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A Case of Hyperphosphatemia and Elevated Fibroblast Growth Factor 23: A Brief Review of Hyperphosphatemia and Fibroblast Growth Factor 23 Pathway

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INTRODUCTION

Phosphate homeostasis is a complex process involving communication among bones, kidneys, and other organ systems.[1,2] This is tightly regulated through parathyroid hormone (PTH) and bone-derived fibroblast growth factor 23 (FGF23).[1–3] Disruption of either pathway has been associated with a number of disorders resulting in hypo- or hyperphosphatemia.[1] These disorders tend to have profound effects on the human body through demineralization or ectopic calcifications/stone production.[1]

The role of PTH in phosphate regulation has been well described.[4] Hypocalcemia or hyperphosphatemia stimulates the release of PTH from the parathyroid glands. Within the kidney, PTH activates vitamin D and downregulates phosphate reabsorption. Activated 1,25-hydroxyvitamin D then has an effect on the gastrointestinal tract and promotes phosphate and calcium absorption.[1–4] With the elucidation of the FGF23 pathway, phosphate regulation has now been further clarified (Figure 1).[5] FGF23 is released from bone during periods of hyperphosphatemia or elevated PTH or 1,25 hydroxyvitamin D levels.[5,6] Within the kidney, FGF23, in conjunction with its critical co-receptor Klotho, decreases reabsorption of phosphate and inhibits conversion of 25-hydroxyvitamin D to 1,25 hydroxyvitamin D by 1α-hydroxylase.[6] It also inhibits PTH release from the parathyroid glands, thereby regulating both PTH and vitamin D.[4] Loss of FGF23 function on target organs results in increased phosphate reabsorption and decreased excretion from the kidneys, leading to hyperphosphatemia, higher levels of 1,25-hydroxyvitamin D, and low levels of 25-hydroxyvitamin D as a result of consumption caused by unregulated conversion and reduced PTH secretion from the parathyroid glands.[4–6]

We present here a patient with a rather unusual presentation of hyperphosphatemia and her subsequent evaluation.

CASE PRESENTATION

Clinical History

A 7-year-old girl with a history of cerebral palsy and severe developmental delay was admitted to the hospital because of failure to thrive with a weight of 11.5 kg. On admission, phosphorus level was 3.8 mg/dl (3.7–5.4). After initiation of nasogastric feeds, her phosphorus level dropped to 3.1 mg/dl, likely secondary to refeeding syndrome.

Initial Laboratory Data

Over the next 4 days, her phosphorus level rose to 7.9 mg/dl without exogenous supplementation. Creatinine was 0.27 mg/dl (0.2–0.6), and calcium was 8.9 mg/dl (8.5–10.2) (Table 1). Findings from renal ultrasound examination were normal, and a radiographic survey revealed no tooth anomalies or calcific masses. She did

