Renal Phosphate Wasting in Fibrous Dysplasia of Bone Is Part of a Generalized Renal Tubular Dysfunction Similar to That Seen in Tumor-Induced Osteomalacia

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ABSTRACT

Fibrous dysplasia (FD) of bone is characterized by focal replacement of normal bone and marrow with abnormal bone and fibrous tissue. It arises from postzygotic activating mutations of the GNAS1 gene. Hypophosphatemia due to renal phosphate wasting has been reported in association with FD as a part of the McCune-Albright Syndrome (MAS), which is characterized by FD, skin hyperpigmentation, and precocious puberty. To date, the prevalence and mechanism of phosphate wasting has not been well studied. We evaluated 42 patients with FD/MAS. Serum and urine samples were tested for indices of mineral metabolism, amino acid handling, and markers of bone metabolism. Twenty (48%) patients had some degree of renal phosphate wasting. Nephrogenous cyclic adenosine monophosphate (cAMP) was normal in FD patients, suggesting that the underlying cause of phosphate wasting is not the presence of activating GNAS1 mutations in the kidney. In addition, there was evidence of a more generalized renal tubulopathy as represented by the presence of abnormal vitamin D metabolism, proteinuria in 36 (86%) patients, and aminoaciduria in 39 (94%) patients. Renal phosphate wasting significantly correlated with the degree of bone involvement, as assessed by serum and urine markers of bone metabolism, suggesting that a circulating factor produced by FD bone and impacting on the kidney may be the mechanism. These data show that phosphaturia as part of a generalized renal tubulopathy represents the most common extraskeletal manifestation of FD and that the observed tubulopathy is similar to that seen in tumor-induced osteomalacia (TIO). (J Bone Miner Res 2001;16:806–813)

Key words: fibrous dysplasia, phosphaturia, tumor-induced osteomalacia, phosphatonin, McCune-Albright syndrome

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INTRODUCTION

Fibrous dysplasia (FD) of bone is characterized by replacement of normal bone and bone marrow by a benign fibroosseous tissue.\(^{(1,2)}\) It may exist at a single skeletal site (monostotic fibrous dysplasia [MFD]), at multiple skeletal sites (polyostotic fibrous dysplasia [PFD]), or as part of the McCune-Albright syndrome (MAS). MAS is a rare disease classically characterized by the triad of PFD, café-au-lait pigmentation of the skin, and precocious puberty (PP).\(^{(3,4)}\) The molecular basis of the disease is a postzygotic activating mutation of the GNAS1 gene, which codes for the G protein, Gs.\(^{(5,6)}\) Gs is a key regulator of the cyclic adenosine monophosphate (cAMP) signaling cascade, and certain mutations of this protein (G227L, R201C, R201H, R201S, and R201G) cause persistent activation of adenyl cyclase and increased production of intracellular cAMP.\(^{(7)}\) It has been postulated that germ line mutations are lethal.\(^{(8)}\) The postzygotic occurrence of the mutations results in somatic mosaicism, with patients presenting with a broad spectrum of affected tissues. If the mutation occurs early in development, multiple tissues are involved, and the complete syndrome, MAS, results. Limited tissue involvement may result if the mutation occurs later in development. This may be the case in isolated pituitary adenomas that harbor GNAS1 mutations.\(^{(9)}\) If the mutation occurs only in skeletal tissues, the result is MFD or PFD.

Rickets and/or osteomalacia have been reported anecdotally as a rare complication of MAS, with approximately 12 cases in the literature.\(^{(10–17)}\) One case of osteomalacia has been reported in association with isolated FD.\(^{(13)}\) The rickets/osteomalacia in FD has been shown to be associated with hypophosphatemia and renal phosphate wasting.\(^{(12,14,18)}\) It has been suggested that renal phosphate wasting in FD/MAS may be caused by either renal tubule cells bearing Gs mutations or, alternatively, to the presence of a circulating phosphaturic factor produced by the dysplastic bone.\(^{(20)}\) However, there is little evidence to support either possibility to date.

In an effort to establish the prevalence and etiology of phosphate wasting in FD/MAS, we performed an evaluation of mineral metabolism in 42 consecutive patients with FD/MAS. Because phosphaturia often coexists with additional abnormalities in renal function in other diseases such as tumor-induced osteomalacia (TIO), X-linked hypophosphatemia, and the Fanconi’s syndrome, we also investigated other aspects of renal tubular function including vitamin D metabolism and urinary protein and amino acids.

