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**Institutionen för klinisk vetenskap, intervention och teknik,  
Enheten för medicinska njursjukdomar**

# The role of FGF23/Klotho in mineral metabolism and chronic kidney disease

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligens försvaras i 9Q Månen, plan 9, Alfred Nobels Allé 8, KI Campus Huddinge

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## ABSTRACT

Chronic kidney disease (CKD) is a global health burden of growing incidence and prevalence. As renal function declines disturbances in mineral metabolism, such as hyperphosphatemia and secondary hyperparathyroidism, inevitably develop. These metabolic changes are closely associated with poor prognosis and survival. The bone-derived hormone fibroblast growth factor-23 (FGF23) and its co-receptor Klotho represent a novel endocrine axis regulating mineral metabolism in health and disease. FGF23-Klotho signalling inhibits renal phosphate reabsorption and activation of vitamin D, and reduces secretion of parathyroid hormone (PTH). Serum levels of FGF23 rise at early stages of CKD, presumably due to increased phosphate load, and numerous studies identify elevated FGF23 as a predictor of adverse clinical outcome. In contrast, tissue expression of Klotho decreases in parallel with CKD progression and reaches low or undetectable levels in end-stage renal disease. Importantly, mice lacking Klotho develop numerous complications associated with accelerated ageing, and many patients with advanced CKD, a state of Klotho deficiency, display a similar senescent-like phenotype. Altogether, FGF23 excess and lack of Klotho may be key pathogenic factors in CKD. In the present thesis we sought to elucidate the role of renal and parathyroid FGF23-Klotho signalling in physiology and in CKD.

In **Study I** we investigate Klotho levels in surgically resected parathyroid tissue specimen from CKD patients with secondary hyperparathyroidism, and find diminished Klotho expression paralleling the decline in renal function. Further, we demonstrate that FGF23 dose-dependently suppresses Klotho in bovine parathyroid cell culture, indicating a ligand-receptor regulatory process.

In **Study II** we generate parathyroid-specific Klotho knockout mice (*PTH-KL<sup>-/-</sup>*) using Cre-Lox recombination. *PTH-KL<sup>-/-</sup>* mice display a normal gross phenotype with a preserved calcium-PTH axis. Their PTH response is similar to wild-type mice when treated with FGF23 or challenged with renal failure. Yet, FGF23 treatment activates the MAPK pathway in wild-type mice but not in *PTH-KL<sup>-/-</sup>* mice. Importantly, blocking of calcineurin with cyclosporine A abolishes the FGF23-mediated PTH suppression in *PTH-KL<sup>-/-</sup>* mice, whereas wild-type mice remain responsive. Thus, we identify a novel calcineurin-dependent pathway in the parathyroid glands that, in the absence of Klotho, mediates acute suppression of PTH secretion by FGF23.

In **Study III** we develop a novel, non-surgical, mouse model of tubulointerstitial nephropathy. By adding various concentrations of adenine to the diet we define an adjustable protocol for inducing and maintaining uremia in mice.

In **Study IV** we generate distal tubule-specific Klotho knockout mice (*Ksp-KL<sup>-/-</sup>*). In contrast to systemic Klotho knockout mice, *Ksp-KL<sup>-/-</sup>* mice are fertile with a normal gross phenotype. Adult *Ksp-KL<sup>-/-</sup>* mice are hyperphosphatemic, indicating attenuated effects of FGF23 on proximal tubular phosphate handling. Further, FGF23 is higher in *Ksp-KL<sup>-/-</sup>* mice than in wild-type mice with matched serum phosphate, suggesting phosphate-independent regulation of FGF23 in *Ksp-KL<sup>-/-</sup>* mice.

Collectively, the studies presented in this thesis identify several novel and critical aspects of FGF23-Klotho signalling and function in health and disease, and provide important tools allowing for continuous investigation.