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SHORT COMMUNICATION

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Hyperphosphatemic familial tumoral calcinosis caused by a mutation in *GALNT3* in a European kindred

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Abstract Hyperphosphatemic familial tumoral calcinosis (HFTC) is an autosomal recessive metabolic disorder characterized by extensive phenotypic and genetic heterogeneity. HFTC was shown recently to result from mutations in two genes: *GALNT3*, coding for a glycosyltransferase responsible for initiating O-glycosylation, and *FGF23*, coding for a potent phosphaturic protein. All *GALNT3* mutations reported so far have been identified in patients of either Middle Eastern or African-American extraction, corroborating numerous historical reports of the disorder in Africa and in the Middle East. In the present study, we describe a patient of Northern European origin displaying typical features of HFTC. Mutation analysis revealed that this patient carries a homozygous novel nonsense mutation in *GALNT3* predicted to result in the synthesis of a significantly truncated protein. The present results expand the spectrum of known mutations in *GALNT3* and demonstrate the existence of HFTC-causing mutations in this gene outside the Middle Eastern and African-American populations.

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Introduction

Familial tumoral calcinosis (FTC) is a severe metabolic disorder characterized by extraosseous calcium phosphate deposition in the skin, muscle, joints, and visceral organs, resulting in incapacitating joint pain, secondary skin infections and various organ dysfunction (Metzker et al. 1988). It is often associated with hyperphosphatemia (Smack et al. 1996), and is then termed hyperphosphatemic familial tumoral calcinosis (HFTC; MIM211900). HFTC has been shown to result from loss-of-function mutations in at least two genes: *GALNT3* coding for UDP-*N*-acetyl-alpha-D-galactosamine: polypeptide *n*-acetylgalactosaminyltransferase 3 (ppGalNacT3) (Topaz et al. 2004; Ichikawa et al. 2005), a glycosyltransferase, which initiates O-glycosylation (Ten Hagen et al. 2003); and *FGF23* (Benet-Pagès et al. 2005; Larsson et al. 2005; Araya et al. 2005; Chefetz et al. 2005) coding for fibroblast growth factor 23 (FGF23), a potent phosphaturic protein (Berndt et al. 2005).

Interestingly, mutations in *GALNT3* have been identified so far only in patients of Middle Eastern or African-American origin. This corroborates previous historical clinical reports pointing to the prevalence of FTC in these regions (McClatchie and Bremner 1969; Hawass et al. 1988; Jain 1989). The existence of various trade routes between Africa and the Middle East during many centuries has been invoked to explain the peculiar geographical distribution of FTC. However, different HFTC-causing mutations have been identified in African-American and Middle Eastern patients (Topaz et al. 2004). In the present report, we describe the identification of a novel *GALNT3* mutation in a patient of European extraction with features typical of HFTC, demonstrating the existence of *GALNT3* mutations outside the Middle Eastern and African populations.

Materials and methods

Patient and control individuals

Blood samples were collected after having obtained written consent from each participant according to a protocol reviewed and approved by the local Helsinki Committee and by the Israeli Ministry of Health. Genomic DNA was isolated from blood samples using the salt chloroform extraction method.

Mutational analysis

All exons and exon-intron boundaries of the *GALNT3* and *FGF23* genes were PCR-amplified as previously described (Frishberg et al. 2005; Benet-Pagès et al. 2005).

To verify Q592X, a 496 bp PCR fragment encompassing exon 9 was amplified using primers 5′-GGCTATTGTATCGTCTATCAC-3′ and 5′-GATATA TTCTCTTATCACATGGG-3′, and digested in the presence of *Xba*I.

Results

Clinical findings

A 32-year-old male individual of Northern European descent was referred with hyperphosphatemia and periarticular calcification. His parents are unaware of any familial relationship. His parents and his healthy brother have normal calcium, phosphate and PTH levels.

Since childhood, this patient has suffered from recurrent episodes of conjunctival irritation and arthralgia. He damaged two front teeth in an accident when he was 9 years old. The dentist's attempt to root fill the teeth was unsuccessful due to obliterated pulp cavities. Later assessment of the patient revealed thin dental enamel, short blunt roots, taurodontism of the pre-molar/molar teeth and obliteration of the pulp cavities in most teeth (Fig. 1a).

Radiological examination revealed periarticular calcifications near the acromio-clavicular joints and both elbow joints (Fig. 1b). Ophthalmological examination disclosed whitish "salt-like" deposits on the conjunctiva as previously described (Chefetz et al. 2005). Of note, no cutaneous calcifications were observed.

