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Clinical Variability of Familial Tumoral Calcinosis Caused by Novel *GALNT3* Mutations

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Abstract

The *GALNT3* gene encodes GalNAc-T3, which prevents degradation of the phosphaturic hormone, fibroblast growth factor 23 (FGF23). Biallelic mutations in either *GALNT3* or *FGF23* result in hyperphosphatemic familial tumoral calcinosis or its variant, hyperostosis-hyperphosphatemia syndrome. Tumoral calcinosis is characterized by the presence of ectopic calcifications around major joints, whereas hyperostosis-hyperphosphatemia syndrome is characterized by recurrent long bone lesions with hyperostosis. Here we investigated four patients with hyperphosphatemia and clinical manifestations including tumoral calcinosis and/or hyperostosis-hyperphosphatemia syndrome to determine underlying genetic cause and delineate phenotypic heterogeneity of these disorders. Mutational analysis of *FGF23* and *GALNT3* in these patients revealed novel homozygous mutations in *GALNT3*. Although the presence of massive calcifications, cortical hyperostosis, or dental anomalies was not shared by all patients, all had persistent hyperphosphatemia, as well as inappropriately normal 1,25-dihyroxyvitamin D [1,25$(OH)_2$D]. Three of the patients also had confirmed low circulating intact FGF23 concentrations. The four novel *GALNT3* mutations invariably resulted in hyperphosphatemia due

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to low intact FGF23, but other clinical manifestations were variable. Therefore, tumoral calcinosis and hyperostosis-hyperphosphatemia syndrome represent a continuous spectrum of the same disease caused by increased phosphate levels, rather than two distinct disorders.

Keywords

Tumoral calcinosis; hyperostosis-hyperphosphatemia syndrome; *GALNT3*; *FGF23*; phosphate

INTRODUCTION

Hyperphosphatemic familial tumoral calcinosis (OMIM 211900) is an autosomal recessive disorder, caused by deleterious mutations in three different genes: *FGF23*, encoding a potent phosphaturic hormone, fibroblast growth factor 23 [Benet-Pagès et al. 2005; Larsson et al. 2005b]; *GALNT3*, encoding GalNAc-T3 [Ichikawa et al. 2005; Topaz et al. 2004]; or Klotho (*KL*), encoding an FGF23 co-factor required to activate FGF receptors [Ichikawa et al. 2007b]. Biochemical features of tumoral calcinosis include hyperphosphatemia due to increased renal phosphate reabsorption, and elevated or inappropriately normal 1,25-dihyroxyvitamin D [1,25$(OH)_2$D]. However, circulating calcium and parathyroid hormone (PTH) are usually normal. The disease is characterized by ectopic calcifications in soft tissues around major joints. Vascular calcifications also occur in some patients. Other reported features of familial tumoral calcinosis include angioid streaks of the retina [Ghanchi et al. 1996; McPhaul and Engel 1961], dental abnormalities [Dumitrescu et al. 2009; Laleye et al. 2008; Lyles et al. 1985], and testicular microlithiasis [Campagnoli et al. 2006; Shetty et al. 2009]; however, it is not clear whether these findings are common in tumoral calcinosis.

Recent studies indicate that tumoral calcinosis shares a genetic etiology with hyperostosis-hyperphosphatemia syndrome (OMIM 610233), which is characterized by recurrent bone lesions with hyperostosis. Typical radiographic features of affected bones include cortical hyperostosis, diaphysitis, and periosteal apposition. As in tumoral calcinosis, the biochemical hallmark of hyperostosis-hyperphosphatemia syndrome is persistent hyperphosphatemia and abnormal 1,25$(OH)_2$D levels. Some patients with tumoral calcinosis were reported to have skeletal features consistent with hyperostosis-hyperphosphatemia syndrome [Clarke et al. 1984; Narchi 1997; Wilson et al. 1989], but only recently we learned that mutations in the *GALNT3* gene are also responsible for hyperostosis-hyperphosphatemia syndrome [Frishberg et al. 2005; Ichikawa et al. 2007a; Olauson et al. 2008]. In fact, identical *GALNT3* mutations may cause either tumoral calcinosis or hyperostosis-hyperphosphatemia syndrome [Frishberg et al. 2005].

Initial reports of tumoral calcinosis occurred predominantly in patients of African and Middle-eastern backgrounds. However, it is becoming increasingly apparent that tumoral calcinosis also affects other ethnicities. Herein, we report four novel mutations in the *GALNT3* gene found in patients of various ethnic origins. Our findings shed light on the biochemical basis and clinical variability of tumoral calcinosis and hyperphosphatemia-hyperostosis syndrome.

SUBJECTS AND METHODS

Subjects

The study was approved by the institutional review board of Indiana University-Purdue University Indianapolis and committee of Necker Hospital. Written informed consents were obtained from all subjects or their parents prior to participation in the study.

Mutational Analysis

Genomic DNA was isolated from leukocytes using standard procedures. All coding exons and their adjacent intronic sequences in *GALNT3* and *FGF2*3 were amplified by PCR, using Multiplex PCR Kit (QIAGEN Inc., Valencia, CA). Approximately 100 ng of purified PCR products were directly sequenced from PCR primers, using Big-Dye Terminator Cycle Sequencing Kit and the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Primer sequences available upon request.