MATERIALS AND METHODS

FD/MAS patients

Forty-two patients who were enrolled in an Institutional Review Board (IRB)–approved study (98-D-0145) of the natural history of FD/MAS at the National Institute of Dental and Craniofacial Research of the National Institutes of Health were studied. The diagnosis of FD was confirmed by some combination of clinical history, radiographic findings, histopathological findings, and, when needed, sequence analysis of the appropriate region of the GNAS1 gene.\(^{(1,2,5)}\) Only patients in whom the diagnosis was clearly established were included in the analysis.

All patients were maintained on a hospital diet. The average daily intake of the following nutrients were as follows: calcium, 850 mg (range, 690–1066 mg); phosphorus, 1312 mg (range, 1065–1744 mg); potassium, 3596 mg (range, 3107–4595 mg); sodium, 5111 mg (range, 4282–6412 mg).

At the time of evaluation, four of the patients in this series were children (<18 years old), yet had PP, which leads to advanced bone age, and were skeletally mature. In statistical analyses these four patients were analyzed according to their bone age, as assessed by the method of Gruelich and Pyle, rather than chronological age.

Hyperparathyroid patients

Nephrogenous cAMP was measured in a randomly selected group of 10 patients with primary hyperparathyroidism (PHPTH) and were seen under a different IRB-approved protocol for the treatment of PHPTH (91-DK-0085).

Serum and urinary measures

All measurements of compounds in sera were performed in duplicate on specimens collected on successive days at 8 a.m. after an overnight fast while on a hospital diet. Urine measurements were performed on aliquots taken from 24-h collections, which were collected coincident with the serum samples and had been refrigerated throughout the collection. Measurements of serum and urinary creatinine, calcium, phosphorus, protein, amino acids, parathyroid hormone (PTH), vitamin D metabolites, and others were all assessed by standard commercially available techniques.

Renal phosphate handling was assessed by calculating the maximum rate of reabsorption of phosphate relative to the glomerular filtration rate (TmP/GFR) from 24-h urine collections, an adaptation of the technique and nomogram of Bijovet.\(^{(27)}\) Age- and gender-appropriate norms for TmP/GFR were taken from the literature.\(^{(28)}\) Vitamin D\(_3\) metabolites, 25-hydroxyvitamin D\(_3\) [25(OH)\(_2\)D\(_3\)] and 1,25-dihydroxyvitamin D\(_3\) [1,25(OH)\(_2\)D\(_3\)], were assayed using commercially available high-pressure liquid chromatography (HPLC) and radioreceptor assays, both with a percent CV (%CV) of 12% (Mayo Medical Laboratories, Rochester, MN, USA). The N-terminal segment of bone collagen (N-telopeptide) was measured using a commercially available ELISA with a %CV of 10% (Mayo Medical Laboratories, Rochester, MN, USA). Collagen cross-linking elements (pyridinoline [PYD] and deoxypyridinoline [DPYD]) were measured by commercially available assays using an HPLC technique with a %CV of 4.7% (Mayo Medical Laboratories). Bone-specific alkaline phosphatase was measured using a Beckman 6300 amino acid analyzer (Beckman, Palo Alto, CA, USA) equipped with a lithium 10-cm column according to an established method.\(^{(29)}\) Nephrogenous cAMP was
assayed with a commercially available HPLC technique with a %CV of <10% (Mayo Medical Laboratories).

Statistical analysis

Bivariate correlations among numerically continuous variables were computed using the Pearson or Spearman r, and simple linear regression lines were plotted. Correlations also were computed controlling for (partialing out) age. Two-way analysis of variance (ANOVA) was performed to compare means between gender and age groups with a test for interaction. Values of p ≤ 0.05 were considered statistically significant. No Bonferroni adjustments were performed. All analyses were performed using SAS version 6.12 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Patient demographics

Patient demographics are presented in Table 1. The patients studied had a spectrum of bone disease that ranged from mild MFD to severe PFD (with nearly every bone involved, panostotic). Ages ranged from 5 to 57 years. There were 25 female and 17 male patients—36 whites, 2 blacks, 3 Hispanics, and 1 Asian.

