

PTH Regulation by the Klotho/FGF23 Axis in CKD



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The Parathyroid Gland

The parathyroid glands, first discovered by the Uppsala anatomist Ivar Sandstrom, appear as a pair of inferior and a pair of superior “bumps” on the dorsal side of the thyroid in adults [1]. The superior and inferior parathyroid glands are found in symmetrical positions in 80% and 70% of subjects, respectively. The parathyroid glands develop from the third and fourth pharyngeal pouches in humans between the fifth and twelfth weeks of gestation [2].

Normal adult parathyroid glands have a characteristic light yellow or red-brownish color and a soft consistency. Most glands are oval or bean-shaped, round, elongated, and bloated, and are multi lobulated. The normal gland size is 2–5 mm and weighs below 60 mg [3–5]. Autopsy studies revealed that 3–6% of normal individuals without hyperparathyroidism had fewer than four parathyroid glands and more than four glands were present in 2.5–6.7% of normal subjects, a condition called supernumerary glands. Supernumerary glands are defined as glands weighing more than 5 mg and located distant from the normal position and are usually found located in the thymus. Supernumerary glands occur in more than 10% of patients with multiple endocrine neoplasia (MEN) type 1 or secondary hyperparathyroidism (SHPT) [4–10].

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The inferior thyroid artery primarily supplies the parathyroid glands, which is the primary vascular supply to both upper and lower parathyroid glands in 76–86% of subjects [11]. The parathyroid glands located low in the mediastinum may be supplied by a thymic branch of the internal mammary artery or by a branch of the aortic arch.

Physiology of the Parathyroid Glands

The main function of the parathyroid glands is to produce parathyroid hormone (PTH). PTH is one of three key hormones modulating calcium and phosphate homeostasis; being the other two calcitriol (1,25-dihydroxyvitamin D) and fibroblast growth factor 23 (FGF23) [12].

PTH is synthesized as a 115-amino acid polypeptide called pre-pro-PTH, which is cleaved within parathyroid cells at the amino-terminal portion, first to pro-PTH (90 amino acids) and then to PTH (84 amino acids). The 84-amino acids form is the stored, secreted and biologically active hormone. The biosynthetic process is estimated to take less than one hour while the secretion by exocytosis takes place within seconds after induction of hypocalcemia. Once secreted, PTH is rapidly cleared from plasma through uptake mainly by the liver and kidney: 1–84 PTH is cleaved into active amino- and inactive carboxyl-terminal fragments that are then cleared by the kidney. Intact PTH has a plasma half-life of two to four minutes while the carboxyl-terminal fragments have half-lives that are 5–10 times greater [13].

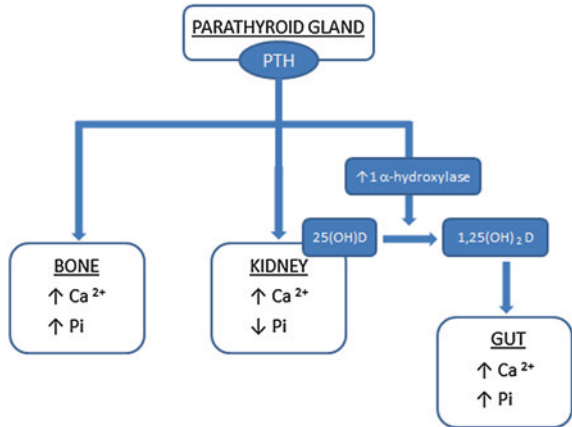
PTH Functions

The principal function of PTH is to maintain the extracellular fluid calcium concentration within a narrow normal range. The hormone acts directly on bone and kidney and indirectly on the intestine through its effects on synthesis of 1,25 OH₂D to increase serum calcium concentration; in turn, PTH production is closely regulated by the concentration of serum ionized calcium (Fig. 1).

In particular the increase in PTH release raises serum calcium concentration toward normal ranges in three ways:

- increased bone resorption, which occurs within minutes after PTH secretion increases
- increased intestinal calcium absorption mediated by increased production of calcitriol, the most active form of vitamin D, which occurs at least a day after PTH secretion increases
- decreased urinary calcium excretion due to stimulation of calcium reabsorption in the distal tubule, which occurs within minutes after PTH secretion increases.

Fig. 1 PTH actions



On a more chronic basis, PTH also stimulates the conversion of calcidiol (25-hydroxyvitamin D) to calcitriol in renal tubular cells, thereby stimulating intestinal calcium absorption as well as bone turnover. Calcitriol inhibits PTH secretion through an indirect negative feedback which consists in its positive action on calcium levels; it also has a direct inhibitory action on PTH biosynthesis and parathyroid cell proliferation [14].

Skeletal Actions of PTH

PTH acts on bone, the main reservoir of calcium, to release calcium in two different phases [15]. The immediate effect of PTH is to mobilize calcium from skeletal stores that are readily available and in equilibrium with the extracellular fluid. Secondly, PTH stimulates the release of calcium and phosphate by activation of bone resorption.

