FGF23-mediated regulation of systemic phosphate homeostasis: is Klotho an essential player?

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Razzaque MS. FGF23-mediated regulation of systemic phosphate homeostasis: is Klotho an essential player? *Am J Physiol Renal Physiol* 296: F470–F476, 2009. First published November 19, 2008; doi:10.1152/ajprenal.90538.2008.—Understanding the physiological regulation of mineral ion metabolism is essential for determining the pathomechanisms of skeletal, vascular, and renal diseases associated with an abnormal regulation of calcium and phosphate homeostasis. Normal calcium and phosphate balance is delicately maintained by endocrine factors that coordinate to influence the functions of the intestine, bone, parathyroid gland, and kidney. Under physiological conditions, the kidneys play an important role in maintaining normal mineral ion balance by fine-tuning the amount of urinary excretion of calcium and phosphate according to the body's needs. Fibroblast growth factor (FGF)23 regulates urinary phosphate excretion to maintain systemic phosphate homeostasis. The exact mode of action of the phosphaturic effects of FGF23 is not fully understood and is an intense area of research. Studies suggest, however, that FGF23, by interacting with FGF receptors, can initiate downstream signaling events and that Klotho, a transmembrane protein, facilitates the interaction of FGF23 with its receptor. FGF23 can inhibit the activities of 1-α-hydroxylase and sodium-phosphate cotransporter in the kidney to influence the overall systemic phosphate balance. This article briefly summarizes how FGF23 might coordinately regulate systemic phosphate homeostasis and how Klotho is involved in such regulation.

vitamin D; mineral metabolism; kidney; calcification

THE MAIN FUNCTIONS of the kidney are to maintain water, electrolyte, and mineral ion balance and eliminate metabolic waste. Most chronic kidney diseases (CKD), without therapeutic intervention, usually progress to irreversible renal failure (45, 52, 61) and affect water, electrolyte, and mineral ion balance. Phosphorus is widely distributed in the body and has numerous essential biological functions, including contributing to bone formation, cell signaling, energy metabolism, and nucleic acid synthesis (14). More than 80% of the total phosphate in the body is present in the bone (Table 1). A very small amount of phosphate ($\sim$0.1%) exists as inorganic phosphate (P_i) anions, either as ions or complexed with cations, such as Ca^{2+} and Mg^{2+}.

A significant amount of intestinal dietary phosphorus absorption partly occurs at the distal portion of the duodenum. Vitamin D helps intestinal phosphorus absorption by facilitating active uptake (6, 25). After intestinal absorption, circulating phosphorus is taken up by the cells that need it, accumulates in the bone matrix protein, and enters the kidney. About 95% of the filtered phosphate in the kidney is reabsorbed at the proximal tubule. Phosphate transport across the proximal tubule epithelial cells is mostly driven by a high extracellular sodium gradient. This gradient is thought to be maintained by membrane-associated Na^+-K^+-ATPase. Recently, it has been claimed that Klotho, a transmembrane protein, directly affects Na^+-K^+-ATPase activity to increase the Na^+ gradient and drive transepithelial calcium transport in the choroid plexus and the kidney (39). It is worth mentioning that the model of Klotho activating Na^+-K^+-ATPase as a means to increase transepithelial calcium transport is provocative, but increasing transport of a single ion by increasing the overall workhorse of the cell is a highly unusual phenomenon. Additional studies are needed to settle this unresolved issue (47).

