Finally, the study by Khan et al.\(^2\) provides an interesting example of the multiple dilemmas that scientists face when attempting to explain the pathogenesis of renal diseases on the basis of isolated ex vivo or in vitro studies. For instance, the lack of glomerular gp120 binding to Gb\(_3\) should be interpreted with caution because these studies do not mimic the natural mechanism by which HIV-1 interacts with glomerular endothelial cells. Learning how HIV-1 or gp120 may be able to pass or interact with glomerular endothelial cells to reach podocytes and tubular epithelial cells would be an essential step to improve our understanding of HIV-associated nephropathy and HIV-HUS. As discussed above regarding children with Stx-HUS, previous ex vivo and in vitro studies provide similarly conflicting results regarding the pathogenesis of HIV-HUS. For example, the expression of DARC in glomerular endothelial cells is upregulated in renal sections harvested from children with HIV-HUS.\(^1\) However, cultured primary human glomerular endothelial cells do not express DARC protein. In contrast, cultured primary human renal glomerular endothelial cells express CXCR4 protein and can fuse with cells expressing HIV-gp120 by a CD4-independent mechanism.\(^9\) These findings suggest that HIV-1 can interact with glomerular endothelial cells via CXCR4, although CXCR4 cannot be detected in renal glomeruli from children with HIV-HUS (P.E.R. unpublished observations). In a similar manner, going back to the pathogenesis of Stx-HUS, renal glomerular endothelial cells from primates are Gb\(_3\)-negative in situ but express Gb\(_3\) in culture and are sensitive to Stx-induced cytopathology.\(^4\) Finally, Khan et al.\(^2\) showed less VT staining in adult glomeruli compared with pediatric glomeruli and found that the VT binding to adult glomeruli could be ‘unmasked’ by removal of the lipid composition of the plasma membranes with detergents or cholesterol-removing drugs. Overall, these findings suggest that the adult glomerular VT binding sites might be protected from toxin binding by unknown factors that are susceptible to detergent extraction, and they explain the age-and glomeruli-restricted pathology of Stx-HUS.

In summary, Khan et al.\(^2\) have refocused the pathological paradigm of Stx-HUS on glomerular endothelial cells and explained why young children develop Stx-HUS. Their findings may also improve our understanding of the pathogenesis of HIV nephropathy and HIV-HUS. Hopefully, more studies will follow their lead and confirm or expand these relevant clinical observations.

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Ablation of klotho and premature aging: is 1,25-dihydroxyvitamin D the key middleman?

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The reversal of soft-tissue abnormalities and prolonged lifespan observed in klotho\(^{-/-}\) mice following genetic inactivation of 1\(\alpha\)-hydroxylase underscores the pathophysiological role of 1,25-dihydroxyvitamin D in mediating some of the premature aging-like features observed in klotho\(^{-/-}\) mice.\(^5\)


Mutation in the klotho gene of mice (kl/kl) was first reported by Kuro-o et al. in 1997, characterized by the mice’s distinct features similar to those observed in the course of the aging process in humans.\(^1\) The associated syndrome observed in kl/kl mice was recapitulated in klotho knockout mice (klotho\(^{-/-}\)) described by Tsujiikawa et al., and the phenotypes were described as premature aging-like rather than natural aging.\(^2\) Over-expression of klotho in mice, on the other hand, resulted in an extension of lifespan. More important, the tissue-specific in vivo

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expression of klotho was downregulated in the course of aging. Collectively, these reports are in accordance with the notion of klotho being a senescence-related gene.

After its discovery, the klotho gene received considerable attention, leading to exciting findings on its tissue-specific localization and its potential molecular mechanisms in governing the aging machinery. Klotho protein is expressed as (1) a transmembrane form that can further undergo proteolytic cleavage, and (2) a secreted form generated by alternative RNA splicing, which is then exported out of the cells into the circulation. The transmembrane form of Klotho protein, instead of being present on the cell surface, was found to localize in the endoplasmic reticulum, endosomes, and Golgi apparatus, associating with its binding protein, z1-Na+/K+ ATPase. Although the exact mechanism remains elusive, it is speculated that Klotho, especially the secreted form, exerts its effect via not only autocrine but also paracrine and/or endocrine signaling, since klotho ablation results in plethoric aging-like syndrome despite its tissue-specific expression.

