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FGF23–parathyroid interaction: implications in chronic kidney disease

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Over the past few years there have been considerable advances in our understanding of the physiological regulation of mineral homeostasis. One of the most important breakthroughs is the identification of fibroblastic growth factor 23 (FGF23) and its role as a key regulator of phosphate and 1,25-dihydroxyvitamin D metabolism. FGF23 exerts its biological functions by binding to its cognate receptor in the presence of Klotho as a cofactor. FGF23 principally acts on the kidney to induce urinary phosphate excretion and suppresses 1,25-dihydroxyvitamin D synthesis, thereby indirectly modulating parathyroid hormone secretion. FGF23 also acts directly on the parathyroid to decrease parathyroid hormone synthesis and secretion. In patients with chronic kidney disease, FGF23 levels increase progressively to compensate for phosphate retention, but these elevated FGF23 levels fail to suppress the secretion of parathyroid hormone, particularly in the setting of uremia. Recent data suggest that this parathyroid resistance to FGF23 may be caused by decreased expression of Klotho–FGFR1 complex in hyperplastic parathyroid glands. This review summarizes recent insights into the role of FGF23 in mineral homeostasis and discusses the involvement of its direct and indirect interaction with the parathyroid gland, particularly focusing on the pathophysiology of secondary hyperparathyroidism in chronic kidney disease.

Kidney International (2010) **77,** 292–298; doi:10.1038/ki.2009.466; published online 9 December 2009

KEYWORDS: chronic kidney disease; FGF23; Klotho; parathyroid hormone; vitamin D

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Received 1 September 2009; revised 18 October 2009; accepted 20 October 2009; published online 9 December 2009

The parathyroid gland plays an important role in the regulation of mineral homeostasis by cross talking with other organs such as the kidney and bone. Fluctuation in extracellular calcium ion levels is sensed by the parathyroid calcium-sensing receptors (CaSRs) and subsequently regulates the synthesis and secretion of parathyroid hormone (PTH).[1] PTH acts on the bone to increase the efflux of calcium and phosphate, and acts on the kidney to reduce urinary calcium excretion, inhibit phosphate reabsorption, and stimulate the production of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). The increased $1,25(OH)_2D$ acts on the small intestines to increase dietary absorption of calcium and phosphate and also acts on the parathyroid gland to inhibit PTH synthesis thereby closing a negative feedback loop.[2,3] These biological effects of $1,25(OH)_2D$ are mediated by binding of $1,25(OH)_2D$ to its specific receptor, the vitamin D receptor (VDR), and subsequent regulation of the transcription by promoter vitamin D response elements.[4] The effect of PTH to promote phosphate excretion offsets the $1,25(OH)_2D$-mediated dietary phosphate absorption and the PTH-mediated phosphate efflux from bone. Increase in serum phosphate also directly stimulates the secretion of PTH through a putative extracellular phosphate sensor, which in turn acts on the kidney to promote phosphaturia and thereby prevents hyperphosphatemia.[5]

Recently, fibroblastic growth factor 23 (FGF23), a novel bone-derived hormone, has been identified that has a physiological role in regulating mineral homeostasis.[6] FGF23 principally functions as a hormone regulating phosphaturia and renal $1,25(OH)_2D$ production,[6–8] suggesting that this circulating factor also mediates secretion of PTH at least indirectly by regulating serum phosphate and $1,25(OH)_2D$ levels. Importantly, recent experimental studies have shown that FGF23 also exerts a direct effect on the parathyroid gland to suppress PTH synthesis and secretion,[9,10] further enhancing our understanding of the mechanisms that govern mineral metabolism in normal physiology and various disorders. In this review, we present recent insights into the role of FGF23 in mineral homeostasis and discuss its direct and indirect interaction with the parathyroid gland. We also examine the involvement of FGF23–parathyroid interaction in the pathophysiology of mineral disturbances, particularly focusing on secondary

hyperparathyroidism (SHPT) in patients with chronic kidney disease (CKD).

