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α Klotho and Vascular Calcification: An Evolving Paradigm

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Abstract

Purpose of Review—Cardiovascular disease remains the single most serious contributor to mortality in chronic kidney disease (CKD). Although conventional risk factors are prevalent in CKD, both cardiomyopathy and vasculopathy can be caused by pathophysiologic mechanisms specific to the uremic state. CKD is a state of systemic α Klotho deficiency. While the molecular mechanism of action of α Klotho is not well understood, the downstream targets and biologic functions of α Klotho are astonishingly pleiotropic. An emerging body of literature links α Klotho to uremic vasculopathy.

Recent Findings—The expression of α Klotho in the vasculature is controversial due to conflicting data. Regardless of whether α Klotho acts a circulating or resident protein, there are good data associating changes in α Klotho levels with vascular pathology including vascular calcification and *in vitro* data of direct action of α Klotho on both the endothelium and vascular smooth muscle cells in terms of cytoprotection and prevention of mineralization.

Summary—It is critical to understand the pathogenic role of α Klotho on the integral endothelium-vascular smooth muscle network rather than each cell type in isolation in uremic vasculopathy, as α Klotho can serve as a potential prognostic biomarker and a biological therapeutic agent.

Keywords

chronic kidney disease; α Klotho; vascular calcification; vascular smooth muscle cells; endothelium

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Conflicts of interest

There are no conflicts of interest.

Introduction

α Klotho was discovered when the serendipitous disruption of its locus led a phenotype of premature multi-organ degeneration and failure [1]. In addition its role as an anti-ageing molecule, the documented downstream actions of α Klotho encompasses an impressively wide range of cell functions including energy metabolism, inhibition of Wnt signaling, antioxidation, modulation of ion transport, and regulation of mineral metabolism [2]. α Klotho is a member of a more extended family of three paralogs (α , β , and γ Klotho) but thus far only α Klotho has been shown to exist in the circulation to exert distant effects as an endocrine substance [3■]. Because the kidney is likely the major source of circulating α Klotho, α Klotho is a major regulator of mineral metabolism which is grossly deranged in chronic kidney disease (CKD), and the strikingly similar cardiovascular lesions seen in genetic α Klotho deficiency and CKD, the focus of α Klotho biology has shifted from aging-related to more nephrocentric issues [4, 5]. We will summarize recent findings of the relationship between α Klotho and the vascular calcification in CKD and propose a plausible yet evolving model of the pathogenic role of α Klotho in this dire complication.

Vascular calcification in CKD

Uremic soft tissue and vascular calcification was described by Virchow over one and a half century ago and he proposed that calcium from bone is metastatically relocated to soft tissue as “*Kalk Metastasen*” [6]. Nearly half a century ago, Parfitt gave a detailed account on uremic soft tissue calcification [7]. Although the proposed mechanisms of calcification seem anachronistic and superficial by today’s standards, the description of the lesions is very accurate and Parfitt importantly commented that of all the different ectopic sites, arterial calcification was universal in all patients [7]. CKD has reached epidemic proportions in many countries and a 2013 examination of the National Vitals Statistics showed that if one lives to his or her seventies in the United States, the chance of developing Stage 3 or higher CKD is staggeringly high at over 50% [8■]. In a recent analysis of nearly 4,000 subjects from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) trial, cardiovascular mortality over 7 years was increased by the presence of CKD (hazard ratio 2.24) much more significantly than either diabetes, metabolic syndrome or smoking (hazard ratio ~1.45) after multivariable adjustment [9]. A seminal report from the National Kidney Foundation over one and one half decade ago showed that the mortality from cardiovascular disease in a 20 year old patient on dialysis is the same as an 80 year old person not on dialysis, and the traditional Framingham risk factors cannot account for this grim outcome [10]. Unfortunately, we have not progressed very far since that report. It is true that traditional cardiovascular risk factors are highly prevalent in CKD and clinicians are working hard to control them but in addition, one should be cognizant of an expanding list of uremia-specific cardiovascular risk factors that require attention at various levels of the experimental and clinical fronts- a few examples are asymmetrical dimethylarginine, endothelin, homocysteine, advanced oxidation protein products, advance glycosylation end-products, indoxyl sulfate, fibroblast growth factor (FGF)-23, phosphate, and α Klotho deficiency whose relationship to uremic vasculopathy which will be discussed below.

