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Familial Tumoral Calcinosis: From Characterization of a Rare Phenotype to the Pathogenesis of Ectopic Calcification

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Abstract

Familial tumoral calcinosis (FTC) refers to a heterogeneous group of inherited disorders characterized by the occurrence of cutaneous and subcutaneous calcified masses. Two major forms of the disease are now recognized. Hyperphosphatemic FTC has been shown to result from mutations in three genes: fibroblast growth factor-23 (*FGF23*), coding for a potent phosphaturic protein, *KL* encoding Klotho, which serves as a co-receptor for FGF23, and *GALNT3*, which encodes a glycosyltransferase responsible for FGF23 *O*-glycosylation; defective function of any one of these three proteins results in hyperphosphatemia and ectopic calcification. The second form of the disease is characterized by absence of metabolic abnormalities, and is, therefore, termed normophosphatemic FTC. This variant was found to be associated with absence of functional SAMD9, a putative tumor suppressor and anti-inflammatory protein. The data gathered through the study of these rare disorders have recently led to the discovery of novel aspects of the pathogenesis of common disorders in humans, underscoring the potential concealed within the study of rare diseases.

Familial tumoral calcinosis (FTC) represents a clinically and genetically heterogeneous group of inherited diseases manifesting with dermal and subcutaneous deposition of calcified materials. In recent years, the pathogenesis of these disorders has been elucidated, not only leading to delineation of the major pathways responsible for regulating extraosseous calcification, but also shedding new light on the pathogenesis of pathologies as common as renal failure and autoimmune diseases.

FAMILIAL TUMORAL CALCINOSIS: HISTORICAL PERSPECTIVE

The first clinical description of familial tumoral calcinosis (FTC) dates back to 1898 when two French dermatologists, Giard and Duret, reported for the first time the cardinal features of the disorder under the name endotheliome calcifié (Giard, 1898; Duret, 1899). The disease was later described in the German literature as lipocalcinogranulomatosis or Teutschlaender's disease after the German dermatologist who studied a large number of such cases (Tseutschlaender, 1935). The term tumoral calcinosis was coined in the American literature by Inclan *et al*. (1943). Inclan *et al*. were also the first to accurately differentiate this inherited disease from other related but acquired conditions, which later

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CONFLICT OF INTEREST

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became known as metastatic calcinosis and dystrophic calcinosis. Subsequently, numerous reports, initially emanating from travelers who had returned from Africa, indicated that the disease is apparently prevalent in this region of the world (McClatchie and Bremner, 1969; Owor, 1972). Despite these advances, it was not until 1996 that Smack *et al.* formally established the distinction between normophosphatemic (NFTC) and hyperphosphatemic FTC (HFTC) (Smack *et al*., 1996), a classification that proved to be instrumental in guiding the molecular studies that eventually led to the identification of the genetic basis of FTC.

FTC: EPIDEMIOLOGICAL, CLINICAL, AND BIOCHEMICAL FEATURES

As mentioned above, FTC has been mainly (but not exclusively) reported in Africa, the Middle-East, and in populations originating from these regions of the world. Its exact prevalence is unknown, but it is considered to be exceedingly rare. Although the disease was initially reported to be inherited in an autosomal dominant fashion (Lyles *et al*., 1985), the recent identification of the genetic defects underlying all forms of FTC definitely established an autosomal recessive mode of inheritance (Topaz *et al*., 2004; Benet-Pages *et al*., 2005; Ichikawa *et al*., 2005).

Serum phosphate levels demarcate the two major subtypes of FTC: HFTC (MIM211900) and NFTC (MIM610455; Sprecher, 2007). Although these two disorders were initially considered part of a clinical continuum (Metzker *et al*., 1988), it is today clear that they represent distinct entities, not only at the metabolic level, but also at the clinical, epidemiological, and genetic (see below) levels.

HFTC is generally characterized by a relatively late onset from the first to the third decade of life (Prince *et al*., 1982; Metzker *et al*., 1988; Slavin *et al*., 1993), although appearance of calcified nodules has been reported as early as at 6 weeks of age (Polykandriotis *et al*., 2004). It has been mainly reported in individuals of Middle Eastern and African origins (Metzker *et al*., 1988; Slavin *et al*., 1993). Many patients are often incidentally diagnosed while undergoing radiographic investigation for unrelated reasons. Slowly growing calcified masses develop mainly at periarticular locations, with a predilection for skin areas overlying large joints. The hips are most often involved. These calcified masses are initially asymptomatic, but progressively reach large sizes (up to 1.5 kg), thereby interfering with movements around the joints (Figure 1a). Ulceration is usually accompanied by intolerable pain and is occasionally associated with secondary infections, rarely reported as a cause of death (Sprecher, 2007). In some affected individuals, extracutaneous signs may predominate. Dental manifestations, including pulp calcifications and obliteration of the pulp cavity, may be prominent (Burkes *et al*., 1991; Campagnoli *et al*., 2006; Specktor *et al*., 2006); testicular microlithiasis has been reported as part of the disease (Campagnoli *et al*., 2006); angioid streaks and corneal calcifications have been observed as well (Ghanchi *et al*., 1996); and bone manifestations (diaphysitis and hyperostosis) may be more common than initially thought (Clarke *et al*., 1984; Mallette and Mechanick, 1987; Ballina-Garcia *et al*., 1996). HFTC has been reported in association with pseudoxanthoma elasticum (Mallette and Mechanick, 1987). Recently, a long-term follow-up study of one of the largest HFTC kindreds described revealed salient features of the disease, including overall good prognosis, lack of efficient treatments for the disorder apart from surgical removal of calcified tumors (most patients undergo over 20 major operations during their life time), and possible association with hypertension and pulmonary restrictive disease (Carmichael *et al*., 2009). Medical treatment is notoriously frustrating. Isolated reports have suggested benefit from combined treatment of HFTC with acetazolamide and sevelamer hydrochloride, a non-calcium phosphate binder (Garringer *et al*., 2006; Lammoglia and Mericq, 2009).

