Letter

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A novel homozygous variant in exon 10 of the *GALNT3* gene causing hyperphosphatemic familial tumoral calcinosis in a family from North India

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SUMMARY Hyperphosphatemic familial tumoral calcinosis (HFTC) is an extremely rare autosomal recessive disorder caused by variants in the *GALNT3* (N-acetylgalactosaminyltransferase 3), *FGF23* (Fibroblast Growth Factor-23) and *αKL* (α-Klotho) genes, which results in progressive calcification of soft tissues. We describe the case of a 9-year-old girl who presented with recurrent hard nodular swellings on her feet and knees which intermittently discharged chalky white material. Her younger brother also had a similar condition. Both siblings showed hyperphosphatemia, but the parents' biochemical parameters were normal. The histological features of the material aspirated from a skin lesion were consistent with tumoral calcinosis. Sanger sequencing identified a novel homozygous non-synonymous sequence variant in exon 10 of the *GALNT3* gene (NM_004482.3:c.[1681T>A];[1681T>A], NP_004473.2:p.[Cys561Ser];[Cys561Ser] in the proband and her affected brother. The parents were heterozygous carriers for the same sequence variant. In conclusion, we report a new variant in the *GALNT3* gene that caused HFTC in a North Indian family.

Keywords hyperphosphatemic familial tumoral calcinosis, calcinosis cutis, *GALNT3* gene, novel variant, Indian family

Hyperphosphatemic familial tumoral calcinosis (HFTC) is a rare disorder of phosphate metabolism caused by mutations in genes related to Fibroblast Growth Factor-23 (FGF23), which include *FGF23* itself, an FGF23-glycosylating enzyme, N-acetylgalactosaminyltransfer ase 3 (*GALNT3*), and the FGF23 co-receptor α-Klotho (*αKL*) (*1*). The altered gene function decreases FGF23 synthesis or activity and causes increased renal tubular reabsorption of phosphate, thereby increasing blood calcium-phosphate product, which leads to predisposition for soft-tissue calcification (*2*). The most common manifestation of HFTC is calcinosis cutis, which appears clinically as firm, otherwise asymptomatic, white, yellowish or flesh-colored papules, plaques, or nodules. The clinical course is often associated with excretion of chalky material, pain, itching, ulceration, or infection of the lesions (*1*).

Only about 75 patients of genetically confirmed HTFC have been reported (*1*). The majority (about 80%) have mutations in the *GALNT3* gene followed by the *FGF23* gene (about 20%) and the *KL* gene (*1*). Most described patients were of African or Middle East origin, with few cases in Caucasians and Asians (*1-6*). Of about 60 patients reported with the *GALNT3* gene mutations, there is one report of two siblings from India (*7*).

A 9-year-old girl presented with recurrent hard nodular swellings that intermittently discharged chalky white material. The first lesion was noticed at age 4 years on the left knee, which gradually increased in size and ruptured spontaneously. Similar lesions appeared on the right knee and both feet over the next year. At age 5 years, she underwent excision of foot lesions, and was subsequently referred to us for repeated recurrences. There were no dental problems, and pain or redness at the site of lesions. She belonged to a hilly hamlet of the North-Indian state of Himachal Pradesh and was born to non-consanguineous parents. There was no family history of such skin lesions.

Examination showed multiple, small, hard, non-tender masses on the feet and knees, along

Table 1. Results of laboratory investigations of the index patient

Parameter	Patient's value	Reference range
Serum phosphorus	7.5 mg/dL	4.5-5.6 mg/dL
Serum calcium	9.0 mg/dL	9-11 mg/dL
Alkaline phosphatase	216 U/L	50-160 U/L
Serum creatinine	0.5 mg/dL	0.3-0.8 mg/dL
Parathyroid hormone	23.77 pg/mL	10-65 pg/mL
Plasma c-FGF23	2612.7 RU/mL	Upto 125 RU/mL
25-hydroxyvitamin D	11.9 ng/mL	20-100 ng/mL
1, 25-dihydroxyvitamin D	68 nmol/L	50-150 nmol/L
Total leucocyte count	8,800/mm3	4,000-11,000/mm3
ESR	10 mm/hr	0-20 mm/hr
C-reactive protein	1.2 mg/dL	< 0.5 mg/dL
Renal TRP	88%	> 85%
TmP/GFR ratio	3.0 mg/dL	2.9-6.5 mg/dL

c-FGF23, c-terminal fibroblast growth factor 23; ESR, erythrocyte sedimentation rate; TmP/GFR, tubular maximum reabsorption of phosphorus/glomerular filtration rate; TRP, tubular reabsorption of phosphate.