Laboratory work-up disclosed elevated serum phosphate (1.9–2.18 mmol/l), normal or slightly raised serum calcium (2.5–2.8 mmol/l), normal ionized calcium (1.30–1.32 mmol/l) and PTH (3.5–5.1 pmol/l) levels (Immulite 2000 Intact PTH; Diagnostic Products, Los Angeles, CA; ref: 0.7–5.7 pmol/l). Measured 24 h creatinine clearance and calcium excretion were normal. Phosphate excretion was reduced (6 mmol/24 h), phosphate clearance was 2.51 ml/min yielding a fractional renal tubular reabsorption of phosphate of 98% (normal 82–90%); 1.25-$(OH)_2$D (106 pmol/l, ref: 50–145 pmol/l) and 25-(OH)-D (45.1 nmol/l, ref: 30–150 nmol/l) were normal. Phosphate-restricted diet and antacids (but not treatment with sevelamer hydrochloride) for 6 months lowered phosphate levels to high normal levels without alleviating clinical signs.

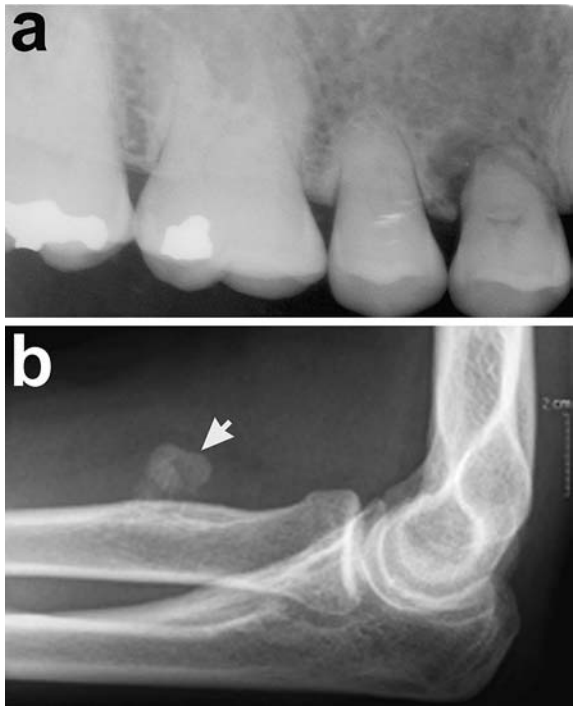

Fig. 1a,b Clinical features. **a** Dental radiograph demonstrating sclerotic teeth with blunt roots and obliterated pulp cavity. **b** Radiographs of the left elbow disclosing large calcifications

Mutation analysis

Since *GALNT3* mutations had never previously been described in European patients, we initially established the entire coding sequence of *FGF23* in this patient. No deleterious sequence alterations in *FGF23* were identified.

We then sequenced the ten coding exons of *GALNT3* in the patient. We identified a C > T transition at position 1,774 of the cDNA sequence (starting from ATG). This mutation was found to be carried in a heterozygous state by his parents (Fig. 2a). Using a PCR-RFLP assay, we confirmed segregation of the mutation in the family (Fig. 2b). We also excluded the mutation from a pool of 124 chromosomes derived from healthy unaffected individuals.

The mutation is predicted to result in the substitution of a stop codon for a glutamine residue at position 592 of the ppGalNacT3 amino acid sequence (Q592X) and thus to result in the synthesis of a truncated protein lacking a significant part of its carbohydrate-binding domain (Fig. 2c).

