artery ultrasonography was normal, as well as coronary computed tomography angiography (calcium score = 0).

Conclusion: The long-term follow-up of our JMC patient has provided insight on therapeutic strategies to control hypercalciuria, on the potential effects of alendronate on FGF23 levels, and on the lack of detectable cardiovascular disease at young adulthood after prolonged exposure to hypercalcemia. (J Clin Endocrinol Metab 97: 1098–1103, 2012)
vating mutations occurring elsewhere in the protein have been associated with Blomstrand chondrodysplasia (MIM 215045), Eiken syndrome (MIM 600002), and primary failure of tooth eruption (MIM 125350).

Fibroblast growth factor 23 (FGF23) is an important regulator of urinary phosphate excretion, largely regulated by serum phosphate levels and calcitriol (4). Elevated FGF23 serum levels have been found in an infant JMC patient despite hypophosphatemia and normal calcitriol levels, leading to the hypothesis that PTH1R signaling could regulate FGF23 levels (5). Indeed, recent experimental data in mice have suggested that PTH itself might regulate FGF23 levels directly (6) and that PTH1R signaling in osteocytes results in increased FGF23 expression in vitro and in vivo (7).

Serum calcium levels have been positively associated with cardiovascular risk (8), and recently, calcium supplementation without vitamin D has been associated with increased risk of myocardial infarction (9). Indeed, states of chronic hypercalcemia such as primary hyperparathyroidism have been previously associated with increased cardiovascular risk (10). JMC represents a natural model of persistent hypercalcemia and, therefore, increased cardiovascular morbidity could be expected in this group but has not been extensively explored.

Here, we report the management of hypercalcemia and hypercalciuria in the long-term follow-up of an adult JMC patient in whom FGF23 levels were found to be within the normal range, and no evidence of detrimental cardiovascular repercussion of hypercalcemia was seen.

**Case Report**

A female offspring of nonconsanguineous parents presented at birth with muscular hypotonia, brachycephaly, and softened skull. Diagnosis of JMC was made when she was 1 yr old, based on skeletal abnormalities and biochemical determinations (data not available). Currently, the patient is 27 yr old, has two healthy sisters, and reports no similar cases in the family.

The patient was referred to our institution (Hospital das Clínicas, University of São Paulo School of Medicine, São Paulo, Brazil) at the age of 5 yr due to bone pain, walking difficulties, and short stature. Her height was 87.5 cm ($SD = -4.16$), and weight was 15.7 kg ($SD = -1.12$). Upon physical examination, several stigmas and skeletal abnormalities were detected, such as macrocephaly, facial hypertelorism, unproportional short stature with short limbs, brachydactyly, and deformities in superior limbs (wrist widening), inferior limbs (genu varum), and in the rib cage. Biochemical analysis showed high total calcium serum levels, normal serum phosphorus levels, and preserved renal function despite hypercalciuria (Table 1). At that point, determination of serum PTH levels was not available, but her urinary cAMP was elevated (5.1 nmol/mg creatinine; normal range, 2.7–4.1). Radiological evaluation revealed shortening of long bones, including phalanges and metacarpus, as well as irregularity and widening of the metaphyses although the epiphyses appeared normal (Fig. 1). Based on this initial assessment, the clinical diagnosis of JMC was made.

**Table 1.** Serial laboratory evaluation of the JMC patient

<table>
<thead>
<tr>
<th>Normal range</th>
<th>5</th>
<th>19</th>
<th>20–25</th>
<th>26</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>8.6–10.2</td>
<td>11.0</td>
<td>10.6</td>
<td>9.0–10.4</td>
<td>9.8</td>
</tr>
<tr>
<td>iCa (mg/dl)</td>
<td>4.6–5.3</td>
<td>5.7</td>
<td>5.4–6.0</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>uCa (mg in 24 h)</td>
<td>&lt;4 mg/kg/d</td>
<td>7.2</td>
<td>8.2</td>
<td>4.2–6.7</td>
<td>5.4</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>11–62</td>
<td>&lt;11</td>
<td>&lt;11</td>
<td>&lt;11</td>
<td>&lt;11</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.5–1.4</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4–0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>3.9</td>
<td>3.1</td>
<td>2.4–3.5</td>
<td>2.9</td>
<td>2.7</td>
</tr>
<tr>
<td>1,25-(OH)2D (pg/ml)</td>
<td>(3.4–6.2)</td>
<td>(2.3–4.6)</td>
<td>(2.3–4.6)</td>
<td>(2.3–4.6)</td>
<td>(2.3–4.6)</td>
</tr>
<tr>
<td>FGF23 (pg/ml)</td>
<td>18–72</td>
<td>26–41 (n = 5)</td>
<td>33</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>AP (U/liter)</td>
<td>379 (up to 150)</td>
<td>207 (up to 104)</td>
<td>66</td>
<td>96–134</td>
<td>61</td>
</tr>
<tr>
<td>P1NP (ng/ml)</td>
<td>15.1–58.6</td>
<td>65.4–90.1</td>
<td>32.4</td>
<td>27.8</td>
<td></td>
</tr>
<tr>
<td>Oc (ng/ml)</td>
<td>11–43</td>
<td>55.5–73.6</td>
<td>46</td>
<td>40.2</td>
<td></td>
</tr>
<tr>
<td>CTx (ng/ml)</td>
<td>Up to 0.57</td>
<td>0.56–0.69</td>
<td>0.43</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$Normal range values vary with age and are shown in parentheses under the measurements.