The molecular mechanism of renal phosphate reabsorption in the tubules is a complex process, in which sodium-phosphate (NaP_i) cotransporters that are located in the luminal side of the proximal tubule epithelial cells play an important role (63). Both NaP_i-2a and NaP_i-2c transporters, in a sodium-dependent manner, help to recycle filtrated phosphate back into the proximal tubule epithelial cells (11, 37). Parathyroid hormone (PTH) and vitamin D actively contribute to the mineral ion transport process by influencing the biological activities of the membrane structures and transport molecules, including the NaP_i and calcium-permeable transient receptor potential V (TRPV) family proteins (18, 63). For instance, PTH can suppress the reabsorption of phosphate in the proximal tubules and thus can induce an increase of urinary phosphate excretion by reducing NaP_i-2a and NaP_i-2c activities. PTH can also mobilize phosphorus from the bone into the circulation, pos-

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Table 1. *Approximate distribution of phosphorus in a healthy 70-kg adult*

	Phosphorus
Total body content	~700 g
In bone	~80%
In viscera	~10.9%
In skeletal muscle	~9%
In extracellular fluid	~0.1%
Intracellular-to-extracellular ratio	~100:1

From Reference 14.

sibly by enhancing osteoclastic bone resorption (62). Moreover, PTH, by inducing the renal expression of 1-α-hydroxylase, can increase the production of the active vitamin D metabolite 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], which can enhance intestinal phosphorus absorption. A major breakthrough in the active regulation of phosphate homeostasis was achieved with the identification of a potent phosphotonin, named fibroblast growth factor (FGF)23 (1, 67).

Fibroblast Growth Factor 23

FGF23 is a protein of ~30 kDa that is proteolytically processed to generate smaller NH$_2$-terminal (18 kDa) and COOH-terminal (12 kDa) fragments. The NH$_2$-terminal fragment of FGF23 contains the FGF receptor (FGFR)-binding domain. In vivo studies using synthetic peptides have indicated that neither of the processed NH$_2$-terminal or COOH-terminal fragments of FGF23 can influence serum phosphate levels (58). FGF23 can decrease the activity of NaP$_i$-2a and NaP$_i$-2c cotransporters to reduce renal phosphate reabsorption and thereby can increase urinary phosphate excretion (55). Similarly, FGF23 can suppress the expression of 1-α-hydroxylase to reduce the production of the active vitamin D metabolite 1,25(OH)$_2$D (55). Moreover, FGF23 can also induce 24-hydroxylase, which degrades 1,25(OH)$_2$D (55). Since 1,25(OH)$_2$D can enhance intestinal phosphate absorption, FGF23, by reducing vitamin D activities, can influence intestinal phosphate absorption (in addition to its urinary phosphate-wasting effects). The FGF23-mediated regulation of phosphate homeostasis is fairly independent of calcium homeostasis. Genetically engineered FGF23 mouse models provided convincing in vivo evidence of the phosphaturic activities of FGF23 (3, 8, 56, 59) and gave an experimental explanation of the clinical symptoms that have been noted in various human diseases, associated with the abnormal regulation of FGF23 (1, 4, 29, 57, 58).

FGF23 and Human Diseases

Human *FGF23* was identified in patients as a gene associated with autosomal dominant hypophosphatemic rickets (ADHR). Gain-of-function mutations of the *FGF23* gene in patients with ADHR are associated with urinary phosphate wasting, which causes rickets in the bone (1). The excessive amount of FGF23 that is produced by the tumor cells is responsible for the clinical symptoms of hypophosphatemia that are seen in patients with tumor-induced osteomalacia (TIO) (57). FGF23 has also been speculated to have a pathogenic role in patients with McCune-Albright syndrome and is believed to be responsible for hypophosphatemia in these patients (53). X-linked hypophosphatemia (XLH) is a common cause of vitamin D-resistant rickets/osteomalacia, which is caused by inactivating mutations in PHEX (a phosphate-regulating gene that is homologous to endopeptidases on the X chromosome) (19). The high level of circulating FGF23 in most patients with XLH induces excessive urinary phosphate wasting, thereby producing clinical symptoms associated with hypophosphatemia (23). Recently, mutations in *dentin matrix protein-1* (*DMP-1*) were found in patients with autosomal recessive hypophosphatemic rickets/osteomalacia (ARHR) (33). The clinical symptoms of hypophosphatemia in patients with ARHR are mostly caused by high circulating levels of FGF23.