NFTC seems to be even less prevalent than HFTC, with only six families reported to date (Topaz *et al*., 2006; Chefetz *et al*., 2008). The disease often initially manifests with a nonspecific erythematous rash and mucosal inflammation during the first year of life (Metzker *et al*., 1988; Katz *et al*., 1989). Inflammatory manifestations in the oral cavity can be particularly debilitating (Gal *et al*., 1994). This eruption heralds the progressive, but often rapid, development of small, acrally located calcified nodules, which almost invariably lead to ulceration of the overlying skin and discharge of chalky material (Figure 1b). Here too, unremitting pain and infection are major causes of morbidity.

Histopathologically (Figure 1c), two major stages in calcified tumor formation have been recognized: an active phase characterized by the presence of multinucleated giant cells and macrophages surrounding calcified deposits in the dermis, and a chronic or inactive phase associated with dense fibrous tissue (Veress *et al*., 1976). Biochemical analysis of extruded calcified masses indicated that they are mainly composed of calcium hydroxyapatite with amorphous calcium carbonate and calcium phosphate (Boskey *et al*., 1983).

As mentioned above, metabolic abnormalities are the major features distinguishing HFTC from NFTC. HFTC patients invariably show elevated levels of circulating phosphate (in the range of 5 to 7 mg dl^{-1}, normal levels = 2.5–4.5mg dl^{-1} or 0.80–1.44 mmol), which are due to decreased fractional phosphate excretion through the kidney proximal tubules (White *et al*., 2006). Calcium levels are typically normal in HFTC, and 1,25-dihydroxyvitamin-D levels are normal or inappropriately elevated (Steinherz *et al*., 1985). In contrast, NFTC patients do not show any metabolic abnormality (Metzker *et al*., 1988; Topaz *et al*., 2006). In fact, these differences are very much reminiscent of the dichotomous nature of acquired calcinosis (Touart and Sau, 1998). Here, calcinosis can either manifest as a result of an underlying metabolic disorder as in chronic renal failure, or develop as a reaction to tissue damage, as in autoimmune diseases (Figure 1d) or as in atherosclerosis (Touart and Sau, 1998). When cutaneous calcinosis is due to deranged phosphate or calcium metabolism (e.g., chronic renal failure), it is termed metastatic calcinosis; by contrast, when it is secondary to tissue damage (e.g., autoimmune disease), it is known as dystrophic calcinosis (Touart and Sau, 1998). At this regard, clinical and metabolic features of HFTC very much resemble metastatic calcinosis, while NFTC models dystrophic calcinosis, thus suggesting that elucidation of the molecular basis of FTC may shed light on the pathogenesis of these two major forms of acquired calcinosis. This assumption was the impetus that drove a large international effort aimed at deciphering the molecular cause and biochemical mechanisms underlying the various forms of FTC.

MOLECULAR GENETICS OF HFTC

We owe to the study of rare diseases the discovery of many key physiological pathways (Antonarakis and Beckmann, 2006). In less than 5 years, the study of FTC has revealed a surprisingly intricate regulatory network of proteins responsible for regulation of phosphate homeostasis and extraosseous calcification (Sprecher, 2007; Chefetz and Sprecher, 2009).

Using homozygosity mapping, HFTC was initially mapped to 2q24–q31 and found to be associated with mutations in *GALNT3*, gene encoding a glycosyltransferase termed UDP-*N*-acetyl-α-D-galactosamine-polypeptide *N*-acetylgalactosaminyltransferase-3 (ppGalNacT3; Topaz *et al*., 2004). ppGalNacT3 catalyses the initial step of *O*-glycosylation of serine and threonine residues, and is ubiquitously expressed (Ten Hagen *et al*., 2003; Wopereis *et al*., 2006). ppGalNacT3 is one of a family of 24 acetylgalactosaminyltransferases, which are expressed in a tissue-specific manner and play an important role in the development and maintenance of a variety of tissues (Ten Hagen *et al*., 2003). Most congenital disorders of glycosylation result from impaired *N*-glycosylation and are characterized by pleiotropic

clinical manifestations (Freeze, 2006); in this regard, HFTC is unique in that it results from abnormal *O*-glycosylation and seems to be associated exclusively with ectopic calcification.