α Klotho levels in CKD

The features of hyperphosphatemia, high FGF23, short life expectancy, and vascular calcification are shared between the α Klotho-deficient mice and CKD [2, 3, 11, 12]. Serum α Klotho levels is decreased in adult CKD stage 2 and linearly declines with eGFR [13]. After multivariable adjustment, serum α Klotho is associated independently with eGFR in full range of CKD [14] [15]. However, the commercial α Klotho assay kits have not been uniform [16] often yielding inconsistent results of circulating α Klotho in CKD (reviewed [3, 5, 12]). The published data of serum/plasma Klotho in the last 3 years and its relationship to cardiovascular disease and CKD is summarized in Table 1.

Because α Klotho levels are affected by many factors including hormones (1,25-dihydroxy-vitamin D3, FGF23, and PTH), mineral parameters (plasma Ca and Pi), medications (statins [26], renin-angiotensin system (RAS) blockers [19, 27], peroxisome proliferator-activated receptor agonists (PPAR) γ [28]), and age, in addition to kidney function, interpretation of plasma α Klotho levels in CKD/end-stage renal disease (ESRD) requires caution. Data in CKD patients addressing correlations between α Klotho and vasculopathy is currently based on a single assay and appears suboptimal at present. Low serum α Klotho was associated with arterial stiffness, but not with endothelial dysfunction in CKD patients (Table 1) [22]. Low serum α Klotho is independently associated with severity of coronary artery disease in patients with normal kidney function [17]. A definitive conclusion could not be made until one can understand all the variables that affect circulating α Klotho levels and more reliable and accurate assays for α Klotho are available.

Endothelial: vascular smooth muscle complex- an inseparable functional network

While the study of endothelial (EC) or vascular smooth muscle cell (VSMC) in isolation permits more definitive conclusions, it is important to note that these cells do not exist in isolation. Blood vessels are composed of endothelial cells, mural cells (smooth muscle cells and pericytes), their shared basement membrane and extracellular matrix. The endothelium lines the entire cardiovascular and lymphatic system [29]. It is not only a barrier but contributes to physiological functions [30–32]. The endothelium can be a primary source of pathophysiology, a secondary player that amplifies pathology, or as a by-standing victim. Intact structure and function of the endothelium is indispensable for maintenance of the integrity of the vasculature. When co-cultured with EC, VSMCs, express more vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF)- β than VSMC cultured in isolation. ECs when co-cultured with VSMCs express higher VEGF than in singly cultured EC, suggesting that VSMC and EC work synergistically [33] (Figure 1 **left panel**). ECs also released a variety of growth factors and cytokines such as von Willebrand factor (VWF) [34], nitric oxide (NO) [35], and prostacyclin to modulate VSMC function (Figure 1 **left panel**) [30, 33]. Increasing attention is devoted to the role of endothelium in modulation of vascular smooth muscle cells.

Endothelial cells damaged by uremic milieu [36] or high phosphate [37] show an increase in apoptosis, reactive oxygen species, impaired nitric oxide production, and pro-inflammatory

cytokines, pro-fibrotic growth factors, and pro-angiogenic growth factors; all of them do not only induce endothelial dysfunction, but also potentially stimulates VSMC proliferation and dedifferentiation [30, 38] (Figure 1 **right panel**). Evidence of endothelial effects on vascular calcification was provided when vascular endothelium was shown to contribute to osteoprogenitor cells in vascular calcification. Yao and colleagues used transgenic mice with EC lineage reporter and found that the endothelium is a source of osteoprogenitor cells in vascular calcification (Figure 1 **right panel**) [39■■■]. Therefore, endothelium directly contributes to vascular calcification via endothelial-mesenchymal transition. In addition, endothelium also stimulates VSMC to initiate or participate in vascular calcification. It is well known that high Pi [11, 40–42] and other uremic insults such as indoxyl sulfate [43], FGF-23 [44■] could induce calcification in VSMC; but whether and how impaired VSMC alters EC is yet to be defined (Figure 1 **right panel**).

αKlotho action on endothelium

Compared to the amount of investigative work devoted to the VSMC (see below), the study of αKlotho action on the endothelium is rather modest. One should briefly note that one enigma in αKlotho biology is how this 130 kD protein leaves the endovascular space to reach its target organs which are impressively widespread in locale. In the renal peritubular capillary, αKlotho appears to have an unparalleled ability to exit the peritubular but not the glomerular capillary, through yet undefined mechanisms [45, 46]. Unless αKlotho can perform this feat in every capillary bed as it does in the peritubular capillary (Figure 1 **middle panel**), it will be a formidable challenge indeed for it to act on its myriads of target organs. One possible model that can potential bypass the access problem, or at least provide a parallel pathway, is that αKlotho may act on the endothelium and produce a secondary effect thereafter via endothelial-vascular smooth muscle cross-talk (Figure 1 **middle panel**). Or αKlotho produced in VSMC and regulates VSMC function as autocrine mode or/and regulates EC as paracrine mode (Figure 1 **middle panel**). This is only a conjecture that needs exploration.

