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Pathophysiology of the Chronic Kidney Disease – Mineral Bone Disorder (CKD-MBD)

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Abstract

Purpose of review—The causes of excess cardiovascular mortality associated with chronic kidney disease (CKD) have been attributed in part to the CKD-mineral bone disorder syndrome (CKD-MBD), wherein, novel cardiovascular risk factors have been identified. The causes of the CKD-MBD are not well known and they will be discussed in this review

Recent findings—The discovery of WNT (portmanteau of wingless and int) inhibitors, especially Dickkopf 1 (Dkk1), produced during renal repair as participating in the pathogenesis of the vascular and skeletal components of the CKD-MBD implied that additional pathogenic factors are critical, and whose discovery lead to the finding that activin A is a second renal repair factor circulating in increased levels during CKD. Activin A derives from peritubular myofibroblasts of diseased kidneys, wherein it stimulates fibrosis, and decreases tubular klotho expression. The type 2 activin A receptor, ActRIIA, is induced by CKD in atherosclerotic aortas specifically in vascular smooth muscle cells (VSMC). Inhibition of ActRIIA signaling by a ligand trap inhibited CKD induced VSMC dedifferentiation, osteogenic transition and atherosclerotic calcification. Inhibition of ActRIIA signaling in the kidney decreased renal fibrosis and proteinuria.

Summary—These studies demonstrate that circulating renal repair factors are causal of the CKD-MBD and CKD associated cardiovascular disease, and identify ActRIIA signaling as a therapeutic target in CKD that links progression of renal disease and vascular disease.

Keywords

CKD-MBD; activin; dickkopf 1; klotho; FGF23; parathyroid hormone; renal osteodystrophy; vascular calcification

Introduction

Kidney diseases are associated with an extremely high mortality, which is related to their production of cardiovascular disease [1]. The kidney disease produced increase in

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cardiovascular risk even extends to type 2 diabetes, where the presence of mild to moderate kidney disease increases atherosclerotic cardiovascular disease risk by 87% [2]. The causes of the increased cardiovascular risk associated with kidney diseases partly reside in the chronic kidney disease – mineral bone disorder (CKD-MBD) syndrome [3]. Three novel cardiovascular risk factors (hyperphosphatemia, vascular calcification, and elevated fibroblast growth factor 23 (FGF23) levels) have been discovered within the CKD-MBD [4-6], and their risk factor status confirmed in the general population [7-9]. The CKD-MBD begins early in CKD (stage 2) [10-13] consisting of vascular dedifferentiation/calcification, an osteodystrophy, loss of klotho and increased FGF23 secretion [10], and progress into its causes have been made [13-16], but they are mostly unknown.

Discoveries in the Pathogenesis of the CKD-MBD

Multiple investigators and we have shown that kidney diseases reactivate developmental programs involved in nephrogenesis during disease stimulated renal repair [17-21]. Among the nephrogenic factors reactivated in renal repair, the Wnt (portmanteau of Wingless and Integrated) family is critical for tubular epithelial reconstitution [20-22]. In the control of Wnt function, canonical signaling transcriptionally induces the expression of a family of Wnt inhibitory proteins which are secreted proteins that serve to restrict the distances of Wnt stimulation to autocrine or paracrine factors [23-27]. The Wnt inhibitors are circulating factors, and the family includes the Dickkopfs (Dkk). We have shown that various forms of kidney disease increase renal expression of Wnt inhibitors and increase their levels in the circulation [14, 18].

Neutralization of a key Wnt inhibitor elevated in the circulation in CKD, Dkk1, inhibited CKD induced vascular dedifferentiation, vascular calcification, and renal osteodystrophy [14]. This effect was surprising since Wnt signaling in the vascular smooth muscle is implicated in stimulating osteoblastic transition and vascular calcification [28, 29]. However, recent studies demonstrate that Dkk1 mediated inhibition of aortic Wnt7b stimulates smad mediated aortic endothelial-mesenchymal transition (EndMT) and vascular calcification [30]. EndMT is a developmental physiologic process involved in the development of the cardiac valves, the cardiac septum and the aortic root [31, 32], and it may [33] or may not [34] contribute to cardiac fibrosis in various adult disease states. Since EndMT is a process driven by smad transcription factors activated by factors in the transforming growth factor beta (TGF β) superfamily [35], we investigated whether other factors involved in attempted renal repair during kidney disease derive from the TGF β superfamily and are increased in the circulation during CKD. Of the TGF β superfamily members, activin, a known renal developmental factor and circulating hormone, was the primary candidate [19, 36].