One of the hallmarks reported in both kl/kl and klotho−/− mice is the strikingly high level of 1,25-dihydroxy vitamin D (1,25(OH)2D3), the activated form of vitamin D, and 1α-hydroxylase (1α(OH)ase), its rate-limiting synthesizing enzyme, associated with mineral ion and hormone imbalance. Hypercalcemia and hyperphosphatemia are believed to be repercussions of enhanced intestinal absorption and/or renal reabsorption stimulated by high 1,25(OH)2D3 activity, whereas the lower-than-normal parathyroid hormone (PTH) expression and circulation levels are most likely due to a negative-feedback signal exerted by both high 1,25(OH)2D3 and calcium levels. The abnormal serum level of 1,25(OH)2D3 in the advent of defective klotho expression was propounded as the main culprit mediating most, if not all, of the aging-like peculiarities, especially those related to mineral ion homeostasis. To further dissect the pathological role of 1,25(OH)2D3 in this context, Ohnishi et al. (this issue) generated klotho−/−/1α(OH)ase−/− double-knockout mice to abolish 1α(OH)ase, thus eliminating 1,25(OH)2D3 in klotho−/− mice. Indeed, this work showed that eliminating 1,25(OH)2D3 by genetic inactivation of 1α(OH)ase reversed or abated typical features routinely observed in klotho−/− mice, including calcium and phosphate levels and the ensuing ectopic calcification, PTH level, multiple organ atrophy, and growth retardation. Although klotho−/−/1α(OH)ase−/− double-knockout mice did not thrive, as was evident by their reduced body weight and slight growth retardation as compared with related wild-type littermates, the obliteration of the aging-like features observed in klotho−/− mice should be attributed to 1,25(OH)2D3 wipeout.

Given the remarkably similar phenotypes observed in klotho−/− and Fgf-23−/− knockout mice, and that both models are characterized by noticeably high levels of 1α(OH)ase and 1,25(OH)2D3, it was proposed that Klotho and fibroblast growth factor-23 (FGF-23), a secreted protein playing a central role in phosphate homeostasis, share the vitamin D signaling pathway. The reversal of aging-like syndrome reported by Ohnishi et al. in klotho−/−/1α(OH)ase−/− mice is found to be consistent with, albeit not as thorough and extensive as, the result seen with Fgf-23−/−/1α(OH)ase−/− double-knockout mice. Indeed, there is compelling evidence that Klotho acts as a cofactor, converting canonical FGF receptors into specific receptors of FGF-23, thus augmenting FGF-23 signaling. It was also recently reported that, in Fgf-23−/−/klotho−/− double mutants, Klotho is required in FGF-23-mediated phosphate and vitamin D homeostasis; this reiterates the intimate relationship between Klotho and FGF-23. These reports are congruent with the observations of Ohnishi et al., who report an increase in sodium–phosphate cotransporter (NaPi2a) expression associated with apparent hyperphosphatemia in klotho−/− mice despite elevated FGF-23, suggesting a defect in FGF-23-mediated phosphaturic action following klotho ablation.

The persistently high level of 1α(OH)ase in both klotho−/− and Fgf-23−/− knockout mice suggested that Klotho, either in conjunction with FGF-23 or via other signaling pathways, may be one of the central inhibitory regulators of 1,25(OH)2D3 synthesis. The ablation of 1α(OH)ase in klotho−/− mice, however, reversed the serum level of FGF-23, implicating regulation of Fgf-23 expression by 1,25(OH)2D3. Notably, it was recently reported that besides jointly regulating vitamin D homeostasis, the FGF-23–Klotho signaling also stimulates proliferation and prevents vitamin D-induced apoptosis, via Ras and phosphatidylinositol-3-kinase transduction pathways. This should, at least in part, explain the tissue atrophy observed in klotho or Fgf-23 knockout mice. Although current in vivo evidence does not support Klotho-independent activity of FGF-23, the significantly high level of FGF-23 in klotho−/− mice, besides the risk of being cytotoxic, might activate an alternative non-vitamin D-related pathway(s) yet to be identified, giving rise to certain anomalies.

Alongside the diminished FGF-23 level, several other biochemical parameters, including serum levels of calcium, phosphate, and PTH, were reversed in klotho−/− mice with 1α(OH)ase ablation, raising the possibility that the rescue stems from reversal of multiplex factors rather than from the absence of 1,25(OH)2D3 itself. In an earlier study, Tsujiwaka et al. reported that the reversal of aging-like features in klotho−/− mice was similarly achieved by feeding the mice a low-vitamin D diet. Tissue calcification was completely prevented in klotho−/− mice on either a low-vitamin D or a low-calcium diet but persisted in mice fed a low-phosphate diet, and the groups on low-calcium and low-phosphate diets did not maintain normal body weight, in contrast to those on a low-vitamin D diet. These findings implied that mineral adjustment in itself might not reverse the aging-like peculiarities as efficiently and effectively as limiting of the 1,25(OH)2D3 level in klotho−/− mice.