PTH concentrations were determined by an immunoradiometric assay recognizing the intact 1-84 PTH (ELISA-PTH kit; CIS Biointernational, Gif-sur-Yvette, France). Serum levels of intact FGF23 were determined by sandwich ELISA for human FGF23 (Kainos, Inc., Tokyo, Japan). Total P1NP, N-MID Oc and β-CTx were determined by electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). BP, Bisphosphonate (alendronate 10 mg/d); Ca, total calcium; iCa, ionized calcium; uCa, urinary calcium; Cr, creatinine; P, phosphorus; 1,25-(OH)2D, calcitriol; FGF23, intact FGF23 (n = number of measurements); AP, alkaline phosphatase.
Molecular analysis of PTH1R was performed in genomic DNA samples obtained from the patient and her parents. Institutional review board approval and written informed consent were obtained before sample collection. Exons 7 (codon 223), 12 (codon 410), and 13 (codon 458) of PTH1R were PCR-amplified as previously described (3), and direct automated sequencing was performed. A heterozygous adenosine to guanine variant at position 668 of the coding region (c.668A/H11022G) was found in exon 7 (Fig. 2A). This substitution was confirmed by restriction fragment length polymorphism using the restriction endonuclease SphI and was not found in the patient’s parents (Fig. 2B). The c.668A/G variant leads to a change of amino acid at residue 223 of the protein from histidine to arginine (p.H223R) and has been previously described as a cause of JMC (1), confirming the diagnosis in our patient.

The patient underwent a series of orthopedic surgeries between the ages of 12 and 16 yr to correct inferior limb deformities and to optimize final stature (total gain, 12 cm; final height, 125 cm). When she reached 18 yr of age, renal ultrasound revealed nonobstructive unilateral nephrolithiasis, probably as the result of chronic hypercalciuria. To attempt to reduce the hypercalciuria, alendronate (10 mg/d) was introduced at 20 yr of age. Three years later, extracorporeal shock wave lithotripsy was performed due to an asymptomatic increase in the number and size of kidney stones. Hypercalciuria was reduced but not normalized by treatment with alendronate; for this reason, 12.5 mg/d of hydrochlorothiazide (HCTZ) was prescribed at age 26 and was subsequently increased to 25 mg/d, leading to successful normalization of calciuria (Table 1).

Serum alkaline phosphatase levels were consistently elevated until 20 yr of age and normalized after the onset of bisphosphonate treatment. Determination of serum levels of FGF23, procollagen type 1 amino terminal propeptide (P1NP), osteocalcin (Oc), and carboxy-terminal collagen crosslinks (CTx) became available in our institution when the patient was 22 yr old and was already using alendronate 10 mg/d. In this condition, FGF23 levels were repeatedly within the normal range, and P1NP, Oc, and CTx levels were also predominantly normal (Table 1). Of note, dual-energy x-ray absorptiometry follow-up revealed that bone mineral density remained stable under treatment with alendronate.

Considering that chronic persistent hypercalcemia could be associated with increased cardiovascular risk, the patient was investigated for coronary and carotid-vertebral vascular disease at 26 yr of age. Carotid and vertebral arteries appeared normal under ultrasonographic Doppler examination, and 320-row multidetector coronary computed tomography angiography (CTA) with calcium-score screening did not detect the presence of calcium within the coronary arteries (score = 0) or any degree of luminal reduction (Fig. 3).

Discussion

We report the long-term follow-up of a patient with JMC from 5 to 27 yr of age.

Very few reports in the literature discuss treatment options for patients with JMC. Most of these focused on the reduction of hypercalcemia and hypercalciuria aiming to ameliorate nephrocalcinosis and/or nephrolithiasis to spare renal function. Parfitt et al. (11) reported a case with early-onset nephrocalcinosis in whom a 6-month course of
oral phosphate at 5.5 yr of age failed to reduce hypercalcemia. The same patient presented with nephrolithiasis at a later age and was treated with a 10-d course of hydrocortisone (150 mg/d) and a 7-d course of calcitonin (200 U/d), which also failed to reduce the hypercalcemia. Schipani et al. (3) reported daily therapy with intranasal salmon calcitonin for a JMC patient from 2 to 7 yr of age with no measurable effects on biochemical parameters. In that patient, treatment with alendronate (10 mg, twice weekly), initiated at 7 yr of age due to significant nephrocalcinosis, led to the normalization of serum calcium levels and reduced urinary calcium excretion.