PCR-RFLP analysis of healthy individuals was performed to verify absence of the sequence variations found in Patients 1 and 4. *GALNT3* exons 2 and 10 were amplified by PCR and then incubated with five units of restriction endonucleases BstXI and KpnI (New England Biolabs, Beverly, MA), respectively. The digested PCR products were then electrophoresed in 2% agarose gels and visualized under the UV light. PCR-RFLP analysis was not performed for the exon 3 and intron 8 sequence variations found in Patients 2 and 3 due to paucity of appropriate ethnicity-matched healthy cohorts.

Laboratory measurements

Clinical laboratory measurements were performed using standard methods. Plasma FGF23 concentrations were measured using two different assays according to the manufacturers' instructions. Intact FGF23 was measured using FGF23 ELISA Kit (Kainos Laboratories Inc., Tokyo, Japan). FGF23 was also measured using Human FGF23 (C-Term) ELISA Kit (Immutopics International, San Clemente, CA), which detects both intact FGF23 and C-terminal FGF23 fragments.

RESULTS

Patient 1

A 13-year-old Turkish girl presented with a painful firm mass over the left lateral gluteal region, which was surgically excised. The lesion recurred and was excised again at ages 15 and 17 years. When she was 26 years old, the lesion reappeared as a small nodule, which gradually expanded to become a large mass of about 10 cm in diameter (Fig 1-1a). At the center of the lesion there was a skin erosion of 3×2 cm with an exposed area of gritty whitish material.

She had no other medical conditions. The patient was the fourth of six children born to a known consanguineous marriage. Her grandfathers were first cousins. However, there was no family history of kidney stones, calcifications, or systemic illness. Physical examination revealed a hard, tender, irregular mass of about 10 cm in the left gluteal region (Fig 1-1a). Tenderness of the left hip abductor muscles limited active abduction, while the passive hip joint mobility was unaffected. She had a palpable hard 7 cm non-tender mass in the right popliteal fossa, with full extension of the knee but with mild limitation in flexion (Fig 1-1b). Vital signs were normal. There was no lymphadenopathy, and the remainder of the physical examination was within normal limits.

She had hyperphosphatemia (6.2 mg/dl), while serum calcium, uric acid, alkaline phosphatase, creatinine and blood urea nitrogen were within the normal range (Table I). An appropriate sample was not available for measurement of FGF23. Complete blood count showed no abnormality. Skeletal radiographs revealed heterogeneous juxta-articular calcified masses adjacent to the greater trochanter on both hips, in the right popliteal fossa, and on the medial aspect of the right foot, adjacent to the first toe (Fig 1-1a-b). Adjacent joints and the contiguous bones were unaffected. The lesions at the right hip, knee and foot were asymptomatic.

The patient was hospitalized for the fourth resection of the left hip mass. The excised mass was composed of a whitish gritty material with an incomplete fibrous covering. Histopathological preparation demonstrated calcium deposits surrounded by dense fibrous tissue and occasional histiocytes. Following the operation, the patient did not have any significant complications until the left hip lesion recurred 18 months later.

Genetic analysis of the *GALNT3* gene demonstrated a G>A transition in exon 2 (c. 485G>A), which resulted in a substitution of arginine 162 to glutamine (p.R162Q) (Fig 2). The mutation was not found in more than 90 healthy Caucasian samples (data not shown).

Patient 2

A five-year-old girl presented with a six month history of painful left lower leg swelling. Her previous medical history was negative, and her parents were first degree cousins from Sri-Lanka with unremarkable family history. Tibial radiographs demonstrated circumferential endosteal and periosteal bone proliferation with patchy sclerosis of the medullary canal, consistent with hyperostosis (Fig 1-2a). Multifocal osteomyelitis was considered. Biopsy demonstrated reactive non-specific new bone, fibroblastic stroma infiltrated by lymphocytes and polynuclear cells. Cultures grew *Staphylococcus aureus*, and antibiotics were administered. After treatment, there was no radiographic change in the tibia, but technetium MDP bone scan demonstrated markedly increased uptake in tibia, femur and forearm.

Nine months later, a painful mass in the left elbow developed and then fifteen months later, a similar mass developed in the right elbow. Radiographs demonstrated peri-articular calcified lesions (Fig 1-2b). The diagnosis of tumoral calcinosis was considered. Biochemical evaluation revealed elevated serum phosphorus (7.2 mg/dl) (Table I). Other biochemical evaluation included normal serum and urine calcium, alkaline phosphatase, and PTH. Her 25-hydroxyvitamin D [25(OH)D] was low (7 ng/ml) with inappropriately normal $1,25(OH)_2D$ (48 pg/ml). Elevated concentrations of C-terminal FGF23 fragments (563 RU/ml) with concomitant low intact FGF23 (11 pg/ml) were compatible with the diagnosis of tumoral calcinosis.

