Citation: IBMS BoneKEy **9**, Article number: 211 (2012) | doi:10.1038/bonekey.2012.211

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COMMENTARY

FGF23 taken to heart: lessons from FGF23 neutralizing antibodies and global FGFR inhibition

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IBMS BoneKEy **9,** Article number: 211 (2012) | doi:10.1038/bonekey.2012.211; published online 14 November 2012

Commentary on: Faul C, Amaral AP, Oskouei B, Hu M-C, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St John Sutton M, Ojo A, Gadegbeku C, Seno di Marco G, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro-o M, Kusek JW, Keane MG, Wolf M. FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011;121:4393–4408 and Shalhoub V, Shatzen EM, Ward SC, Davis J, Stevens J, Bi V, Renshaw L, Hawkins N, Wang W, Chen C, Tsai M-M, Cattley RC, Wronski TJ, Xia X, Li X, Henley C, Eschenberg M, Richard WG. FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. *J Clin Invest* 2012;122:2543–2553.

High levels of serum phosphorus, calcium, PTH and FGF23 are all associated with increased cardiovascular morbidity and mortality in chronic kidney disease (CKD) patients. Too low levels of phosphorus, calcium and PTH are also predictive of increased mortality in these patients.[1] There is a cascade of factors involved in the pathogenesis of CKD mineral and bone disorders, and unraveling them and their interactions is a demanding challenge.[2] For instance, the high levels of FGF23 are now considered to be of primary importance in this cascade, which, together with PTH, responds in an ill-understood manner to cause a phosphaturia. FGF23 acts to decrease *PTH* gene expression, but in advanced CKD there is downregulation of its receptor complex in the parathyroid and the glands are no longer responsive to FGF23.[3,4] In turn, the high levels of PTH due to the secondary hyperparathyroidism of CKD are essential for the expression of the excessively high levels of FGF23 in experimental CKD.[5] High levels of PTH have long been known to be a uremic toxin with direct deleterious effects on the heart.[6] Do the excessively high FGF23 levels in CKD themselves directly act on the heart cells to cause left ventricular hypertrophy (LVH)? Of late, there have been two exciting articles in the *Journal of Clinical Investigation* looking at this question and, inevitably, with strikingly different yet complementary results reflecting the different tools that were available to the different investigators.

Faul *et al.*[7] convincingly showed that patients and rats with uremia because of 5/6 nephrectomy with developing CKD have LVH, which correlates with increasing serum FGF23 levels. Shalhoub *et al.*[8] present similar findings and emphasize that the LVH correlated with high serum phosphorus levels, low calcitriol and serum calcium, as well as high PTH and FGF23 levels. Faul *et al.*[7] then relied on cardiomyocytes in primary culture as well as cell lines to show that despite these heart cells not expressing klotho, which together with FGFR1c is the FGF23 receptor, they respond to FGF23. FGF23 added to the cells activates a signal transduction, which is different from the classical FGF23 signaling as well as different from canonical FGF2 signaling. Moreover, the FGF23-induced increase in cell size and signal transduction was prevented by the addition of an fibroblast growth factor receptor (FGFR) inhibitor, PD173074.[7] Convincingly, rats with experimental uremia after 5/6 nephrectomy developed LVH, and this was attenuated by pharmacological inhibition of FGFR by PD173074.[7] On the basis of this and other evidence, they propose that FGF23 in high concentrations as in CKD directly activates an alternative signal transduction pathway leading to LVH. The formal proof would be dependent upon showing that an FGF23 antibody prevented the LVH of experimental CKD, as well as *in vitro* that it prevented activation of their proposed alternative pathway. However, this antibody was not available to these investigators.

Shalhoub *et al.*[8] had the resources of Amgen laboratories available and developed a rat monoclonal antibody to FGF23 (FGF23-ab). They showed that the antibody specifically bound FGF23 and prevented the action of FGF23 to activate a reporter gene. Given as single injections to mice and rats, the FGF23-ab increased serum phosphorus, indicating effective inhibition of the phosphaturia due to FGF23. Their antibody, given to rats with experimental uremia due to 5/6 nephrectomy, effectively increased serum calcium, phosphorus and calcitriol, and, consequent to the raised calcium and calcitriol, led to the expected decrease in serum PTH. However, in sharp contrast to what may have been expected from the studies of Faul *et al.* their antibody did not correct the LVH in the CKD rats nor did it lead to a decrease in the genetic markers of LVH.[8] In fact, there was a very high mortality in their animals given the FGF23 antibody representing the complications of inhibiting FGF23's metabolic actions and the subsequent high serum phosphorus and calcitriol.

So where does this leave us? Well it is clear that no patient is going to receive the FGF23 antibody, which hastens mortality in experimental CKD. Moreover, Shalhoub's studies[8] conclusively show that an FGF23 antibody does not correct the LVH of CKD. So how can we best explain Faul's seminal studies that FGF23 directly acts on heart cells to cause LVH?[7] Well, first, studies using cardiomyocytes in either primary culture or in cell lines are complicated by the fact that they soon develop the genotype and phenotype of fibroblasts rather than heart cells despite still expressing certain cardiomyocyte gene markers.[9,10] Their response to FGF23 in high concentrations as *in vitro* or by direct intracardiac injection may represent a secondary response of mobilized canonical FGFs, and not necessarily FGF2, which they studied *in vitro*. For instance, injected FGF23 could feasibly act on the FGF23 receptor complex, FGFR1–klotho, of the blood vessels of heart or conceivably of other target tissues that may then release a factor, such as an FGF, which acts on the cardiomyocytes through a canonical FGFR.[11] The combination of epidemiological, clinical, *in vivo* and *in vitro* data provide a compelling case that FGF23 is associated with the LVH of CKD. However, these data do not indicate the causal role of FGF23 in LVH.

The apparent paradoxes in the result will provide a fruitful inspiration for further creative studies. There are many other paradoxes in biology, which have provided the bases for new discoveries. For instance, chronic hypocalcemia leads to very high levels of serum PTH despite the many-fold increase in calcitriol levels, which should have inhibited the *PTH* gene transcription. A partial answer to this paradox was that hypocalcemia increased nuclear calreticulin levels in the parathyroid, which prevented the action of calcitriol on the PTH promoter.[12] The failure of FGF23 to decrease PTH expression and parathyroid cell proliferation cited above is another example.[4]

The truth will come out, if not in the wash then at least in the rinse. This is the beauty of science and surely the stimulus to further exciting studies. The gauntlet has been raised and all of you fortune-seekers should put your shoulders to the grind-stone and develop new tools and different approaches to provide answers, and then more questions as to whether the LVH of CKD is due to the vascular changes secondary to the high serum phosphorus, high PTH and FGF23, and low calcitriol as well as the myriad other abnormal factors inherent to CKD, or whether FGF23 itself is the lone culprit. The Faul *et al.* studies are groundbreaking, but back to the drawing board to research FGF23's role in causing LVH. It would be in the interest of the scientific endeavor that the FGF23 antibody developed in Bill Richards' lab at Thousand Oaks was made available to other researchers, such as Myles Wolf in Miami who is the senior author in the Faul study, as well as others interested in taking up the challenge. Good science and a modicum of good luck will surely provide us with another enthralling episode in the quest to understand FGF23's role in the heart. These two researchers are well positioned to receive the baton, make impressive strides and pass it on to others. The questions are inherent to understanding the hugely increased cardiovascular mortality in CKD patients, and only by well-validated research can the field be laid to develop effective therapeutic strategies for our patients.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported in part by the Harold and Ethel Pupkewitz Renal Research Fund.

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