FIBROBLAST GROWTH FACTOR 23

FGF23 is a 32-kDa protein that is secreted mainly by osteocytes in bone.[11] It was first cloned in mice as a new member of FGF family[12] and subsequently identified as a causative humoral factor for autosomal-dominant hypophosphatemic rickets/osteomalacia[13] and tumor-induced osteomalacia.[14] It induces urinary phosphate excretion by suppressing the expression of sodium/phosphate cotransporter.[7,8] It also suppresses $1,25(OH)_2D$ by inhibition of 1α-hydroxylase (CYP27B1) that converts 25-hydroxyvitamin D (25(OH)D) to $1,25(OH)_2D$ and stimulation of 24-hydroxylase (CYP24) that converts $1,25(OH)_2D$ to inactive metabolites in the proximal tubule of the kidney.[7,8] Circulating FGF23 level ranges 25–50 pg/ml in normal subjects using an intact FGF23 assay, whereas this level is increased to 10- to 20-fold in patients with hypophosphatemic rickets syndromes.[13,14] Excess FGF23 results in renal phosphate wasting, inappropriately low levels of $1,25(OH)_2D$, and osteomalacia.[15–18] In contrast, depletion of FGF23 leads to hyperphosphatemia, excessive levels of $1,25(OH)_2D$, and soft tissue calcification.[19–23] FGF23 is proteolytically processed to generate N-and C-terminal fragments. Neither of the processed N-or C-terminal fragments of FGF23 can exert its hormonal effects.[24] Indeed, in patients with hereditary diseases of FGF23 deficiency, augmented production of C-terminal fragments fails to ameliorate hyperphosphatemia and hypervitaminosis D.[21–23]

FGF23 exerts its biological functions by binding to its cognate fibroblastic growth factor receptor (FGFR) in the presence of its coreceptor Klotho.[25,26] Klotho is a transmembrane protein that determines the tissue specificity of FGF23. The N-terminal region of FGF23 binds to and activates FGFR whereas the C-terminal region of FGF23 is necessary for interaction with Klotho.[27,28] These facts clearly explain why *Klotho* null mice display a phenotype identical to that of *Fgf23* null mice, both of which are characterized by premature aging-related phenotypes associated with hyperphosphatemia and paradoxically high $1,25(OH)_2D$ levels.[19,29] Indeed, the administration of FGF23 to either *Klotho* knockout mice or *Klotho/Fgf23* double knockout mice does not produce its phosphaturic and $1,25(OH)_2D$-inhibitory effect.[30] Recent studies also showed that genetic inactivation of *Klotho* in either *Hyp* mice, a murine homolog of X-linked hypophosphatemic rickets, or *Fgf23* transgenic mice resulted in hyperphosphatemia and increased $1,25(OH)_2D$ levels, a phenotype consistent with *Fgf23*-ablated mice, further emphasizing the *in vivo* importance of Klotho in FGF23 action.[31,32]

Among multiple FGFR isoforms, Klotho can bind with FGFR1c, 3c, and 4 and increase their affinity to FGF23 *in vitro*.[27] However, a functional analysis has shown that Klotho-dependent FGF23 signaling defined by upregulation of the gene early growth-responsive 1 (*Egr-1*) is restricted to interaction with FGFR1c.[28] Recent *in vivo* studies have also shown that neither FGFR3 nor FGFR4 is the principal mediator of the FGF23 effects in the kidney,[33] supporting the possibility that FGFR1c is the physiologically relevant target for FGF23 *in vivo*. It is, however, still unclear how in the kidney FGF23 exerts its physiological effects on the proximal tubule despite the highest expression of Klotho–FGFR1c complexes in the distal tubule. FGF23 actions on the proximal tubule may be mediated by FGF23 stimulation of the distal tubule and subsequent distal–proximal tubule interaction, but further researches are needed to confirm this possibility.