Renal phosphate wasting

Given the age- and gender-specific differences in TmP/GFR, the results of TmP/GFR testing are grouped and displayed accordingly (Fig. 1). The normal ranges are derived from the literature with the normal ranges (mean ± 2 SD) indicated. Twenty patients (48%) had a reduced TmP/GFR and thus some degree of phosphate wasting. The correlation between TmP/GFR and serum phosphorus was strong (r = 0.79 for children and r = 0.89 for adults, data not shown). However, in 13 of the 20 patients with a low TmP/GFR (65%), the serum phosphorus was within the age-specific normal range (data not shown). Using a two-way ANOVA for TmP/GFR, there was a statistically significant interaction between gender and TmP/GFR in the 26- to 45-year age group; all of the males in this group had a low TmP/GFR and 8 of the 9 females had a TmP/GFR within the normal range.

Vitamin D metabolism

In normal subjects, serum phosphorus is an important regulator of the 1,25(OH)2D3 concentration, and an inverse relationship exists between the two variables; that is, lower serum phosphate concentrations are associated with higher 1,25(OH)2D3 concentrations. In our population, the opposite was observed. There was a statistically significant positive correlation between serum phosphorus and 1,25(OH)2D3 levels (Fig. 2). Of the 42 patients, 2 patients (5%) had undetectable serum 1,25(OH)2D3 levels (<5 pg/ml) on repeated measurement.

Proteinuria and aminoaciduria

Because proteinuria and aminoaciduria have been associated with other renal tubulopathies such as TIO and the Fanconi syndrome, we performed assays to determine if they also occur in patients with FD. Thirty-seven patients (86%) showed some degree of proteinuria (Fig. 3). The highest urinary protein observed was 291 mg/24 h, reflecting a relatively mild degree of proteinuria. The electrophoretic pattern revealed a low molecular weight distribution, consistent with a tubular proteinuria (data not shown). Although the concentrations of serum amino acids were in the normal range, 39 patients (94%) showed some degree of aminoaciduria. Only 3 patients (7%) had a normal urinary amino acid screen (Table 2). Table 3 is a representative profile of urinary amino acids of 1 patient with aminoaciduria. This shows the finding of a generalized aminoaciduria.

<table>
<thead>
<tr>
<th>TABLE 1. PATIENT DEMOGRAPHICS</th>
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<tbody>
<tr>
<td>Children</td>
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<tr>
<td>Male</td>
</tr>
<tr>
<td>age range: 5–51 years</td>
</tr>
<tr>
<td>mean: 20.8 ± 14.5 years</td>
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<tr>
<td>Female</td>
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<tr>
<td>age range: 7–57 years</td>
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<tr>
<td>mean: 24.6 ± 13.3 years</td>
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<tr>
<td>Total</td>
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<tr>
<td>Diagnosis</td>
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<tr>
<td>PFD</td>
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<tr>
<td>FD and café-au-lait or MAS</td>
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<td>and endocrinopathy</td>
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<td>Total</td>
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* Chronological age < 18 years.
not suggestive of defects in specific amino acid transport systems.

In addition to the finding of aminoaciduria, chromatography of urinary amino acids in patients with FD showed a distinct pattern, different from that of a normal control subject (Fig. 4). Unknown compounds were consistently detected in the urine of affected FD patients who displayed aminoaciduria (peaks labeled with asterisks in Fig. 4). To exclude the possibility that the aberrant peaks simply represented an elevated concentration of physiological amino acids with a similar or overlapping retention time, samples were reanalyzed using a different HPLC-based method in which the elution profile for the amino acids is different. These additional analyses confirmed that the unusual peaks were not physiological amino acids. Further characterization of the modified amino acids represented by these peaks is ongoing.

**Nephrogenous cAMP**

It has been suggested that renal phosphate wasting sporadically observed in MAS patients may be the result of the presence of Gsα mutations in renal tubular cells, and that the presence of mutation in the kidney would result in an elevation of nephrogenous cAMP, as in hyperparathyroidism.\(^{19}\) The mean (±SD) basal nephrogenous cAMP in the FD patients was in the normal range and significantly less than a group of PHPTH patients (\(p<0.05\); Fig. 5).

**Correlation between bone disease activity and TmP/GFR**

By analogy to TIO, the renal phosphate wasting in FD also may be caused by secretion of a circulating factor.\(^{20}\) If so, we hypothesize that markers of bone metabolism, used here as an indicator of bone disease activity, would be correlated negatively with TmP/GFR. The range, mean, and SD of various markers of bone metabolism (alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin, N-telopeptide, and PYD and DPYD cross-links), as well the correlation of the markers to TmP/GFR, are presented in Table 4. To control for the fact that markers of bone metabolism in children are normally elevated relative to adults, the correlations between bone markers and TmP/GFR were calculated controlling for (partialing out) age (Table 4). Once age is adjusted for, there is a statistically significant negative correlation between the markers of bone metabolism to TmP/GFR (\(r=-0.366\) to \(-0.640\); \(p=0.02\) to \(<0.001\)). Thus, the higher level of bone activity, the lower the TmP/GFR and the greater the degree of renal phosphate wasting.