It is known that osteoblasts and not osteoclasts express PTH receptors, so osteoblasts are the main target for bone remodeling. However, osteoclasts are indirectly activated in this process [16]. In fact, under PTH stimulation, pre-osteoblasts mature into bone-forming osteoblasts that produce collagen and subsequently mineralize matrix [17]. Since the remodeling unit is always coupled, once pre-osteoblasts are stimulated, they release cytokines that can activate osteoclasts resulting in bone resorption.

Thus, osteoclast formation requires an interaction with osteoblasts, which may depend upon cell to cell contact or regulators of osteoclast formation such as RANK (the receptor activator of nuclear factor kappa-B), osteoprotegerin, and RANK ligand (RANKL) [18]. PTH, then, can increase osteoclasts in number and activity indirectly through its effects on RANKL and osteoprotegerin [19].

New evidences suggest that PTH can actually bind osteoclasts through a different and incompletely characterized PTH receptor with specificity for the carboxyl-terminal region of PTH (C-PTHrPs) [16].

The net effect of PTH on bone can vary according to the severity and chronicity of the PTH excess. Chronic exposure to high serum PTH concentrations, typical of primary or secondary hyperparathyroidism, results in net bone reabsorption, whereas intermittent administration of recombinant human PTH (both full-length 1–84 or a 1–34 amino acids fragment) has been seen to stimulate bone formation more than resorption. Moreover, specific elements of the PTH molecule seem essential for bone anabolism. PTH fragments 1–31 and 1–34 retain all of the biologic activity of the intact peptide; while amino-terminal truncation of the first two amino acids of PTH eliminates most of the cyclic adenosine monophosphate (cAMP) signaling, a pathway that seems important for the anabolic effect of PTH on bone [20, 21].

Renal Actions of PTH

PTH stimulates calcium and phosphate reabsorption in the kidney and promotes the activation of calcidiol to calcitriol leading to an increase of intestinal calcium absorption and ultimately calcium blood levels.

Reabsorption of calcium—Filtered calcium is reabsorbed by a great part of the nephron. The mechanisms by which it is reabsorbed and their regulation, vary according to the function of location along the nephron. Most filtered calcium is reabsorbed passively in the proximal tubule due to the favorable electrochemical gradients created by sodium and water reabsorption. In contrast, calcium transport is actively regulated in the distal nephron according to the needs of the organism. This takes place in the cortical thick ascending limb of the loop of Henle (cTAL), as well as in the distal convoluted tubule (DCT) and adjacent connecting segment (a small segment between the distal tubule and cortical collecting tubule). PTH acts at both cTAL and DCT in the distal tubule to stimulate calcium reabsorption. Thus, if PTH secretion falls appropriately after an increase in serum ionized calcium, the ensuing fall in tubular calcium reabsorption and the increase in calcium excretion will contribute to restoring normocalcemia. High serum calcium itself also contributes to the calciuresis, acting via the CaSR [22, 23].

Reabsorption of phosphate—PTH, along with fibroblast growth factor 23 (FGF23), is a key hormonal determinant of serum phosphate concentration. It inhibits mostly proximal but also distal tubular reabsorption of phosphorus. This effect is primarily mediated by decreased activity, internalization and degradation of the Npt2A and Npt2C, sodium-phosphate cotransporters in the luminal membrane of the proximal tubules that mediate tubular reabsorption of phosphate.

Synthesis of calcitriol—PTH stimulates the synthesis of 1-alpha hydroxylase in the proximal tubules and, therefore, the conversion of calcidiol to calcitriol. Approximately 50 percent of patients with primary hyperparathyroidism show high serum calcitriol concentrations as a result of this action of PTH. PTH also decreases the activity of a 24-hydroxylase that inactivates calcitriol. This is an extremely important action of PTH in maintaining calcium homeostasis in states of vitamin D deficiency.

Other Actions of PTH

The possibility that PTH acts on other tissues has been raised in the attempt to explain certain clinical manifestations of primary and secondary hyperparathyroidism. Some experimental studies have found effects of PTH on the intestine, liver, adipose tissue, cardiovascular function, and neuromuscular function. Impaired glucose tolerance and alterations in lipid metabolism reminiscent of the metabolic syndrome have been described in hyperparathyroidism. Similarly, patients with primary hyperparathyroidism have an increased incidence of hypertension, left ventricular hypertrophy, and neuromuscular abnormalities. In addition, chronic excess of PTH, along with the associated elevations in the level of FGF23, has been implicated in the pathogenesis of vascular calcification and hypertension in patients with CKD [24, 25]. These atypical effects of PTH may be mediated by differing PTH moieties and/or differing PTH receptors [16]. Moreover, the extent to which these abnormalities improve after parathyroidectomy varies considerably, and whether they are actually directly caused by PTH excess is uncertain.