More than 10 mutations have so far been reported in *GALNT3* (Topaz *et al*., 2004; Ichikawa *et al*., 2005, 2006; Campagnoli *et al*., 2006; Garringer *et al*., 2006, 2007; Specktor *et al*., 2006; Barbieri *et al*., 2007; Dumitrescu *et al*., 2009; Laleye *et al*., 2008); all of these are predicted or have been found (Topaz *et al*., 2005) to result in loss of function of ppGalNacT3. Deleterious alterations in *GALNT3* were also found to underlie at least one additional autosomal recessive syndrome, hyperostosis–hyperphosphatemia syndrome (MIM610233), which, as HFTC, is also associated with elevated levels of serum phosphate (Melhem *et al*., 1970; Altman and Pomerance, 1971; Mikati *et al*., 1981). This syndrome manifests with episodes of excruciating pain associated with swelling, along the long bones. Despite the fact that HFTC and hyperostosis–hyperphosphatemia syndrome manifest phenotypically in two different tissues, skin and bone, mutations in the same gene, *GALNT3*, and in one instance, the very same mutation (Frishberg *et al*., 2005), were found to underlie both syndromes (Ichikawa *et al*., 2007a; Dumitrescu *et al*., 2009; Olauson *et al*., 2008; Gok *et al*., 2009). Interestingly, coexistence of the two diseases in one family has been reported (Narchi, 1997; Nithyananth *et al*., 2008). In fact, phenotypic heterogeneity seems to be quite characteristic of the HFTC group of diseases, with a widely variable spectrum of disease severity and tissue involvement (Sprecher, 2007).

Large-scale screening of HFTC families revealed that *GALNT3* mutations cannot be found in all HFTC families. In parallel, several groups noticed that HFTC represents in many ways the metabolic mirror image of another rare disorder known as hypophosphatemic rickets (Bastepe and Juppner, 2008). Three genetic types of hypophosphatemic rickets have been described: X-linked, autosomal recessive, and autosomal dominant hypophosphatemic rickets, which were shown to be caused by mutations in *PHEX*, *DMP1*, and fibroblast growth factor-23 (*FGF23*), respectively (Juppner, 2007; Bastepe and Juppner, 2008; Shaikh *et al*., 2008). *FGF23* encodes the FGF23, a potent phosphaturic protein responsible for promoting phosphate excretion through kidney (Strom and Juppner, 2008). DMP1 and PHEX function as negative regulators of the activity of FGF23 (Quarles, 2008). FGF23 mainly originates from mineralized tissues, although it has also been detected in other tissues such as kidneys, liver, and brain (Yoshiko *et al*., 2007). Recent data indicate that FGF23 signals through the FGFR1(IIIc) receptor, which is converted into a functional FGF23 receptor by Klotho (Urakawa *et al*., 2006), a molecule previously shown in mice to regulate aging-related processes (Kurosu and Kuro-o, 2008). Despite the fact that activating mutations in FGFR3 have been linked to hypophosphatemia (Quarles, 2008), it seems that this receptor does not mediate the renal effects of FGF23 (Liu *et al*., 2008).

Assessment of HFTC patients without *GALNT3* mutations revealed that while dominant gain-of-function mutations in FGF23 result in hypophosphatemic rickets, recessive loss-of-function mutations in the same gene cause HFTC (Araya *et al*., 2005; Benet-Pages *et al*., 2005; Chefetz *et al*., 2005; Larsson *et al*., 2005b; Lammoglia and Mericq, 2009; Masi *et al*., 2009). HFTC-causing mutations in *FGF23* are associated with a variety of biochemical abnormalities such as abnormal secretion of the intact molecule from the Golgi (Benet-Pages *et al*., 2005) and decreased FGF23 stability (Larsson *et al*., 2005a; Garringer *et al*., 2008).

FGF23 activity is partially processed intracellularly by subtilisin-like proprotein convertases at a consensus sequence between residues R179 and S180 (Benet-Pages *et al*., 2004). Dominant mutations associated with hypophosphatemic rickets affect the proteolytic site of FGF23, preventing proper degradation of the molecule (Shimada *et al*., 2002). In contrast, HFTC-causing mutations in FGF23 have been shown to result in enhanced proteolytic

processing of FGF23 (Larsson *et al*., 2005a; Garringer *et al*., 2008). In addition, a recent study showed that a missense mutation in *KL*, encoding Klotho, also results in a phenotype very much resembling HFTC (Ichikawa *et al*., 2007b). This mutation was found to result in decreased expression of Klotho and concomitant decreased FGF23 signaling, leading to a severe form of HFTC characterized by widespread cutaneous and visceral calcifications, diffuse osteopenia, and sclerodactyly (Ichikawa *et al*., 2007b).