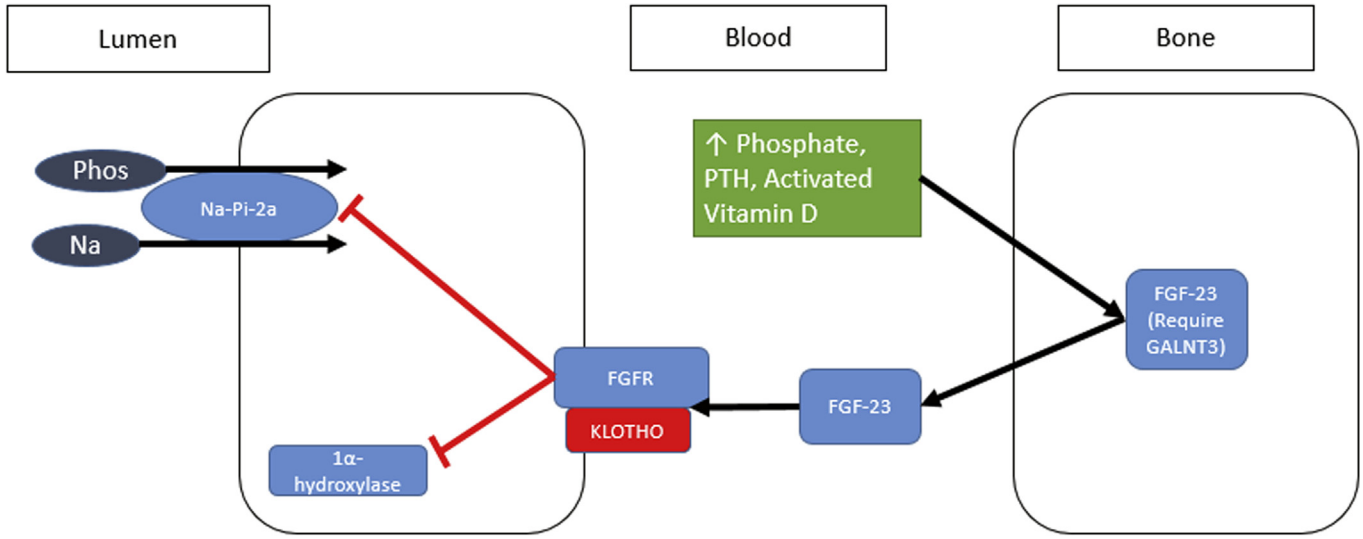

Figure 1. Fibroblast growth factor 23 (FGF23) signaling pathway. FGF23 is induced by hyperphosphatemia, increased parathyroid hormone (PTH), and increased activated vitamin D. After it acquires protection from proteolysis before secretion by GALNT3, FGF23 signaling relies on the FGF receptor (FGFR) and its co-receptor Klotho. Activation of the FGFR leads to the inhibition of the sodium-phosphate cotransporter (Na-Pi-2a), leading to decreased phosphate reabsorption. FGFR-Klotho also inhibits the conversion of 25-hydroxyvitamin D to 1,25 hydroxyvitamin D, leading to decreased activated vitamin D. Not shown here are FGF23's actions on the parathyroid gland where it decreases PTH secretion as well. Na, sodium; Phos, phosphorus.

not receive phosphate-containing enemas or any other sources of exogenous phosphate.

Additional Workup

An evaluation was initiated to investigate potential causes of her hyperphosphatemia. PTH was 54 pg/ml (15–65), 25-hydroxyvitamin D level was undetectable, and 1,25-hydroxyvitamin D was 157 pg/ml (24–86). Maximal tubular reabsorption of phosphate/glomerular filtration rate was elevated at 6.99 mg/dl (2.97–4.45) with a tubular reabsorption of phosphate >102%.

FGF23 circulating levels can now be measured with immunometric assays that detect either the active intact hormone (iFGF23), inactive C-terminal fragments (cFGF23), or both.[3] The level of iFGF23 was 250 pg/ml (10–50), and the level of cFGF23 was 523 RU/ml (20–108).

Genetic analysis was performed by the University of Michigan on a peripheral blood sample, and all genes implicated in FGF23 pathway dysfunction were examined. The analysis revealed 10 total polymorphisms (Table 2). Two polymorphisms involved FGF23–glycosylating enzyme (GALNT3), while 8 involved Klotho. The GALNT3 polymorphisms were ruled out based on our FGF23 assay. The elevated cFGF and iFGF levels are not indicative of a GALNT3 mutation; rather, these results suggest a dysfunction in Klotho. Six of the 8 Klotho polymorphisms have been previously described in a patient with infantile hypercalcemia hypercalciuria and in 2 supercentenarians. These patients do not have findings similar to those in our patient.[7,8] The remaining 2 Klotho polymorphisms, although unreported in relation to human disease, are unlikely culprits, given their frequent occurrence in the general population.

Table 1. Laboratory results

Parameter	Patient results	Normal range
Serum phosphorus (mg/dl)	On admission: 3.8 Initial feeding: 3.1 After feeding: 7.9	3.7–5.4
Serum creatinine (mg/dl)	0.27	0.2–0.6
Calcium (mg/dl)	8.9	8.5–10.2
Additional workup		
PTH (pg/ml)	54	15–65
25-Hydroxyvitamin D (mg/dl)	Undetectable	20–100
1,25-Hydroxyvitamin D (pg/ml)	157	24–86
TmP/GFR (mg/dl)	6.99	2.97–4.45
TRP (%)	>102	—[a]
Intact FGF23 (pg/ml)	250	10–50
C-terminal FGF23 (RU/ml)	523	20–108

FGF, fibroblast growth factor; PTH, parathyroid hormone; TmP/GFR, maximal tubular reabsorption of phosphate/glomerular filtration rate; TRP, tubular reabsorption of phosphate.
[a]Normal value ranges with phosphate level indicating hyperphosphatemia should be associated with a low tubular reabsorption of phosphate.

Diagnosis

FGF23 pathway dysfunction with classic biochemical profile and atypical physical and radiologic presentation.

Clinical Follow-up

The patient is currently doing well, and her phosphate level has ranged from 3.8 to 5.3 mg/dl with the help of a phosphate binder over the past 2 years of follow-up. She continues to have no evidence of calcifications, likely because of tight control of her phosphate levels.

