Hyperphosphatemic Familial Tumoral Calcinosis in Two Siblings with a Novel Mutation in GALNT3 gene: Experience from Southern Turkey

Kışla Ekinci RM et al. A novel mutation in GALNT3 gene

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What is already known on this topic?

Mutations in FGF23, KL and GALNT3 genes cause a rare disorder, called Hyperphosphatemic familial tumoral calcinosis (HFTC). Patients with HFTC constantly present hyperphosphatemia and tumor like soft tissue calcifications. The management of HFTC mainly targets pain control and phosphate depletion.

What this study adds?

Our report introduces two HTFC patients with a novel homozygote GALNT3 mutation to the literature. We wish to emphasize that physicians should pay attention to this rare condition in differential diagnosis of calcinosis, thus appropriate follow-up and treatment can be performed.

Abstract

Inactivating autosomal recessive mutations in both FGF23, KL and GALNT3 genes lead to a rare disorder, hyperphosphatemic familial tumoral calcinosis (HFTC). Patients with HFTC constantly present hyperphosphatemia and tumor like soft tissue calcifications. Although 78% of patients develop their first symptoms between 2-13 years of age, diagnosis is usually delayed until adulthood. Some individuals with the same genetic defect overlap a condition named Hyperphosphatemic hyperostosis syndrome (HHS). Herein we report two siblings suffering from periarticular warm, hard and tender subcutaneous masses. Subcutaneous calcifications were present on X-ray and biopsy results were consistent with calcinosis in both patients. Laboratory results showed marked hyperphosphatemia and elevated renal tubular phosphate reabsorption, normal renal function tests and serum 25 hydroxyvitamin D levels. Thus, we suspected HFTC and performed next generation sequencing for GALNT3 gene, mostly causative in the literature. A novel homozygote P85Rfs*6 (c.254_255delCT) mutation in GALNT3 gene was identified in both siblings. Our report introduces two new patients to the knowledge about a rare genetic disease and suggests that small deletions in GALNT3 gene may be related with HFTC phenotype. Increased knowledge of physicians about this disease and studying phenotype-genotype correlation with more patients are needed to confirm our suggestion.

Introduction

Hyperphosphatemic familial tumoral calcinosis (HFTC) is a very rare disorder of phosphate homeostasis resultant from decreased Fibroblast growth factor 23 (FGF23) synthesis or activity (1). **FGF23** gene encodes this protein which inhibits sodium phosphate cotransporter in proximal renal tubules and 25-hydroxyvitamin D 1- α -hydroxylase expression, by its co-receptor Klotho (KL). GALNT3 gene codes UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3) enzyme, which protects intact FGF23 from breakdown and inactivation by posttranslational **glycosylation** (2). Inactivating autosomal recessive mutations in both **FGF23**, **KL** and **GALNT3** genes lead to increased renal tubular phosphate reabsorption and usually elevated 1,25-dihydroxyvitamin D₃ (**1-25 OH2D3**), promoting gastrointestinal absorption of calcium and phosphorus (1,3,4).

Patients with HFTC constantly present hyperphosphatemia and tumor like soft tissue calcifications. Although 78% of patients develop their first symptoms between 2-13 years of age, diagnosis is usually delayed until adulthood. Some individuals with the same genetic defect overlap a condition named Hyperphosphatemic hyperostosis syndrome (HHS) which was formerly described as a distinct entity (5). Here we report childhood onset HFTC in two siblings with a novel homozygote *GALNT3* mutation.

Case Report

Patient 1

Previously healthy 6 years old female patient suffered from pain and swelling on her left elbow. Due to the limitation of movement of the elbow, surgery was performed in another medical center at the age of 8 years. Excisional biopsy revealed well-circumscribed subcutaneous tissue including widespread dystrophic calcification and multinuclear giant cells. She was referred to our practice after the recurrence of calcinosis in bilateral elbows and right upper thigh at 10 years-old-age. She was born to a first-degree cousin marriage and past medical history revealed no myositis, skin lesions or renal disease. Physical examination was remarkable for **calcinosis** in the left elbow and warm, hard and tender masses in the right elbow and right upper thigh, with approximately 3 cm and 6 cm diameters respectively (Figure 1). Laboratory results showed marked hyperphosphatemia, normal serum creatinine, 25 Hydroxyvitamin D and Parathormone (PTH) levels and elevated ratio of tubular maximum reabsorption of phosphorus/glomerular filtration rate (TmP/GFR), consistent with HFTC (Table 1). Direct radiographs demonstrated radiopaque soft tissue masses around elbow bilaterally and right upper femur diaphysis (Figure 2). Bone mineral density Z score was found 0.7. Dental and ophthalmological examination showed no involvement. Milimetric calcified plaques were present inside of the right lower eyelid. A novel homozygote P85Rfs*6 (c.254_255delCT) mutation in exon 1 of the *GALNT3* gene was detected in next generation sequencing (NGS). In-silico analyses was performed with Mutation Taster, which confirmed that the mutation led to frameshift and premature stop codon. Both parents were also heterozygous carriers for the same mutation.

Patient 2

Nine years old female patient was simultaneously referred to our department with her older sister, Patient 1. She had similar but milder complaints for last 2 years including swelling of the left elbow which required surgery due to the joint contracture and recurrence in bilateral elbows thereafter. Direct radiographs demonstrated radiopaque soft tissue masses around elbow bilaterally (Figure 2). Dental and ophthalmological examination showed no involvement. Hyperphosphatemia, elevated TmP/GFR ratio, family history, biopsy result and same homozygote P85Rfs*6 (c.254_255delCT) mutation in *GALNT3* gene ensured the HFTC diagnosis. Figure 3 shows the pedigree of our patients and NGS results of our patients. A written informed consent was obtained from the parents of the patients.