Activin is increased in the circulation by CKD associated with increased expression of activin in the kidney [37]. Surprisingly, the activin type 2A receptor (ActRIIA) was induced by CKD in the aortic vascular smooth muscle and not the endothelium. We demonstrated that inhibiting ActRIIA signaling using an ActRIIA ligand trap blocked CKD stimulated vascular smooth muscle osteoblastic transition, vascular calcification, and inhibited renal fibrosis [37]. We found that inhibiting activin signaling decreased renal Wnt activation and

circulating Dkk1. As a result a composite vascular effect of indirectly increasing endothelial Wnt signaling through loss of Dkk1 in the circulation, and decreased vascular smooth muscle Wnt activation by blocking direct activin VSMC effects was produced by the ActRIIA ligand trap.

Key Pathogenic Components of the CKD-MBD: *DKK1 and Activin*

Much work needs to be done to characterize the effects of renal injury and the various kidney diseases on the production and maintenance of circulating factors causing cardiovascular and skeletal diseases, bringing the preclinical studies to human pathobiology. Although elevations in plasma DKK1, sclerostin (another Wnt inhibitor elevated in CKD and partly of renal origin) and activin have been found in human kidney diseases [37, 38], these studies are preliminary and need confirmation and characterization.

FGF23

FGF23 is the original phosphatonin (hormone regulating phosphate excretion) discovered in studies of autosomal dominant hypophosphatemic rickets and oncogenic osteomalacia [39]. FGF23 is produced by osteocytes and osteoblasts, and it represents direct bone-kidney and bone-parathyroid connections in the multiorgan systems biology involved in the CKD-MBD [40]. Circulating FGF23 levels rise after mild renal injury and progressively increase several fold during the course of CKD due to increased osteocyte secretion as well as decreased catabolism by the injured kidney. FGF23 levels rise prior to changes in calcium, phosphorus, or PTH levels and are now recognized as one of the earliest detectable biomarkers of the CKD-MBD [11, 41].

FGF23 levels have been associated with cardiovascular risk in CKD, and kidney transplant loss and mortality [42, 43]. In humans and animal models, Faul et al demonstrated that FGF23 is not only a biomarker associated with cardiovascular risk in CKD, but is also a direct pathogenic factor causing left ventricular hypertrophy (LVH) through activation of the calcineurin-NFAT pathway in cardiac myocytes [44].

Recently, the pathogenic nature of circulating FGF23 has become more intriguing. Andrukhova et al showed that FGF23 directly regulates the abundance of the thiazide-sensitive sodium-chloride transporter (NCC) in the distal convoluted tubule, leading to increased distal sodium reabsorption, effective circulating volume expansion, hypertension, and cardiac hypertrophy, effects that were abrogated by a thiazide diuretic [45]. Interestingly, these FGF23-mediated effects on cardiovascular pathophysiology were augmented in animal models ingesting a low sodium diet (which stimulates aldosterone secretion), raising the possibility of an interaction between FGF23 and the renin-angiotensin-aldosterone axis in CKD-stimulated cardiovascular disease. Furthermore, Humalda et al demonstrated that humans with higher baseline FGF23 levels had a reduced antiproteinuric response to dietary sodium restriction and ACE inhibitor therapy, which has been associated with heightened cardiovascular and end-stage renal disease (ESRD) risk in CKD [46]. Andrukhova et al also demonstrated that FGF23 promotes calcium reabsorption through stimulation of the apical calcium entry channel, TRPV5, in the distal tubule [47]. Because the calcium entry channel is also regulated by klotho [48], the Andrukhova et al

findings [45, 47] raise the issue of the mechanism of klotho's actions. Are they direct through glucuronidase activity and FGF23 independent, or as the FGF23 co-receptor and FGF23 dependent?

Klotho

FGF23 signaling through FGF receptors typically requires the co-receptor function of membrane-bound α klotho. Alpha klotho is highly expressed in very few tissues and defines the targets of FGF23 as the proximal and distal renal tubules, the parathyroid glands and the brain [49]. Klotho also circulates as a physiologically active hormone after either being cleaved at the cell surface by ADAM-10 and -17 in the distal renal tubule. Alternative splicing of the *klotho* gene transcript produces a soluble protein with only one klotho domain of unknown function [50]. Cleaved klotho directly regulates calcium and phosphorus excretion in the kidney and participates in systemic mineral homeostasis by regulating 1-alpha hydroxylase activity, PTH and FGF23 secretion [51, 52]. Klotho expression is significantly reduced by kidney injuries such as acute kidney injury, glomerulonephritis, calcineurin inhibitor use and chronic allograft injury [53]. We have shown that the reduction of klotho is in part related to activating and ActRIIA signaling [37]. The resulting klotho deficiency limits its regulation of FGF23 production and leaves hyperphosphatemia as the principal regulator of FGF23 secretion in CKD. Furthermore, the loss of membrane-bound klotho expression limits FGF23-stimulated signal transduction through FGF receptor/klotho complexes. One result is the loss of negative feedback to FGF23 secretion and the continual production of FGF23 and secretion by the osteocyte. In late CKD, the very high levels of FGF23 permit anomalous FGF receptor activation independent of Klotho and result in unique FGF23-stimulated pathologies such as cardiac myocyte hypertrophy [44]. In addition, recent mechanistic studies have directly linked klotho deficiency with cardiovascular disease including vascular calcification, vascular stiffness, and uremic vasculopathy [13].