In the case presented here, asymptomatic nephrolithiasis was detected when the patient was 18 yr old, leading to the introduction of 10 mg/d of alendronate, which resulted in reduction but not normalization of calciuria (Table 1). Therefore, at 26 yr of age, 12.5 mg/d of HCTZ was added and subsequently increased to 25 mg/d, leading to successful normalization of calciuria. To our knowledge, this is the first report of successful normalization of calciuria in a JMC patient with bisphosphonate and thiazide diuretic combination therapy. Considering the detrimental consequences of sustained hypercalciuria in these patients, this treatment option could be considered in other JMC cases.

Our patient bears a de novo heterozygous p.H223R mutation in PTH1R. This substitution was the first activating mutation in PTH1R to be described in association with JMC and has been subsequently identified in a number of cases (1–3, 5, 12). In vitro studies have confirmed that this substitution results in constitutive ligand-independent intracellular activation of cAMP signaling (13). A recent report by Brown et al. (5) of a patient with JMC also bearing the p.H223R mutation has described elevated serum FGF23 levels in the presence of hypophosphatemia during the first 2 yr of life. Although the iron status of the patient was not available and recent evidence suggests that iron deficiency might lead to increased FGF23 in certain situations (14, 15), this finding has indicated that PTH1R signaling may constitute an additional mechanism of regulation of FGF23 levels. This emerging concept has been corroborated by recent experimental data in mice, where paradoxically low Fgf23 levels were found in parathyroidectomized mice despite hyperphosphatemia (6) and constitutively activated PTH1R signaling in osteocytes from transgenic animals resulted in increased expression of Fgf23 (7).

Serum FGF23 levels, repeatedly measured in our patient from 24 yr of age onward, were always within the normal range (Table 1). Concomitant serum phosphorus levels were within the low normal range, and calcitriol levels were also normal. However, all FGF23 measurements were made during treatment with alendronate, which seemed to effectively reduce bone turnover as shown by reduction of serum alkaline phosphatase. Therefore, we suppose that alendronate reduced FGF23 levels through the reduction of bone turnover. Data on the potential effects of bisphosphonates on FGF23 levels are scarce and conflicting. Treatment with alendronate has been shown to modestly increase Fgf23 levels in oophorectomized female mice, whereas no such influence was seen in male animals (16). In humans, a decline in FGF23 preceding changes in serum phosphate levels was recently observed after iv pamidronate in the treatment of osteogenesis imperfecta (17). Accordingly, reports of reduction of FGF23 levels after treatment of primary hyperparathyroidism (18) and Graves’ disease (19), conditions that are also marked by high bone turnover, support the hypothesis that reduction of bone turnover might lead to a reduction of FGF23 levels.

Epidemiological data have associated serum calcium levels to cardiovascular risk, both with values within the normal range (8, 20) and in hypercalcemic states (10, 21, 22). Moreover, a recent metaanalysis found an increased risk of myocardial infarction in association with calcium supplementation without vitamin D (9). Our JMC patient represents a natural model of chronic hypercalcemia; therefore, to determine the potential cardiovascular implications of such long-term exposure to increased serum calcium levels, she was investigated with carotid/vertebral and coronary imaging. The results of this investigation were surprisingly normal. In fact, no calcium deposits were detected within the coronary arteries by CTA. Although coronary CTA is not frequently performed in individuals in the third decade of life, asymptomatic coronary artery calcification has been detected by electron
beam CTA in a majority of patients with end-stage renal disease between 20 to 30 yr of age (23). The absence of coronary calcification in our patient may suggest that normal low serum phosphorus levels have protected her from detrimental cardiovascular repercussions of hypercalcemia. Indeed, recent data have emerged of a prominent association of serum phosphorus with cardiovascular risk in individuals with or without cardiovascular disease (24, 25) and in chronic kidney disease (26). Perhaps actively investigating the potential cardiovascular repercussion of more prevalent genetic hypercalcemic states, such as familial hypocalciuric hypercalcemia, may help clarify this issue in the future.

In conclusion, in a JMC patient bearing a de novo heterozygous p.H223R mutation in PTH1R, we demonstrate potential effects of alendronate on FGF23 levels and effective control of hypercalciuria with combined oral bisphosphonate and thiazide diuretic therapy. In addition, the absence of cardiovascular repercussion in this patient, despite long-term exposure to sustained hypercalcemia, may shed light on the complex interplay between serum calcium and phosphorus levels in association with cardiovascular risk.

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References