The study of the action of αKlotho on the endothelium was initiated soon after its discovery. The homozygous αKlotho hypomorphic mice have decreased vasodilation in response to acetylcholine [47]. The Otsuka Long-Evans Tokushima Fatty rat had diminished renal αKlotho expression and impaired aortic relaxation in response to acetylcholine, and both could be attenuated with thiazolidinedione [48]. A similar rescue can also be achieved by viral delivery of αKlotho [49]. This finding inferred but did not prove that circulating αKlotho takes responsible for the normal vasorelaxation due to regulation of NO production in vascular endothelium (Figure 1 **middle panel**).

In cultured EC (Figure 1), treatment with αKlotho alleviated the induction of adhesion molecules [50]; prevented the increases in reactive oxygen species (ROS) activity, mitochondrial potential and cell apoptosis induced by tumor necrosis factor (TNF)-α [51]. αKlotho is also able to ameliorate the indoxyl sulfate-induced endothelial dysfunction and reduce ROS and NO release and monocyte chemoattractant protein-1 expression in EC (Figure 1 **middle panel**) [52]. The most definitive study to date of direct effects of αKlotho on the endothelium was conducted by Kusaba and coworkers [53]. αKlotho-deficient mice

have increased VEGF-mediated calcium influx, down-regulation of vascular endothelial (VE)-cadherin, increased apoptosis and excessive permeability of vessels. The KL2 domain of α Klotho protein binds directly to VEGF receptor 2 and endothelial transient-receptor potential Ca^{2+} channel 1 on the extracellular side and promote their co-internalization thereby reducing the Ca^{2+} -activated and caspase-mediated destruction of catenin and VE-cadherin on the cell surface (Figure 1 **middle panel**).

α Klotho action on vascular smooth muscle

α Klotho mRNA was found in murine aorta with the highly sensitive RT-PCR assay [1]. Subsequently, its present in aorta was confirmed independently by RT-PCR in human [41, 54] and rat [55, 56■■■]. In contrast, α Klotho protein in the aorta was only detected by a few laboratories [41, 44, 55] (Table 2). Very clear negative results were also obtained [38, 50].

The association of α Klotho deficiency with vascular calcification was documented in α Klotho hypomorphic mice [1] and transgenic overexpression or viral delivery of α Klotho rescued the phenotype [61–63]. Medial vascular calcification is one of the most prominent features in both CKD and α Klotho-deficient animals. Mice overexpressing α Klotho having high circulating α Klotho had much less vascular calcification after CKD induction, while mice with low circulation α Klotho had more severe vascular calcification [11]. These whole animal data support but do not prove a causal relation of α Klotho deficiency with vascular calcification. Thus one still does not know if α Klotho plays a direct role in vasculature.

Theoretically, elucidation of α Klotho expression and location in the aorta is of fundamental importance to α Klotho biology in the vasculature. Pragmatically, it is also an important question for therapeutics. If resident α Klotho in the aorta protects the vasculature against vascular calcification as a transmembrane protein or in a paracrine or autocrine manner, up-regulation of native vascular α Klotho expression would be the therapeutic objective for prevention or treatment of vascular calcification [41]. In contrast, if circulating α Klotho protects against vascular calcification in an endocrine manner, then soluble α Klotho would be potential therapeutic agent. Lau and colleagues showed that vitamin D receptor activators *in vivo* increased serum α Klotho concentration, but not in aorta, but still reduced vascular calcification [57]. Specific Klotho deletion in vascular smooth muscle further challenged the concept that α Klotho in VSMC plays an important role [56], since there was no enhanced vascular calcification in mice with specific α Klotho deletion in VSMC [56]. Furthermore, increased α Klotho mRNA was found in calcified aorta in *Enpp1*^{-/-} mice [59] that have extensive ectopic calcification, and seemingly α Klotho protein is also increased in atherosclerotic arteries [54]. These findings (assuming not confounded by reagent problems) are against resident α Klotho being protective. Intraperitoneal administration of a large amount of recombinant α Klotho, identical to extracellular domain of membrane α Klotho, [60] α Klotho gene delivery [62], and increased of endogenous circulating α Klotho [57] all reduced vascular calcification and improved endothelial functions [49], supporting that soluble α Klotho plays a pivotal role in protection of vasculature against calcification in the manner of endocrinal factor (Table1). It is important to note that this does not rule out a simultaneous effect of resident α Klotho protein in the vasculature.