Hyperphosphatemia

As renal injury decreases the number of functioning nephrons, phosphate excretion is maintained by reducing the tubular reabsorption of filtered phosphate in the remaining nephrons under the influence of FGF23 and PTH [54]. The effects of FGF23 on phosphate excretion are limited by proximal tubular klotho deficiency in CKD, and PTH becomes the major adaptive mechanism maintaining phosphate homeostasis. In stage 4-5 CKD (GFR < 30% of normal), this adaptation is no longer adequate and hyperphosphatemia develops despite high PTH and FGF23 levels [54].

CKD contributes to hyperphosphatemia and vascular calcification through inhibition of skeletal function. Bone resorption increases phosphate release to the plasma and decreases phosphate deposition resulting in increased serum phosphorus levels [55]. Hyperphosphatemia stimulates osteoblastic transition in the vasculature and directly contributes to extraskeletal mineralization through an elevated calcium-phosphorus product [56].

Hyperphosphatemia exerts other important effects in the CKD-MBD axis. In the kidney, hyperphosphatemia suppresses 1-alpha-hydroxylase activity that further contributes to calcitriol deficiency [57]. In the parathyroid gland, hyperphosphatemia directly stimulates parathyroid cells independent of calcium and calcitriol levels, producing nodular hyperplasia and increasing PTH secretion [58]. In the skeleton, phosphorus stimulates FGF23 secretion from osteocytes [59, 60].

Vitamin D deficiency

In early CKD, the physiologic actions of FGF23 secretion from the osteocyte include inhibition of 1-alpha-hydroxylase and stimulation of 24-hydroxylase in proximal renal tubules, thereby decreasing calcitriol production and producing 25-hydroxyvitamin D deficiency [61]. As CKD advances, the decrease in functioning nephron mass combined with hyperphosphatemia and increased FGF23 levels results in calcitriol (1,25-hydroxyvitamin D) deficiency as well [62]. Calcitriol deficiency decreases intestinal calcium absorption leading to hypocalcemia and diminishes tissue levels of vitamin D receptors, which in the parathyroid gland results in resistance to calcitriol-mediated regulation and stimulation of PTH secretion leading to secondary hyperparathyroidism [63].

Hyperparathyroidism

PTH regulates secretion of FGF23 and is required for the early stimulation of FGF23 secretion [64], which is the earliest detected abnormality of the CKD-MBD [41]. Thus, there is a regulation of PTH secretion early in CKD that remains to be clarified. As CKD progresses, components of the CKD-MBD result in increased production of PTH and nodular hyperplasia of the parathyroid glands. Sustained elevation in PTH levels, while adaptive to maintain osteoblast surfaces, are associated with an abnormal phenotype of osteoblast function and osteocyte stimulation with relatively less type 1 collagen and more RANKL ligand production than anabolic osteoblasts [65]. New studies discussed below indicate that other factors such as FGF23 and activin may impact osteoblast function besides PTH, and produce the mineralization disorder of CKD changing the material properties of bone. The outcome is a high turnover renal osteodystrophy, excess bone resorption, skeletal frailty and elevated fracture risk [66].

Renal osteodystrophy

With progressive loss of renal function, cancellous bone volume may be increased along with a loss of cortical bone, but this is in part due to deposition of woven immature collagen fibrils instead of lamellar mature fibrils. Thus, bone strength suffers despite an apparent increase in mass detected by dual energy x-ray absorptiometry (DXA) [67]. Patients with advanced CKD could have a loss or gain in bone volume depending on overall bone balance. When the bone balance is positive, osteosclerosis may be observed when osteoblasts are active in depositing new bone composed primarily of immature woven collagen. However, this scenario is rare in the 21st century due to improved therapy of secondary hyperparathyroidism. When the bone balance is negative both cortical and cancellous bone loss occurs, resulting in osteopenia or osteoporosis detected by DXA. The prevalence of osteoporosis in CKD patients exceeds that of the general population and is a major public health concern in CKD [68]. With high-turnover renal osteodystrophy, as in