PATHOGENESIS OF HFTC

In summary, (1) HFTC can be caused by mutations in at least three genes (*GALNT3*, *FGF23*, and *KL*); (2) loss-of-function sequence alterations in *GALNT3* are associated with two distinct phenotypes, HFTC and HSS; and (3) loss-of-function mutations in *FGF23* also cause HFTC, while dominant gain-of-function mutations in the same gene are associated with hypophosphatemic rickets (2000), which is also due to mutations in *DMP1* (Feng *et al*., 2006; Lorenz-Depiereux *et al*., 2006) and *PHEX* (Table 1 and Strom and Juppner, 2008). These findings raised the possibility that these various molecules may be part of a single physiological pathway. Recent data lend support to this hypothesis (Figure 2).

For decades, phosphate homeostasis has been thought to be maintained through concerted actions of parathyroid hormone, which inhibits renal phosphate reabsorption and mobilizes bone calcium and phosphate, and 1,25-hydroxyvitamin-D, which increases phosphate transport across small intestine (Schiavi and Moe, 2002; Quarles, 2008). More recently, a group of proteins, known as phosphatonins, has been shown to regulate phosphate levels in the circulation independently of calcium (Schiavi and Moe, 2002). FGF23 is the phosphatonin that has been best characterized so far.

As mentioned above, FGF23 signaling is dependent on the presence of Klotho (Kurosu and Kuro, 2009). Thus FGF23 mainly targets organs expressing Klotho such as parathyroid glands and kidneys (Razzaque and Lanske, 2007).

FGF23 inhibits phosphate reabsorption through the major sodium-phosphate transporters, NaPi-IIa and NaPi-IIc, thus allowing efficient counter-regulation of excessive circulating levels of phosphate (Saito *et al*., 2003; Razzaque, 2009). FGF23 also suppresses CYP27B1, which catalyzes 1-α-hydroxylation of 25-hydroxy-vitamin-D and activates CYP24, which inactivates 1,25-hydroxyvitamin-D (Liu and Quarles, 2007). Interestingly, 1,25-dihydroxyvitamin-D was found to upregulate FGF23 expression in bone, thereby establishing a novel endocrine feedback loop between these two hormones (Kolek *et al*., 2005; Saito *et al*., 2005). Not only does FGF23 deficiency alter phosphate homeostasis, it also leads to abnormal mineralization, suggesting that FGF23 plays a role in bone formation (Sitara *et al*., 2008), perhaps in a paracrine manner since osteocytes in bone are the major source of FGF23 (Yoshiko *et al*., 2007). The clinical phenotype associated with decreased Klotho activity very much overlaps with that exhibited by FGF23-deficient individuals, except for the fact that HFTC caused by *KL* mutations is associated with hypercalcemia as well as elevated serum levels of parathyroid hormone and FGF23 (Ichikawa *et al*., 2007b). This may in fact relate to the fact that Klotho plays major direct (e.g., regulation of the secretion of the parathyroid hormone) and indirect (e.g., decreased 1,25-dihydroxyvitamin-D_3 activity) roles in the maintenance of calcium homeostasis (Nabeshima and Imura, 2008). Of note, independently of regulation of phosphate and calcium homeostasis, Klotho plays a number of other important roles such as controlling insulin signaling (Razzaque and Lanske, 2007; Kurosu and Kuro-o, 2008; Nabeshima and Imura, 2008). Taken altogether, these data suggest that FGF23 mainly serves as a counter-regulatory hormone to offset the effect of excessive 1,25-hydroxy-vitamin-D.

As mentioned above, activity of FGF23 is mainly modulated by a poorly understood process of proteolysis at a furin-like convertase sequence motif. PHEX and DMP1 may promote the activity of the subtilisin-like protease(s) responsible for mediating FGF23 cleavage (Quarles, 2008). More interestingly, FGF23 was found to be a substrate for ppGalNacT3-mediated *O*-glycosylation. ppGalNacT3 was found to catalyze the glycosylation of the recognition site of subtilisin-like proprotein convertase, thereby protecting FGF23 from proteolysis (Kato *et al*., 2006). FGF23 secretion was also found to be dependent on ppGalNacT3-mediated *O*-glycosylation (Kato *et al*., 2006). Thus, since loss-of-function mutations in *GALNT3*, *FGF23*, and *KL* all result in decreased activity of FGF23, it is not surprising that they also yield overlapping phenotypes (Table 1).

Currently very little is known about the factors regulating the expression of *GALNT3*. Phosphate was found to stimulate the expression and/or activity of ppGalNacT3 (Chefetz *et al*., 2009) as well as FGF23 (Saito *et al*., 2005), while it induced a marked decrease in the activity of 1-α-hydroxylase, thereby decreasing the amount of active vitamin-D_3 (Perwad *et al*., 2005). Since 1,25-dihydroxyvitamin-D was shown to decrease the expression of *GALNT3* (Chefetz *et al*., 2009), this complex feedback loop enables for restraining the activity of FGF23 once it is no more required, namely when phosphate level is back to normal.