Patients in advanced stages of CKD have high serum levels of FGF23, partly due to decreased renal clearance of FGF23. The additional cause of increased levels of FGF23 in CKD patients may be a compensatory response to the hyperphosphatemia. Of relevance, human studies have shown that a dietary phosphorus load can increase serum FGF23 levels (2, 10). CKD patients are unable to excrete urinary phosphate, despite high serum FGF23 levels (13), and develop hyperphosphatemia, although they tend to have a lower level of 1,25(OH)$_2$D, and suffer from hyperparathyroidism. Whether the low levels of 1,25(OH)$_2$D and secondary hyperparathyroidism that are observed in patients with CKD are influenced by increased levels of circulating FGF23 is a complex issue. Since FGF23 has a counterregulatory effect on vitamin D (30), the elevated level of FGF23 in patients with CKD has the potential to reduce vitamin D activity; this effect may eventually facilitate the development of compensatory secondary hyperparathyroidism. Furthermore, calcitriol therapy in patients with CKD may also contribute to increased serum levels of FGF23 (42), because studies have shown that elevated serum 1,25(OH)$_2$D can induce an increase in the serum level of FGF23 (54). The exact pathological significance of elevated serum levels of FGF23 in patients with CKD is not clear, and the question of whether increased levels of FGF23 can exert additional toxic effects, independent of other known risk factors of CKD patients (e.g., race, diabetes, hypertension, and advanced age) will need additional clinical studies.

In addition to the several hypophosphatemic diseases that are caused by an increased activity of FGF23, there are also human diseases that are caused by a reduced activity of FGF23. For instance, patients with familial tumoral calcinosis (FTC) usually develop hyperphosphatemia and ectopic calcification due to loss-of-function mutations in the *FGF23* gene (4). Similarly, mutations in the *GALNT3* (UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetyl-galactosaminyl transferase 3) gene are also found in patients with FTC (12). GALNT3, a Golgi-associated enzyme, can selectively *O*-glycosylate a furinlike convertase recognition site in FGF23, and thereby can allow secretion of intact FGF23. Mutations in the *GALNT3* gene can impair *O*-glycosylation of the FGF23 protein; defects in glycosylation may result in rapid processing of the FGF23 protein, which may be the cause of the lower levels of the intact FGF23 protein found in patients with FTC (15). The abnormally high serum levels of phosphate in patients with FTC, due to the reduced activity of the human FGF23, resemble the significantly high serum levels of phosphate that are found in mice without Fgf23 activity [*Fgf23*-knockout (*Fgf23$^{-/-}$*) mice] (8, 32). Moreover, there are strikingly similar physical, morphological, biochemical, and molecular phenotypes in *Fgf23$^{-/-}$* and *klotho*-knockout

($klotho^{-/-}$) mice; these similarities led to the identification of Klotho as a cofactor for FGF23 and its receptor interactions (26, 28, 48, 50).

Klotho

The Klotho protein contains a putative signal sequence at its NH_2 terminus and a single transmembrane domain near its COOH terminus that is believed to anchor the protein to the membrane (39). The *klotho* gene encodes a type 1 membrane protein, which is predicted to be present on the cell surface of Klotho-expressing cells. The human *Klotho* gene has five exons and can generate two transcripts. The full-length transcript is 5.2 kb and encodes a 130-kDa membrane protein; once the short transmembrane domain is removed, this membrane form can be released into circulation. A recent study has suggested that A Disintegrin and Metalloproteinases (ADAM)-10 and ADAM-17 are capable of cleavage of Klotho from the plasma membrane, and that insulin can stimulate Klotho shedding (5). An alternative mRNA splicing event can generate another transcript, which codes for the NH_2-terminal region of Klotho and creates a protein with a molecular mass of ~65–70 kDa. Human *Klotho* has ~86% homology to mouse *klotho* and has been mapped to chromosome 13q12 (34). Klotho expression has been detected mostly in the distal convoluted tubules of the kidney, the parathyroid gland, and the epithelium of the choroid plexus in the brain (34). The exact role of Klotho in systemic regulation of mineral ion metabolism is not clear, other than that it gives the tissue specificity for FGF23 action. The question of whether there is a functional difference between the membrane form and the secreted form of Klotho needs additional study.