**Significant negative findings**

In this series of patients, there was no evidence of magnesium, or calciuria as is seen in PHPTH. Serum bicarbonate and urinary pH were normal in all patients. Except for one 57-year-old patient with type 2 diabetes mellitus, there was no evidence of glucosuria. Therefore, many of the
components of a generalized renal tubulopathy that are seen in cases of renal tubular damage, such as an acquired Fanconi syndrome, were absent. Furthermore, there was no evidence of cumulative or progressive renal damage, as indicated by the lack of change in GFR, or association between GFR and age.

**DISCUSSION**

Before this study, the prevalence of either hypophosphatemia and/or renal phosphate wasting in association with FD had not been examined closely. In a large series of patients with FD, we have established that renal phosphate wasting is a common finding in FD. Twenty of the 42 patients (48%) in our series show some degree of phosphate wasting. Of note is the fact that the serum phosphorus concentration of 13/20 (65%) patients with a low TmP/GFR was in the normal range, indicating that calculation of the TmP/GFR is necessary to establish phosphate wasting. We also found that the renal phosphate wasting is part of a more general proximal renal tubulopathy as manifested by altered vitamin D metabolism, proteinuria, and aminoaciduria. This is the first report indicating that a proximal renal tubulopathy is a common feature in patients with FD.

Renal phosphate wasting—endogenous Gsα mutation or phosphaturic factor?

The etiology of the phosphate wasting observed in association with FD is debated. Because Gsα mutations have been found in kidney tissue in severe, neonatal lethal MAS cases, it has been suggested that the abnormality is en-

<table>
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<th>Table 3. Urinary Amino Acids in a Patient with FD</th>
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<tr>
<td><strong>Amino acid</strong></td>
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<tr>
<td>Taurine</td>
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<td>Threonine</td>
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<td>Serine</td>
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<td>Asparagine</td>
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<td>Glutamic acid</td>
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<td>Glutamine</td>
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<td>Glycine</td>
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<tr>
<td>Alanine</td>
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<tr>
<td>Citrulline</td>
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<tr>
<td>α-Aminodic acid</td>
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<tr>
<td>Valine</td>
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<tr>
<td>Cystine</td>
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<tr>
<td>Leucine</td>
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<td>α-Aminobutyric acid</td>
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<sup>a</sup> Units = μmol/24 h.  
<sup>b</sup> Beyond normal range.
1,25(OH)2 D3. Under normal physiological conditions, low renal proximal tubule, and thus serum levels of a serum phosphorus stimulates 1 correlation with lower serum 1,25(OH)2 D3. This suggests that there is an alteration of 1α-hydroxylase activity in FD. Supporting the hypothesis that fibrous dysplastic bone elaborates a phosphaturic factor, is the report of a patient with FD and phosphate wasting in whom the phosphate wasting corrected after extensive resection of FD tissue. The fact that markers of bone metabolism, as a surrogate marker for FD tissue burden, significantly correlate with TMP/GFR (Table 4) also supports the hypothesis that the etiology of the phosphate wasting is a phosphaturic factor elaborated by FD bone.

Based on characterization of patients with TIO, current thinking suggests that the abnormal phosphate metabolism is mediated by a systemic factor “phosphatonin” and that abnormalities in its metabolism result in the generation of systemic osteomalacia. This putative phosphate-regulating factor has not yet been isolated, and it is not known what cell type produces it, although bone-forming cells are likely candidates. Mutated cells within FD lesions may produce abnormal levels of this factor; alternatively, they may deregulate its production by normal osteogenic cells or cells elsewhere in the body. Another possibility is that the hypermetabolic fibrous dysplastic bone produces a factor(s) toxic to the kidney.