At the age of 11, she is growing along the 75^{th} centile for height but continues to have 2 or 3 episodes a month with fever, local inflammation, pain and tender swelling over the long bones. Hearing is not impaired, and no dental abnormalities are noted. She had undergone multiple excisions for the massive juxta-articular calcific deposit in the left elbow. To date, her serum phosphate and areas of calcinosis have not responded clinically to attempted treatment with oral sevelamer (a non-calcium phosphate binder) and methotrexate. Family members tested were free of clinical or laboratory abnormalities related to tumoral calcinosis.

Mutational analysis of this patient revealed a homozygous deletion of C in exon 3 of the *GALNT3* gene (c.677delC) (Fig 2). This frame shift mutation is predicted to cause premature termination and produce significantly truncated GALNT3 protein with 277 amino acids (instead of 633 amino acids synthesized from the wild-type allele).

Patient 3

A 15-year-old Lebanese male presented with abnormal gait and hip pain following strenuous sport activity. Radiographs showed femoral epiphysiolysis with severe coxa valga (Fig 1-3a). There was no known history for consanguinity. Screening for lysosomal storage disease was negative. Surgical correction of the coxa valga was performed because of the risk for slipped capita femora epiphysis. Three months later, he presented with severe right tibia pain and localized swelling in the distal third of the leg. X-rays revealed periosteal

appositions and diaphyseal hyperostosis without distinguishable medullary canal (Fig 1-3b). No ectopic calcification was demonstrated. Magnetic resonance imaging (MRI) showed inflammatory process and technetium scintigraphy displayed localized increased uptake at the tibia diaphysis. The bone biopsy eliminated osseous malignant processes, showing non-specific fibrous tissue with lymphocytic peri-vascular infiltration of the periosteum. A blood count and serum calcium were normal, but no serum phosphorus measurement was performed during this first hospitalization. His symptoms gradually resolved with analgesics and restriction of physical activities.

Nine months later, following a fishing trip, he developed similar pain in the opposite tibia. Radiographs showed new mild broadening of the left diaphysis, with linear periosteal reaction but without any ectopic calcification (Fig 1-3c). This left tibial local hyperostosis was not present on the previous radiographs. The original right tibial lesion had normalized (Fig 1-3d). Hyperphosphatemia (7.9 mg/dl) was detected with inappropriately elevated tubular reabsorption of phosphate (TRP) of 96 %. Other biochemical evaluation included normal serum and urine calcium, alkaline phosphatase, and PTH (Table I). 25(OH)D and $1,25(OH)_2D$ were also normal. The C-terminal FGF23 concentration was elevated (958 RU/ml) with inappropriately normal intact FGF23 (26 pg/ml). Symptoms improved in a few weeks with paracetamol, ibuprofen and leg immobilization using a splint. Two years later, he remains asymptomatic.

The patient was homozygous for a G>A transition in the donor splice site of intron 8 (c. 1392+1G>A or IVS8+1G>A) of the *GALNT3* gene (Fig 2). An *in silico* analysis of the intronic mutation, using NNSPLICE version 0.9 (http://www.fruitfly.org/seq_tools/splice.html) predicts complete disruption of the donor splice site, likely resulting in skipping of the adjacent exon 8 or activation of cryptic donor splice site(s).

Patient 4

A Greek woman initially presented at 8 years of age with sudden onset of a painful inflammatory mass in the muscles medial to the upper right tibia, accompanied by a low fever. The symptoms improved with acetylsalicylic acid. One month later she had removal of an approximately 6 cm tibial lesion involving both cortical and trabecular bone. Histologically, the lesion incorporated mature trabeculae, some loose fibroblastic tissue, some areas with increased concentrations of osteoblasts producing osteoid, small collections of inflammatory cells, and cortical bone. Mature cortical bone was noted along with intact periosteum but increased subperiosteal ossification. The pathologic interpretation was one of possible osteoid osteoma. Post-operatively, she was free of symptoms and the right leg bony lesion was not evident on later radiographs (ages 15 and 20).

At age 14 an approximately 5 cm subcutaneous calcified lesion was removed from her external upper right thigh, followed by removal of a smaller (2cm) calcified mass in her left hand (metacarpal region). Histologically nodular masses containing deposits of calcium salts were separated and surrounded by dense fibrous connective tissue with abundant histiocytes and polynuclear giant osteoclasts, consistent with tumoral calcinosis. The only reported biochemical abnormality was hyperphosphatemia.

At age 15 she was referred for evaluation and management of recurrent calcified masses and hyperphosphatemia. She had been otherwise healthy without a history of inflammatory conditions or kidney disorders. The parents originate from two small villages (4 km apart) in Greece, but there is no known consanguinity. Her 14 year old brother was healthy. The patient's mother had three sisters and one brother. Her second maternal aunt died from unknown causes in infancy. The patient's father is healthy and has five siblings (two sisters

and three brothers). There is no family history of similar calcified masses or mineral disorders.