The inactivation of Klotho function in the mouse results in hyperphosphatemia and hypervitaminosis D (Fig. 1), which leads to the development of phenotypes resembling extensive premature aging-like features, including infertility and a shortened life span, in the mutant mouse (26). Interestingly, the phenotype of *klotho*-ablated mice resembles that of *Fgf23*-ablated mice (41, 48, 50); this observation led to the identification of the FGF23 signaling pathways.

FGF23 Signaling

FGFs are circulatory factors that can be grouped into several subfamilies (Table 2). Most of the FGFs are able to bind to cell surface FGFRs to activate downstream signaling events with diverse biological consequences. FGF11 to FGF14, however, are unable to bind and activate FGFRs (22); these FGFs are also termed "FGF homologous factors" (43). FGF23 is a member of the FGF19 subfamily. Studies have shown that FGF23 needs Klotho as a cofactor to interact with the FGFRs (Fig. 2). FGF23 has been shown to bind to multiple FGFRs, including FGFR1c, FGFR3c, and FGFR4 (Table 3) (9, 27, 38, 64). A recent study, however, claimed that neither FGFR3 nor FGFR4 is the principal mediator of the FGF23 effects in vivo (31). Klotho appears to allow FGF23 to bind to its receptor complex with much higher affinity than to FGFR alone. Furthermore, FGF23, in the presence of Klotho, can activate downstream signaling events, as indicated by the activation of early growth response element-1 (Egr-1) and the phosphorylation of FGF receptor substrate-2a, extracellular signal-regu-
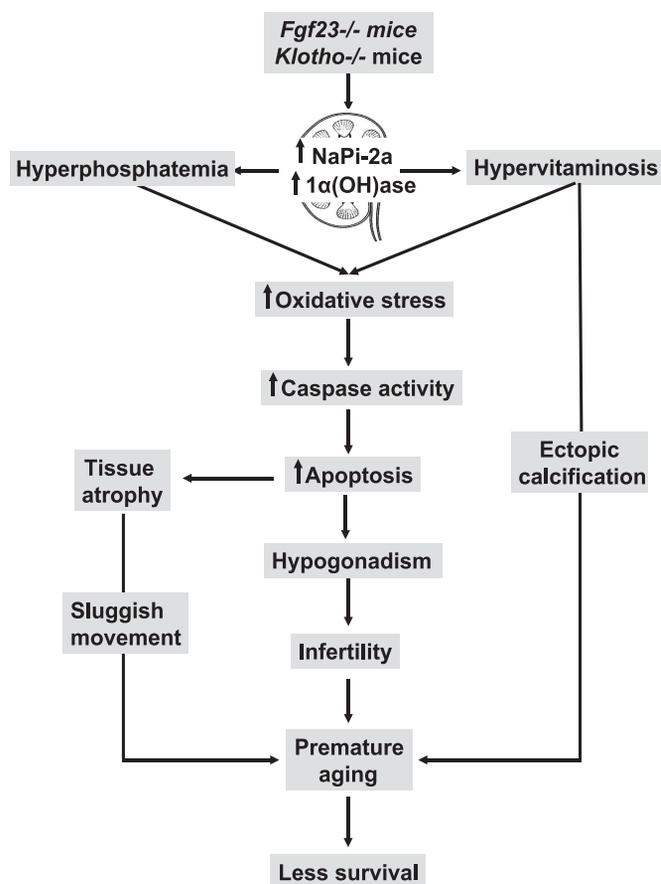