To avoid the complexity of systemic administration and secondary effects, soluble α Klotho protein was added to or transfected into VSMCs. Either maneuver effectively attenuated high phosphate-induced osteogenic transition and abolished calcification of VSMC suggesting that α Klotho might be a direct vascular protective protein (Figure 1 **middle panel**) [11, 41]. However, one study did not see any effect of α Klotho protein on FGF23 and high phosphate-mediated calcification in either human and mouse VSMCs [58]. The differential effects of α Klotho on high phosphate-induced calcification *in vitro* may be due to differences in VSMCs or to different α Klotho protein preparations used. Surprisingly, deleterious effect of α Klotho on vascular calcification was found in one study in cultured rat VSMC where FGF23 accelerated high Pi-induced calcification only when α Klotho was overexpressed (Table 1) [44]. Thus the *in vitro* system intended to generate definitive data on the direct effects of α Klotho has resulted in a conflicting database. The reasons for the disparity are unclear but different VSMCs preparations and induction of calcification, and different ways of administering α Klotho are possibilities.

Conclusion

Endothelial dysfunction/stiffness is associated with increased risk of cardiovascular events and mortality in CKD patients [64]. The endothelium is constantly bathed in blood and is clearly a potential target of uremic toxicity. CKD patients had lower flow-mediated dilation (FMD) which correlated with higher C-reactive protein and increased left ventricular mass index (LVMI) [65] and there is some improvement in FMD after kidney transplant [66]. The relationship between LVMI and FMD remained significant after adjusting for age, diabetes, and smoking. Recently, the endothelium was shown to be a source of osteo-progenitor cells in diabetic mice [39] (Figure 1 **right panel**). The potential role of damaged endothelium in mediation of osteogenic transition and vascular calcification is proposed in Figure 1 **right panel** and remains to be tested. The disturbed interplay of endothelium and vascular smooth muscle cells in vascular calcification in CKD has not been clearly addressed. The protection of endothelium may be a novel therapeutic target for treatment of vascular calcification in CKD as for atherosclerosis [67]. Soluble α Klotho may have potential action on modulation of endothelium and smooth muscle cells individually through regulation of VEGF signaling, NO production and neutralization of pro-inflammatory cytokine and pro-fibrogenic factors (Figure 1 **middle panel**). Conceivably, healthy endothelium potentially protects smooth muscle cells from vascular calcification promoted by uremic milieu including high phosphate and high FGF23.

Studies of endothelial or vascular smooth muscle cells in isolation may not fully represent the system and intact animal studies are important but limited in dissecting out the role of individual players. Co-culture of vascular smooth muscle and endothelial cells may be a viable intermittent system that will help understand the interactions and how α Klotho action maintains normal structure and function of vasculature. Despite controversies of α Klotho expression and its action on cultured cells, the current wisdom still favors, although not prove, a pathogenic role of α Klotho deficiency in vascular calcification. We propose that the endothelium-vascular smooth muscle complex may be a potential target of α Klotho in vascular calcification in CKD.

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Key points

- Despite mixed results of plasma α Klotho levels and aortic α Klotho expression in CKD, majority of clinical and experimental results supported that α Klotho deficiency is associated with vascular calcification.
- Soluble α Klotho protein has been shown to protect endothelial cell from high phosphate and oxidative stress, and block osteogenic transition of smooth muscle cell induced by high phosphate.
- Normal interplay between endothelial cells and smooth muscle cells is indispensable for maintenance of normal vascular structure and function even though detailed molecular mechanisms remain to be illustrated.
- Administration of soluble α Klotho and/or modulators of α Klotho expression may hold promise as therapeutic approaches for management of vascular calcification in CKD/ESRD patients.

