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Autoimmune hyperphosphatemic tumoral calcinosis in a patient with FGF23 autoantibodies

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Hyperphosphatemic familial tumoral calcinosis (HFTC)/hyperostosis-hyperphosphatemia syndrome (HHS) is an autosomal recessive disorder of ectopic calcification due to deficiency of or resistance to intact fibroblast growth factor 23 (iFGF23). Inactivating mutations in FGF23, N-acetylgalactosaminyltransferase 3 (GALNT3), or KLOTHO (KL) have been reported as causing HFTC/HHS. We present what we believe is the first identified case of autoimmune hyperphosphatemic tumoral calcinosis in an 8-year-old boy. In addition to the classical clinical and biochemical features of hyperphosphatemic tumoral calcinosis, the patient exhibited markedly elevated intact and C-terminal FGF23 levels, suggestive of FGF23 resistance. However, no mutations in FGF23, KL, or FGF receptor 1 (FGFR1) were identified. He subsequently developed type 1 diabetes mellitus, which raised the possibility of an autoimmune cause for hyperphosphatemic tumoral calcinosis. Luciferase immunoprecipitation systems revealed markedly elevated FGF23 autoantibodies without detectable FGFR1 or Klotho autoantibodies. Using an in vitro FGF23 functional assay, we found that the FGF23 autoantibodies in the patient’s plasma blocked downstream signaling via the MAPK/ERK signaling pathway in a dose-dependent manner. Thus, this report describes the first case, to our knowledge, of autoimmune hyperphosphatemic tumoral calcinosis with pathogenic autoantibodies targeting FGF23. Identification of this pathophysiology extends the etiologic spectrum of hyperphosphatemic tumoral calcinosis and suggests that immunomodulatory therapy may be an effective treatment.

Introduction

Fibroblast growth fact 23 (FGF23) is a phosphate- and 1,25(OH)2 vitamin D-regulating (1,25D-regulating) hormone produced by osteoblasts and osteocytes (1). FGF23 acts via FGF receptor 1 (FGFR1) coupled with the coreceptor KL to reduce expression of sodium phosphate cotransporters (NaPi-2a and -2c) and renal 25-hydroxy vitamin D 1-α-hydroxylase (2). FGF23 lowers serum phosphate and 1,25D levels by its actions on the kidney to reduce renal tubular reabsorption of phosphate (TRP) and 1,25D production. Excess FGF23 has been implicated in a number of hypophosphosphemic disorders, such as tumor-induced osteomalacia (3), X-linked hypophosphatemic rickets (4), and autosomal dominant hypophosphatemic rickets (5).

Hyperphosphatemic familial tumoral calcinosis (HFTC)/hyperostosis-hyperphosphatemia syndrome (HHS) (OMIM 211900) is a disorder of FGF23 deficiency or resistance. Affected individuals develop ectopic calcifications called tumoral calcinosis and/or diaphyseal hyperostosis, which manifests clinically in the long bones as diaphyseal pain and swelling (6). Characteristic dental findings of HFTC/HHS include shortened roots with dilacerations, thistle-shaped dental pulps, pulp chamber and root canal obliteration, and pulp stones (7). In addition, some patients experience systemic inflammation.

HFTC/HHS is an autosomal recessive disease, and to date, causal mutations in 3 genes have been identified: FGF23 (12p13.3) (8), UDP-GalNAc-polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3) (2q24-q31) (9), and Klotho (KL) (13q12) (10). Mutations in FGF23 and GALNT3 result in premature cleavage of biologically inactive intact FGF23 (iFGF23) into inactive fragments, while mutations in KL interrupt FGF23 signaling, causing FGF23 resistance. Lack of iFGF23 results in hyperphosphatemia, due to increased TRP and elevated or inappropriately normal 1,25D production, which promotes gastrointestinal absorption of phosphorus and calcium. The net effect is an increase in the calcium x phosphate product, leading to tumoral calcinosis.