secondary hyperparathyroidism with osteitis fibrosa, bone resorption rates are in excess of bone formation and osteopenia progressing to osteoporosis may result [69]. With low-turnover renal osteodystrophy, as occurs with over-treatment of secondary hyperparathyroidism that inappropriately suppresses PTH-stimulated skeletal remodeling, both bone formation and resorption rates may be reduced although resorption is still in relative excess and loss of bone mass occurs [70]. Therefore, osteoporosis may be observed with either high-turnover or low-turnover renal osteodystrophy. The impact of this phenomenon extends far beyond bone health in CKD, as excessive bone resorption rates contribute to hyperphosphatemia with stimulation of heterotopic mineralization including vascular calcification [56]. This disrupted systems biology links kidney, skeletal, and parathyroid dysfunction to cardiovascular risk and mortality through the CKD-MBD.

Cardiovascular disease

The CKD-MBD is a contributing factor to vascular stiffness and calcification that increases the systolic blood pressure, pulse wave velocity, and left ventricular mass, all of which are surrogates for cardiovascular risk in the general population and in those with CKD [71]. Structural and functional abnormalities of the vasculature are seen in early CKD, including vascular stiffness and endothelial dysfunction that progress to vascular calcification, a common phenomenon in the aging general population that is accelerated in CKD to the highest level seen in clinical medicine. Vascular calcification further intensifies vascular stiffness and promotes the development of LVH, all processes that contribute to cardiovascular risk and excess cardiac mortality in native and transplant CKD.

In animal models with mild renal insufficiency (equivalent to human stage 2 CKD), we have demonstrated that expression of proteins involved in the contractile apparatus of aortic smooth muscle cells are decreased, reflecting a dedifferentiated state of the vasculature in early CKD [14]. Within the developmental program of mesenchymal stem cells and early vascular progenitors, dedifferentiated vascular smooth muscle cells are susceptible to osteoblastic transition, which contributes to vascular calcification in CKD. Osteoblastic transition of vascular smooth muscle cells produces CKD-stimulated calcification of atherosclerotic plaques as well as the tunica media, resulting in either neointimal or medial vascular calcification [72].

Emerging Concepts in the Systems Biology of the CKD-MBD: The Wnt pathway and reactivation of renal repair mechanisms in the CKD-MBD

Kidney injuries produce circulating signals that directly affect the vasculature, the myocardium and the skeleton. These signals are derived from reactivation of developmental programs of nephrogenesis in an attempt at kidney repair, which are typically silent in the normal adult kidney [14]. The classic example is the reactivation of the Wnt pathway that controls tubular epithelial proliferation and polarity during nephrogenesis and is a driving force in renal fibrosis [21]. Activation of the canonical Wnt pathway increases expression of downstream transcriptional targets, including Wnt inhibitors that function in a negative feedback loop to autoregulate Wnt activation. These Wnt inhibitors include Dickkopf-1 (Dkk1), soluble frizzled related proteins, Wnt-modulator in surface ectoderm (Wise), and

sclerostin among others. While Wnts are strictly autocrine/paracrine factors, the Wnt inhibitors also function as circulating systemic factors [73]. The role of Wnt in renal development largely precedes the invasion of the microcirculation forming the glomerulus and the peritubular capillaries. Therefore, while the Wnt inhibitors did not evolve as circulating factors produced by the normal kidney, during kidney injury and repair they are released into the systemic circulation and may inhibit the physiologic roles of Wnt in extrarenal tissues [14].

We and others have recently shown this “unintended” systemic inhibition of Wnt activity stimulated by kidney disease to have major consequences in the skeleton and vasculature. In animal models of early CKD, incomplete recovery from acute kidney injury led to increased expression of Wnt inhibitors (e.g., Dkk1, sclerostin) in the injured kidney and increased levels in the systemic circulation [14]. The skeleton was affected through both changes in remodeling (decreased bone formation rates) and in secretory properties of the osteocytes (increased FGF23 secretion). The cardiovascular system was affected through loss of vascular contractile machinery and dedifferentiation of vascular smooth muscle cells, stimulation of osteoblastic transition and vascular calcification, and promotion of cardiac hypertrophy [14, 37]. Neutralization of circulating Dkk1 using a monoclonal antibody resulted in increased bone formation rates and bone volume, improved vascular function, and decreased osteoblastic transition and vascular calcification [14].