Table 2. Results of genetic analysis

Gene	Nucleotide	Protein	dbSNP No.	Nonreference allele frequency
Klotho	c.273T>C (hetero)	p.Asp91=	rs2772364	C:98.464% (11143/113172)
Klotho	c.ex2-46 C>G (hetero)	Intronic variant	rs9536313	G: 14.270% (17944/125748)
Klotho	c.1054 T>G (hetero)	p.Phe352Val	rs9536314	G: 14.257% (18022/126406)
Klotho	c.1109 G>C (hetero)	p.Cys370Ser	rs9527025	C: 14.258% (18023/126402)
Klotho	c.1155 G>A (hetero)	p.Lys385	rs9527026	A: 12.999% (4889/5008)
Klotho	c.2619 T>C (homo)	p.Asn873=	rs649964	C: 97.624% (102157/125262)
Klotho	c.2701+22 T>A (hetero)	Intronic	rs650439	A: 81.555% (102157/125262)
Klotho	c.2702-72 C>A (hetero)	Intronic	rs78603971	A: 50.000% (1/2)
GALNT-3	c.688+32 C>G (hetero)	Intronic	rs114169299	G: 99.54% (4985/5008)
GALNT-3	c.688+40 T>C (homo)	Intronic	rs2113840	C: 99.581% (4987/5008)

dbSNP, Single Nucleotide Polymorphism database; GALNT3, fibroblast growth factor 23–glycosylating enzyme.
Eight Klotho and 2 GALNT3 polymorphisms were identified by genetic analysis. Nonreference allele frequencies were derived from the Exome Aggregation Consortium (ExAC) browser. The GALNT3 nonreference alleles are not likely related to our patient's presentation, given our fibroblast growth factor 23 assay results. Six of the 8 Klotho polymorphisms have been previously reported in cases unrelated to tumoral calcinosis. The remaining polymorphisms (c.ex2-46 C>G and c.2702-72 C>A) have not been reported in relation to any human disease; however, they occur at a high frequency in the population and therefore are not likely the cause of our patient's presentation.

METHODS

A peripheral whole blood sample was collected in tubes containing ethylenediamine tetraacetic acid. Genomic DNA was extracted by using the Gentra Puregene blood purification kit (Qiagen, Hilden, Germany) according to the protocol suggested by the manufacturer.

Primers for all exons and exon/intron boundaries of *KL* (NM_004795), *FGF23* (NM_020638), and *GALNT3* (NM_004482) genes were designed using Primer 3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) and purchased from Integrate Device Technologies (IDT, Coralville, Iowa).

All exons were amplified using a polymerase chain reaction with HotStar Taq polymerase mixture (Qiagen). Polymerase chain reaction products were submitted to the DNA Sequencing Core (University of Michigan) for direct Sanger sequencing. Sequences were analyzed using the software Sequencer (Gene Code, Ann Arbor, Michigan) and compared with the reference sequence (GRCh38/hg38) of each exon for each gene involved in hyperphosphatemic familial tumoral calcinosis.

DISCUSSION

The hyperphosphatemia evaluation begins by differentiating pseudohyperphosphatemia from true hyperphosphatemia (Table 3).[9,10] Pseudohyperphosphatemia is most commonly associated with increased paraproteins in conditions including multiple myeloma and Waldenstrom macroglobulinemia.[11,12] It may also be associated with hyperlipidemia and hyperbilirubinemia.[9] In our patient there was no evidence of lymphoproliferative disorder, abnormal lipids, or abnormal liver function, essentially ruling out pseudohyperphosphatemia.

True hyperphosphatemia occurs under 1 of the following conditions: increased phosphate load, large extracellular shift, kidney dysfunction (acute or chronic), or increased tubular reabsorption/decreased excretion.[3,9] Increased phosphate load was ruled out in our patient. She presented in a malnourished state and did not follow a diet high in phosphate or receive any treatments with a high phosphate content, such as enemas or laxatives.[13] In fact, she experienced refeeding syndrome at the beginning of her clinical course. She had no evidence of acidosis, making extracellular shift highly unlikely. Her normal creatinine level, urine output, and renal ultrasound findings effectively rule out kidney dysfunction as the cause of her hyperphosphatemia.

Therefore increased tubular reabsorption is the likely cause of her hyperphosphatemia. This is supported by her high maximal tubular reabsorption of phosphate/glomerular filtration rate, as well as tubular reabsorption

Table 3. Teaching points: causes of hyperphosphatemia

Pseudohyperphosphatemia
Multiple myeloma
Waldenstrom macroglobulinemia
Hyperlipidemia
Hyperbilirubinemia
High-dose liposomal amphotericin B
True hyperphosphatemia
Increased load
Exogenous supplementation
Phosphate-containing laxatives or enemas
Extracellular shift
Enhanced catabolism
Lactic acidosis
Neoplastic disease
Rhabdomyolysis
Acute or chronic kidney disease
Increased reabsorption or decreased excretion
Parathyroid hormone pathway dysfunction
Fibroblast growth factor 23 pathway dysfunction

A brief review of common causes of pseudohyperphosphatemia and true hyperphosphatemia.

of phosphate. The differential diagnosis for increased tubular reabsorption includes hypoparathyroidism, vitamin D toxicity, and disorders of the FGF23 pathway, namely hyperostosis hyperphosphatemia and tumoral calcinosis.[1] Hypoparathyroidism was ruled out because of her normal PTH level. Despite the elevated level of 1,25-hydroxyvitamin D, there was no history of vitamin D supplementation before or during admission, and her 25-hydroxyvitamin D level was not elevated. A normal calcium level also makes vitamin D toxicity less likely.