PTH Regulation and Pathophysiology of Secondary Hyperparathyroidism

PTH secretion is primarily regulated by extracellular calcium, along with extracellular phosphate, calcitriol, and fibroblast growth factor 23 (FGF23).

Extracellular calcium—The relationship between the serum calcium concentration and PTH secretion is described by an inverse, sigmoidal curve, based on studies of calcium-regulated PTH secretion both in vivo and in vitro [13, 26]. In normal individuals, a decrease in serum ionized calcium concentration of as little as 0.1 mg/dL (0.025 mmol/L) produces a large increase in serum PTH concentration within minutes; conversely, an equally small increase in serum ionized calcium rapidly lowers the serum PTH concentration. Particularly, PTH secretion steeply increases to a maximum value of five times the basal rate of secretion as calcium concentration falls from normal to the range of 1.9–2.0 mmol/L (7.5–8.0 mg/dl, measured as total calcium).

Parathyroid cells have many methods of adapting to increased needs for PTH production. The first most rapid (within minutes) being the secretion of preformed hormone in response to hypocalcemia. The second, within hours, are the changes in gene activity and increased PTH-mRNA are induced by sustained hypocalcemia. Finally, protracted challenge leads within days to cellular replication to increase gland mass.

The change in calcium concentration is perceived by an exquisitely sensitive calcium-sensing receptor (CaSR) on the surface of parathyroid cells.

When activated by a small increase in serum ionized calcium, the calcium-CaSR complex acts via one or more guanine nucleotide-binding (G) proteins through second messengers, to inhibit PTH secretion and decrease renal tubular reabsorption of calcium by the CaSR's actions on parathyroid and kidney, respectively. Conversely, the effect of deactivation of the receptor by a small decrease in serum ionized calcium concentration is to stimulate PTH secretion and enhance renal tubular reabsorption of calcium.

Calcium regulates not only the release but also the synthesis and degradation of PTH, in all its molecular forms. During hypocalcemia, intracellular degradation of PTH decreases, and a greater proportion of PTH 1–84 is secreted in relation with other molecular species of the hormone. In comparison, during hypercalcemia, intracellular degradation of intact PTH increases and reduces the availability of biologically active PTH 1–84 for secretion; consequently, mostly biologically inactive carboxyl-terminal fragments of PTH are secreted during hypercalcemia [12, 14, 27]. The ionized fraction of blood calcium is the most important determinant of hormone secretion. Magnesium may influence hormone secretion in the same direction as calcium. As a matter of fact, severe intracellular magnesium deficiency impairs PTH secretion. However, it is unlikely that physiologic variation in magnesium concentration affects PTH secretion.

Extracellular phosphate—Phosphate, like calcium, acts as an extracellular ionic messenger. Hyperphosphatemia, as well as extracellular calcium, calcitriol and FGF23, modulates many parameters of parathyroid function by stimulating PTH secretion, probably in large part by increasing PTH-mRNA stability and promoting parathyroid cell growth [28, 29]. These responses may be mediated, partially by the induction of hypocalcemia owing to the increase in serum phosphate concentration.

However, small elevations in serum phosphate concentrations may not be sufficient to lower serum calcium concentration to a level that stimulates PTH secretion. In addition, there is increasing evidence that hyperphosphatemia, regardless of calcium and calcitriol serum concentrations, directly stimulates PTH synthesis as well as parathyroid cellular proliferation in patients with advanced renal failure (the most common cause of hyperphosphatemia) [28, 29]. The nature of the putative phosphate-sensing mechanism is unknown, however, it has been recently demonstrated that phosphate can bind the CaR and interfere with the physiological inhibitory effect of calcium on PTH secretion (ref).

Calcitriol—Vitamin D is synthesized in the skin, or may be ingested in the diet, and is transported to the liver, where it is metabolized to form 25-hydroxyvitamin D (Fig. 2). 25-hydroxyvitamin D is the main storage form of vitamin D and is the

substrate for the enzyme 1 α -hydroxylase, which converts it into 1,25-dihydroxyvitamin D, the major circulating active metabolite of vitamin D. 1,25-dihydroxyvitamin D is responsible for the effects of vitamin D on calcium and phosphorus metabolism, the maintenance of bone health, and the regulation of the parathyroid glands [30, 31]. Parathyroid cells contain vitamin D receptors, and the PTH gene contains a vitamin D-response element. Calcitriol, by binding to the vitamin D receptor, inhibits PTH gene expression and, therefore, PTH synthesis [32]. Calcitriol also inhibits parathyroid cell proliferation. Some of the actions of calcitriol on parathyroid function are related to its ability to increase the expression of the CaSR.

FGF23—Fibroblast growth factor-23 (FGF23) is a 251-amino-acid protein (molecular weight: 26 kDa) that was found to be synthesized and secreted by osteocytes and osteoblasts [33]. FGF23 binds to and activates FGFR1, which is functional only if co-expressed with the Klotho transmembrane protein, as a Klotho-FGF receptor complex. When stimulated by phosphate increase and calcitriol elevation, FGF23 directly increases urinary fractional excretion of phosphate (FePi) in the proximal tubule by reducing the expression of type II sodium-phosphate cotransporters (NPT2a and NPT2c) and, indirectly it reduces phosphate absorption in the gut by suppressing 25-hydroxyvitamin D-1 α -hydroxylase (1 α -hydroxylase) activity.