Conclusion and Future Directions

The CKD-MBD defines a disruption in the systems biology between the injured kidney, skeleton, and cardiovascular system that has a profoundly negative impact on survival in CKD. Recent translational discoveries have introduced a new paradigm where kidney injury directly leads to skeletal and cardiovascular injury through the production of pathogenic circulating factors during attempted renal repair, including molecules that inhibit the canonical Wnt pathway and stimulate endothelial-to-mesenchymal transition, both processes that have been implicated in chronic allograft injury as well as cardiovascular disease. Future studies must clarify whether incomplete recovery from acute kidney injury is sufficient to stimulate these disturbances in the kidney-skeletal-cardiovascular axis that contribute to decreased patient and allograft survival. This would identify the early CKD-MBD as an important therapeutic target for improving long-term outcomes in CKD.

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REFERENCES

* of special interest

** of outstanding interest

1. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney Disease as a Risk Factor for Development of Cardiovascular Disease. *Circulation*. 2003; 108:2154–2169. [PubMed: 14581387]
2. Papademetriou V, Lovato L, Doumas M, et al. Chronic kidney disease and intensive glycemic control increase cardiovascular risk in patients with type 2 diabetes. *Kidney Int*. 2015; 87:649–659.
* This paper establishes that mild to moderate diabetic nephropathy increases cardiovascular risk through stimulation of atherosclerosis. [PubMed: 25229335]
3. Moe S, Drueke T, Cunningham J, et al. Definition, evaluation, and classification of renal osteodystrophy: a position statement from kidney disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2006; 69:1945–1953. [PubMed: 16641930]
4. Block GA, Hulbert-Shearon TE, Levin NW, Port FK. Association of serum phosphorus and calcium X phosphate product with mortality risk in chronic hemodialysis patients: a national study. *Am J Kidney Dis*. 1998; 31:607–617. [PubMed: 9531176]
5. Blacher J, Guerin AP, Pannier B, et al. Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension*. 2001; 38:938–942. [PubMed: 11641313]
6. Gutierrez OM, Mannstadt M, Isakova T, et al. Fibroblast Growth Factor 23 and Mortality among Patients Undergoing Hemodialysis. *New Engl J Med*. 2008; 359:584–592. [PubMed: 18687639]
7. Dhingra R, Sullivan LM, Fox CS, et al. Relations of Serum Phosphorus and Calcium Levels to the Incidence of Cardiovascular Disease in the Community. *Arch Intern Med*. 2007; 167:879–885. [PubMed: 17502528]
8. Matsushita K, Sang Y, Ballew SH, et al. Subclinical Atherosclerosis Measures for Cardiovascular Prediction in CKD. *J Am Soc Neph*. 2014; 26:439–447.
9. Dalal M, Sun K, Cappola AR, et al. Relationship of serum fibroblast growth factor 23 with cardiovascular disease in older community-dwelling women. *Eur J Endocrinol*. 2011; 165:797–803. [PubMed: 21873490]
10. Fang Y, Ginsberg C, Sugatani T, et al. Early chronic kidney disease-mineral bone disorder stimulates vascular calcification. *Kidney Int*. 2014; 85:142–150. * This paper characterizes the CKD-MBD in early CKD in a translational model of type 2 diabetes equivalent to human stage 2 CKD as consisting of vascular smooth muscle dedifferentiation, vascular calcification, loss of renal klotho, elevation of FGF23 and renal osteodystrophy. PTH, Pi, Ca, and calcitriol are normal at this stage. [PubMed: 23884339]
11. Pereira RC, Juppner H, Azucena-Serrano CE, et al. Patterns of FGF-23, DMP1 and MEPE expression in patients with chronic kidney disease. *Bone*. 2009; 45:1161–1168. [PubMed: 19679205]
12. Fang Y, Zhang Y, Mathew S, et al. Early chronic kidney disease (CKD) stimulates vascular calcification (VC) and decreased bone formation rates prior to positive phosphate balance. *J Am Soc Nephrol*. 2009; 20:36A.
13. Hu MC, Shi M, Zhang J, et al. Klotho Deficiency Causes Vascular Calcification in Chronic Kidney Disease. *J Am Soc Nephrol*. 2011; 22:124–136. [PubMed: 21115613]
14. Fang Y, Ginsberg C, Seifert M, et al. CKD-induced Wntless/Integration1 Inhibitors and Phosphorus Cause the CKD-Mineral and Bone Disorder. *J Am Soc Nephrol*. 2014; 25:1760–1763.
** This paper is the first discovery of a renal repair factor, a Wnt inhibitor-Dkk1, causing extrarenal disease, showing that CKD directly causes cardiovascular diseases. The surprising effects of Dkk1 neutralization suggested that CKD caused endothelial - mesenchymal transition which was confirmed in the Agapova et al paper #37. [PubMed: 24578135]
15. de Oliveira RB, Gracioli FG, dos Reis LM, et al. Disturbances of Wnt/ β -catenin pathway and energy metabolism in early CKD: effect of phosphate binders. *Nephrol Dial Transplant*. 2013; 28:2510–2517. [PubMed: 23975746]
16. Sabbagh Y. Repression of osteocyte Wnt/ β -catenin signaling is an early event in the progression of renal osteodystrophy. *J Bone Miner Res*. 2012; 27:1757–1772. [PubMed: 22492547]
17. Surendran K, McCaul SP, Simon TC. A role for Wnt-4 in renal fibrosis. *Am J Physiol Renal Physiol*. 2002; 282:F431–F441. [PubMed: 11832423]

