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.[10] 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endothelial cells via CXCR4, although CXCR4 cannot be detected in renal glomeruli from children with HIV-HUS (P.E.R. unpublished observations).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 glomerular-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.

DISCLOSURE
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REFERENCES

1. Ray PE, Liu X-H. Pathogenesis of Shiga toxin-induced hemolytic uremic syndrome. *Pediatr Nephrol* 2001; **16**: 823–839.
2. Khan F, Proulx F, Lingwood CA. Detergent-resistant globotriaosyl ceramide may define verotoxin/glomeruli-restricted hemolytic uremic syndrome pathology. *Kidney Int* 2009; **75**: 1209–1216.
3. Hughes AK, Stricklett PK, Kohan DE. Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells. *Kidney Int* 1998; **54**: 426–437.
4. Taylor FB, Tesh VL, De Bault L *et al.* Characterization of the baboon responses to Shiga-like toxin: descriptive study of a new primate model of toxic responses to Stx-1. *Am J Pathol* 1999; **154**: 1285–1299.
5. Liu X-H, Lingwood CA, Ray PE. Recruitment of renal tubular epithelial cells expressing verotoxin-1 (Stx-1) receptors in HIV-1 transgenic mice with renal disease. *Kidney Int* 1999; **55**: 554–561.
6. Crublet E, Andrieu J-P, Vives RR *et al.* The HIV-1 envelope glycoprotein gp-120 features four heparan sulfate binding domains including the coreceptor binding site. *J Biol Chem* 2008; **283**: 15193–15200.
7. Viard M, Parolini I, Rawat SS *et al.* The role of glycosphingolipids in HIV-signaling entry and pathogenesis. *Glycoconj J* 2004; **20**: 213–222.
8. Lund N, Branch DR, Mylvaganam M *et al.* A novel soluble mimic of the glycolipid, globotriaosyl ceramide inhibits HIV infection. *AIDS* 2006; **20**: 333–343.
9. Ray PE, Soler Garcia AA, Xu L *et al.* Fusion of HIV-1 envelope-expressing cells to human glomerular endothelial cells through CXCR4-mediated mechanism. *Pediatr Nephrol* 2005; **20**: 1401–1409.
10. Liu X-H, Hadley TJ, Xu X *et al.* Up-regulation of Duffy antigen receptor in children with renal disease. *Kidney Int* 1999; **55**: 1491–1500.

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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 1α-hydroxylase underscores the pathophysiological role of 1,25-dihydroxyvitamin D in mediating some of the premature aging-like features observed in *klotho*$^{-/-}$ mice.

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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 Tsujikawa *et al.*, and the phenotypes were described as premature aging-like rather than natural aging.[2] Overexpression 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.[3] 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 thereupon transported out of the cells into the circulation.[3] 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, α1-Na^+/K^+ ATPase.[4] 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.[3]

One of the hallmarks reported in both *kl/kl* and *klotho*$^{-/-}$ mice is the strikingly high level of 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), the activated form of vitamin D, and 1α-hydroxylase (1α(OH)ase), its rate-limiting synthesizing enzyme, associated with mineral ion and hormone imbalance.[2,3,5] Hypercalcemia and hyperphosphatemia are believed to be repercussions of enhanced intestinal absorption and/or renal reabsorption stimulated by high $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$ and calcium levels.[2] The abnormal serum level of $1,25(OH)_2D_3$ 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.[2,5] To further dissect the pathological role of $1,25(OH)_2D_3$ in this context, Ohnishi *et al.*[5] (this issue) generated *klotho*$^{-/-}$*/1a(OH)ase*$^{-/-}$ double-knockout mice to abolish 1α(OH)ase, thus eliminating $1,25(OH)_2D_3$ in *klotho*$^{-/-}$ mice. Indeed, this work showed that eliminating $1,25(OH)_2D_3$ 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.[5] Although *klotho*$^{-/-}$*/1a(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,[5] the obliteration of the aging-like features observed in *klotho*$^{-/-}$ mice should be attributed to $1,25(OH)_2D_3$ 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 $1,25(OH)_2D_3$,[2,5,6] 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(s).[6,7] The reversal of aging-like syndrome reported by Ohnishi *et al.*[5] in *klotho*$^{-/-}$*/1a(OH)ase*$^{-/-}$ mice is found to be consistent with, albeit not as thorough and extensive as, the result seen with *Fgf-23*$^{-/-}$*/1a(OH)ase*$^{-/-}$ double-knockout mice.[7] 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.[8] It was also recently reported that, in *Fgf-23*$^{-/-}$*/klotho*$^{-/-}$ double mutants, Klotho is required in FGF-23-mediated phosphate and vitamin D homeostasis;[6] this reiterates the intimate relationship between Klotho and FGF-23. These reports are congruent with the observations of Ohnishi *et al.*,[5] 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[2,5,6] suggested that Klotho, either in conjunction with FGF-23 or via other signaling pathways, may be one of the central inhibitory regulators of $1,25(OH)_2D_3$ synthesis. The ablation of *1a(OH)ase* in *klotho*$^{-/-}$ mice, however, reversed the serum level of FGF-23,[5] implicating regulation of *Fgf-23* expression by $1,25(OH)_2D_3$.[7] 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.[9] 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,[5] 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,[5] raising the possibility that the rescue stems from reversal of multiplex factors rather than from the absence of $1,25(OH)_2D_3$ itself. In an earlier study, Tsujikawa *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.[2] 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.[2] These findings implied that mineral adjustment in itself might not reverse the aging-like peculiarities as efficiently and effectively as limiting of the $1,25(OH)_2D_3$ 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.[10] At the subcellular level, Klotho binds to Na^+/K^+ ATPase and thus enhances its trafficking to