Fig. 1. Schematic diagram of possible events leading to premature aging-like phenotypes in $Fgf23^{-/-}$ and $klotho^{-/-}$ mice. The genetic inactivation of either *Fgf23* or *klotho* results in an increased renal expression of sodium-phosphate cotransporter (NaP_i)-2a and 1-α-hydroxylase [1α(OH)ase], which leads to hyperphosphatemia and hypervitaminosis D in these mutant mice. Such biochemical changes in serum phosphate and 1,25-dihydroxyvitamin D in $Fgf23^{-/-}$ and $klotho^{-/-}$ mice can induce oxidative stress to induce apoptosis and generate hypogonadism and generalized tissue atrophy. Moreover, hyperphosphatemia and hypervitaminosis D can induce vascular calcification (36, 51); the combined effect can result in premature aging-like phenotypes and early death in $Fgf23^{-/-}$ and $klotho^{-/-}$ mice.

lated kinase (ERK), p38, c-Jun NH_2-terminal kinase (JNK), and AKT proteins (16, 27, 64, 68). None of these signaling phosphoproteins was detected in human proximal tubule epithelial cells treated with FGF23 without Klotho. These observations suggest that the FGF23-FGFRs interaction and subsequent signaling activities need Klotho as a cofactor (16, 35).

Cellular, intracellular, transcellular, and pericellular mineral ion transport is achieved through a series of complex events that utilize both active and passive translocation processes. An important question that remains to be solved is how Klotho, produced in the distal tubules, can facilitate the FGF23-mediated phosphate transport process in the proximal tubules. Whether a secretory form of Klotho, released from the distal part of the tubules, can affect the functionality of the proximal part of the tubules is an important unresolved issue.

Genetic mutations in the FGF23 signaling components, such as Klotho or the FGFs, are also linked to diseases associated with altered mineral ion homeostasis and/or dysregulation of vitamin D metabolism. For example, osteoglophonic dysplasia is caused by missense mutations in the ligand-binding and

Table 2. *Members of FGF subfamilies*

FGF1 subfamily	FGF1
	FGF2
FGF4 subfamily	FGF4
	FGF5
	FGF6
FGF7 subfamily	FGF3
	FGF7
	FGF10
	FGF22
FGF8 subfamily	FGF8
	FGF17
	FGF18
FGF9 subfamily	FGF9
	FGF16
	FGF20
FGF11 subfamily	FGF11
	FGF12
	FGF13
	FGF14
FGF19 subfamily	FGF19
	FGF21
	FGF23

FGF, fibroblast growth factor. See References 22 and 43.

Table 3. *Ligand specificities of FGF receptor isoforms*

FGFR1b	FGFR1c	FGFR2b	FGFR2c	FGFR3b	FGFR3c	FGFR4
FGF1	FGF1	FGF1	FGF1	FGF1	FGF1	FGF1
FGF2	FGF2	FGF3	FGF2	FGF9	FGF2	FGF2
FGF3	FGF4	FGF7	FGF4		FGF4	FGF4
FGF10	FGF5	FGF10	FGF6		FGF8	FGF6
	FGF6	FGF20	FGF9		FGF9	FGF8
	FGF19	FGF22	FGF17		FGF17	FGF9
	FGF20		FGF18		FGF18	FGF15
	FGF21		FGF20		FGF20	FGF16
	FGF23				FGF23	FGF17
						FGF18
						FGF19
						FGF21
						FGF23

FGFR, FGF receptor. See References 9 and 38.

Is Klotho Essential for FGF23 Signaling?

Although Klotho-mediated FGF23 signaling is well documented, it is not yet clear whether FGF23 may also have Klotho-independent effects. This possibility was raised by the observation that FGF23 can have effects on cells that do not express Klotho. For instance, FGF23 has been shown to suppress osteoblast differentiation and bone mineralization in fetal rat calvaria cells (65). Similarly, FGF23 was shown to exhibit weak proliferative effects on a murine bone marrow-derived pro-B cell line that was overexpressing FGFRs in the absence of Klotho (69).