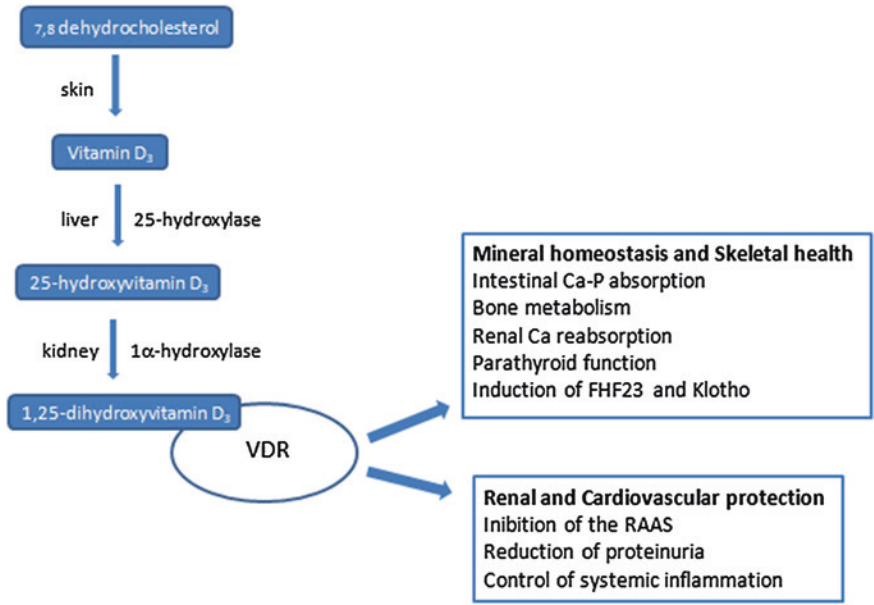


Fig. 2 Beneficial effects of VDR activation. Vitamin D actions require vitamin D activation to its hormonal form, 1,25-dihydroxyvitamin D, by renal and extrarenal 1 α -hydroxylases to bind and activate the vitamin D receptor (VDR). Upon activation, ligand-bound VDR acts as a transcription factor to regulate the expression of genes involved in vitamin D maintenance of mineral homeostasis, skeletal health, renal and cardiovascular protection

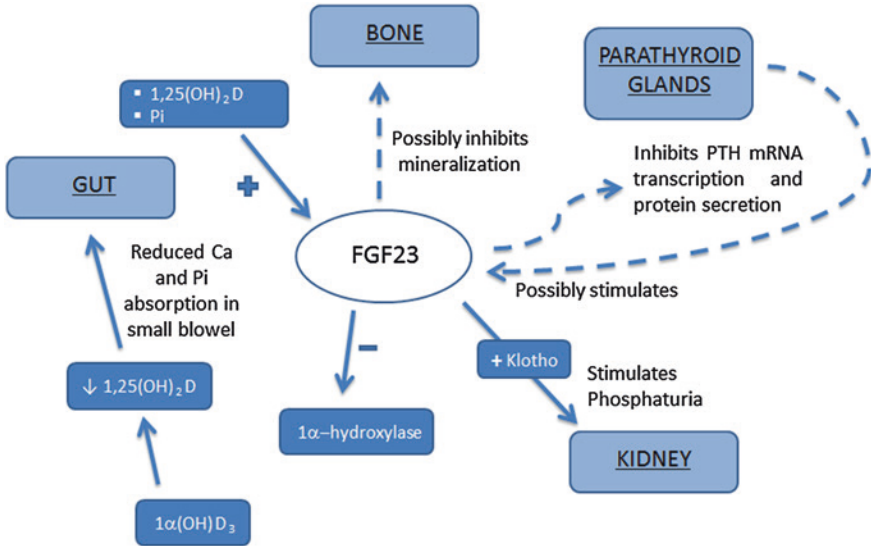


Fig. 3 FGF23 functions

In addition to its phosphaturic action, FGF23 exerts direct actions on parathyroid glands, inhibiting PTH synthesis and secretion [34] (Fig. 3). Therefore, PTH, calcitriol, and FGF23 all participate in maintaining both calcium and phosphate homeostasis.

Klotho

The Klotho gene encodes a single transmembrane protein belonging to glycosidase family 1 and is mainly expressed in the renal tubules [35, 36]. The Klotho protein forms a constitutive complex with several FGF receptor isoforms, such as FGFR1c, 3c, and 4 and markedly increases the specificity of FGFR for FGF23. That is, Klotho functions as a co-receptor for FGF23 [37]. The fact that FGF23 requires Klotho as a co-receptor is demonstrated by the finding that Klotho-deficient mice and FGF23-deficient mice have the same phenotype [38]. Extremely high serum FGF23 concentrations do not have adverse effects on Klotho-deficient mice, indicating that Klotho is required for FGF23 signaling [39].