FGF23 secretion is regulated by several systemic factors. Given the hormonal effect of FGF23 to regulate renal phosphaturia and $1,25(OH)_2D$ production, its secretion should be regulated by serum phosphate and/or $1,25(OH)_2D$ levels. The former possibility is supported by experimental studies showing that dietary phosphate loading in mice leads to an increase in bone FGF23 mRNA and serum FGF23,[34] although this effect is rather modest in humans.[35,36] It is, however, still unclear how extracellular phosphate is sensed by the body and whether this sensing mechanism is involved in the regulation of FGF23 secretion. On the other hand, there is concrete evidence that $1,25(OH)_2D$ stimulates FGF23 production both *in vivo* and *in vitro*, through vitamin D-response elements present in the FGF23 promoter.[37] A clinical study also showed that administration of $1,25(OH)_2D$ to patients undergoing dialysis results in increase in serum FGF23 levels.[38] More recently, estrogen is identified as a novel positive regulator of FGF23 secretion. Estrogens increase FGF23 synthesis both *in vivo* and *in vitro*, which may in part explain the effect of estrogens to decrease serum $1,25(OH)_2D$ and phosphate levels.[39] FGF23 secretion is also regulated by several local bone-derived factors, such as phosphate-regulating gene with homologies to endopeptidases on the X chromosome, dentin matrix acidic phosphoprotein 1 and matrix extracellular phosphoglycoprotein, although their precise interactions remain to be elucidated.[6]

FGF23 AND THE PARATHYROID GLAND

The parathyroid gland also expresses Klotho and FGFR1 and is a target for FGF23. This novel FGF23–parathyroid interaction is primarily suggested by the effects of recombinant FGF23 to upregulate the expression of *Egr-1* in the parathyroid gland of rats.[28] However, until recently, it has been a matter of debate whether FGF23-dependent pathways stimulate or inhibit PTH secretion. In patients and animal models of excess FGF23 production, serum levels of PTH have been variably reported but were most frequently elevated.[15–18] A translocation causing increased Klotho level results in severe hyperparathyroidism associated with increased FGF23 levels.[40] Furthermore, in patients with CKD, elevated FGF23 levels are associated with PTH hypersecretion.[41,42] However, whether the elevated PTH levels seen in these cases are the direct result of increased FGF23 is difficult to discern, because FGF23-mediated reductions in

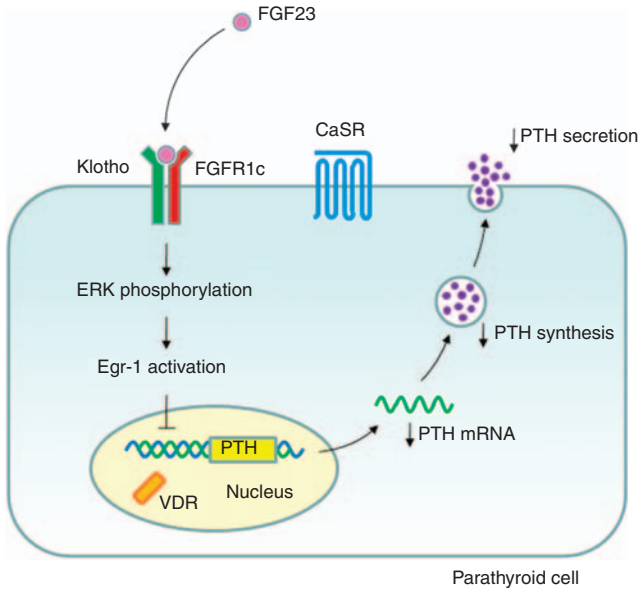

Figure 1 | FGF23 signaling in the parathyroid cell. FGF23 can bind to the Klotho–FGFR1c complex on the surface of the parathyroid cell and thereby triggers downstream signaling. This results in the activation of ERK and the induction of *Egr-1* expression, leading to inhibition of PTH gene expression and secretion. CaSR, calcium-sensing receptor; Egr-1, early growth-responsive 1; ERK, extracellular signal-regulated kinase; FGF23, fibroblastic growth factor 23; FGFR1c, fibroblastic growth factor receptor 1c; PTH, parathyroid hormone; VDR, vitamin D receptor.

$1,25(OH)_2D$ might also have a role in dysregulated PTH secretion.