Figure 1. Proposed model of how Klotho affects endothelial cell (EC) -vascular smooth muscle cell (VSMC) interaction

Left panel: Normal EC-VSMC cross-talk. Normal EC modulates VSMC growth via release of growth factors (black line in left panel). VEGF: Vascular endothelial growth factor. PDGF: platelet-derive growth factor, PGI₂: Prostaglandin I₂, NO: Nitric oxide. VEGF released from pericytes and/or VSMC could also regulate endothelial cell. **Right panel:** In chronic kidney disease, uremic toxins including high plasma Pi, damage EC and induce release of growth factors, pro-inflammatory cytokines and pro-fibrotic factors, which exacerbate EC injury and also induce VSMC transition to osteoblast and promote vascular calcification in medial layer (red solid lines in right panel). Impaired EC also directly contributes to vascular calcification through endo-osteoblast transition (purple line in right panel). Whether de-differentiated or damaged VSMC could further modulate function of endothelial cells is only speculative (red dash line in right panel). **Middle panel:** αKlotho is a vascular protective protein. Whether resident aortic αKlotho protein in VSMC functions as autocrine and/or as paracrine mode to modulate VSMC and/or EC remains to be clarified (blue dash line in middle panel). How αKlotho is able to access to VSMC from the circulation and functions as endocrine factor remain to be defined (green dash line in middle panel). αKlotho, regardless of source could increase NO production from EC and NO consequently modulates VSMC and EC function in an autocrine mode (green solid line in middle panel). αKlotho could protect EC from high phosphate and other uremic toxins and also attenuate oxidative stress and pro-inflammatory cytokines induced cell senescence and apoptosis in VSMC (orange line in middle panel). αKlotho also directly inhibits osteoblast transition induced by hyperphosphatemia and uremic milieu (orange line in middle panel). Current experimental and clinical observations suggest that both EC and VSMC

endothelium may be potential targets of soluble α Klotho to protect the vasculature from vascular calcification in CKD.

Table 1

Circulating α Klotho levels with renal and cardiovascular disease

Circulating α Klotho			Vascular disease				Comments	Citation
Changes	Sample*	Study subjects	CAD	Endothelial dysfunction	Vascular calcification	Arterial stiffness		
↓ in SCAD ↑↑ in NSCAD	Serum	371 CAD eGFR 60 ml/min/1.73m ²	↓ in severe CAD	N/A	N/A	N/A	↓ serum α Klotho independently associated with the presence of severe CAD	[17 ■ ■ ■]
↓ protein ↓ mRNA	Serum Kidney	14 CKD 1-5 43 HD	N/A	N/A	N/A	N/A	Serum α Klotho lower in HD than compared to pre-HD Renal α Klotho correlated with serum α Klotho	[18 ■ ■ ■]
↓	Serum	243 CKD 1-5, no healthy subjects	N/A	N/A	N/A	N/A	Lower serum α Klotho associated with more advanced CKD.	14
↓	Plasma	76 DM2 Ser < 1.7 mg/dl	N/A	N/A	N/A	↓ ↓ (not by multivariate analysis)	Arterial stiffness not correlated with serum α Klotho by multivariate analysis RAS blocker increased plasma α Klotho and decreased aortic-pulse wave velocity	19
No change	Plasma	321 CKD 2-4	N/A	N/A	N/A	N/A	α Klotho not ↓ with ↓ GFR and not correlated with CV outcome	20
↓	Serum	87 CKD 1-5 21 healthy	N/A	N/A	N/A	N/A	α Klotho ↓ linearly with ↓ eGFR	15
↓	Plasma	154 children: 69 CKD 1-3, 13 CKD 4-5, 28 Dialysis, 44 functioning graft (CKD 1-3)	N/A	N/A	N/A	N/A	α Klotho ↓ in advanced CKD (4-5) and dialysis compared with α Klotho in CKD 1-3 and functioning graft with CKD (1-3).	[21 ■ ■]
↓	Serum	114 CKD 1-5	N/A	↓ ↓ (not by multivariate analysis)	↓ ↓ (not by multivariate analysis)	↓ ↓	↓ α Klotho independently associated with arterial stiffness	[22 ■ ■]
No change	Serum	36 PD	N/A	N/A	N/A	N/A	Urine but not serum α Klotho ↓ and correlated with residual GFR	[23 ■ ■ ■]
↓	Serum	292 CKD 1-5	N/A	N/A	N/A	N/A	α Klotho ↓ beginning in CKD 2	13
Not ↓ in CKD ↓ in ADPKD	Serum	32 CKD 1-2, 99 ADPKD 1-2, 20 healthy	N/A	N/A	N/A	N/A	α Klotho not ↓ early CKD, but in ADPKD with stage 1-2, and serum α Klotho values correlated inversely with cyst volume and kidney size	24
↑	Serum	30 CKD 10 healthy	N/A	N/A	N/A	N/A	Serum α Klotho ↑ in CKD	25