Klotho is not a kidney-specific protein: Klotho mRNA is also expressed at high levels in the parathyroid gland, whereas it is barely expressed in the thyroid gland, intestinal tract, and liver. Immunohistochemical analyses have confirmed the specific localization of Klotho to the parathyroid gland and have also shown the presence of FGFR1 and FGFR3 in parathyroid tissue. These results provide further evidence that the parathyroid gland is the target organ of FGF23, due to

the presence of FGFR-Klotho receptor complexes. Klotho is also expressed in other organs such as the brain, but it is particularly present in the distal convoluted tubules, in the proximal convoluted tubules and in the inner medullary collecting duct of the kidney [40]. In the kidney Klotho binds to the FGF receptor (FGFR) which in turn binds to FGF23 leading to the formation of a Klotho/FGFR/FGF23 complex with subsequent activation of FGF23 signal transduction. Klotho is required to convert different types of FGFR (FGFR1c, FGFR3c, FGFR4) into specific receptors for FGF23, increasing the affinity for it and not for other FGFs [41, 42].

There is also a soluble circulating form of Klotho which can still bind FGFRs but also functions as a humoral factor with pleiotropic activities including regulation of oxidative stress, growth factor signaling, and ion homeostasis [43]. Soluble Klotho can be generated in two ways: the extracellular domain of transmembrane Klotho can be shed by secretases and released into the circulation as cleaved Klotho; alternatively secreted Klotho can be generated by alternative transcript splicing. Interestingly soluble Klotho can bind to FGFR-FGF23 to form the active receptor complex but it seems to prevent high FGF23-induced effects [44]. In physiological conditions the kidney is the main responsible for the maintenance of Klotho homeostasis: it produces and releases cleaved Klotho and it also clears it from the blood through transcytosis in renal tubules. In patients with chronic kidney disease (CKD), as expected, Klotho levels are much lower than in healthy people and Klotho mRNA expression in the kidney decreases as GFR declines.

Klotho indirectly regulates PTH production through modulation of plasma levels of FGF23, active vitamin D and phosphate. In addition, membrane Klotho may have a direct effect on PTH production and release. Quantitative RT-PCR experiments have shown that Klotho mRNA is expressed at high levels in the parathyroid gland, whereas it is barely expressed in the thyroid gland, intestinal tract, and liver. Immunohistochemical analyses have confirmed the specific localization of Klotho to the parathyroid gland and have also shown the presence of FGFR1 and FGFR3 in parathyroid tissue. These results provide further evidence that the parathyroid gland is the target organ of FGF23, due to the presence of FGFR-Klotho receptor complexes. In physiological conditions FGF23 directly decreases PTH production and it also increases Klotho in the parathyroid gland, which facilitates its suppression of PTH production. Moreover, high vitamin D levels increase Klotho expression, which in turn promotes phosphaturia and suppresses 1,25 Vitamin D production [45].

Parathyroid Resistance to PTH and FGF23 Action in CKD

During CKD, the decrease in nephron number should activate the FGF23/ α Klotho endocrine axis [46]. FGF23 is strictly involved in renal phosphate handling. When nephronic mass and GFR decrease, serum phosphate load on nephrons increases. Even in normo-phosphatemic CKD patients a positive phosphate balance occurs.

To counterbalance a phosphate overload, both FGF23 and PTH increase. Probably, CKD is the most common cause of chronically elevated serum FGF23 levels [47]; circulating FGF23 levels increase in parallel with the progressive fall in GFR and they can reach high and maladaptive concentrations in ESRD patients [48].

Klotho deficiency plays a major role in these responses. Klotho mRNA expression decreases progressively with GFR impairment, defining a reduction in serum klotho levels, starting from CKD stage 1. Serum Klotho reduction precedes serological elevation of FGF23, PTH and P. The progressive reduction of Klotho expression in renal and parathyroid tissue defines a FGF23 resistance state and an improvement in FGF23 synthesis [49].

Ben-Dov et al. reported Klotho expression in the parathyroid gland and showed that FGF23 significantly reduces *PTH* gene expression and PTH secretion by activating the mitogen-activated protein kinases (MAPK) pathway [50] or secondly through the Klotho-independent phospholipase C gamma (PLC γ)-dependent activation of the nuclear factor of activated T-cells (NFAT) cascade [51]. Experimental stimulation of activated FGF23 resulted in a rapid reduction in PTH expression and decreased PTH secretion. The decrease in serum PTH levels induced by FGF23 is partially reversed by MEK inhibition, indicating that the MAPK pathway is important for the suppression of PTH secretion by parathyroid FGF23-Klotho receptor complex signaling. FGF23 inhibits vitamin D metabolism, reduces serum phosphorus levels, and acts directly on the parathyroid gland to lower serum PTH levels. Thus, FGF23 targets the parathyroid gland and is involved in endocrine control signaling between bone cells and the parathyroid gland. Paradoxically, despite very high serum FGF23 levels in dialysis patients, PTH does not necessarily decrease [52, 53]. When FGF23 is administered to normal rats for 2 consecutive days, *PTH* mRNA significantly decreases in the parathyroid gland. However, in uremic rats with hyperparathyroidism, administration of FGF23 does not reduce *PTH* mRNA levels [27]. In vitro, the hyperplastic parathyroid gland secretes more PTH than the normal gland, but the addition of a significantly higher concentration of FGF23 does not reduce PTH secretion.