On examination, she was a healthy, fully developed female of normal height (159 cm) and weight (58 kg). She had scars from previous surgeries. Palpation of the right thigh scar revealed a 1.5 cm hard mass, suggesting a remnant or a recurrence of the previous mass. A 1.5 cm area of vitiligo on her left abdomen was observed. She has all her teeth, but those of the front of the upper jaw were repaired after an injury at the age of 5. Dental radiograph revealed absence of pulp chambers and root canals, and short bulbous roots in almost all teeth with only a few exceptions (Fig 1-4). MRI of the head indicated very small calcifications of the basal ganglia.

Laboratory testing at age 15-16 revealed hyperphosphatemia (6.5-6.8 mg/dl), and inappropriately increased $1,25(OH)_2D$ (57 pg/ml), but low 25(OH)D (17 ng/ml) (Table I). She had normal serum calcium (9.5-10.0 mg/dl), magnesium (1.8-1.9 mg/dl), uric acid (4.6 mg/dl) and intact PTH (31-46 ng/ml). Renal TRP was inappropriately elevated (96.3%) in the setting of hyperphosphatemia. Based on the findings of soft tissue calcification, hyperphosphatemia, and inappropriately normal $1.25(OH)_2D$, the diagnosis of hyperphosphatemic tumoral calcinosis was made. C-terminal FGF23 concentrations were elevated (1066 RU/ml), while intact FGF23 concentration was inappropriately low normal (20 pg/ml).

She was advised to avoid phosphate rich foods, and phosphate binding therapy began with aluminum hydroxide 800 mg three times daily and acetazolamide 250 mg daily. Initially, the patient's compliance was poor. The mass grew further, and at age 16, three calcified masses (12 × 5, 10 × 5, and 5 × 5 cm) were removed. The histology was identical to the previously removed mass. She developed mild anemia (HCT 36%, serum ferritin 14 ng/ml), and oral ferrous sulfate was prescribed. During the next two years her medication compliance improved, and her serum phosphorus decreased slightly. However, she remained hyperphosphatemic (5.6-6.0 mg/dl) with elevated TRP (87-94%).

At age 19 a trial of oral risedronate 35 mg weekly began with the goal of additional improvement in the biochemical profile. At age 20, risedronate and acetazolamide were discontinued, and the dose of aluminum hydroxide was reduced. Addition of probenecid for eight months did not change the biochemical profile, except for a small reduction of serum uric acid to 2.8 mg/dl. However, during the last 6 years of treatment there has been no recurrence of calcified masses as demonstrated by skeletal survey radiographs. Recent ophthalmological examination was also negative for angioid streaks of the retina.

Mutational analysis of the *GALNT3* gene showed a homozygous mutation in exon 10 (c. 1720T>G), which affects amino acid coding (p.C574G) (Figure 2). The patient's asymptomatic parents and brother were heterozygous for this nucleotide substitution. This change was not found in the first maternal aunt and 92 healthy white Americans by PCR-RFLP analysis (data not shown).

DISCUSSION

In this study, we identified four novel homozygous mutations in the *GALNT3* gene, which led to tumoral calcinosis in three patients and hyperostosis-hyperphosphatemia syndrome in three patients. No *FGF23* mutations were detected. As demonstrated in this and other reports, inactivating mutations in the *GALNT3* gene cause variable clinical manifestations, including massive calcifications around the major joints, dental and ophthalmic anomalies, and lesions in the long bone. The clinical findings of tumoral calcinosis can be as benign as eyelid calcifications [Ichikawa et al. 2006] or as severe as generalized vascular calcifications

[Chefetz et al. 2005; Larsson et al. 2005b]. Clinical variability is also observed within hyperostosis-hyperphosphatemia syndrome. Although it is unknown at this time if coxa valga with chronic epiphysiolysis is a part of hyperostosis-hyperphosphatemia syndrome, to our knowledge, this unique skeletal feature is not previously reported. However, the most consistent clinical finding of tumoral calcinosis and hyperostosis-hyperphosphatemia syndrome is the presence of hyperphosphatemia, caused by low circulating intact FGF23. Although these diseases have been described as "allelic" [Frishberg et al. 2005], features of both conditions can manifest in the same patient, as in Patients 2, 4 and three recently reported patients [Benet-Pagès et al. 2005; Dumitrescu et al. 2009; Joseph et al. 2010]. Patient 4 initially presented with hyperostosis-hyperphosphatemia syndrome (age 8) and later with tumoral calcinosis (age 14). Therefore, these two entities represent a continuous spectrum of the same disease that is caused by loss of GALNT3 function and subsequent hyperphosphatemia.

The patient with solely hyperostosis-hyperphosphatemia syndrome (Patient 3) appeared to have a mild presentation of the disease with only two attacks of acute swelling of the long bones before the age of 18 years. As cortical hyperostosis is associated with other diseases, the condition of this patient was initially mistaken for osteosarcoma, which emphasizes the importance of serum phosphate measurements in evaluating diaphyseal hyperostosis. The cause of disease onset is unknown, but for this patient each episode was associated with an unusual amount of physical activity.