A major question, which remained largely unanswered, is why does HFTC-associated ectopic calcification mainly affect cutaneous and subcutaneous tissues in the face of hyperphosphatemia affecting equally all tissues. Recent data have implicated FGF7, another phosphatonin, which is secreted in response to hyperphosphatemia (characteristically found in HFTC; Carpenter *et al*., 2005), is produced exclusively in the dermis (auf demKeller *et al*., 2004), and is capable of triggering the activation of matrix metalloproteinases (MMPs; Putnins *et al*., 1995; Shin *et al*., 2002; Simian *et al*., 2001). Interestingly, MMPs have been shown to mediate ectopic calcification in vascular tissues (Satta *et al*., 2003; Qin *et al*., 2006). Accordingly, fibroblasts derived from HFTC patients or normal fibroblasts knocked down for *GALNT3* showed increased activation of several key MMPs (Chefetz *et al*., 2009).

MOLECULAR GENETICS OF NFTC

The fact that NFTC has so far been reported exclusively among Yemenite Jews suggested that a founder mutation may underlie the disease in this community, which in turn allowed homozygosity mapping to be used to locate the disease gene. Through study of five affected kindreds, the disease gene was found to map to 7q21, confirming at the genetic level the fact that NFTC and HFTC (which maps to 2q) are two separate non-allelic disorders, despite clinical similarities between them (Topaz *et al*., 2006). Candidate gene screening revealed initially a missense mutation in *SAMD9* segregating with the disease phenotype in all families (Topaz *et al*., 2006). The mutation was found to result in SAMD9 protein degradation, suggesting a loss-of-function effect (Topaz *et al*., 2006). In agreement with these results, a second nonsense mutation was later identified in an additional kindred of Jewish Yemenite origin (Chefetz *et al*., 2008). Although at first, identification of two mutations in *SAMD9* causing a similar phenotype may have been expected, these data are surprising given the rarity of the disease and the very small size of the Jewish Yemenite population. In fact, the occurrence of two independent genetic events within a very close and small population may reflect a selection effect (Chefetz *et al*., 2008). Unfortunately, very little is currently known about the physiological function of SAMD9 so that no functional rationale has been provided so far to explain this effect.

PATHOGENESIS OF NFTC

SAMD9 is a 1,589-amino-acid cytoplasmic protein ubiquitously expressed in all adult and fetal tissues except for fetal brain and skeletal muscle (Topaz *et al*., 2006; Li *et al*., 2007). Apart from an N-terminal SAM domain, it lacks homology to any known family of proteins.

The fact that calcification in NFTC is often preceded by an inflammatory rash (Metzker *et al*., 1988; Katz *et al*., 1989) suggests a role for SAMD9 in the regulation of inflammation within the skin. In line with this hypothesis, tumor necrosis factor-α as well as osmotic shock were found to induce SAMD9 expression in a p38- and NF-κB-dependant manner (Chefetz *et al*., 2008). This indicates that SAMD9 may be involved in tumor necrosis factor-α-induced cell apoptosis. It is, therefore, of interest to note that the expression of SAMD9 was found to be significantly downregulated in a large proportion of tumors of various origins (Li *et al*., 2007). Decreased expression of SAMD9 was found to be associated with increased cell proliferation and decreased apoptosis (Li *et al*., 2007). In addition, transfection of tumor cells with SAMD9 was accompanied by increased apoptotic activity, decreased cell invasion in an *in vitro* assay, and decreased cancer cell proliferation (Li *et al*., 2007). Tumor cells overexpressing SAMD9 formed tumor of a lower volume as compared with wild-type cells (Li *et al*., 2007). Altogether, these data indicate that SAMD9 may function as a tumor-suppressor gene. In support of this hypothesis, recent data show that myelodysplatic syndrome, acute myeloid leukemia, and juvenile myelomonocytic leukemia are associated with a microdeletion on 7q21.3 spanning *SAMD9*, and its paralog, *SAMD9L* (Asou *et al*., 2009). How are these two putative functions of SAMD9 (anti-inflammatory and tumor-suppressive) mechanistically related is not yet known.

RELEVANCE TO ACQUIRED DISEASES

The data reviewed above indicate the importance of ppGalNacT3, FGF23, Klotho, and SAMD9 in the prevention of abnormal calcification in peripheral tissues. Their involvement in the pathogenesis of relevant human pathologies is currently the focus of intense research efforts.

A growing body of evidence points to extraosseous calcification as a major cause or predictor of morbidity and mortality in humans. Vascular calcification has been shown to quite accurately predict mortality, mainly due to cardiovascular causes, in large cohorts (Thompson and Partridge, 2004; Barasch *et al*., 2006). Among factors that have been known for long to be associated with the propensity to develop cardiovascular diseases, are elevated levels of circulating phosphate (Kestenbaum *et al*., 2005; Tonelli *et al*., 2005; Giachelli, 2009), which has been shown to induce ectopic calcification (Jono *et al*., 2000). Hyperphosphatemia is most likely acting independently of more conventional cardiovascular risk factors (Lippi *et al*., 2009), suggesting that interventions specifically directed at attenuating extraosseous calcification may provide substantial additional benefit to individuals at risk (Schmitt *et al*., 2006). Several studies have asked whether the newly discovered regulators of phosphate homeostasis have a predictive value of their own for pathologies known to be associated with elevated levels of circulating phosphate. Although FGF23 levels were found to also correlate with mortality among patients undergoing hemodialysis, total-body atherosclerosis in the general population, and progression of chronic renal disease, they were found to predict outcome independently of phosphate levels (Fliser *et al*., 2007; Gutierrez *et al*., 2008; Mirza *et al*., 2009); in contrast, FGF23 levels were found to be associated with renal stone formation only if due to increased urinary excretion of phosphate (Rendina *et al*., 2006).