18. Surendran K, Schiavi S, Hruska KA. Wnt-dependent- β -catenin signaling is activated after unilateral ureteral obstruction, and recombinant secreted frizzled-related protein 4 alters the progression of renal fibrosis. *J Am Soc Nephrol*. 2005; 16:2373–2384. [PubMed: 15944336]
19. Maeshima A, Nojima Y, Kojima I. The role of the activin-follistatin system in the developmental and regeneration processes of the kidney. *Cytokine & Growth Factor Reviews*. 2001; 12:289–298. [PubMed: 11544099]
20. Terada Y, Tanaka H, Okado T, et al. Expression and Function of the Developmental Gene Wnt-4 during Experimental Acute Renal Failure in Rats. *J Am Soc Nephrol*. 2003; 14:1223–1233. [PubMed: 12707392]
21. Kawakami T, Ren S, Duffield JS. Wnt signalling in kidney diseases: dual roles in renal injury and repair. *J Pathol*. 2013; 229:221–231. [PubMed: 23097132]
22. Rinkevich Y, Montoro Daniel T, Contreras-Trujillo H, et al. In Vivo Clonal Analysis Reveals Lineage-Restricted Progenitor Characteristics in Mammalian Kidney Development, Maintenance, and Regeneration. *Cell Reports*. 2014; 7:1270–1283. [PubMed: 24835991]
23. Niida A, Hiroko T, Kasai M, et al. DKK1, a negative regulator of Wnt signaling, is a target of the β -catenin/TCF pathway. *Oncogene*. 2004; 23:8520–8526. [PubMed: 15378020]
24. Niehrs C. Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene*. 2006; 25:7469–7481. [PubMed: 17143291]
25. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature*. 2003; 423:409–414. [PubMed: 12717450]
26. Chamorro MN, Schwartz DR, Vonica A, et al. FGF-20 and DKK1 are transcriptional targets of β -catenin and FGF-20 is implicated in cancer and development. *The EMBO Journal*. 2004; 24:73–84. [PubMed: 15592430]
27. Gonzalez-Sancho JM, Aguilera O, Garcia JM, et al. The Wnt antagonist DICKKOPF-1 gene is a downstream target of β -catenin/TCF and is downregulated in human colon cancer. *Oncogene*. 2004; 24:1098–1103. [PubMed: 15592505]
28. Al-Aly Z, Shao JS, Lai CF, et al. Aortic Msx2-Wnt Calcification Cascade Is Regulated by TNF- α Dependent Signals in Diabetic Ldlr / Mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2007; 27:2589–2596.
29. Shao JS, Cheng SL, Pingsterhaus JM, et al. Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. *Journal of Clinical Investigation*. 2005; 115:1210–1220. [PubMed: 15841209]
30. Cheng S-L, Shao J-S, Behrmann A, et al. Dkk1 and Msx2-Wnt7b Signaling Reciprocally Regulate the Endothelial-Mesenchymal Transition in Aortic Endothelial Cells. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2013; 33:1679–1689.
31. Eisenberg LM, Markwald RR. Molecular Regulation of Atrioventricular Valvuloseptal Morphogenesis. *Circulation Research*. 1995; 77:1–6. [PubMed: 7788867]
32. Camenisch TD, Molin DGM, Person A, et al. Temporal and Distinct TGF β Ligand Requirements during Mouse and Avian Endocardial Cushion Morphogenesis. *Developmental Biology*. 2002; 248:170–181. [PubMed: 12142029]
33. Zeisberg EM, Tarnavski O, Zeisberg M, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med*. 2007; 13:952–961. [PubMed: 17660828]
34. Moore-Morris T, Guimar xE, et al. Resident fibroblast lineages mediate pressure overload-induced cardiac fibrosis. *The Journal of Clinical Investigation*. 2014; 124:2921–2934. [PubMed: 24937432]
35. Cooley BC, Nevado J, Mellad J, et al. TGF- β Signaling Mediates Endothelial-to-Mesenchymal Transition (EndMT) During Vein Graft Remodeling. *Science Translational Medicine*. 2014; 6:227ra234. * This paper establishes EndMT in neointimal formation which we have shown to be stimulated in CKD. It also shows that the smad pathway activated by activin is involved.
36. Ying S-Y. Inhibins, Activins, and Follistatins: Gonadal Proteins Modulating the Secretion of Follicle-Stimulating Hormone. *Endocrine Reviews*. 1988; 9:267–293. [PubMed: 3136011]
37. Agapova OA, Fang Y, Sugatani T, et al. Kidney disease produced circulating renal repair factors cause vascular disease. *Circ Res*. 2015 Submitted. ** This paper is the first demonstration that CKD increases circulating activin levels and vascular activin receptor type 2A signaling producing