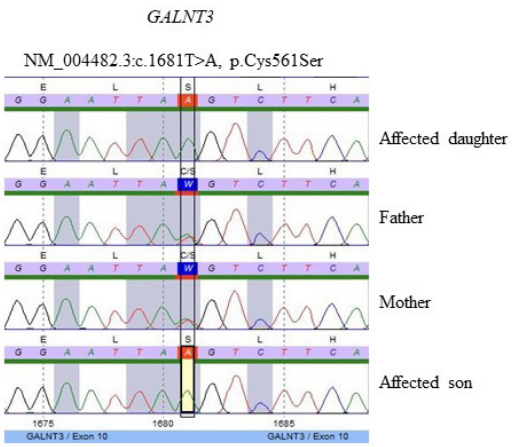

Figure 1. Mutation analysis of the *GALNT3* gene in two affected siblings and their parents. The mutation is indicated above the electropherograms. The affected patients are homozygous for a non synonymous sequence variant in exon 10 of the *GALNT3* gene. The parents are heterozygous carriers.

with an incision scar on the left foot. Her dental, ophthalmological, and systemic examinations were normal. The results of laboratory investigations are shown in Table 1. The younger sibling also showed abnormal serum biochemical parameters (phosphorus 8.1 mg/dL, calcium 8.7 mg/dL, alkaline phosphatase 202 U/L) but normal parathyroid hormone levels (44.94 pg/mL). The parents' biochemistry was normal. Cytological examination of the thick cheese-like material aspirated from one of the skin lesions showed extensive amorphous calcified deposits with granular calcification and clusters of benign epithelial cells of adnexa.

All relevant ethical guidelines have been followed for data collection and reporting. We obtained consent and assent from parents and children respectively, and approval from the Departmental Review Board for reporting data. Genomic DNA was extracted from leucocytes in the peripheral blood of the children and their parents. The 10 coding exons (exons 2-11) of the *GALNT3* gene were amplified by using PCR. Sanger sequencing identified a novel homozygous non-synonymous sequence variant in exon 10 of the *GALNT3* gene (NM_004482.3:c.[1681T>A];[1681T>A], NP_004473.2:p.[Cys561Ser];[Cys561Ser] in both affected siblings (Figure 1). The parents were heterozygous carriers for the same sequence variant (Figure 1). The detected variant is absent from > 251,000 control alleles of the gnomAD browser (*http://gnomad.broadinstitute.org*), which is comprised of exome and genome data from different populations (18,392 East Asian alleles and 30,610 South Asian alleles). This is not published in the literature and has not been reported as clinically relevant in other patients. The c.1681T>A variant is predicted to result in substitution of an evolutionarily highly conserved amino acid that is possibly involved in the formation of an intramolecular disulfid bridge between amino acid Cys561 and Cys574 of the GALNT3 protein. Both children were placed on a low phosphate diet with aluminium hydroxide and sevelamer. The frequency of new lesions decreased and the serum phosphorus concentrations returned to normal levels.

About 40 pathogenic variants in the *GALNT3* gene described so far include missense, nonsense, splice site and frameshift variants in addition to insertions and deletions (*1-7*). Our patients have a missense variant in the *GALNT3* gene while both their parents are heterozygous, and hence, carriers for the disease. Pathogenic variants in the *GALNT3* gene result in a defective GALNT3 protein that is unable to *O*-glycosylate FGF23 (*2*). Therefore, the FGF23 protein is readily cleaved into biologically inactive *N*-terminal and *C*-terminal fragments (c-FGF23). The loss of FGF23 activity leads to hyperphosphatemia typical of HFTC (*1*). The circulating concentrations of c-FGF23 are increased whereas the intact FGF23 (i-FGF23) remains low or inappropriately normal for the level of hyperphosphatemia (*1*).

Differential diagnoses of HFTC include progressive osseous heteroplasia, Cole disease, benign tumoral calcinosis, porphyria cutanea tarda, normophosphatemic FTC, fibrodysplasia ossificans progressiva, iatrogenic tumoral calcinosis and connective tissue disease-associated tumoral calcinosis, which all have normophosphatemia (*1,8*). Very rarely, cutaneous or tendinous xanthomas of homozygous familial hypercholesterolemia needs differentiation from HFTC lesions (*9,10*). Diagnoses of chronic renal failure and pseudohypoparathyroidism were excluded, in our patient, using appropriate biochemical and hormonal investigations.

The phenotypic variability in HFTC is well known (*4*). Family members, in particular siblings, with the same *GALNT3* pathogenic variants, genetic background, and similar biochemical parameters may show marked

variation in disease severity and clinical course (*4*). The younger sibling of our proband showed milder manifestations and disease course.

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