These data suggest a potential benefit for novel therapies based on modulation of the activity of the various molecules known to play a role in the pathogenesis of inherited FTC.

Tetracyclines have been shown to ameliorate the course of ectopic calcinosis (Robertson *et al*., 2003; Boulman *et al*., 2005) as well as to inhibit the activity of MMPs (Kim *et al*., 2005; Qin *et al*., 2006), which may play a role in the pathogenesis of FTC (Chefetz *et al*., 2009). Interestingly, we were recently able to show that doxycycline significantly upregulates the expression of ppGalNacT3 (Chefetz and Sprecher, 2009), suggesting the need to assess the efficacy of tetracyclines in disorders associated with deranged activity of the ppGalNacT3–FGF23 axis.

CONCLUSIONS

The study of FTC phenotypes has led to delineation of novel biochemical pathways of importance for maintenance of phosphate balance. It exemplifies remarkably the immense potential concealed within the study of rare diseases, for only through the study of human subjects could the function of ppGal-NacT3, FGF23, and Klotho have been delineated. Indeed, although ppGal-NacT3-deficient mice feature a normal lifespan, show hyperphosphatemia, and inappropriately normal levels of 1,25-dihydroxyvitamin-D_3, in analogy with what is seen in HFTC patients, the mice do not show ectopic calcifications and males are infertile, which are not features of HFTC in humans (Ichikawa *et al*., 2009). In contrast to humans, FGF23-deficient mice have a shortened lifespan and show growth retardation, infertility, muscle atrophy, hypoglycemia, and visceral calcification (Kuro-o *et al*., 1997; Shimada *et al*., 2004). In fact FGF23 mice resemble very much Klotho-deficient mice (Kuro-o *et al*., 1997), even though Klotho deficiency manifests differently in humans than FGF23 deficiency (Ichikawa *et al*., 2007b). More interestingly, SAMD9 does not exist at all in the murine genome (Li *et al*., 2007). Thus, through study of an exceedingly rare phenotype the major regulatory molecules of critical importance for maintenance of phosphate homeostasis and prevention of ectopic calcification have been discovered and are on the verge of being used to refine therapeutic approaches to much more common ailments in humans such as renal failure and autoimmune diseases.

Abbreviations

FGF23	fibroblast growth factor-23
FTC	familial tumoral calcinosis
HFTC	hyerphosphatemic FTC
MMP	matrix metalloproteinase
NFTC	normophosphatemic FTC
ppGalNacT3	polypeptide *N*-acetylgalactosaminyltransferase-3

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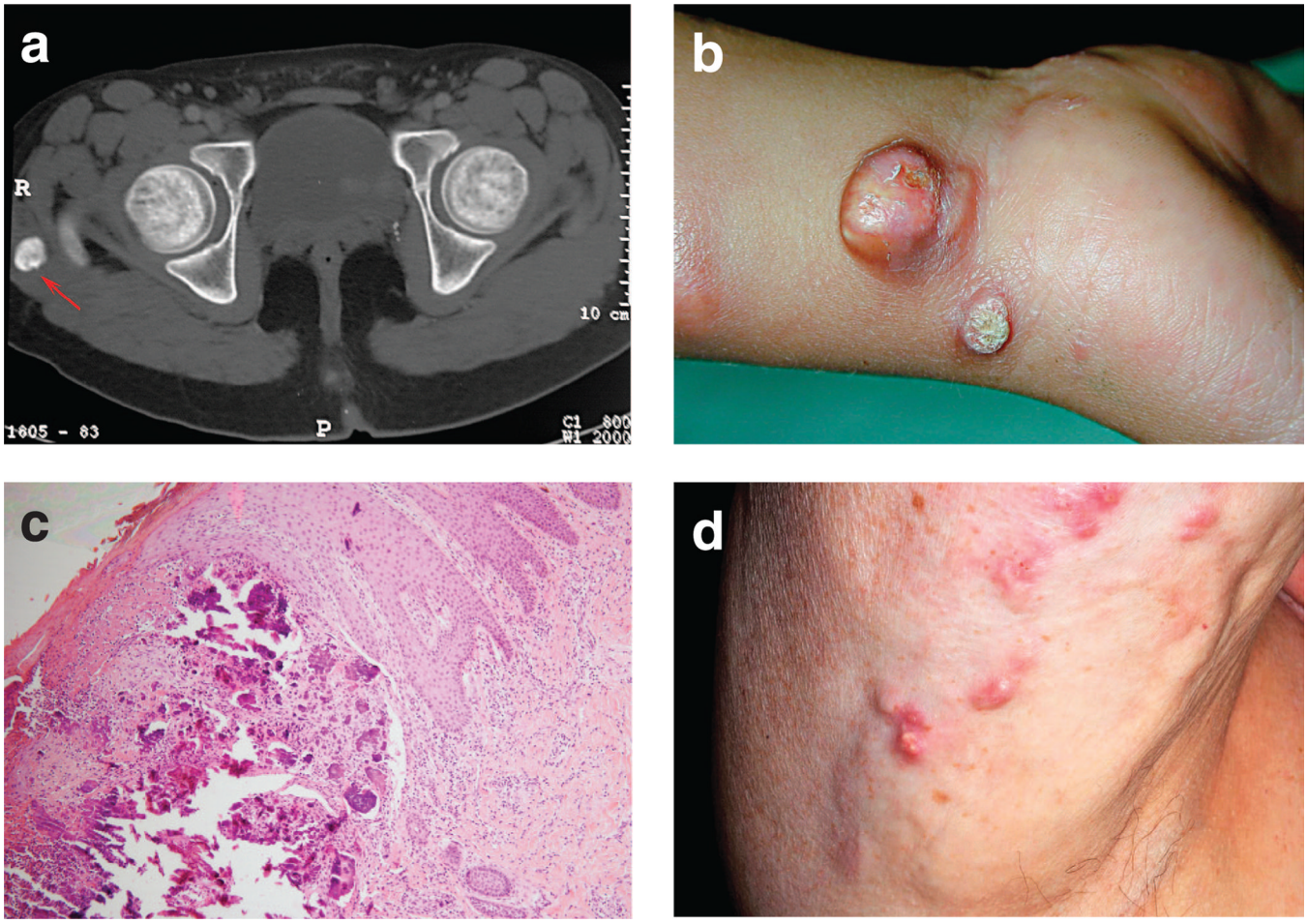