Hyperostosis hyperphosphatemia and familial tumoral calcinosis are rare autosomal recessive disorders that result from a disruption of the FGF23 pathway.[14] Recent evidence suggests the 2 diseases are not distinct disorders but rather part of a spectrum of clinical manifestations.[14,15] Classically, hyperostosis hyperphosphatemia presents with episodes of long bone swelling and cortical hyperostosis.[14] Tumoral calcinosis typically presents with ectopic calcifications of soft tissues.[14–16] Even in patients with tumoral calcinosis, the physical manifestations exist on a spectrum, and patients present with a wide range of findings, from minor localized calcifications to diffuse generalized calcifications.[14,15] Some patients have also presented with symptoms consistent with both disorders.[14,15] The most consistent findings among all patients with hyperostosis hyperphosphatemia and tumoral calcinosis are hyperphosphatemia and an abnormal 1,25-hydroxyvitamin D level.[14,15]

Three abnormalities in the FGF23 pathway have been identified as being associated with tumoral calcinosis and hyperostosis hyperphosphatemia. These include (i) loss-of-function mutations in FGF23; (ii) mutations in Klotho, a required FGF23 co-receptor; and (iii) mutations in GALNT3, an enzyme responsible for preventing proteolysis of FGF23.[14–16] Lack of intact FGF23 pathway activity leads to increased phosphate reabsorption as a result of decreased inhibition of sodium-phosphorus transporters (Figure 1). All 3 abnormalities have been implicated in tumoral calcinosis, whereas only GALNT3 has been implicated in hyperostosis hyperphosphatemia.

The FGF23 assay can be used to differentiate among the various etiologies of tumoral calcinosis by allowing examination of active intact FGF23 versus inactive cFGF23.[14,17] Mutations in FGF23 itself or in GALNT3 would lead to nonfunctional FGF23, with a low iFGF23 level and an elevated cFGF23 level. A Klotho mutation, on the other hand, has normally functioning FGF23 but lacks a key co-receptor that is required for FGF23 function.[14,17] Studies of Klotho-deficient mice have revealed phenotypic and biochemical abnormalities similar to those in FGF23-deficient mice.[18,19] Klotho deficiency would present with elevated iFGF23 and cFGF23 levels as the body overproduces FGF23 because of lack of feedback.[17] Although our patient's FGF23 assay suggests a Klotho dysfunction, our genetic analysis revealed no causal mutations in our patient within Klotho.

Our patient's case is unique, given her initial presentation and the eventual manifestation of her underlying disorder. Although findings from her physical and radiologic examinations are inconsistent with tumoral calcinosis and hyperostosis hyperphosphatemia, her biochemical profile is consistent with these disorders. The similarity of her biochemical profile to that of another patient with a Klotho mutation described in a previous report, as well as her FGF23 assay results are, highly suggestive of a Klotho mutation.[17] However, the results of genetic analysis do not indicate any dysfunction in Klotho. We speculate that she has a disruption along the FGF23 pathway at the receptor level. Klotho-FGFR1c (fibroblast growth factor receptor1c–splicing form) is present in the parathyroid glands and distal tubules of the kidney, through which FGF23 reduces PTH secretion from the parathyroid glands, inhibits 1,25-hydroxyvitamin D production, and decreases phosphate reabsorption from the kidneys.[20] The possible disruption at Klotho-FGFR1c could be partial in the parathyroid glands and complete in the kidneys, accounting for the biochemical picture in our patient—a higher normal PTH level despite hyperphosphatemia and high levels of 1,25- hydroxyvitamin D. This would account for the similarities between our patient and a patient with Klotho deficiency, as well as the FGF23 assay results.[14–17]

A nonfunctioning receptor would lead to a biochemical presentation similar to that of Klotho deficiency. She likely has a novel mutation in the FGF receptor or downstream signaling pathways, representing a case that falls toward the tumoral calcinosis end of the FGF23 pathway disorder spectrum. Without physical manifestations of her hyperphosphatemia, it is difficult to identify exactly where along the spectrum she falls. We suspect her malnutrition was actually protective in terms of calcification, thus leading to her unusual presentation. Because her phosphate level is being closely monitored and remains in a normal range, we do not expect her to exhibit physical symptoms that would allow us to make a definitive clinical diagnosis at this time.

DISCLOSURE

All the authors declared no competing interests.

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