Discussion

Tumoral calcinosis (TC) is a condition in which calcium crystals accumulate in soft tissues, particularly in periarticular regions. HFTC is the autosomal recessive inherited form of TC with hyperphosphatemia and normal renal functions. Differential diagnosis includes chronic renal failure, hypervitaminosis D, primary hyperparathyroidism, connective tissue diseases including particularly dermatomyositis and scleroderma. HFTC is very rare, and **almost all of the information is based on case reports** (5-10). Homozygote mutations on *GALNT3*, *FGF23* and *KL* genes were found in patients with HHS phenotype. HHS is characterized by painful diaphyseal hyperostosis and may overlap TC phenotype in some cases. One study speculated that nonsense and missense *GALNT3* mutations are related with TC and HHS phenotypes respectively (11). Indeed, majority of reported *GALNT3* mutations are missense or nonsense, only five distinct small deletion was identified in HFTC patients, according to The Human Gene Mutation Database (HGMD). Small deletions were reported to cause only TC phenotype in literature, including our patient either (8,12,13).

Besides subcutaneous calcifications, patients often present with dental abnormalities and occasionally anemia, low grade fever, regional lymphadenopathy, splenomegaly, amyloidosis, chronic recurrent osteomyelitis (CRMO), eyelid calcifications (14-16). Vascular calcifications may rarely occur and cause significant morbidity (17). Some HFTC patients develop hyperphosphatemia several years after the onset of dental abnormalities and calcinosis (11). Eyelid calcification was present in one of our patients, however other clinical properties have not been occurred yet.

The management of HFTC mainly targets pain control and phosphate depletion in the literature. Surgery is not recommended due to recurrences, until the calcinosis cause restricted joint movements. Phosphate restricted diet and phosphate binders are the mainstays of the medical treatment (18). A calcium-free phosphate binder, Sevelamer alters the absorption of phosphorus from intestine. Although Sevelamer and dietary phosphate restriction lead complete or partial recovery in calcinosis, recurrence have been reported due to possible self-discontinuation or ineffectiveness of the drug in a significant proportion of patients. Other agents including acetazolamide, probenocid and topical sodium thiosulfate shown beneficial with variable outcomes (8,19-21).

The limitation of our study was the unavailability of serum 1-25 OH₂D₃ levels in both our patients. However, the patients had other clinical and laboratory results consistent with HFTC diagnosis and we think that elevated serum 1-25 OH₂D₃ levels are only supportive in diagnosis of HFTC beside molecular studies. In conclusion, we report two siblings with novel homozygote **GALNT3** mutation representing HFTC phenotype. HFTC is a rare cause of tenderness and pain around the joint in pediatric population and should be kept in mind in differential diagnosis of arthritis. Our report introduces two new patients to the knowledge about a rare genetic disease and we wish to highlight the need for attention to this rare disorder. We speculate that small deletions in **GALNT3** gene may be related with HFTC phenotype. However, this could be confirmed only with genotype-phenotype correlation studies including long term outcomes of more patients in the future.

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Table 1. Clinical characteristics, laboratory and genetic results of the patients with Hypertrophic Familial Tumoral Calcinosis		
Parameters	Patient 1	Patient 2
Gender (Male/Female)	F	F
Age at calcinosis onset (year)	6	7
Age at HFTC diagnosis (year)	10	9
Dental involvement	No	No
Eyelid calcifications	Yes	No
Arterial calcifications on echocardiography	No	No
Serum phosphorus (normal, 3.7-5.6 mg/dL)	7.6	9.25
Serum calcium (normal, 9-11 mg/dL)	9.5	11
Serum creatinine (normal, 0.3-0.7 mg/dL)	0.3	0.4
Alkaline phosphatase (normal, 50-160 U/L)	97	100
Parathormone (normal, 10-65 pg/ml)	26	19.3
25-hydroxyvitamin D (normal, 20-100 ng/mL)	39.3	41.8
Erythrocyte sedimentation rate (normal, 0-20 mm/h)	31	13
C-reactive protein (normal, <0.5mg/dl)	1.4	0.9
Leukocyte count (normal, 4000-10000/mm ³)	9300	6380
Renal Tubular reabsorption of phosphate (normal, >85%)	97.8	96.9
TmP/GFR ratio (normal, 2.9-6.5 mg/dl)	7.34	7.73
Bone mineral density Z score	0.7	0.0
GALNT3 gene	P85Rfs*6 homozygote	P85Rfs*6 homozygote
HFTC; Hypertrophic Familial Tumoral Calcinosis, TmP/GFR; tubular maximum reabsorption of phosphorus/glomerular filtration rate, GALNT3; Polypeptide N-Acetylgalactosaminyltransferase 3

Figure 1. **Calcinosis** in the left elbow of Patient 1 (A). Subcutaneous mass around the left elbow of Patient 2. (B)

Figure 2. Radiographic findings of our patients with Hyperphosphatemic Familial Tumoral Calcinosis. Two giant radiopaque soft tissue masses around the left femur neck of Patient 1 in anteroposterior X-ray (A). Lateral view of the left elbow of the patient 1, revealing subcutaneous calcifications (B). Anteroposterior X-ray view of upper extremities shows radiopaque mass around elbow joint of Patient 2 (C). Subcutaneous calcified mass behind the olecranon is identified on X-ray, lateral view of the left elbow (D).

Figure 3. Genetic pedigree of the Hyperphosphatemic Familial Tumoral Calcinosis patients is presented in A. Next generation sequence view of the variant identified in the two cases showed in B (Patient 1) and C (Patient 2).