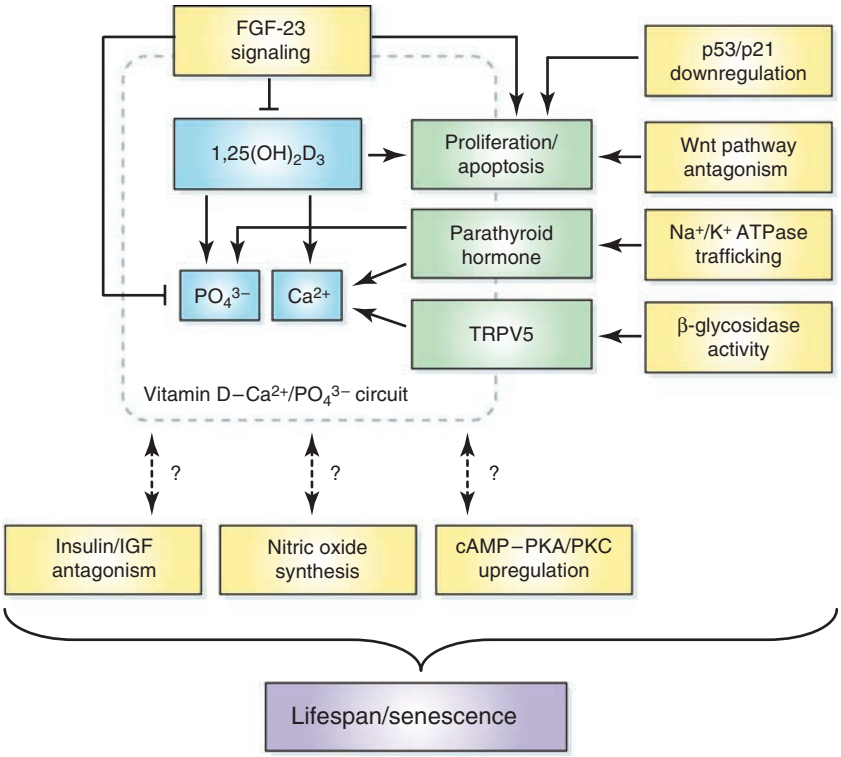

Figure 1 | Klotho's activity within and beyond the vitamin D–Ca^{2+}/PO_4^{3-} circuit. Klotho regulates various pathways/effectors (yellow) in an autocrine, paracrine, or endocrine manner. FGF-23 signaling intersects the vitamin D–Ca^{2+}/PO_4^{3-} circuit, modulating the downstream Ca^{2+}/PO_4^{3-} homeostasis (blue) by negatively regulating $1,25(OH)_2D_3$ 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.

the cell surface to carry out its activity in response to low extracellular calcium levels.[4] 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.[4] The disruption of tubular calcium reabsorption via TRPV5 and the significance of Klotho–Na^+/K^+ ATPase association under hypercalcemic conditions following Klotho deficit, 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;[3] (2) antagonizing the insulin–insulin-like growth factor signaling pathway and therefore improving resistance to oxidative stress, leading to increased survival;[3] (3) upregulating nitric oxide synthesis, which contributes to normal endothelial function;[3] (4) downregulating proapoptotic protein p53 and thus preventing senescence;[3] and (5) activating protein kinase A and protein kinase C, and affecting downstream targets of these protein kinases thereafter.[3] 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 $1,25(OH)_2D_3$—taking into consideration both its calcemic and its non-calcemic regulatory actions[11]—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).[4,8–10] 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*$^{-/-}$/*1a(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 $1,25(OH)_2D_3$ 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.

DISCLOSURE
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REFERENCES

1. Kuro-o M, Matsumura Y, Aizawa H *et al.* Mutation of the mouse *klotho* gene leads to a syndrome resembling ageing. *Nature* 1997; **390**: 45–51.
2. Tsujikawa H, Kurotaki Y, Fujimori T *et al.* Klotho, a gene related to a syndrome resembling human premature aging, functions in a negative regulatory circuit of vitamin D endocrine system. *Mol Endocrinol* 2003; **17**: 2393–2403.
3. Wang Y, Sun Z. Current understanding of klotho. *Ageing Res Rev* 2009; **8**: 43–51.
4. Imura A, Tsuji Y, Murata M *et al.* α-Klotho as a regulator of calcium homeostasis. *Science* 2007; **316**: 1615–1618.
5. Ohnishi M, Nakatani T, Lanske B *et al.* Reversal of mineral ion homeostasis and soft-tissue calcification of klotho knockout mice by deletion of vitamin D 1α-hydroxylase. *Kidney Int* 2009; **75**: 1166–1172.
6. Nakatani T, Sarraj B, Ohnishi M *et al. In vivo* genetic evidence for klotho-dependent, fibroblast growth factor 23 (Fgf23) -mediated regulation of systemic phosphate homeostasis. *FASEB J* 2009; **23**: 433–441.
7. Razzaque MS, Sitara D, Taguchi T *et al.* Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. *FASEB J* 2006; **20**: 720–722.
8. Urakawa I, Yamazaki Y, Shimada T *et al.* Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* 2006; **444**: 770–774.
9. Medici D, Razzaque MS, Deluca S *et al.* FGF-23-klotho signaling stimulates proliferation and prevents vitamin D-induced apoptosis. *J Cell Biol* 2008; **182**: 459–465.
10. Chang Q, Hoefs S, van der Kemp AW *et al.* The β-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science* 2005; **310**: 490–493.
11. Campbell MJ, Adorini L. The vitamin D receptor as a therapeutic target. *Expert Opin Ther Targets* 2006; **10**: 735–748.