Recently, the direct effect of FGF23 on the parathyroid gland has been uncovered by two independent research groups.[9,10] One study showed that FGF23 acts directly on the parathyroid to activate the mitogen-activated protein kinase pathway and thereby decrease PTH gene expression and secretion both *in vivo* in rats and *in vitro* in rat parathyroid cultures.[9] Another study used primary cultures of bovine parathyroid cells and showed that FGF23 decreases PTH secretion, which may be partly mediated by increased parathyroid CYP27B1 expression.[10] These data conclusively show that FGF23 acts directly on the parathyroid gland to decrease PTH secretion (Figure 1). Importantly, this direct effect of FGF23 to inhibit PTH secretion is in contrast to its indirect effect by inhibition of renal $1,25(OH)_2D$ production, suggesting that the systemic effect of FGF23 on parathyroid function can be varied under different conditions. This complex regulation of PTH secretion by FGF23 may in part explain the discrepancy in PTH levels observed in humans and experimental animals of FGF23 excess (Figure 2).

ROLE OF FGF23 IN CKD

Traditionally, the disturbed mineral and bone homeostasis in CKD has been viewed from the perspective of the PTH-vitamin D axis, where the progressive reduction in $1,25(OH)_2D$ synthesis as a result of decreased renal mass along with a functional inhibition of CYP27B1 by hyperphosphatemia has a major role in the pathogenesis of SHPT.[43] However, the observation that $1,25(OH)_2D$ levels begin to decline long before the development of hyperphosphatemia give cause to reconsider the traditional view of SHPT. In this regard, the identification of FGF23 provides important implications for the understanding of abnormal mineral metabolism in CKD. In patients with CKD, circulating levels of FGF23 are progressively increased as kidney function declines.[44] This increase in serum FGF23 levels cannot be explained solely by the accumulation of C-terminal fragments, because a subsequent study showed a similar increase by use of the intact assay that exclusively detects the full-length, biologically active molecule.[45] Thus, it is reasonable to consider that in patients with CKD, FGF23 production is increased to counteract chronic phosphate retention by promoting urinary phosphate excretion in the face of reduced nephron mass.

Notably, in this setting, a previous study has shown that FGF23 was a strong independent predictor of diminished $1,25(OH)_2D$ levels even after adjustment for renal function, serum phosphate levels, and 25(OH)D levels.[46] This finding suggest that in patients with CKD, increased FGF23 to maintain neutral phosphate balance results in suppression of renal $1,25(OH)_2D$ production and thereby triggers the early development of SHPT. In advanced stage of CKD, dietary phosphate may overcome such compensation for decreased nephron mass despite markedly elevated FGF23 levels. This results in overt hyperphosphatemia and $1,25(OH)_2D$ deficiency, both of which further stimulate PTH secretion and parathyroid cell proliferation, leading to the development of uremic parathyroid hyperplasia.[47]

FGF23 also is important in the pathogenesis of post-transplant hypophosphatemia. This complication has been long thought to be caused by tertiary hyperparathyroidism,[48] but this PTH-centric view cannot explain the concomitant $1,25(OH)_2D$ deficiency and the development of urinary phosphate wasting even in the absence of elevated PTH levels. In this context, recent data suggest that FGF23 rather than PTH is the primary factor accounting the syndrome of posttransplant hypophosphatemia and inappropriately low $1,25(OH)_2D$ levels.[49–52] In the pretransplant period, FGF23 secretion is extremely increased to counteract chronic phosphate retention but fails to exert its hormonal effects in the absence of functioning kidney. However, after kidney transplantation, this excess FGF23 acts on the allograft to promote phosphaturia and suppress $1,25(OH)_2D$ production. Importantly, this FGF23-centric view may imply the limitation of current management strategies using high doses of phosphate supplements and active vitamin D sterols, both of which further stimulate FGF23 secretion.[49] In this regard, it is promising that the use of recently developed anti-FGF23 monoclonal antibodies is therapeutically effective for *Hyp* mice.[53] This insight may open new therapeutic strategies of FGF23-related hypophosphatemic disorders including post-transplant hypophosphatemia.

