

EDITORIAL

Klotho, Spinning the Thread of Life: an Anti-Ageing Gene

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ABSTRACT

Klotho, named after the ancient Greek goddess *Klotho*, the spinner (of life), is an aging suppressor or longevity-related gene, discovered in 1997. A defect in the *Klotho* gene expression in mice leads to phenotypes resembling human premature or accelerated aging syndromes, with a short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema, while *Klotho* overexpression is associated with extended longevity. The *Klotho* gene encodes a transmembrane protein expressed mainly in the kidney, the parathyroid gland and the choroid plexus. The *Klotho* protein has multiple regulating functions, can serve as a useful biomarker and may have potential therapeutic applications, and thus an emerging role in reno-cardio-vascular disease, which is briefly explored in this overview.

According to ancient Greek mythology, *Klotho* or *Clotho* (in Greek: Κλωθώ), the Spinner, is the youngest of the 3 Fates or Moires: *Klotho* who spins, *Lachesis* who measures, and *Atropos* who cuts the thread of life. *Klotho* was in charge of spinning the thread of human life. She also made critical decisions, as to when a person was to be born, saved or die, thus in essence controlling people's lives. *Klotho* is also the name given to an aging suppressor or longevity-related gene, discovered in 1997.¹⁻³ A defect in the *Klotho* gene expression in mice leads to phenotypes resembling human premature or accelerated aging syndromes, with a short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema, while *Klotho* overexpression is associated with extended longevity. *Klotho* knock-out mice develop osteopenia and vascular or other ectopic calcifications, similar to those seen in chronic kidney disease (CKD). They also have shorter lifespan and senescent changes in many other organs and tissues, including the heart, lungs, muscles, skin, thymus, gonads, hearing, and motor neurons. The *Klotho* gene encodes a (130-kDa) single-pass transmembrane protein expressed mainly in the kidney, the parathyroid gland and the choroid plexus,⁴ and to a lesser extent in placenta, prostate and small intestine. The *Klotho* protein has multiple (pleiotropic) regulating functions with an emerging role in cardiorenal disease.⁵

There are two separate forms of *Klotho* protein, membrane (beta) *Klotho* and secreted (alpha) *Klotho*.^{2,3} Membrane *Klotho* forms a complex with fibroblast growth factor (FGF) receptors and functions as an obligate co-receptor for FGF-23, a bone-derived hormone (the major phosphatonin) that induces phosphate excretion into urine. Mice lacking *Klotho* or FGF-23 not only develop phosphate retention but are

ABBREVIATIONS

CKD = chronic kidney disease
FGF = fibroblast growth factor

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also afflicted by a premature-aging syndrome, pointing to a link between phosphate metabolism and aging. Secreted α -Klotho functions as a humoral factor that regulates activity of multiple glycoproteins on the cell surface, including ion channels and growth factor receptors such as insulin/insulin-like growth factor-1 receptors, and inhibits acute kidney injury, vascular calcification, renal fibrosis, and cancer metastasis in an FGF-23-independent manner. The activity of this extracellular domain of Klotho increases the expression of antioxidant enzymes and provides cell and organism resistance to oxidative stress.

Klotho is expressed in areas involved with calcium regulation, predominantly in the kidney distal convoluted tubules, but also in the brain choroid plexus (which produces cerebrospinal fluid) and the parathyroid gland. Klotho acts as a cofactor for interaction of FGF23 with FGF R1. This interaction negatively regulates 1 α hydroxylase, the rate limiting enzyme in the synthesis of 1,25(OH) $_2$ D $_3$ (vitamin D). Klotho deficient mice show severe hyperphosphatemia and ectopic calcification of soft tissues due to excess vitamin D. Although the *klotho* gene was first reported as having anti-ageing properties in mice, human *Klotho* gene polymorphisms have been linked with reduced longevity.

