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A homozygous missense mutation in human *KLOTHO* causes severe tumoral calcinosis

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Familial tumoral calcinosis (OMIM 211900) is a rare autosomal recessive disorder, characterized by ectopic calcifications and hyperphosphatemia. The known genetic causes of tumoral calcinosis are biallelic inactivating mutations in the genes encoding fibroblast growth factor 23 (*FGF23*)[1-4] or UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (*GALNT3*)[5-8]. FGF23 is a hormone that promotes renal phosphate excretion by decreasing phosphate reabsorption in the proximal tubule and also reduces circulating $1,25(OH)_2D$ by both decreasing biosynthesis and increasing metabolism of $1,25(OH)_2D$[9]. GALNT3 is a Golgi-associated enzyme that initiates *O*-glycosylation of mature polypeptides. This enzyme selectively *O*-glycosylates a furin-like convertase recognition sequence in FGF23, thereby preventing proteolytic processing of FGF23 and allowing secretion of intact FGF23[10]. Therefore, dysfunction of either FGF23 or GALNT3 decreases circulating intact, bioactive FGF23, which leads to hyperphosphatemia and ultimately to tumoral calcinosis.

Recent studies have shown that FGF23 requires an additional co-factor, Klotho (KL), to bind and signal through its cognate fibroblast growth factor receptors (FGFRs)[11,12]. Diminished *Kl* expression in mice results in a phenotype characterized by osteopenia, skin and muscle atrophy, pulmonary emphysema, infertility, hypoactivity, ectopic vascular and soft-tissue calcifications, and death by 60 days of age, which was interpreted as a premature aging phenotype[13]. Biochemical abnormalities of *Kl*-deficient mice include severe hyperphosphatemia, hypercalcemia, hypoglycemia and increased serum levels of $1,25(OH)_2D$[13-15]. Of significance, the *Kl*-deficient phenotype largely overlaps with the phenotype of FGF23-null mice[12,16,17], indicating functional crosstalk between KL and FGF23 and underscoring the observed direct interactions between KL, FGF23, and its cognate FGFRs[11,12].

A 13-year-old girl presented with severe vascular and soft-tissue calcifications, including dural and carotid artery calcifications. This patient exhibited multiple defects in mineral ion homeostasis with marked hyperphosphatemia and hypercalcemia, as well as elevated serum levels of parathyroid hormone (PTH) and FGF23. However, there were no features of premature aging. Mutational analysis of *FGF23*, *GALNT3*, and *KL* in the patient revealed a homozygous missense mutation (His193Arg) in the *KL* gene. Mapping of His193Arg mutation onto the crystal structure of myrosinase, a plant homologue of KL, reveals that this histidine residue is at the base of the deep catalytic cleft and mutation of this histidine to arginine should destabilize the putative glycosidase domain (KL1) of KL, thereby attenuating production of membrane-bound and secreted KL. Indeed, compared to wild-type KL, expression and secretion of His193Arg KL were markedly reduced *in vitro*, resulting in diminished ability of FGF23 to bind and signal via its cognate FGFRs. Taken together, our clinical and molecular findings provide the first evidence that loss-of-function mutations in human *KL* impair FGF23 bioactivity and lead to severe tumoral calcinosis, underscoring the essential role of KL in FGF23-mediated phosphate and vitamin D homeostasis in humans.

The authors have no conflict of interest.

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