Galitzer et al. showed that rats with chronic kidney disease (CKD) are resistant to FGF23 in the parathyroid gland, indicating that CKD correlates with the down-regulation of parathyroid Klotho and FGFR expression and FGF23 signaling [13]. *PTH* mRNA expression is known to be increased in CKD rats. Then, as CKD progresses into secondary hyperparathyroidism, Klotho and FGFR protein and mRNA levels decrease. In experiments investigating the response to FGF23 in the parathyroid gland of CKD rats, FGF23 administration did not decrease PTH expression in rats with advanced CKD at 6 weeks. Thus, Klotho and *FGFR* mRNA levels decrease in the parathyroid glands of CKD rats and Klotho and FGFR expression decrease in cultured glands in vitro. These results indicate that the increase in PTH expression in secondary hyperparathyroidism and the decrease in Klotho and FGFR expression are related to the resistance of the CKD rat parathyroid gland to FGF23.

Patients with secondary hyperparathyroidism maintain high serum PTH concentrations, but patients with uremia show extremely high FGF23 levels. To clarify the mechanism of resistance to FGF23 in uremic patients, Komaba et al.

investigated the expression of Klotho and FGFR in hyperplastic parathyroid tissue by immunohistochemistry [54]. Klotho and FGFR levels are significantly reduced in the hyperplastic parathyroid glands of uremic patients, when compared to normal tissues. These trends are more pronounced in tissues with advanced disease, accompanied by nodular hyperplasia.

These results answered some of the questions regarding the mechanism whereby serum PTH levels are resistant to extremely high serum FGF23 levels. Furthermore, it was shown that decreased levels of the Klotho-FGFR complex in the hyperplastic parathyroid gland may contribute to this resistance mechanism. This could explain the emergence of frameworks of SHPT. Despite its direct inhibitory action on the parathyroid, FGF23 also contributes to the progression of secondary hyperparathyroidism via reduction of renal calcitriol synthesis and subsequent decrease in active intestinal calcium and phosphate absorption [55].

References

1. Sandstrom IV. *Omen ny kortel hos menniskan och atskilige baggdjur lakarefore rings* (ed). Upsala. 1880;441–71.
2. Burke JF, Chen H, Gosain A. Parathyroid conditions in childhood. *Semin Pediatr Surg.* 2014;23:66–70.
3. Scharpf J, Randolph G. Thyroid and parathyroid glands, chapter 33. In: KJ Lee's, Chan Y, Goddard JC, editors. *Essential otolaryngology*. 11th ed. McGraw Hills Company Ltd. 2015.
4. Akerstrom G, Malmaeus J, Bergstrom R. *Surgical anatomy of human parathyroid glands*. Surgery. 1984;95:14–21.
5. Tominaga Y. Embryology and anatomy of parathyroid gland. In: Tominaga Y, editor. *Surgery for hyperparathyroidism—focusing on secondary hyperparathyroidism*. Japan: TOKYO IGAKUSYA; 2017. p. 1–8.
6. Wang C. The anatomic basis of parathyroid surgery. *Ann Surg.* 1976;183:271–5.
7. Gilmour JR. The gross anatomy of the parathyroid glands. *J Pathol.* 1938;46:1098.
8. Numano M, Tominaga Y, Uchida K, et al. Surgical significance of supernumerary parathyroid glands in renal hyperparathyroidism. *World J Surg.* 2000;24:1330–4.
9. Pattou FN, Pellissier LC, Noel C, et al. Supernumerary parathyroid glands: frequency and surgical significance in treatment of renal hyperparathyroidism. *World J Surg.* 2000;24:1330–4.
10. Edis AJ, Purnell DC, van Heeden JA. The undecoded “parathyroid”. An occasional cause of failed neck exploration for hyperparathyroidism. *Ann Surg.* 1979;190:64–8.
11. Alveryd A. Parathyroid glands in thyroid surgery. I. Anatomy of parathyroid glands. II. Postoperative hypoparathyroidism—Identification and autotransplantation of parathyroid glands. *Acta Chir Scand.* 1968;389:1–120.
12. Hasegawa H, Nagano N, Urakawa I, et al. Direct evidence for a causative role of FGF23 in the abnormal renal phosphate handling and vitamin D metabolism in rats with early-stage chronic kidney disease. *Kidney Int.* 2010;78:975–70.
13. Galitzer H, Ben-Dov IZ, Silver J, et al. Parathyroid cell resistance to fibroblast growth factor 23 in secondary hyperparathyroidism of chronic kidney disease. *Kidney Int.* 2010;77:211–8.
14. Portale AA, Booth BE, Halloran BP, et al. Effect of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J Clin Invest.* 1984;73:1580–9.