Chronic kidney disease (CKD) has been suggested as a state of Klotho deficiency in the kidney, plasma, and urine.^{6,7} Secreted Klotho protein has FGF-23-independent phosphaturic and calcium-conserving effects via its paracrine action on the proximal and distal tubules, respectively.⁸ On the other hand, Klotho deficiency causes phosphate retention and accelerated ageing. As Klotho declines in CKD, it causes FGF-23 resistance and induces FGF-23 and parathyroid hormone increases, and hypovitaminosis D. Klotho downregulation appears to be an early biomarker for kidney dysfunction and mineral dysregulation, may play a pathogenetic role in the progression of CKD, and it may also be responsible for vascular calcification, which is one of the principal complications of CKD. The anti-calcification effect of Klotho may possibly be related to a phosphaturic action, the preservation of glomerular filtration rate (GFR), and a direct effect on soft tissues including the vascular smooth muscle. Thus, in clinical practice, Klotho can serve as an early and sensitive biomarker of CKD. Maintaining normal phosphate levels with use of phosphate binders in patients with CKD with declining Klotho expression is expected to ameliorate mineral and vascular derangements. Furthermore, Klotho replacement therapy or manipulation of up-regulation of endogenous Klotho may slow progression of CKD and also prevent and/or reverse its complications.⁷

Fibroblast growth factor 23 (FGF-23) is a recently discovered secretory hormone, mainly produced by osteocytes, with main functions the inhibition of renal tubular phosphate reabsorption and the suppression of circulating vitamin D levels by decreasing synthesis and enhancing catabolism of vitamin D.⁹⁻¹¹ FGF-23 participates in the bone/kidney axis that

protects the organism from excess vitamin D and coordinates renal phosphate handling with bone mineralization/turnover. Abnormalities of FGF-23 production underlie many inherited and acquired disorders of phosphate homeostasis. Recent studies have shown that the function of FGF-23 is dependent on interaction with Klotho, as an obligate co-receptor, which binds FGF-23 and then activates FGF receptors.¹⁰ These proteins appear responsible for maintaining mineral-ion homeostasis, but also regulate cell survival, proliferation and vitamin D metabolism. Hereditary disorders that exhibit high serum FGF-23 levels are associated with phosphate wasting and impaired bone mineralization, whereas defects in either FGF-23 or Klotho are associated with phosphate retention and a premature-aging syndrome.³ The aging-like phenotypes in Klotho-deficient or FGF-23-deficient mice can be managed by targeting and treating hyperphosphatemia with dietary or genetic manipulation, suggesting a novel concept that phosphate retention accelerates aging, as seen in CKD. Interestingly, a recent study indicated that elevated FGF-23 levels were independently of klotho associated with left ventricular hypertrophy (LVH) in a large, racially diverse CKD cohort.¹² In addition to FGF-23, there are other endocrine FGFs that have been recognized as hormones that regulate a variety of metabolic processes. FGF-19 is secreted from the intestine during food intake and acts on liver to suppress bile acid synthesis. FGF-21 is secreted from the liver during fasting and acts on adipose tissue to promote lipolysis and responses to fasting. One critical feature of endocrine FGFs is that they require the Klotho gene family of transmembrane proteins as coreceptors to bind their related FGF receptors and exert their biological actions.

Klotho is an antiageing protein that confers resistance to oxidative stress and several pathological conditions predisposing to cardiovascular-renal damage.⁵ Klotho is essential in calcium-phosphate metabolism and the maintenance of vascular integrity; it offers cardiorenal protection. Reduced levels of soluble Klotho are detected in the early stages of cardiovascular-renal disease; thus, Klotho might be considered as a useful biomarker that predicts atherosclerosis and vascular calcification.

In a cohort of 804 adults of ≥ 65 years of the InCHIANTI study, a longitudinal population-based study of aging in Tuscany, Italy, plasma Klotho was an independent predictor of all cause mortality, with participants in the lowest tertile of plasma klotho (< 575 pg/mL) having an increased risk of death compared with participants in the highest tertile of plasma klotho (> 763 pg/mL; hazards ratio 1.78).¹³ The same authors reported similar findings in a larger cohort of 1023 individuals, aged 24-102 years, whereby higher plasma klotho concentrations were independently associated with a lower likelihood of having cardiovascular disease.¹⁴

In a recent study,¹⁵ thoracic aorta specimens from 44 patients who underwent elective cardiac surgery, and thrombus

material from 2 patients with acute coronary syndrome, were tested for FGF-23-Klotho system expression. It was found that human vascular tissue expresses members of the FGF23-Klotho system, indicating that it can be a direct target organ for FGF-23. In addition, Klotho expression was detected in occlusive coronary thrombi. These findings suggest a putative role of FGF23-Klotho axis in human vascular pathophysiology and cardiovascular disease. Another most recent study reported endogenous Klotho expression in human arteries, *in vivo*, and in human aortic smooth muscle cells, *in vitro*.¹⁶ The authors maintained that vascular Klotho deficiency in CKD, promoted by chronic metabolic stress factors found in CKD, may be a possible explanation for accelerated vascular aging with calcification observed in these patients. They also showed that high levels of FGF-23 in CKD cannot be vasculoprotective because Klotho/FGF-receptor deficiency mediates resistance and that vitamin D receptor activator therapy mediates vascular protection by enhancing vascular Klotho expression and rendering vascular cells FGF-23 responsive.