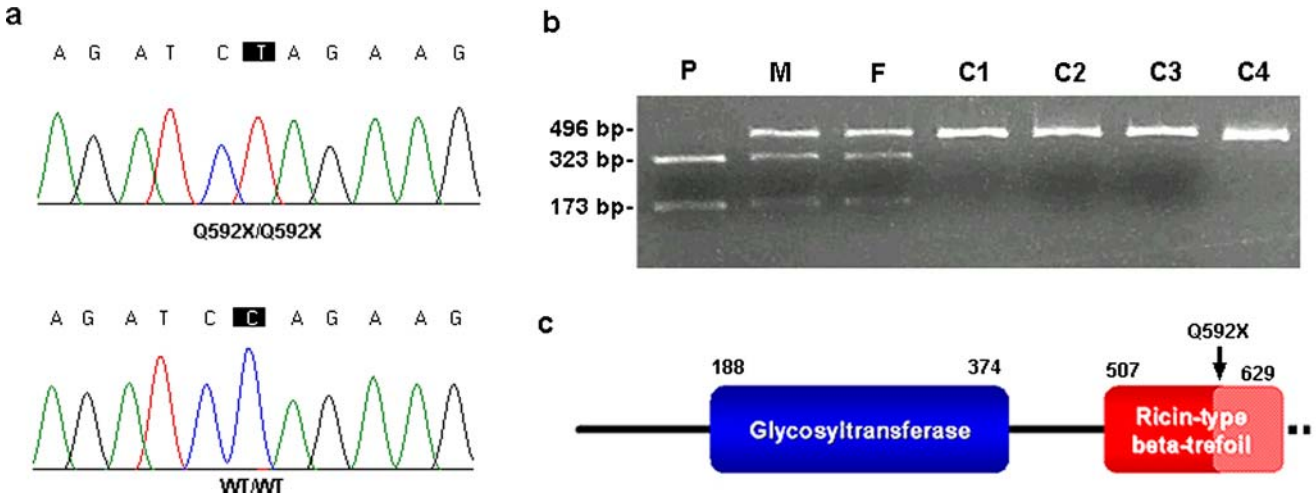

Fig. 2a–c Mutation analysis. **a** Direct sequencing reveals a homozygous C → T transition at position 1,774 of the *GALNT3* cDNA sequence (*upper panel*). The wildtype sequence is given for comparison (*lower panel*). **b** To confirm C1774T, a 496 bp fragment encompassing *GALNT3* exon 9 was PCR-amplified and digested with *Xba*I endonuclease. Since C1774T creates a recognition site for *Xba*I endonuclease, the patient (*P*) displays PCR fragments of 323 bp and 173 bp, healthy individuals (C1–C4) display an undigested 496 bp fragment only, whereas the mother (*M*) and father (*F*) of the patient display all fragment types. **c** The mutation results in the substitution of a stop codon for a glutamine residue at position 592 as indicated along a scheme of the ppGalNacT3 molecule. The lighter shaded area corresponds to the truncated part of the enzyme

Discussion

In the present study, we report the first case of HFTC caused by a mutation in *GALNT3* in a patient of non-African or non-Middle-Eastern origin. This finding suggests that this disease may be more common and more widely distributed than initially thought.

Hyperphosphatemic familial tumoral calcinosis is characterized by marked phenotypic heterogeneity. Although most patients display periarticular calcifications, other manifestations, such as visceral or mucosal involvement, are not invariably seen (Gal et al. 1994; Topaz et al. 2004; Chefetz et al. 2005). Apart from severe joint disease, the present case was remarkable for prominent dental anomalies. Dental abnormalities in HFTC patients have been documented in a number of previous reports and have been found to be associated with mutations in both *GALNT3* and *FGF23* (Ichikawa et al. 2005; Benet-Pages et al. 2005). Some investigators have even suggested that dental findings, including short bulbous roots and obliteration of the pulp cavities as seen in the present case, may serve as a useful phenotypic marker for FTC (Burkes et al. 1991).

GALNT3 codes for the widely expressed ppGalNacT3 glycosyltransferase (Ten Hagen et al. 2003). The mechanisms underlying the role of ppGalNacT3 in maintaining phosphate homeostasis are still elusive. However, the fact that mutations in *GALNT3* and in *FGF23* result in a similar phenotype (Topaz et al. 2004; Benet-Pagès et al. 2005), and the fact that FGF23 levels are perturbed in patients with ppGalNacT3 deficiency (Topaz et al. 2004) indicate that the two proteins participate in a common regulatory pathway. FGF23 decreases circulating phosphate levels by downregulation of NaPiIIa (Shimada et al. 2005), the major phosphate transporter in the renal proximal tubule, by inhibition of NaPiIIb, responsible for trans-intestinal phosphate transport (Miyamoto et al. 2005), and by down-regulation of 1-alpha-hydroxylation of 25-hydroxycholecalciferol (Inoue et al. 2005). Proteolytic degradation of FGF23 is believed to play a major role in regulating these activities (Saito et al. 2003). However, recent data suggest that FGF23 is also subject to O-glycosylation (Fukumoto 2005), and it is therefore tempting to speculate that ppGalNacT3-mediated O-glycosylation may be involved in the regulation of FGF23 activity. Other potential targets for ppGalNacT3-mediated O-glycosylation include FGF23 putative receptors (Yu et al. 2005) and phosphate transporters (Berndt et al. 2005).

To summarize, we have described a novel mutation in *GALNT3* causing HFTC in a patient of European origin. These findings add to the expanding spectrum of genetic alterations in this gene and are in line with a growing number of reports pointing to the role played by impaired glycosylation in the pathogenesis of genetic diseases (Vogt et al. 2005).

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