Persistent hyperphosphatemia, with elevated or inappropriately normal $1.25(OH)_2D$, is invariably found in all reported tumoral calcinosis patients due to inactivating mutations in *GALNT3* or *FGF23*. The hyperphosphatemia in these patients results from a decrease in intact bioactive FGF23, which normally inhibits renal phosphate reabsorption and $1,25(OH)_2D$ synthesis. Since GALNT3 is a glycosylating enzyme that protects FGF23 from proteolytic processing, in the absence of normal GALNT3 function, intact FGF23 is cleaved before secretion, leading to accumulation of fragmented FGF23 [Kato et al. 2006]. Importance of GALNT3 for FGF23 secretion is also confirmed by a recently developed animal model of tumoral calcinosis, the *Galnt3* knockout mouse [Ichikawa et al. 2009]. In this animal model, hyperphosphatemia appropriately induced *Fgf23* gene expression, but only a small fraction of intact Fgf23 was secreted. Similar to *GALNT3* mutations, *FGF23* mutations causing tumoral calcinosis also destabilize FGF23 proteins, thereby reducing intact FGF23 concentrations [Larsson et al. 2005a]. Therefore, low circulating intact FGF23 is the common culprit regardless of mutations in *GALNT3* or *FGF23*.

Of the mutations described in this report, the location of the c.485G>A (p.R162Q) mutation is particularly intriguing. This mutation occurred adjacent to the location of another reported mutation, c.484C>T (p.R162X), from two other families with tumoral calcinosis [Ichikawa et al. 2005; Topaz et al. 2004]. Although this finding could be coincidental and patients are limited, identification of these mutations suggests that this region of the *GALNT3* gene may be susceptible to increased mutation rate.

Since the identification of *GALNT3* mutations in tumoral calcinosis [Topaz et al. 2004], a total of 21 different *GALNT3* mutations have been reported in the literature (Table II). This study reports four new mutations, increasing the total to 25. In contrast, mutations in the two other genes associated with tumoral calcinosis are rarer. There are seven reported *FGF23* mutations, and there is only one report of *KL* mutation to date causing tumoral calcinosis. Although *GALNT3* is a larger gene than *FGF23* and additional patients are needed, it appears that *GALNT3* is the most prevalent genetic cause of tumoral calcinosis. Interestingly, bone lesions seen in hyperostosis-hyperphosphatemia syndrome are mostly associated with mutations in *GALNT3* (Table II).

In summary, we identified four novel mutations in the *GALNT3* gene in patients with tumoral calcinosis and hyperostosis-hyperphosphatemia syndrome. The present study supports the concept that these two disorders are clinical variants of the same disease. We propose that tumoral calcinosis and hyperostosis-hyperphosphatemia syndrome be considered one disease process and simply referred to as familial hyperphosphatemic tumoral calcinosis. Further studies are warranted to determine how GALNT3 deficiency and subsequent FGF23 insufficiency result in massive calcifications in some patients, but cortical hyperostosis in the others.

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REFERENCES

Araya K, Fukumoto S, Backenroth R, Takeuchi Y, Nakayama K, Ito N, Yoshii N, Yamazaki Y, Yamashita T, Silver J, Igarashi T, Fujita T. A novel mutation in fibroblast growth factor 23 gene as a cause of tumoral calcinosis. J Clin Endocrinol Metab. 2005; 90:5523–5527. [PubMed: 16030159]

Barbieri AM, Filopanti M, Bua G, Beck-Peccoz P. Two novel nonsense mutations in GALNT3 gene are responsible for familial tumoral calcinosis. J Hum Genet. 2007; 52:464–468. [PubMed: 17351710]

Benet-Pagès A, Orlik P, Strom TM, Lorenz-Depiereux B. An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia. Hum Mol Genet. 2005; 14:385–390. [PubMed: 15590700]

Bergwitz C, Banerjee S, Abu-Zahra H, Kaji H, Miyauchi A, Sugimoto T, Juppner H. Defective O-glycosylation due to a novel homozygous S129P mutation is associated with lack of fibroblast growth factor 23 secretion and tumoral calcinosis. J Clin Endocrinol Metab. 2009; 94:4267–4274. [PubMed: 19837926]

Campagnoli MF, Pucci A, Garelli E, Carando A, Defilippi C, Lala R, Ingrosso G, Dianzani I, Forni M, Ramenghi U. Familial tumoral calcinosis and testicular microlithiasis associated with a new mutation of GALNT3 in a white family. J Clin Pathol. 2006; 59:440–442. [PubMed: 16567474]

Chefetz I, Heller R, Galli-Tsinopoulou A, Richard G, Wollnik B, Indelman M, Koerber F, Topaz O, Bergman R, Sprecher E, Schoenau E. A novel homozygous missense mutation in FGF23 causes Familial Tumoral Calcinosis associated with disseminated visceral calcification. Hum Genet. 2005; 118:261–266. [PubMed: 16151858]

Clarke E, Swischuk LE, Hayden CK Jr. Tumoral calcinosis, diaphysitis, and hyperphosphatemia. Radiology. 1984; 151:643–646. [PubMed: 6718723]

Dumitrescu CE, Kelly MH, Khosravi A, Hart TC, Brahim J, White KE, Farrow EG, Nathan MH, Murphey MD, Collins MT. A case of familial tumoral calcinosis/hyperostosis-hyperphosphatemia syndrome due to a compound heterozygous mutation in GALNT3 demonstrating new phenotypic features. Osteoporos Int. 2009; 20:1273–1278. [PubMed: 18982401]