The potential use of FGF-23 – Klotho as an antiageing therapy is tempting; however, there is also lurking risk.¹⁰ Although increased serum levels of active vitamin D are clearly responsible for several ageing-like phenotypes, including tissue atrophy, moderate production of active vitamin D is still

essential for normal bone mineralization. Also, vitamin D deficiency has been linked to increased risk in development of various forms of cancer or other diseases such as multiple sclerosis, diabetes mellitus, rheumatoid arthritis, osteoarthritis, hypertension, and stroke. Therefore, it is clear that a balance of moderate levels of systemic active vitamin D is essential for maintaining overall health and longevity. Nevertheless, Klotho pathways remain as potential targets for anti-ageing interventions and reno-cardiovascular regulation and homeostasis.^{5,17} Prevention of Klotho decline and supplementation of Klotho can be a novel therapeutic strategy for many age-related diseases.^{18,19} In a recent animal study, it was demonstrated that the administration of a vitamin D analogue and/or an angiotensin receptor blocker (olmesartan) improved chronic renal failure and up-regulated the *klotho* gene in the kidney.²⁰ In particular, the combination therapy of the 2 drugs provided the most effective renal protection.

Although the seminal discovery of Klotho, the anti-ageing gene, may not have still increased our longevity, however, it has shed new light into our understanding of the role of the Klotho protein and mineral homeostasis in the pathogenesis of reno-cardio-vascular disease (Fig. 1). Our task still remains to unravel more secrets of function and potential therapeutic applications for this spinner of life in future studies.

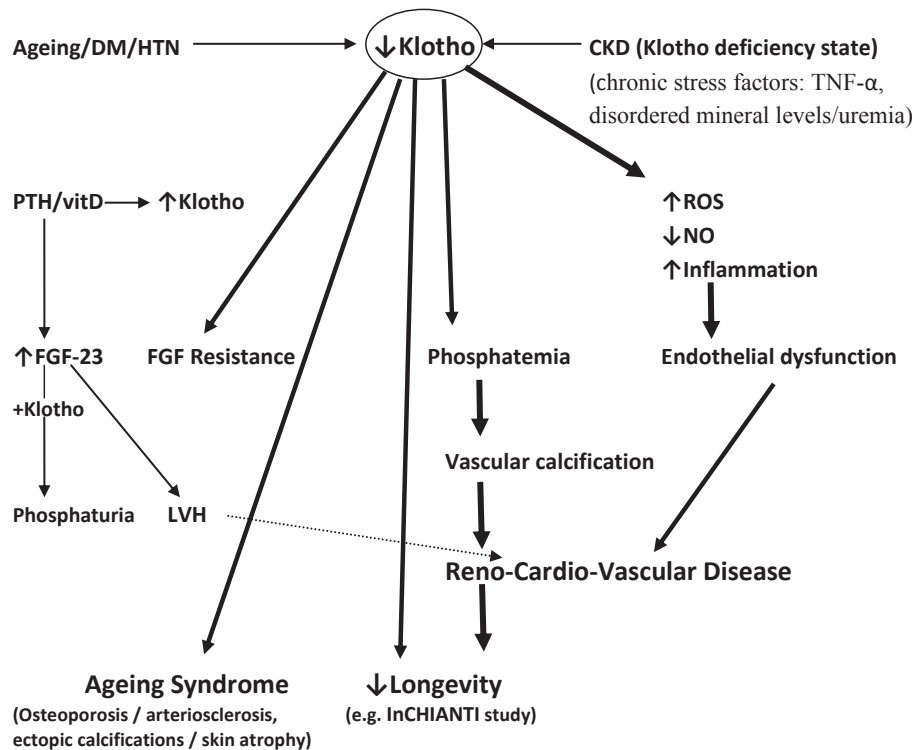


FIGURE 1. Putative role of **Klotho** involvement in *reno-cardio-vascular disease*. CKD = chronic kidney disease; DM = diabetes mellitus; FGF = fibroblast growth factor; HTN = hypertension; LVH = left ventricular hypertrophy; NO = nitric oxide; PTH = parathyroid hormone; ROS = reactive oxygen species; vitD = vitamin D.

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