vascular effects related to activin and Wnt signaling. Activin in the kidney stimulates Wnt signaling producing increased circulating Dkk1. This produces a complex systemic effect of Wnt inhibition and activin signaling.

38. Seifert ME, De las Fuentes L, Rothstein M, et al. Effects of phosphate binder therapy on vascular stiffness in the early chronic kidney disease. *Amer J Nephrol*. 2013; 38:158–167. [PubMed: 23941761]
39. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet*. 2000; 26:345–348. [PubMed: 11062477]
40. Liu S, Tang W, Zhou J, et al. Distinct roles for intrinsic osteocyte abnormalities and systemic factors in regulation of FGF23 and bone mineralization in Hyp mice. *AJP - Endocrinology and Metabolism*. 2007; 293:E1636–E1644.
41. Isakova T, Wahl P, Vargas GS, et al. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int*. 2011; 79:1370–1378. [PubMed: 21389978]
42. Wolf M, Molnar MZ, Amaral AP, et al. Elevated Fibroblast Growth Factor 23 is a Risk Factor for Kidney Transplant Loss and Mortality. *Journal of the American Society of Nephrology*. 2011; 22:956–966. [PubMed: 21436289]
43. Fliser D, Kollerits B, Neyer U, et al. Fibroblast Growth Factor 23 (FGF23) Predicts Progression of Chronic Kidney Disease: The Mild to Moderate Kidney Disease (MMKD) Study. *Journal of the American Society of Nephrology*. 2007; 18:2600–2608. [PubMed: 17656479]
44. Faul C, Amaral AP, Oskoue B, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest*. 2011; 121:4393–4408. [PubMed: 21985788]
45. Andrukhova O, Slavic S, Smorodchenko A, et al. FGF23 regulates renal sodium handling and blood pressure. *EMBO Molecular Medicine*. 2014; 6:744–759. * This paper and #47 raise the question of the mechanism of *klklo* actions as indirect/co-receptor for FGF23 or direct, through its *gluronidase* and *siladase* activities. [PubMed: 24797667]
46. Humalda JK, Lambers Heerspink HJ, Kwakernaak AJ, et al. Fibroblast Growth Factor 23 and the Antiproteinuric Response to Dietary Sodium Restriction During Renin-Angiotensin-Aldosterone System Blockade. *American Journal of Kidney Diseases*. 2015; 65:259–266. [PubMed: 25278093]
47. Andrukhova O, Smorodchenko A, Egerbacher M, et al. FGF23 promotes renal calcium reabsorption through the TRPV5 channel. 2014 * See above for #45.
48. Chang Q, Hoefs S, van der Kemp AW, et al. The α -glucuronidase *klklo* hydrolyzes and activates the TRPV5 channel. *Science*. 2005; 310:490–493. [PubMed: 16239475]
49. Kurosu H, Ogawa Y, Miyoshi M, et al. Regulation of fibroblast growth factor-23 signaling by *Klotho*. *J Biol Chem*. 2006; 281:6120–6123. [PubMed: 16436388]
50. Chen C-D, Podvin S, Gillespie E, et al. Insulin stimulates the cleavage and release of the extracellular domain of *Klotho* by ADAM10 and ADAM17. *Proceedings of the National Academy of Sciences*. 2007; 104:19796–19801.
51. Sakan HNK, Asai O, Imura A, Tanaka T, et al. Reduced Renal α -*Klotho* Expression in CKD Patients and Its Effect on Renal Phosphate Handling and Vitamin D Metabolism. *PLoS ONE*. 2014; 9(1):e86301. [PubMed: 24466013]
52. Smith RC, O'Bryan LM, Farrow EG, et al. Circulating α -*Klotho* influences phosphate handling by controlling FGF23 production. *J Clin Invest*. 2012; 122:4710–4715. [PubMed: 23187128]
53. Hu M-C, Shi M, Zhang J, et al. *Klotho* deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective. *Kidney Int*. 2010; 78:1240–1251. [PubMed: 20861825]
54. Silver J, Rodriguez M, Slatopolsky E. FGF23 and PTH—double agents at the heart of CKD. *Nephrology Dialysis Transplantation*. 2012; 27:1715–1720.
55. Davies MR, Lund RJ, Mathew S, Hruska KA. Low turnover osteodystrophy and vascular calcification are amenable to skeletal anabolism in an animal model of chronic kidney disease and the metabolic syndrome. *J Am Soc Nephrol*. 2005; 16:917–928. [PubMed: 15743994]
56. Mathew S, Tustison KS, Sugatani T, et al. The mechanism of phosphorus as a cardiovascular risk factor in chronic kidney disease. *J Am Soc Nephrol*. 2008; 19:1092–1105. PMID:PMC2396927. [PubMed: 18417722]