Frishberg Y, Topaz O, Bergman R, Behar D, Fisher D, Gordon D, Richard G, Sprecher E. Identification of a recurrent mutation in GALNT3 demonstrates that hyperostosis-hyperphosphatemia syndrome and familial tumoral calcinosis are allelic disorders. J Mol Med. 2005; 83:33–38. [PubMed: 15599692]

Garringer HJ, Fisher C, Larsson TE, Davis SI, Koller DL, Cullen MJ, Draman MS, Conlon N, Jain A, Fedarko NS, Dasgupta B, White KE. The role of mutant UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyltransferase 3 in regulating serum intact fibroblast growth factor 23 and matrix extracellular phosphoglycoprotein in heritable tumoral calcinosis. J Clin Endocrinol Metab. 2006; 91:4037–4042. [PubMed: 16868048]

Garringer HJ, Malekpour M, Esteghamat F, Mortazavi SMJ, Davis SI, Farrow EG, Yu X, Arking DE, Dietz HC, White KE. Molecular genetic and biochemical analyses of FGF23 mutations in familial tumoral calcinosis. Am J Physiol Endocrinol Metab. 2008; 295:E929–937. [PubMed: 18682534]

Garringer HJ, Mortazavi SMJ, Esteghamat F, Malekpour M, Boztepe H, Tanakol R, Davis SI, White KE. Two novel GALNT3 mutations in familial tumoral calcinosis. Am J Med Genet A. 2007; 143:2390–2396. [PubMed: 17853462]

Ghanchi F, Ramsay A, Coupland S, Barr D, Lee WR. Ocular tumoral calcinosis. A clinicopathologic study. Arch Ophthalmol. 1996; 114:341–345. [PubMed: 8600898]

Gok F, Chefetz I, Indelman M, Kocaoglu M, Sprecher E. Newly discovered mutations in the GALNT3 gene causing autosomal recessive hyperostosis-hyperphosphatemia syndrome. Acta Orthop. 2009; 80:131–134. [PubMed: 19297793]

Ichikawa S, Guigonis V, Imel EA, Courouble M, Heissat S, Henley JD, Sorenson AH, Petit B, Lienhardt A, Econs MJ. Novel GALNT3 mutations causing hyperostosis-hyperphosphatemia syndrome result in low intact fibroblast growth factor 23 concentrations. J Clin Endocrinol Metab. 2007a; 92:1943–1947. [PubMed: 17311862]

Lammoglia JJ, Mericq V. Familial tumoral calcinosis caused by a novel FGF23 mutation: response to induction of tubular renal acidosis with acetazolamide and the non-calcium phosphate binder sevelamer. Horm Res. 2009; 71:178–184. [PubMed: 19188744]

Larsson T, Davis SI, Garringer HJ, Mooney SD, Draman MS, Cullen MJ, White KE. Fibroblast growth factor-23 mutants causing familial tumoral calcinosis are differentially processed. Endocrinology. 2005a; 146:3883–3891. [PubMed: 15961556]

Larsson T, Yu X, Davis SI, Draman MS, Mooney SD, Cullen MJ, White KE. A novel recessive mutation in fibroblast growth factor-23 causes familial tumoral calcinosis. J Clin Endocrinol Metab. 2005b; 90:2424–2427. [PubMed: 15687325]

Lyles KW, Burkes EJ, Ellis GJ, Lucas KJ, Dolan EA, Drezner MK. Genetic transmission of tumoral calcinosis: autosomal dominant with variable clinical expressivity. J Clin Endocrinol Metab. 1985; 60:1093–1096. [PubMed: 3998061]

Masi L, Gozzini A, Franchi A, Campanacci D, Amedei A, Falchetti A, Franceschelli F, Marcucci G, Tanini A, Capanna R, Brandi ML. A novel recessive mutation of fibroblast growth factor-23 in tumoral calcinosis. J Bone Joint Surg Am. 2009; 91:1190–1198. [PubMed: 19411468]

McPhaul JJ Jr. Engel FL. Heterotopic calcification, hyperphosphatemia and angioid streaks of the retina. Am J Med. 1961; 31:488–492. [PubMed: 13774168]

Narchi H. Hyperostosis with hyperphosphatemia: evidence of familial occurrence and association with tumoral calcinosis. Pediatrics. 1997; 99:745–748. [PubMed: 9113957]

Olauson H, Krajisnik T, Larsson C, Lindberg B, Larsson TE. A novel missense mutation in GALNT3 causing hyperostosis-hyperphosphataemia syndrome. Eur J Endocrinol. 2008; 158:929–934. [PubMed: 18322299]

Shetty GM, Murari AS, Shah SV, Dhengle S. Nonfamilial hyperphosphatemic tumoral calcinosis with ulnar neuropathy. Joint Bone Spine. 2009; 76:198–201. [PubMed: 19073370]

Specktor P, Cooper JG, Indelman M, Sprecher E. Hyperphosphatemic familial tumoral calcinosis caused by a mutation in GALNT3 in a European kindred. J Hum Genet. 2006; 51:487–490. [PubMed: 16528452]