Figure 1. Clinical findings in FTC

(**a**) Computed tomographic scan showing a calcified mass in the soft tissue adjacent to the right major Trochanter (red arrow). (**b**) Calcified tumor on the right wrist of a 5-year-old boy with NFTC. (**c**) A skin biopsy obtained from an 8-year-old female patient with NFTC have calcified materials in the upper and middle dermis (hematoxylin and eosin, × 400). (**d**) Calcified nodules in a 70-year-old patient with dermatomyositis and acquired calcinosis cutis.

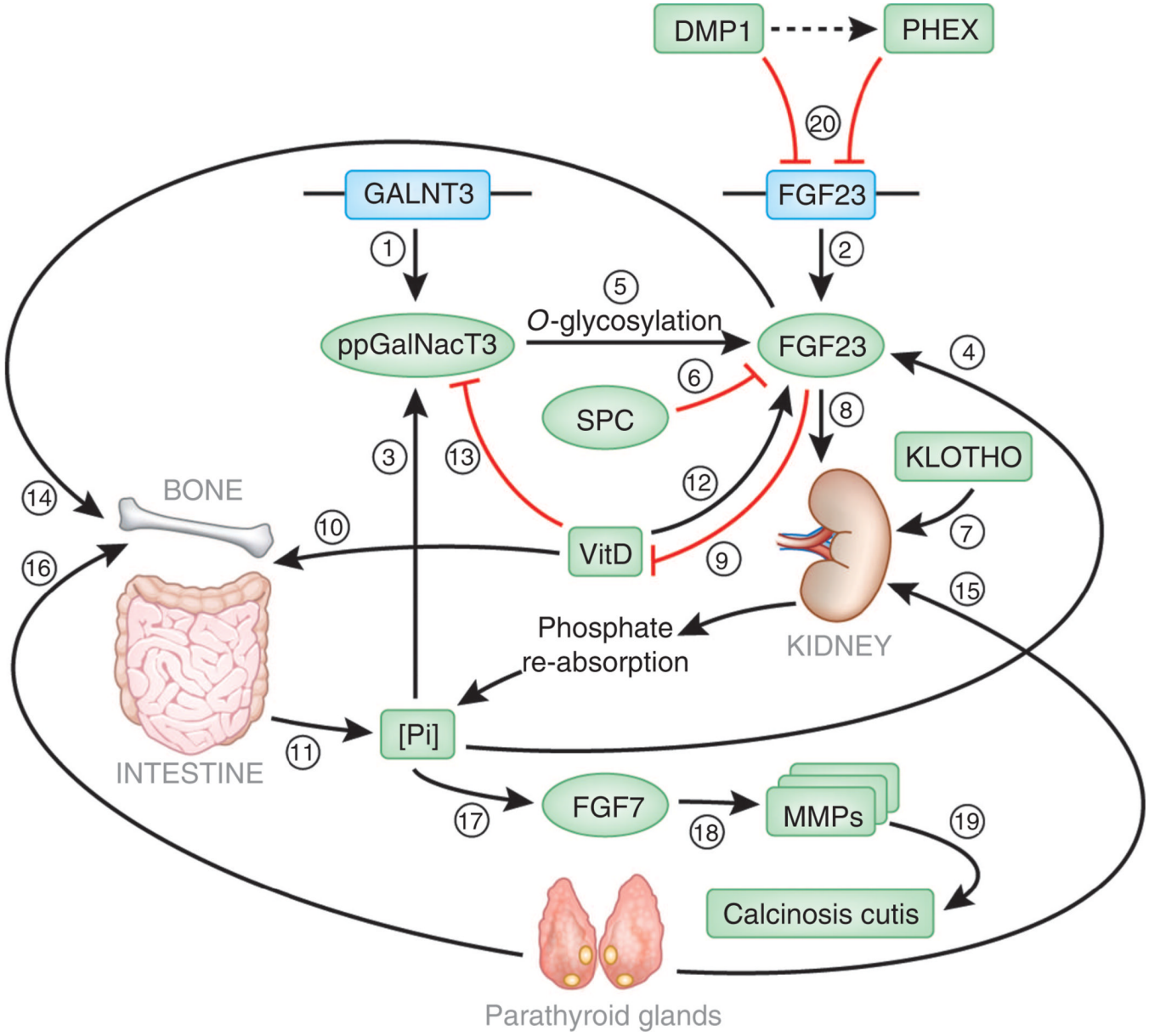

Figure 2. FTC-associated molecules and the regulation of phosphate homeostasis

Two genes have been found to be associated with classical FTC, *GALNT3* encoding ppGalNacT3 (1) and *FGF23* encoding the phosphatonin FGF23 (2). Increased phosphate levels upregulate the activity of ppGAlNacT3 (3) as well as FGF23 (4). ppGalNacT3 then *O*-glycosylates FGF23 (5), thereby protecting it from the proteolytic activity of subtilisin-like proprotein convertases (SPC) (6). FGF23 then signals through its receptor in the presence of Klotho (7), resulting in decreased transport of phosphate through the Na–Pi transporters (8) and inhibiting vitamin-D1 hydroxylation (9). Since 1,25-dihydroxyvitamin-D acts by promoting phosphate absorption through the small intestine (10), the effect of FGF23 on vitamin-D metabolism results in decreased entry of phosphate into the circulation (11). In contrast, 1,25-dihydroxyvitamin-D augments FGF23 signaling (12), but inhibits ppGalNacT3 expression (13), thereby establishing a double-regulatory feedback loop mechanism. Of note, FGF23 influences bone mineralization (14). The entire system is also under the regulation of parathyroid hormone, which promotes phosphate excretion through

kidney (15) and mobilizes phosphate from the bone (16), thus integrating calcium- and phosphate-regulatory systems. As a consequence of hyperphosphatemia, FGF7 is induced (17), which results in expression and activation of several MMPs (18), which are known to mediate ectopic calcification (19). Since FGF7 mainly originates from the dermis, this may explain the propensity of ectopic calcification to develop in the skin in HFTC. Additional elements involved in the regulation of FGF23 activity include PHEX and DMP1, which inhibit FGF23 activity through still poorly understood mechanisms (possibly including a direct interaction between them) (20). Loss-of-function mutations in the genes encoding these two molecules are associated with increased FGF23 activity and hypophosphatemia.