Here, we present what we believe is the first case of autoimmune hyperphosphatemic tumoral calcinosis due to pathogenic FGF23 autoantibodies. Autoantibodies directed against extracellular targets, such as receptors (e.g., nicotinic acetylcholine receptor autoantibodies in myasthenia gravis) or secreted mole-
Table 1. Biochemical results in patient with autoimmune tumoral calcinosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6 yr, 7 mo (initial laboratory evaluation)</th>
<th>7 yr, 3 mo</th>
<th>7 yr, 9 mo</th>
<th>8 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEV</td>
<td>SEV, ACZ</td>
<td>SEV, ACZ</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (3.2–6.3 mg/dl)</td>
<td>7.2</td>
<td>7.9</td>
<td>6.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Calcium (8.2–10 mg/dl)</td>
<td>9.9</td>
<td>9.9</td>
<td>9.7</td>
<td>10.1</td>
</tr>
<tr>
<td>Calcium x phosphorus (~65 mg²/dl² for &lt;12 yr)</td>
<td>71</td>
<td>78</td>
<td>64</td>
<td>56</td>
</tr>
<tr>
<td>1,25(OH)D (24–86 pg/ml)</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>59</td>
</tr>
<tr>
<td>Intact PTH (15–65 pg/ml)</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>11.2</td>
</tr>
<tr>
<td>TRP (85%–95%)</td>
<td>95</td>
<td>97.2</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>TP/GFR (age specific mean 4.4 mg/dl; ref. 24)</td>
<td>6.1</td>
<td>6.5</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>iFGF23 (&lt; 52 pg/ml)</td>
<td>13,000</td>
<td>7,800</td>
<td>6,900</td>
<td></td>
</tr>
<tr>
<td>CFGF23 (3M-17Y ≤ 230)</td>
<td>33,000</td>
<td>33,000</td>
<td>22,400</td>
<td></td>
</tr>
<tr>
<td>CRP (&lt; 4.99 mg/l)</td>
<td>1.7</td>
<td>&lt; 0.15</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>ESR (0–42 mm/h)</td>
<td>37</td>
<td>37</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Glucose (nonfasting &lt; 200 mg/dl)</td>
<td>82</td>
<td>119</td>
<td>81 (fasting)</td>
<td>433</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin A1c (4%–6%)</td>
<td>10.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insulin (2.6–24.9 mcU/ml)</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-peptide (11–5 ng/ml)</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bicarbonate (22–29 mmol/l)</td>
<td>28</td>
<td>20</td>
<td>23</td>
</tr>
</tbody>
</table>

ACZ, acetazolamide; SEV, sevelamer; PTH, parathyroid hormone; TP, tubular maximum reabsorption of phosphorus; GFR, glomerular filtration rate; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

Results and Discussion

This White boy presented at 6 years, 3 months, with pain, swelling, and development of a firm lesion on the lateral right hip. MRI of the lesion revealed a calcified mass in the right gluteus maximus extending into the subcutaneous soft tissue. A biopsy of the lesion showed tumoral calcinosis. Subsequent laboratory evaluation revealed hyperphosphatemia (7.2 mg/dl; normal for age, 3.2–6.3) with normal blood calcium and renal function, consistent with the diagnosis of HFTC/HHS. iFGF23 and C-terminal CFGF23 levels were markedly elevated, at 13,000 pg/ml (normal, <52) and 33,000 RU/ml (normal for age, <230) (Table 1), respectively, consistent with CFGF23 resistance. The patient was started on the phosphate binder sevelamer and a low-phosphate diet. Sequencing of FGF23, KL, and FGFR1 genes and whole-exome sequencing did not identify gene mutations or genetic variants, including mutations and/or variants in GALNT3, that could explain the clinical calcinosis/phosphate phenotype.

Given the lack of an identifiable genetic cause for hyperphosphatemic tumoral calcinosis and the new diagnosis of T1DM, we hypothesized that the tumoral calcinosis could be autoimmune performed serial dilutions of the patient’s plasma (1:1, 1:10, 1:20, 1:50, 1:100, 1:500, 1:1000) and measured iFGF23 and CFGF23 via second-generation ELISA kits (Immutopics). For both iFGF23 and CFGF23, levels decreased with serial dilution as expected, except for the 1:500 and 1:1000 dilutions of iFGF23, which were below the lower limit of detection of the assay (Supplemental Table 1). The ability to detect dilutions of iFGF23 and CFGF23 via ELISA ruled out the presence of an interfering antibody resulting in the elevated iFGF23 and CFGF23 levels.

Twenty months after initial symptom onset, the patient reported a 2-week history of polyuria, polydipsia, and nocturia, with no change in appetite or weight. He denied dysuria, hematuria, enuresis, or fever. Laboratory evaluation revealed blood phosphorus of 5.6 mg/dl (normal for age, 3–5.7), 1,25D of 39 pg/ml (normal, 24–86), and TRP of 93% (normal, 85%–95% in the setting of normal blood phosphorus). iFGF23 and CFGF23 levels remained markedly elevated at 6900 pg/ml (normal, <52) and 22,400 RU/ml (normal for age <230), respectively. In addition, the subject was hyperglycemic (fasting blood glucose, 433 mg/dl; normal, <120) and insulinopenic (4.1 mcU/ml; normal fasting, 2.6–24.9) with a low C-peptide (0.8 mg/ml; normal, 1.1–1.5). Hemoglobin A1c was elevated at 10.7% (estimated average glucose, 260 mg/dl) (Table 1). The subject had positive islet antigen-2 (IA-2) and antiinsulin antibodies and negative glutamic acid decarboxylase (GAD65) antibodies. Of note, he had a normal fasting blood glucose of 81 mg/dl 3 months prior. These results were consistent with new-onset T1DM. The patient was admitted for initiation of insulin therapy and diabetes education.