Topaz O, Shurman DL, Bergman R, Indelman M, Ratajczak P, Mizrachi M, Khamaysi Z, Behar D, Petronius D, Friedman V, Zelikovic I, Raimer S, Metzker A, Richard G, Sprecher E. Mutations in GALNT3, encoding a protein involved in O-linked glycosylation, cause familial tumoral calcinosis. Nat Genet. 2004; 36:579–581. [PubMed: 15133511]

Wilson MP, Lindsley CB, Warady BA, Johnson JA. Hyperphosphatemia associated with cortical hyperostosis and tumoral calcinosis. J Pediatr. 1989; 114:1010–1013. [PubMed: 2656956]

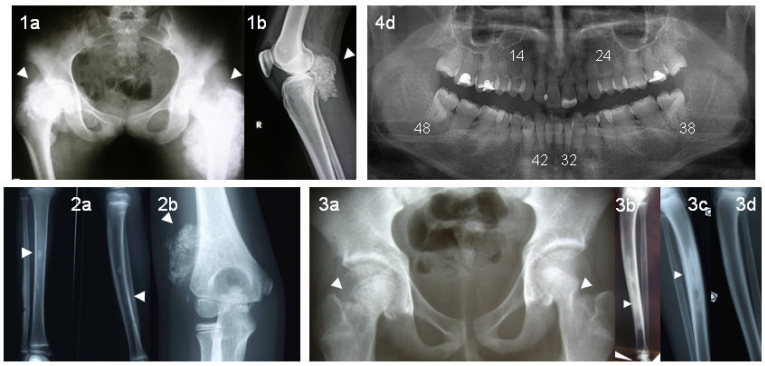

Figure 1.

Plain radiographs of pelvis (1a) and knee (1b) with calcifications in Patient 1. Radiologic findings in Patient 2: Hyperostosis of the long bones with periosteal appositions and metaphyseal densification in diaphysis (2a); right elbow calcification (2b). Radiological findings in Patient 3: Pelvis showing coxa valga and epiphysiolysis (3a); first episode of hyperostosis with abnormal irregular hyperostosis of the right tibial diaphysis (3b); second episode of hyperostosis of the left tibial diaphysis (3c) with amelioration of the right diaphysis hyperostosis (3d). Arrow heads indicate calcifications and hyperostosis. Radiologic features in Patient 4: Dental radiograph demonstrating short bulbous roots in all teeth except teeth 14 and 24, which show complete root resorption. Pulp chambers and root canals are absent in all teeth with the following exceptions: teeth 38 and 48 (regular pulp chamber and root canal) and teeth 32 and 42 (residual root canal in the apical region only). Note that all exceptions are observed in two teeth lying symmetrically. The temporo-mandibular joint area lacked lesions, which are sometimes seen in patients with tumoral calcinosis. Teeth are numbered using the Two-Digit Fédération Dentaire Internationale (FDI) notation for adult teeth.

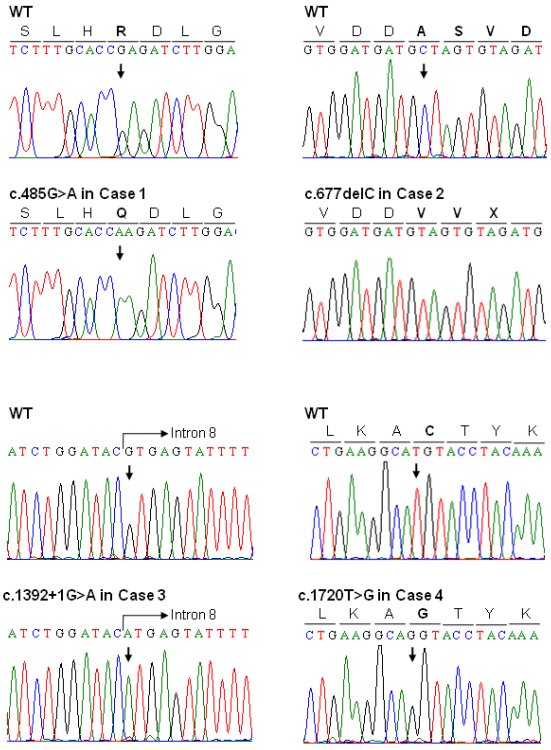

Figure 2.
Mutational analysis of the *GALNT3* gene. Electropherograms of PCR products show wild-type sequences (top) and mutated sequences (bottom) in four patients. Arrows indicate nucleotides involved in the mutations.

Table I

Representative laboratory data of the patients.