Moreover, a recent study has found that $Fgf23^{-/-}$ mice and $Fgf23^{-/-}/NaPi-2a^{-/-}$ double-knockout mice have a similar osteomalacic phenotype (less mineralization of the bone) despite opposing serum phosphate levels (high serum phosphate in $Fgf23^{-/-}$ mice, but low serum phosphate in $Fgf23^{-/-}/NaPi-2a^{-/-}$ mice). This result suggests that there may exist a serum phosphate-independent intrinsic effect of FGF23 on the bone (60). In fact, in vitro studies have suggested that FGF23 treatment can inhibit the mineralization of calvarial osteoblasts. Since Klotho is not expressed in osteoblasts, any effect of FGF23 on these cells would indicate that Klotho-independent effects of FGF23 exist, unless bone cells do express extremely low levels of Klotho that are undetectable with existing tools.

It is, however, necessary to mention that the injection of bioactive FGF23 protein into either $klotho^{-/-}$ mice or $Fgf23^{-/-}/klotho^{-/-}$ double-knockout mice does not produce any obvious changes in the serum levels of phosphate (41), implying that Klotho is essential for the in vivo systemic regulation of phosphate homeostasis. Interestingly, when bioactive FGF23 protein was injected into either wild-type or $Fgf23^{-/-}$ mice, the serum phosphate level was significantly reduced by 12 h after the injection (41). Of course, both wild-type and $Fgf23^{-/-}$ mice have endogenous Klotho therefore, the exogenous injection of FGF23 can influence systemic phosphate homeostasis. On the other hand, in $klotho^{-/-}$ or $Fgf23^{-/-}/klotho^{-/-}$ mice, in the absence of Klotho, FGF23 is unable to exert its phosphaturic effects. It is therefore likely that Klotho is essential for the FGF23-mediated systemic regulation of phosphate homeostasis. As mentioned above, the homozygous loss-of-function mutation in *Klotho* found in a patient with tumoral calcinosis has provided additional evidence for the

transmembrane domains of FGFR1 (66). In addition, a point mutation in the human *Klotho* gene in a 13-year-old patient was found to be associated with ectopic calcification, despite significantly elevated serum levels of FGF23 (20); the lack of Klotho function in this patient can attenuate the ability of FGF23 to exert its phosphate-lowering effects, which can eventually lead to severe vascular and soft tissue calcifications (20). In contrast to the above-mentioned observations, however, a few recent studies have claimed that there are Klotho-independent functions of FGF23 (65).
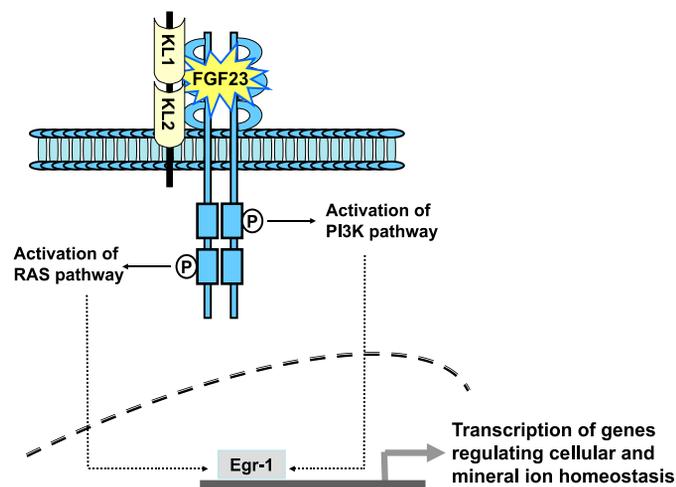

Fig. 2. Simplified schematic diagram of fibroblast growth factor (FGF)23 signaling. FGF23, in the presence of Klotho (KL), can bind to FGF receptors with high affinity to trigger downstream signaling that induces the expression of genes regulating cellular homeostasis and mineral ion homeostasis. PI3K, phosphatidylinositol 3-kinase; Egr-1, early growth response element-1.

dependence of FGF23 on Klotho in the regulation of phosphate and vitamin D homeostasis in humans (20).