15. Almaden Y, Canalejo A, Hernandez A, et al. Direct effect of phosphorus on PTH secretion from whole rat parathyroid glands in vitro. *J Bone Miner Res.* 1996;11:970–6.
16. Moallem E, Kilav R, Silver J, et al. RNA-Protein binding and posttranscriptional regulation of parathyroid hormone gene expression by calcium and phosphate. *J Biol Chem.* 1998;273:5253–9.
17. Stubbs JR, Liu S, Tang W, et al. Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. *J Am Soc Nephrol.* 2007;18:2116–24.
18. Silver J, Sela SB, Naveh-Many T. Regulation of parathyroid cell proliferation. *Curr Opin Nephrol Hypertens.* 1997;6:321–6.
19. Patel SR, Ke HQ, Vanholder R, Koenig RJ, Hsu CH. Inhibition of calcitriol receptor binding to vitamin D response elements by uremic toxins. *J Clin Invest.* 1995;96:50–9.
20. Patel S, Ke HQ, Vanholder R, Hsu CH. Inhibition of nuclear uptake of calcitriol receptor by uremic ultrafiltrate. *Kidney Int.* 1994;46:129–33.
21. Naveh-Many T, Rahamimov R, Livni N, Silver J. Parathyroid cell proliferation in normal and chronic renal failure rats. The effects of calcium, phosphate, and vitamin D. *J Clin Invest.* 1995;96:1786–93.
22. Mathias RS, Nguyen HT, Zhang MYH, Portale AA. Reduced expression of the renal calcium-sensing receptor in rats with experimental chronic renal insufficiency. *J Amer Soc Nephrol.* 1998;9(11):2067–74.
23. Kifor O, Moore FD, Wang P, Goldstein M, Vassilev P, Kifor I, et al. Reduced immunostaining for the extracellular Ca²⁺-sensing receptor in primary and uremic secondary hyperparathyroidism. *J Clin Endocrinol Metab.* 1996;81:1598–606.
24. Roussanne MC, Lieberherr M, Souberbielle JC, Sarfati E, Druke T, Bourdeau A. Human parathyroid cell proliferation in response to calcium, NPS R-467, calcitriol and phosphate. *Eur J Clin Invest.* 2001;31(7):610–6.
25. Chikatsu N, Fukumoto S, Takeuchi Y, Suzawa M, Obara T, Matsumoto T, et al. Cloning and characterization of two promoters for the human calcium-sensing receptor (CaSR) and changes of CaSR expression in parathyroid adenomas. *J Biol Chem.* 2000;275(11):7553–7.
26. Lundgren S, Carling T, Hjälm G, Juhlin C, Rastad J, Pihlgren U, et al. Tissue distribution of human gp330/megalyn, a putative Ca²⁺-sensing protein. *J Histochem Cytochem.* 1997;45:383–92.
27. Canalejo R, Canalejo A, MartinezMoreno JM, RodriguezOrtiz ME, Estepa JC, Mendoza FJ, et al. FGF23 fails to inhibit uremic parathyroid glands. *J Amer Soc Nephrol.* 2010;21(7):1125–35.
28. Densmore MJ, Sato T, Mannstadt M, et al. Interrelated role of Klotho and calcium-sensing receptor in parathyroid hormone synthesis and parathyroid hyperplasia. *Proc Natl Acad Sci U S A.* 2018.
29. Fukagawa M, Kaname S-Y, Igarashi T, Ogata E, Kurokawa K. Regulation of parathyroid hormone synthesis in chronic renal failure in rats. *Kidney Int.* 1991;39:874–81.
30. Dusso AS, Pavlopoulos T, Naumovich L, Lu Y, Finch J, Brown AJ, et al. P21 (WAF1) and transforming growth factor- α mediate dietary phosphate regulation of parathyroid cell growth. *Kidney Int.* 2001;59(3):855–65.
31. Cozzolino M, Lu Y, Finch J, Slatopolsky E, Dusso AS. P21 (WAF1) and TGF- α mediate parathyroid growth arrest by vitamin D and high calcium. *Kidney Int.* 2001;60(6):2109–17.
32. Gogusev J, Duchambon P, Stoermann-Chopard C, Giovannini M, Sarfati E, Drüeke TB. De novo expression of transforming growth factor- α in parathyroid gland tissue of patients with primary or secondary uraemic hyperparathyroidism. *Nephrol Dial Transplant.* 1996;11:2155–62.
33. Arcidiacono MV, Cozzolino M, Spiegel N, Tokumoto M, Yang J, Lu Y, et al. Activator protein 2 α mediates parathyroid TGF- α self-induction in secondary hyperparathyroidism. *J Am Soc Nephrol.* 2008;19(10):1919–28.