Table 1

Genetic and phenotypic heterogeneity among disorders of phosphate metabolism associated with abnormal ppGalNacT3 and FGF23 function

Disease	OMIM	Phenotype	Gene (protein)	Protein function
Hyperphosphatemic familial tumoral calcinosis	211900	Calcified masses over large joints, occasionally visceral calcifications	*GALNT3* (ppGalNacT3)	Initiates mucin type *O*-glycosylation
Hyperostosis hyperphosphatemia syndrome	610233	Repeated attacks of painful swellings of the long bones; on radiography, cortical hyperostosis, periosteal reaction, diaphysitis	*GALNT3* (ppGalNacT3)	Initiates mucin type *O*-glycosylation
Hyperphosphatemic familial tumoral calcinosis	211900	Calcified masses over large joints, more frequent visceral calcifications than with *GALNT3* mutations	*FGF23* (FGF23)	Regulates phosphate reabsorption
Autosomal dominant hypophosphatemic rickets	193100	Growth retardation, osteomalacia, rickets, bone pain	*FGF23* (FGF23)	Regulates phosphate reabsorption
Hyperphosphatemic familial tumoral calcinosis	211900	Widespread cutaneous and visceral calcifications, diffuse osteopenia and sclerodactyly	*KL* (Klotho)	Involved in the regulation of phosphate and calcium levels
X-linked hypophosphatemic rickets	307800	Growth retardation, osteomalacia, rickets, bone pain	*PHEX* (PHEX)	Protease, which regulates FGF23 expression and bone mineralization
Autosomal recessive hypophosphatemic rickets	241520	Growth retardation, osteomalacia, rickets, bone pain	*DMP1* (DMP1)	Regulates FGF23 expression and bone mineralization
Epidermal nevus[1]	162900	Hyperkeratotic papules/plaques distributed in a linear or segmental fashion	*FGFR3* (FGFR3)	Serves as receptor for fibroblast growth factors such as FGF1

FGF23, fibroblast growth factor-23; ppGalNacT3, polypeptide *N*-acetylgalactosaminyltransferase-3.

[1] In selected cases of epidermal nevus, increased levels of FGF23 associated with hypophosphatemic rickets are observed due to end-organ gain-of-function mutations.