Nonetheless, under pathological conditions in which there is a higher concentration of FGF23, it may be possible that FGF23 can exert nonspecific effects without Klotho; for example, FGF23 may be able to bind to its receptors with low affinity without Klotho (64). In this regard, the toxic effects of an extremely high serum level of FGF23 in patients with CKD, who have a reduced level of Klotho, need to be studied carefully (21, 46). Of relevance, the expression of *Klotho* mRNA and protein is significantly reduced in kidneys obtained from patients with CKD (24). How elevated serum levels of FGF23 might exert toxic effects in CKD patients whose tissue expression of Klotho is low is an important question, yet to be resolved.

Can FGF23 Be a Therapeutic Target?

Is anti-FGF23 therapy a feasible approach to fine-tune existing treatments for diseases associated with abnormal mineral ion metabolism? The clinical application of a controlled therapeutic inactivation of FGF23 might be of therapeutic benefit for patients with excessive urinary phosphate-wasting diseases, including ADHR, ARHR, and XLH. It is worth mentioning that the current treatment for hypophosphatemic diseases that are caused by genetic defects is mostly symptomatic, such as oral phosphate replacement. The prolonged use of such replacement can lead to the development of secondary hyperparathyroidism. Patients with CKD might benefit from the therapeutic lowering of FGF23, particularly if such an intervention could be provided in the early stages of the disease. Additional studies are needed to determine whether normalizing extremely high serum FGF23 levels in patients with late stages of CKD will reduce the various complications, including renal osteodystrophy. Elevated FGF23 levels in patients with CKD may reduce vitamin D activity by suppressing the expression of 1-α-hydroxylase. Since Klotho is an important component of FGF23 signaling, targeted manipulation of Klotho activity may also reduce the pathological effects of FGF23 and restore mineral balance. It is, however, worth mentioning that despite a number of studies proposing various roles for FGF23 in CKD patients, these studies have generated as many questions as they have answered. Whether a compensatory increased level of FGF23 in CKD patients is a protective response (in the earlier stage of the disease) or a harmful response (in the later stage of the disease) remains an unsettled issue, and any clinical interventions that target FGF23 therapeutically will need thoughtful consideration. In contrast to anti-FGF23 therapy, providing exogenous bioactive FGF23 protein might help restore the phosphate balance and reduce abnormal calcifications in patients with FTC, which is usually caused by the reduced activity of FGF23.

Concluding Remarks

Future studies will determine not only the therapeutic benefit of the manipulation of FGF23 signaling components but also its diagnostic and prognostic significance. The detection of circulatory FGF23 has become an important tool for determining the underlying causes of diseases associated with abnormal mineral ion metabolism. For instance, serum FGF23 levels can be used to differentially diagnose suspected TIO. Studies have suggested that the pretreatment serum level of FGF23 is a good predictor of the effectiveness of a vitamin D therapy in dialysis patients and might also be a useful marker for predicting the development of refractory hyperparathyroidism (40). FGF23 has been proposed to be an important biomarker of mortality in patients with early renal diseases, particularly in patients in whom the serum FGF23 level increased before the development of hyperphosphatemia (17). In light of these findings, it appears likely that FGF23 has diagnostic, prognostic, and therapeutic value.

Recent studies using mouse genetics have significantly enhanced our understanding of the in vivo regulation of phosphate homeostasis (49). The strikingly similar biochemical phenotypes of hypervitaminosis D, reduced serum PTH levels, and hyperphosphatemia in $Fgf23^{-/-}$ and $klotho^{-/-}$ single-knockout mice and $Fgf23^{-/-}/klotho^{-/-}$ double-knockout mice suggest that these factors constitute a common signaling pathway (Fig. 2). These results provide compelling genetic evidence of the in vivo importance of Klotho in regulating systemic phosphate homeostasis with FGF23. Finally, it is necessary to mention that FGF23-mediated functions are mostly Klotho dependent; Klotho, however, also has numerous FGF23-independent functions, including adipogenesis (7). Our challenge will be to use these experimental observations to fine-tune the existing therapeutic options by manipulating the FGF23-Klotho axis to treat patients suffering from the complications of abnormal mineral ion metabolism (44).

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