Even more prevalent than phosphate wasting and rearrangement of vitamin D metabolism is the finding of proteinuria and aminoaciduria in most of the patients in this series. Proteinuria and aminoaciduria also are seen in TIO. The magnitude of the proteinuria was mild (the highest value was 291 mg/24 h) and characterized by the presence of low molecular weight proteins in the urine. Relative to the glomerulonephropathies, in which grams of protein per day can be detected in the urine, the proteinuria in FD is not likely to be of major clinical significance. The aminoaciduria was variable with respect to the number of amino acids in which the concentration was elevated in the urine. The pattern was that of a generalized aminoaciduria, indicating that it was not an isolated transport system that was affected. Interestingly, the aminoaciduria was further characterized by the consistent presence of unknown metabolites in the urine of patients with FD (Fig. 4).

The etiology of the aminoaciduria is not clear. Some have suggested that high PTH and/or low serum vitamin D can directly impact the renal tubule and cause aminoaciduria. Although hyperparathyroidism can be associated with aminoaciduria, this is an uncommon finding and in those cases in which it is present, it usually is an isolated glycinuria. It is not likely that changes in vitamin D metabolism or hyperparathyroidism account for the common place occurrence of aminoaciduria seen in our patients. Although 7 patients (17%) had serum vitamin D levels low enough to induce secondary hyperparathyroidism at the initial evaluation (data not shown), the secondary hyperparathyroidism resolved with vitamin D replacement. The data shown in this study represent evaluations done at least 3 months after vitamin D replacement and normalization of serum PTH.

Further distinguishing these findings from those seen in PHPTH is the relationship between serum phosphorus and 1,25(OH)2D3 levels. Serum phosphorus is a key regulator of 25(OH)D3-1α-hydroxylase (1α-hydroxylase) activity in the renal proximal tubule, and thus serum levels of 1,25(OH)2D3. Under normal physiological conditions, low serum phosphorus stimulates 1α-hydroxylase activity and increases the serum level of 1,25(OH)2D3. In PHPTH, 1,25(OH)2D3 levels are normal or high and maintain an inverse relationship (negative correlation) to serum phosphorus. We found that in FD lower serum phosphorus was correlated with lower serum 1,25(OH)2D3. This suggests that there is an alteration of 1α-hydroxylase activity in FD. A similar observation has been made in TIO and X-linked hypophosphatemia but not in the phosphaturia of hyperparathyroidism.
autosomal dominant hypophosphatemia, and the Fanconi syndrome. In these conditions the renal findings are phosphate wasting, mild proteinuria, aminoaciduria, and normal nephrogenous cAMP.\(^{21,24,35,36}\) The absence of carbonaturia, glucosuria, and acidosis in FD distinguishes it from the Fanconi syndrome. Thus, it appears that the phosphate wasting observed in association with FD is more similar to that seen in the phosphotonin-mediated phosphate wasting syndromes, TIO, and X-linked and autosomal dominant hypophosphatemia and distinct from that seen in either PHPTH or the tubular damage (Fanconi) syndromes.

**Clinical significance**

Given the recent report that FD lesions can be osteomalic,\(^{25}\) the finding of renal phosphate wasting is of clinical significance. Renal phosphate wasting with chronically low serum phosphorus (or perhaps even low to normal levels in growing children) may influence the development of lesions osteomalacia. Undermineralization of FD bone may contribute to the development of the shepherd’s crook deformity of the proximal femur, the quality of fracture healing, and to the rare but serious complication of platybasia of the skull. Therefore, it is critical to assess the phosphate status of patients with FD. We currently prescribe phosphorus supplementation for patients both with low serum phosphorus and with low-normal serum phosphorus but a low TmP/GFR. As in other patients with phosphate wasting syndromes, phosphate supplementation usually results in secondary hyperparathyroidism, the prevention of which is brought about by adding vitamin D (calcitriol) to the regimen. Long-term studies in FD patients are required to determine if raising serum phosphorus levels will improve the quality and mechanical properties of osteomalic bone in FD. Furthermore, it is clinically relevant to determine the presence or absence of phosphate wasting before treatment with bisphosphonates, which have been reported to be beneficial in the treatment of FD, but in certain circumstances, may themselves be associated with mineralization defects.\(^{37}\)

Our data show that a renal tubulopathy manifested by phosphate wasting, altered vitamin D metabolism, low molecular weight proteinuria, and aminoaciduria is common in FD/MAS. In fact, the renal tubulopathy represents the most common metabolic feature in this group of patients. Our findings suggest that the cause may be a circulating factor produced by fibrous dysplastic bone, although the presence of mutations in renal Gs\(^a\) cannot be excluded and may contribute. Further study is needed to determine the precise pathogenetic mechanisms and appropriate clinical care.

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