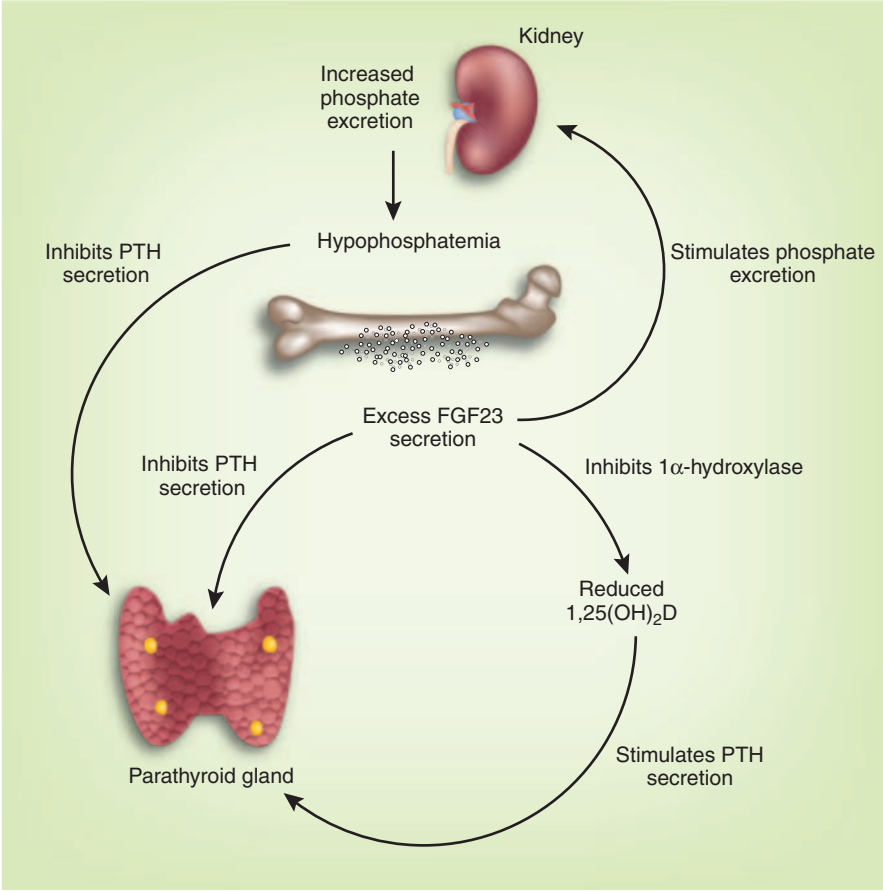

Figure 2 | FGF23–parathyroid interaction in hypophosphatemic disorders due to excess FGF23 secretion. Excess FGF23 acts on the kidney to induce urinary phosphate excretion and suppress renal production of $1,25(OH)_2D$. In this setting, FGF23-mediated hypophosphatemia acts to suppress PTH secretion, whereas FGF23-mediated low $1,25(OH)_2D$ acts to stimulate PTH secretion. FGF23 also acts directly on the parathyroid to inhibit PTH synthesis and secretion. This complex regulation of PTH secretion by FGF23 may explain the discrepancy in PTH levels observed in hypophosphatemic disorders due to excess FGF23 secretion. $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; FGF23, fibroblastic growth factor 23; PTH, parathyroid hormone.

FGF23 AND UREMIC PARATHYROID HYPERPLASIA

Parathyroid hyperplasia is one of the characteristic features of patients with end-stage renal disease.[54] It can be divided into two types with different morphological features, that is, diffuse and nodular hyperplasia. Initially, in patients with CKD, several factors such as phosphate retention, decreased $1,25(OH)_2D$, and resulting hypocalcemia act on the parathyroid cells to proliferate, leading to diffuse hyperplasia. Some cells proliferate more vigorously, forming small nodules each of which is monoclonal in origin. These nodules become grossly enlarged and exhibit a nodular hyperplasia.[55] Importantly, both CaSR and VDR are progressively downregulated in the course of parathyroid hyperplasia,[56–58] which is considered to explain the resistance to active vitamin D therapy in advanced SHPT.