57. Hruska KA, Mathew S, Lund R, et al. Hyperphosphatemia of chronic kidney disease. *Kidney Int.* 2008; 74:148–157. [PubMed: 18449174]
58. Moallem E, Kilav R, Silver J, Naveh-Many T. RNA-Protein Binding and Post-transcriptional Regulation of Parathyroid Hormone Gene Expression by Calcium and Phosphate. *Journal of Biological Chemistry.* 1998; 273:5253–5259. [PubMed: 9478982]
59. Larsson T, Nisbeth U, Ljunggren O, et al. 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; 64:2272–2279. [PubMed: 14633152]
60. Isakova T, Barchi-Chung A, Enfield G, et al. Effects of dietary phosphate restriction and lanthanum carbonate on FGF23 in CKD. *J Am Soc Nephrol.* 2012; 23:P45A.
61. Prié D, Friedlander G. Reciprocal Control of 1,25-Dihydroxyvitamin D and FGF23 Formation Involving the FGF23/Klotho System. *Clinical Journal of the American Society of Nephrology.* 2010; 5:1717–1722. [PubMed: 20798257]
62. KDIGO Clinical Practice Guideline for the Diagnosis E, Prevention, and Treatment of the CKD-MBD. Chapter 4.1: Treatment of CKD-MBD targeted at lowering high serum phosphorus and maintaining serum calcium. *Kidney Int.* 2009; 76:S50–S99.
63. Naveh-Many T, Marx R, Keshet E, et al. Regulation of 1,25-dihydroxyvitamin D3 receptor gene expression by 1,25-dihydroxyvitamin D3 in the parathyroid in vivo. *J Clin Invest.* 1990; 86:1968–1975. [PubMed: 2174913]
64. Meir T, Durlacher K, Pan Z, et al. Parathyroid hormone activates the orphan nuclear receptor Nurrl to induce FGF23 transcription. *Kidney Int.* 2014; 86:1106–1115. * This paper establishes the mechanism of the relationship between PTH and FGF23 in the osteocyte. [PubMed: 24940803]
65. Wesseling-Perry K, Salusky IB. Chronic Kidney Disease: Mineral and Bone Disorder in Children. *Seminars in Nephrology.* 2013; 33:169–179. [PubMed: 23465503]
66. Moe SM, Abdalla S, Chertow GM, et al. Effects of Cinacalcet on Fracture Events in Patients Receiving Hemodialysis: The EVOLVE Trial. *Journal of the American Society of Nephrology.* 2014
67. Malluche HH, Porter DS, Pienkowski D. Evaluating bone quality in patients with chronic kidney disease. *Nat Rev Nephrol.* 2013; 9:671–680. [PubMed: 24100399]
68. Cunningham J, Sprague S, Cannata-Andia J, et al. Osteoporosis in chronic kidney disease. *Am J Kidney Dis.* 2004; 43:566–571. [PubMed: 14981616]
69. Rix M, Andreassen H, Eskildsen P, et al. Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. *Kidney Int.* 1999; 56:1084–1093. [PubMed: 10469378]
70. Coco M, Rush H. Increased incidence of hip fractures in dialysis patients with low serum parathyroid hormone. *Am J Kidney Dis.* 2000; 36:1115–1121. [PubMed: 11096034]
71. Ix JH, Katz R, De Boer IH, et al. Association of Chronic Kidney Disease with the Spectrum of Ankle Brachial Index: The Cardiovascular Health Study. *Journal of the American College of Cardiology.* 2009; 54:1176–1184. [PubMed: 19761940]
72. Moe SM, Chen NX. Pathophysiology of Vascular Calcification in Chronic Kidney Disease. *Circulation Research.* 2004; 95:560–567. [PubMed: 15375022]
73. Diarra D. Dickkopf-1 is a master regulator of joint remodeling. *Nat Med.* 2007; 13:156–163. [PubMed: 17237793]