At 7 years, 3 months, the patient was referred to the NIH, and acetazolamide was added to promote renal phosphate excretion. After 9 months on this regimen, the right hip tumoral calcinosis was decreased in size on physical exam and repeat radiograph (Figure 1, A–D) and no new lesions had developed. The blood phosphorus fluctuated, but decreased overall, with a nadir of 5.4 mg/dl (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI122004DS1).

Given the considerably elevated iFGF23 and CFGF23 levels in this patient compared with reported cases of HFTC/HHS and lack of an identifiable genetic cause, we questioned whether an interfering antibody led to falsely elevated iFGF23 and CFGF23 levels. Therefore, we...
ELISAs are generated against relatively small, defined polypeptides derived from the 2 ends of the FGF23 protein and would not be influenced by the existing patient’s autoantibodies. Studies with other pathogenic autoantibodies, including pulmonary alveolar proteinosis and membranous nephropathy, have also used similar antibody-based methods to establish that the protein levels of the granulocyte-macrophage CSF (GM-CSF) (15) and anti-phospholipase A2 receptor (PLA2R) (16), respectively, are unchanged or increased in these autoimmune diseases. Finally, our concordant findings from 2 different commercial ELISA assays showing comparable results with elevated FGF23 protein levels potentially support the validity of this approach.

Based on the elevated FGF23 autoantibodies found in the patient, we tested the effect of FGF23 autoantibodies on FGF23 signaling in an in vitro functional assay. Human embryonic kidney cells (HEK293) stably transfected with the full-length transmembrane form of mouse Klotho (17) were treated with vehicle (PBS), recombinant human FGF23 R176Q, or basic FGF (bFGF) in the presence of either 2% plasma from our patient or 2% human control plasma. Recombinant FGF23 increased early growth response 1 (EGR1) mRNA expression in a dose-dependent manner, whereas the patient’s plasma blocked the FGF23-dependent increase in EGR1 mRNA expression. The inhibitory effect of the patient’s plasma on EGR1 mRNA expression was overcome with the addition of 100 ng/ml FGF23 (Figure 3A). Control plasma had no effect on EGR1 mRNA expression at any FGF23 concentration (Figure 3B). Recombinant bFGF increased EGR1 mRNA expression independently of the patient’s plasma (Figure 3C), indicating that autoantibodies present in our patient’s plasma are specific in blocking the action of FGF23.

FGF23 binding to the FGFR1-Klotho receptor complex leads to ERK1/2 phosphorylation. We analyzed FGF23-depen-
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common variable immunodeficiency-associated autoimmune cytopenias (22), and cytotoxic T lymphocyte–associated protein 4 (CTLA4) haploinsufficiency-associated immune dysregulation (23). Evaluation of T and B cell clonality was performed via PCR amplified for detection of immunoglobulin (IgH and IgK loci) and T cell receptor gene rearrangements. No significant T or B cell populations were identified. The subject was screened for autoantibodies associated with other autoimmune diseases and did not demonstrate autoantibodies to thyroglobulin, thyroid peroxidase, tissue transglutaminase, intrinsic factor, parietal cells, and 21-hydroxylase. He had normal immunoglobulin (IgG, IgA, and IgM) levels. Although the exact mechanism involved in the generation of anti-FGF23 autoantibodies in this subject is unknown, we speculate that autoreactive B cells not identified in the immune cell evaluation and/or epitope spreading in the context of T1DM might be responsible. Given the lack of an identifiable target for treatment, immunomodulatory medications were not recommended for this patient.

Here, we describe what we believe is the first case of pathogenic autoantibodies that target FGF23, causing hyperphosphatemic tumoral calcinosis. This observation is supported by identification of elevated levels of FGF23 autoantibodies, which, in vitro, block FGF23 action at the level of the FGFR1-Klotho receptor complex. We speculate that the FGF23 autoantibodies likely sequester FGF23 and prevent it from interacting with its receptor to promote normal signal transduction. The present data depict a pathophys-
iology for hyperphosphatemic tumoral calcinosis and suggest a potential role for immunomodulatory therapy in the treatment of this disease if an immunological target can be identified.

**Methods**

Further information is provided in Supplemental Methods.

**Statistics.** The statistical analyses were performed using 2-way ANOVA, followed by Tukey’s test for multiple comparisons (GraphPad Prism, version 7.02). Data are provided as mean ± SD. A P value of less than 0.05 was considered statistically significant.

**Study approval.** This study was approved by the Institutional Review Board of the NIDCR (NIH). The parents of the subject provided written consent to the study protocol, and the subject provided written assent.

**Author contributions**

RIG and MTC oversaw the study. MSR, RIG, and MTC coordinated the work and prepared the manuscript, which was reviewed, edited and approved by all authors. MSR, CJR, LCG, MTC, and RIG clinically examined the patient, collected blood samples, and prescribed medical treatment. SI, EF, and MJE performed DNA sequencing, genetic analyses, and whole-exome sequencing. PDB performed LIPS experiments. DES and FP performed in vitro assay work.

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