Several observational studies have shown that in patients undergoing dialysis FGF23 levels are extremely increased in association with the severity of SHPT and/or hyperphosphatemia.[38,41,42] Such a marked elevation in FGF23 levels may in part be attributed to active vitamin D treatment,[38] in addition to other possible factors such as impaired renal FGF23 degradation and chronic phosphate retention. It is not clear whether PTH-induced phosphate release from bone stimulates FGF23 secretion. Not surprisingly, the source of increased FGF23 is bone cells, but not parathyroid cells, even in patients with advanced parathyroid hyperplasia.[41] This fact is in line with the observation that parathyroidectomy for patients with advanced parathyroid hyperplasia results in gradual, but not rapid, decline in serum FGF23 levels.[59] The mechanism of this FGF23 decline is not clear, but it may be attributed to decreased serum phosphate levels as a result of increased phosphate influx into bone after parathyroidectomy.

In this context, it is of particular interest that pretreatment serum FGF23 levels predict the effectiveness of active vitamin D therapy and the future development of refractory hyperparathyroidism in dialysis patients with SHPT.[41,42] The mechanism of this finding is not clear; however, recent studies suggest that FGF23 is a more sensitive biomarker of disordered phosphate metabolism than concomitant serum phosphate measurements.[60] Given that high phosphate directly stimulates PTH secretion and parathyroid cell