	Normal range[1]	Patient 1	Patient 2	Patient 3	Patient 4
Age (year)		25	7	16	15[2]
Serum calcium (mg/dl)	8.8-10.8	9.8	9.6	10.6	10.0
Serum phosphorus (mg/dl)	2.7-4.7 (3.6-5.6)	6.2	7.2	7.9	6.8
25(OH)D (ng/ml)	30-80	ND	7	24	17
$1,25(OH)_2D$ (pg/ml)	10-80	ND	48	39	57
Alkaline phosphatase (IU/l)	40-135 (70-300)	87	100	162	136
Creatinine (mg/dl)	0.6-1.4 (0.34-0.93)	0.6	0.6	0.74	0.7
PTH (pg/ml)	10-46	18	18	18	31
Intact FGF23 (pg/ml)	<71	ND	11	26	20
C-terminal FGF23 (RU/ml)	<150	ND	563	958	1066

[1] Normal ranges specific for children are in the parentheses.

[2] Laboratory data are those at age 15 (before treatment), except for intact FGF23 and c-terminal FGF23 (age 20)

ND: Not determined.

SI unit conversions: To convert the values for calcium to mmol/L, multiply by 0.250; to convert the values for phosphorus, multiply by 0.323; to convert the values for 25(OH)D to nmol/L, multiply by 2.496; to convert the values for $1,25(OH)_2D$ to pmol/L, multiply by 2.599; to convert the values for creatinine to μmol/L, multiply by 76.26.

Table II

Mutations causing tumoral calcinosis and/or hyperostosis-hyperphosphatemia syndrome

Phenotype[1]	Gene	Mutation[2]	Predicted Change	Reference
TC	*GALNT3*	c.1524+1G>A	p.K465_Y508del[3]	[Topaz et al. 2004]
TC	*GALNT3*	c.484C>T; c.1524+5G>A	p.R162X; Splicing error	[Topaz et al. 2004]
TC	*GALNT3*	c.516-2A>T	p.C173VfsX4[3]	[Ichikawa et al. 2005]
TC	*GALNT3*	c.484C>T; c.516-2A>T	p.R162X; p.C173VfsX4[3]	[Ichikawa et al. 2005]
TC	*GALNT3*	c.1387A>T	p.K463X	[Campagnoli et al. 2006]
TC	*GALNT3*	c.1774C>T	p.Q592X	[Specktor et al. 2006]
TC	*GALNT3*	c.42_57del	p.R14SfsX8	[Garringer et al. 2006]
TC	*GALNT3*	c.815C>A; c.1076C>A	p.T272K; p.T359K	[Ichikawa et al. 2006]
TC	*GALNT3*	c.1102_1103insT	p.S368FfsX8	[Garringer et al. 2007]
TC	*GALNT3*	c.1460G>A	p.W487X	[Garringer et al. 2007]
TC	*GALNT3*	c.966T>G; c.1441C>T	p.Y322X; p.Q481X	[Barbieri et al. 2007]
TC	*GALNT3*	c.516-2A>T	p.C173VfsX4[4]	[Laleye et al. 2008]
TC	*GALNT3*	c.485G>A	p.R162Q	This study
TC	*FGF23*	c.123C>A	p.H41Q	[Masi et al. 2009]
TC	*FGF23*	c.211A>G	p.S71G	[Larsson et al. 2005b]
TC	*FGF23*	c.287T>C	p.M96T	[Chefetz et al. 2005]
TC	*FGF23*	c.385T>C	p.S129P	[Bergwitz et al. 2009]
TC	*FGF23*	c.386C>T	p.S129F	[Araya et al. 2005]
TC	*FGF23*	c.160C>A	p.Q54K	[Garringer et al. 2008]
TC	*FGF23*	c.367G>T	p.G123W	[Lammoglia and Mericq 2009]
TC	*KL*	c.578A>G	p.H193R	[Ichikawa et al. 2007b]
HHS	*GALNT3*	c.1524+1G>A	p.K465_Y508del[3]	[Frishberg et al. 2005]
HHS	*GALNT3*	c.803_804insC; c.1626+1G>A	p.T269NfsX3; Splicing error	[Ichikawa et al. 2007a]
HHS	*GALNT3*	c.1313G>A	p.R438H	[Olauson et al. 2008]
HHS	*GALNT3*	c.2T>A; c.839G>A	p.M1?; p.C280Y	[Gok et al. 2009]
HHS	*GALNT3*	c.1392+1G>A	Splicing error	This study
TC/HHS	*GALNT3*	c.1312C>T; c.1774C>T	p.R438C; p.Q592X	[Dumitrescu et al. 2009]
TC/HHS	*GALNT3*	c.842A>G; c.1097T>G	p.E281G; p.L366R	[Joseph et al. 2010]
TC/HHS	*GALNT3*	c.677delC	p.A226VfsX3	This study
TC/HHS	*GALNT3*	c.1720T>G	p.C574G	This study
TC/HHS	*FGF23*	c.211A>G	p.S71G	[Benet-Pagès et al. 2005]

[1] TC, tumoral calcinosis; HHS, hyperostosis-hyperphosphatemia syndrome.

[2] Reported according to mutation nomenclature guidelines given by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/): +1 is the A of the ATG start codon of the cDNA sequences (accession numbers: NM_020638 for *FGF23*, NM_004482 for *GALNT3*, and NM_004795 for *KL*). Some of the mutations are described differently from the original publications.

[3] Based on RNA obtained from the affected patient [Topaz et al. 2004].

[4] Based on RNA obtained from the affected patients [Laleye et al. 2008].