Besides participating in vitamin D regulation in conjunction with FGF-23, Klotho has been found to possess pleiotropic effects, some of which may participate directly or indirectly in systemic calcium–phosphate homeostasis. It was reported that the extracellular domain of Klotho closely resembles β-glycosidase, which is capable of hydrolyzing and stabilizing the distal tubular epithelial calcium channel transient receptor potential vanilloid 5 (TRPV5), thus enhancing tubular reabsorption of calcium. At the subcellular level, Klotho binds to Na+/K+ ATPase and thus enhances its trafficking to
was found to facilitate the rapid tuning of which contributes to normal endothelial to oxidative stress, leading to increased sur-
pathway and therefore improving resistance – insulin-like growth factor signaling pathway, thus preventing Wnt-triggered cel-
functions include (1) antagonizing the Wnt that are important for basic physiological
deficit, however, have yet to be addressed.

The consequent increase in Na\(^+\) gradient was found to facilitate the rapid tuning of extracellular concentration of calcium in the choroid plexus and PTH release from the parathyroid gland that subsequently regulates systemic calcium–phosphate balance. The disruption of tubular calcium reabsorption via TRPV5 and the significance of Klotho–Na\(^+\)/K\(^+\) ATPase association under hypercalcemic conditions following Klotho ablation, however, have yet to be addressed.

Other potential actions of Klotho protein that are important for basic physiological functions include (1) antagonizing the Wnt pathway, thus preventing Wnt-triggered cellular senescence; (2) antagonizing the insulin–insulin-like growth factor signaling pathway and therefore improving resistance to oxidative stress, leading to increased survival; (3) upregulating nitric oxide synthesis, which contributes to normal endothelial function; (4) downregulating proapoptotic protein p53 and thus preventing senescence, and (5) activating protein kinase A and protein kinase C, affecting downstream targets of these protein kinases thereafter. Taken together, perturbation of all these mechanistic pathways along with the vitamin D signaling pathway following klotho ablation might, in concert, contribute to the senescence-related features (Figure 1).

In a nutshell, excessive activation of 1,25(OH)\(_2\)D\(_3\), taking into consideration both its calcemic and its non-calcemic regulatory actions may indeed be the primary factor inciting the senescence-related phenotypes that follow klotho ablation, given that effectors and mediators of Klotho, including TRPV5, Na\(^+\)/K\(^+\) ATPase, and FGF-23, have all been reported to intersect with the vitamin D-regulatory system (Figure 1).\(^{4–10}\) The current challenge, nevertheless, is to explain how the divergent targets and pathways of Klotho reconcile with each other. Therefore, although most features of the klotho\(^{-/-}\) mice were seemingly reversed in klotho\(^{-/-}\)/1α(OH)\(_{ase}\)\(^{-/-}\) double-knockout mice, it is imperative to scrutinize whether any defects in klotho\(^{-/-}\) mice were not rescued by genetic inactivation of 1α(OH)\(_{ase}\). This would provide valuable insight into the additional aforementioned functions of Klotho, whether they represent the non-vitamin D-regulatory pathway of Klotho, and their relationship to 1,25(OH)\(_2\)D\(_3\) signaling in the prevention of premature aging. Another critical factor that deserves attention is the putative existence of a Klotho-specific receptor\(^3\) that could potentially link together all the pathways identified to date. The regulation of endogenous Klotho expression by various endogenous and exogenous factors, as well as under certain physiological and pathological conditions,\(^3\) may be crucial clues for further investigations in order to decipher the intricacy of Klotho’s mechanisms.

**Figure 1** Klotho’s activity within and beyond the vitamin D–Ca\(^{2+}\)/PO\(_4\)^{3–} circuit. Klotho regulates various pathways/effectors (yellow) in an autocrine, paracrine, or endocrine manner. FGF-23 signaling intersects the vitamin D–Ca\(^{2+}\)/PO\(_4\)^{3–} circuit, modulating the downstream Ca\(^{2+}\)/PO\(_4\)^{3–} homeostasis (blue) by negatively regulating 1,25(OH)\(_2\)D\(_3\) synthesis, and adjusting PO\(_4\)^{3–} directly, bypassing the vitamin D pathway. Klotho also acts via other transduction pathways that may partially overlap with the vitamin D–Ca\(^{2+}\)/PO\(_4\)^{3–} circuit through specific mediators (green), including parathyroid hormone and transient receptor potential vanilloid 5 (TRPV5). It is plausible that Klotho’s targets cross-talk with each other or with the vitamin D–Ca\(^{2+}\)/PO\(_4\)^{3–} circuit and act synchronically to prevent premature senescence and maintain lifespan. PKA, protein kinase A; PKC, protein kinase C.

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