ADPKD: adult dominant polycystic kidney disease; CAD: coronary artery disease; CKD: chronic kidney disease; CVD: cardiovascular disease; HD: hemodialysis; N/A: information not available; NSCAD: no significant coronary artery disease (0-49% stenosis in any epicardial coronary artery); PD: peritoneal dialysis; SCAD: significant coronary artery disease (≥ 50% stenosis in any epicardial coronary artery); DM2: Type 2 diabetes mellitus

* Performed with commercial ELISA kit

Table 2
αKlotho expression in human and animal aortas and αKlotho effect on vascular calcification *in vivo* and *in vitro*

αKlotho expression		αKlotho effect			Comments	Citation
Aorta	Cultured VSMC	Intact animal	Cultured aorta	Cultured VSMC		
↓mRNA and protein in human CKD	↓mRNA and protein in human VSMC by calcifying media, TNF-α			αKlotho knockdown → ↑ calcification in human VSMC	VDRA → ↑αKlotho turns FGF23 from promotion to suppression of calcification by calcifying media in human cultured VSMC FGF23. αKlotho in aortic ring ↑ by VDRA treatment. <i>Human aorta αKlotho deficiency in CKD can be reversed ex vivo with VDRA and calcification is attenuated</i>	41
No mRNA or protein in mouse aorta	N/A	N/A	N/A	N/A	VDRA <i>in vivo</i> → ↑plasma αKlotho → ↓VC in CKD mice <i>VDRA may be beneficial by increasing circulating αKlotho</i>	57
mRNA but no protein in mouse aorta	mRNA but no protein in bovine VSMC	No VC in SM22-Cre- <i>αKlotho</i> ^{fl/fl} mice			No αKlotho protein in aorta and gene deletion in aorta has no effect FGF23 has not effect on VC <i>in vitro</i> and vascular function <i>ex vivo</i> <i>Aortic αKlotho has no role in VC</i>	[56 ■ ■ ■]
No mRNA in normal and calcified aortas of CKD mice	No mRNA in human and mice			No effect on human and mice VSMC	No αKlotho expression in human and murine aorta and in cultured VSMC <i>No aortic αKlotho expression and no αKlotho effect in vitro</i>	58
↑mRNA and protein in calcified aorta of <i>Enpp1</i> ^{-/-} mice	↑ mRNA and protein in mice by calcified media	N/A	N/A	N/A	Calcification increases αKlotho and FGF23 expression in aorta <i>↑αKlotho associated with ↑VC. Causality not established.</i>	59
mRNA in human aorta, coronary arteries, and thrombus	N/A	N/A	N/A	N/A	<i>αKlotho mRNA detectable in human arteries and thrombi of occlusive coronary disease. Significance unknown.</i>	54
↓mRNA and protein in <i>ldlr</i> ^{-/-} CKD mice	N/A	N/A	N/A	N/A	Low aortic αKlotho but high circulating αKlotho associated with vascular calcification in <i>ldlr</i> ^{-/-} CKD mice <i>αKlotho in the vasculature may be more important</i>	55
N/A	N/A	αKlotho protein injection → ↓ VC in <i>Kl/Kl</i> mice	N/A	N/A	Soluble (extracellular domain) αKlotho improves VC in <i>Kl/Kl</i> mice <i>Circulating αKlotho may be more important</i>	60
N/A	N/A	Tg-Kl → ↓ VC in mice	N/A	αKlotho protein → ↓ VC induced	<i>Soluble αKlotho protein directly protects VSMC from high phosphate induced calcification</i>	11

αKlotho expression		αKlotho effect			Comments	Citation
Aorta	Cultured VSMC	Intact animal	Cultured aorta	Cultured VSMC		
				by high Pi in rat VSMC		
Expression in aorta but not in VSMC	None	N/A	N/A	αKlotho permits FGF23 to induce VC	αKlotho is present in rat aorta, but not in VSMC. Transfected αKlotho is required for FGF23 to enhance Pi induced VC in rat VSMC. <i>No native αKlotho in VSMC but with overexpression, αKlotho worsens VC</i>	[44■]

Enpp1: Ectonucleotidepyrophosphatase/phosphodiesterase 1; K/Kl: homozygous Klothohypomorph; ldlr: LDL receptor; N/A: information not available; Tg-Kl: transgenic Klotho with overexpressing Klotho; VC: vascular calcification; VSMC: vascular smooth muscle cells; VDRA: Vitamin D receptor agonist.