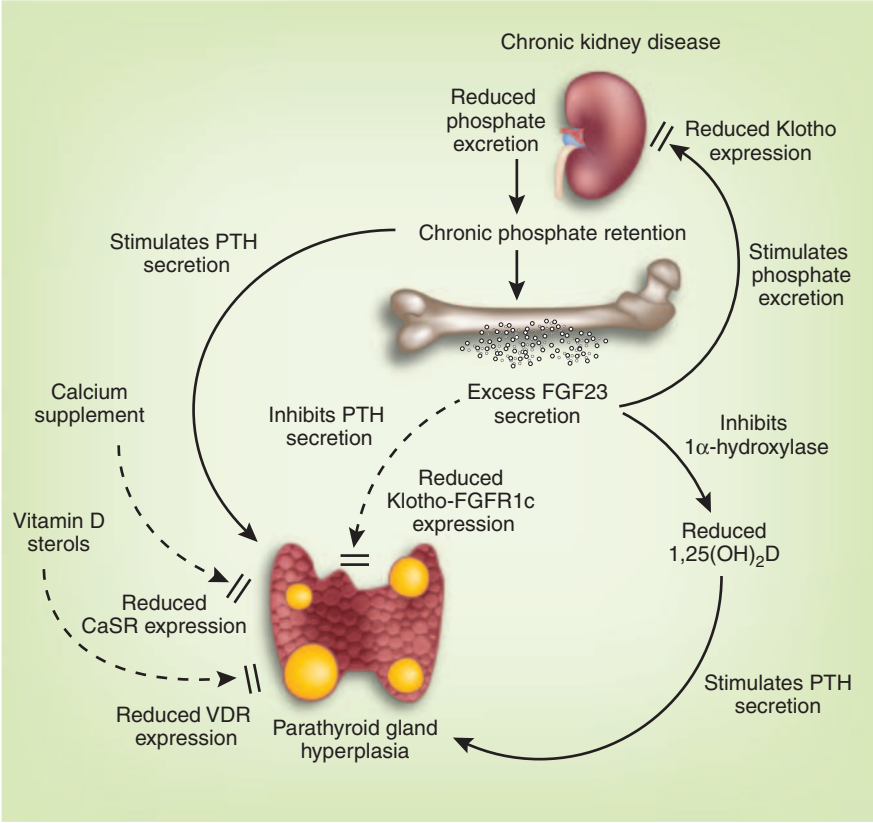

Figure 3 | FGF23–parathyroid interaction in chronic kidney disease. In patients with chronic kidney disease, serum FGF23 is increased to maintain neutral phosphate balance, but this leads to suppression of renal $1,25(OH)_2D$ production and thereby triggers the early development of secondary hyperparathyroidism. Progression to renal failure may overcome this compensation for decreased nephron mass and result in overt hyperphosphatemia, which further stimulates PTH secretion and parathyroid cell proliferation, leading to parathyroid gland hyperplasia characterized by enlargement of the parathyroid glands. In this setting, markedly increased FGF23 should act on the parathyroid as a negative regulator but fails to suppress PTH secretion. This resistance of parathyroid to FGF23 may be caused by downregulation of Klotho–FGFR1c complex. $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; CaSR, calcium-sensing receptor; FGF23, fibroblastic growth factor 23; FGFR1c, fibroblastic growth factor receptor 1c; PTH, parathyroid hormone; VDR, vitamin D receptor.

proliferation,[5] it can be deduced that chronic phosphate retention as reflected by elevated FGF23 levels may contribute to further progression of parathyroid hyperplasia. Another possibility is that high levels of FGF23 at baseline may be a consequence of prolonged active vitamin D administration for severe SHPT, which may lead to future resistance to this therapy. Regardless, additional studies are needed to translate these findings into the development of novel therapeutic approaches. In addition, whether FGF23 stimulation or suppression is beneficial or potentially harmful is also crucial and worthy of further investigation.[61]

Another issue that should be addressed is how to extrapolate recent data showing that FGF23 is a negative regulator of parathyroid function[9,10] in patients with CKD. Specifically in uremic patients undergoing dialysis, the indirect action of FGF23 to promote PTH secretion by decreased $1,25(OH)_2D$ may be less evident, because renal production of $1,25(OH)_2D$ is substantially impaired and active vitamin D sterols are frequently used to control SHPT. Nevertheless, in these patients, PTH secretion remains elevated despite extremely high FGF23 levels.[38,41,42,59] This fact suggests the presence of resistance to FGF23 action in uremic patients. One possible explanation for this resistance is that some uremic toxins that accumulate in renal failure interfere with interaction between FGF23 and Klotho–FGFR1c complex and/or subsequent intracellular signaling, in a manner analogous to erythropoietin resistance in renal anemia and skeletal resistance to PTH in low-turnover bone disease. Another possibility is that the expression of Klotho–FGFR1c complex may be decreased in uremic parathyroid hyperplasia, as has been reported previously in association with CaSR and VDR.[56–58] To investigate this possibility, we have recently examined the expression of Klotho and FGFR1 in surgically excised parathyroid glands of uremic patients. Compared with normal tissue, the expression of Klotho and FGFR1 decreased significantly in hyperplastic parathyroid glands, particularly in glands with nodular hyperplasia.[62] This result suggests that the depressed expression of the Klotho–FGFR1c complex in hyperplastic glands may explain the resistance to extremely high FGF23 levels in uremic patients (Figure 3). Indeed, recent preliminary data also support this possibility by

showing that FGF23 does not inhibit PTH secretion both *in vivo* CKD rats and *in vitro* parathyroid gland cultures from CKD rats (abstract; Rodriguez M, *et al. J Am Soc Nephrol* 2009; **20**: 53A and Galitzer H, *et al. J Am Soc Nephrol* 2009; **20**: 53A). Whether downregulation of the Klotho–FGFR1c complex has a role in the pathogenesis of SHPT and whether this expression is mediated by other factors involved in the disorder would be worthy of future investigation.

CONCLUSION

The identification of FGF23 and the clarification of its role as a key regulator of phosphate and $1,25(OH)_2D$ has greatly enhanced our knowledge of the physiological regulation of mineral homeostasis. These insights have important implications for understanding the pathophysiology of not only rare disorders such as renal phosphate wasting disorders but also more common mineral and bone disorders associated with CKD. Furthermore, the recent data on the direct interaction between FGF23 and the parathyroid gland further stimulate the research interest in the pathogenesis of uremic parathyroid hyperplasia, where PTH synthesis and secretion remains stimulated despite extremely high FGF23 levels. It is hoped that the translation of these insights into new therapeutic agents and biomarkers improves the outcomes of patients suffering from disturbed mineral homeostasis.

DISCLOSURE

The authors declared no competing interests.

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