34. Cordero JB, Cozzolino M, Lu Y, Vidal M, Slatopolsky E, Stahl PD, et al. 1,25-Dihydroxyvitamin D down-regulates cell membrane growth- and nuclear growth-promoting signals by the epidermal growth factor receptor. *J Biol Chem.* 2002;277(41):38965–71.
35. Mian IS. Sequence, structural, functional, and phylogenetic analyses of three glycosidase families. *Blood Cells Mol Dis.* 1998;24:83–100.
36. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature.* 1997;390(6655):45–51.
37. Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M. Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem.* 2006;281:6120–3.
38. Nakatani T, Sarraj B, Ohnishi M, Densmore MJ, Taguchi T, Goetz R, Mohammadi M, Lanske B, Razzaque MS. In vivo genetic evidence for klotho-dependent, fibroblast growth factor 23 (Fgf23)-mediated regulation of systemic phosphate homeostasis. *FASEB J.* 2009;23:433–41.
39. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature.* 2006;444:770–4.
40. Sakaguchi K. Acidic fibroblast growth factor autocrine system as a mediator of calcium-regulated parathyroid cell growth. *J Biol Chem.* 1992;267:24554–62.
41. Matsushita H, Hara M, Endo Y, Shishiba Y, Hara S, Ubara Y, et al. Proliferation of parathyroid cells negatively correlates with expression of parathyroid hormone-related protein in secondary parathyroid hyperplasia. *Kidney Int.* 1999;55(1):130–8.
42. Lewin E, Garfia B, Almaden Y, Rodriguez M, Olgaard K. Autoregulation in the parathyroid glands by PTH/PTHrP receptor ligands in normal and uremic rats. *Kidney Int.* 2003;64(1):63–70.
43. Volovelsky O, Cohen G, Kenig A, Wasserman G, Dreazen A, Meyuhos O, et al. Phosphorylation of ribosomal protein S6 mediates mammalian target of rapamycin complex 1-induced parathyroid cell proliferation in secondary hyperparathyroidism. *J Am Soc Nephrol.* 2016;27(4):1091–101.
44. Gunther T, Chen ZF, Kim J, Priemel M, Rueger JM, Amling M, et al. Genetic ablation of parathyroid glands reveals another source of parathyroid hormone. *Nature.* 2000;406(6792):199–203.
45. Correa P, Akerstrom G, Westin G. Underexpression of Gcm2, a master regulatory gene of parathyroid gland development, in adenomas of primary hyperparathyroidism. *Clin Endocrinol (Oxf).* 2002;57(4):501–5.
46. Morito N, Yoh K, Usui T, Oishi H, Ojima M, Fujita A, et al. Transcription factor MafB may play an important role in secondary hyperparathyroidism. *Kidney Int.* 2018;93(1):54–68.
47. Naveh-Many T, Silver J. Transcription factors that determine parathyroid development power PTH expression. *Kidney Int.* 2018;93(1):7–9.
48. Arnold A, Brown MF, Ureña P, Gaz RD, Sarfati E, Drüeke TB. Monoclonality of parathyroid tumors in chronic renal failure and in primary parathyroid hyperplasia. *J Clin Invest.* 1995;95:2047–54.
49. Chudek J, Ritz E, Kovacs G. Genetic abnormalities in parathyroid nodules of uremic patients. *Clin Cancer Res.* 1998;4:211–4.
50. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuro-o M, Mohammadi M, Sirkis R, Naveh-Many T, Silver J. The parathyroid is a target organ for FGF23 in rats. *J Clin Invest.* 2007;117(12):4003–8.
51. Falchetti A, Bale AE, Amorosi A, Bordi C, Cicchi P, Bandini S, Marx SJ, Brandi ML. Progression of uremic hyperparathyroidism involves allelic loss on chromosome 11. *J Clin Endocrinol Metab.* 1993;76:139–44.

52. Gutierrez O, Isakova T, Rhee E, Shah A, Holmes J, Collerone G, Jüppner H, Wolf M. Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J Am Soc Nephrol.* 2005;16:2205–15.
53. Larsson T, Nisbeth U, Ljunggren O, Jüppner H, Jonsson KB. Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. *Kidney Int.* 2003;64:2272–9.
54. Komaba H, Goto S, Fujii H, et al. Depressed expression of Klotho and FGF receptor 1 in hyperplastic parathyroid glands from uremic patients. *Kidney Int.* 2010;77:232–8.
55. Tahara H, Imanishi Y, Yamada T, Tsujimoto Y, Tabata T, Inoue T, Inaba M, Morii H, Nishizawa Y. Rare somatic inactivation of the multiple endocrine neoplasia type 1 gene in secondary hyperparathyroidism of uremia. *J Clin Endocrinol Metab.* 2000;85:4113–7.