Tying TAZ and Nek1 into Polycystic Kidney Disease through Polycystin 2 Levels

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Polycystic kidney disease (PKD) is one of the most common genetic diseases in the world and is characterized by chronic renal cystic growth and kidney failure in children and adults. The kidney as well as the liver and pancreas undergo a buildup of fluid-filled cysts. The renal cysts arise from the epithelia of the nephrons and renal collecting system. PKD is usually inherited as an autosomal dominant trait through mutations in either the polycystin 1 (PC1; a transmembrane protein mutated in 85% of the patients) or the polycystin 2 (PC2; a nonselective calcium-permeable cation channel protein) gene.[1,2] PC1 and PC2 localize to primary cilia as well as the apical membrane of epithelial cells. Cilium-mediated signaling from PC1 and PC2 is a key determinant of cyst formation,[3] although the exact role of planar cell polarity defects in the initiation of cystogenesis remains unclear.[2,4]

In the story of Goldilocks and the three bears, Goldilocks at each encounter in the bears' house picks the one that is *just right* through trial and error. An understanding of PKD is coming to the same conclusion; the balance between PC1 and PC2 needs to be just right, but trial and error is not the method. Modulating the levels of PC2 that function in kidney primary cilia to monitor mechanical forces must be carefully regulated.[5] The Benjamin laboratory followed the role of two proteins, Nek1 and TAZ, whose individual loss causes cystic kidneys in mice, and they concluded that finding the right balance is important and that these two proteins are regulated by a negative feedback loop.[6]

TAZ was originally characterized as a transcriptional co-activator with a PDZ-binding domain.[7] Mutations of TAZ result in PKD, emphysema, and partial embryonic lethality in mice,[8] and morpholino knockdown causes cystic kidneys in zebrafish.[9] Human TAZ is phosphorylated on at least five serine residues (S66, S89, S117, S311, and S314), but only three are discussed. S89 phosphorylation by the LATS2 kinase from the Hippo tumor pathway allows binding to 14-3-3 and cytoplasmic retention.[10] S314 phosphorylation by casein kinase 1ε (CK1ε) and S311 phosphorylation by LATS2 both are required for binding to $SCF^{\beta\text{-TrCP}}$ E3 ubiquitin ligase and degradation of TAZ by ubiquitination.[11]

The next modulator is Nek1, which is a member of the NimA kinase family. Although NimA was first identified by its role in cell-cycle control in *Aspergillus*, this kinase family is expanded in organisms with cilia.[12] Humans have 11 Nek genes, and several are implicated in ciliary and centrosomal function. Mice with Nek1 mutations develop PKD as well as other cilium-based defects.[13] Patients with autosomal recessive short-rib polydactyly syndrome, Majewski type, which is associated with polycystic kidneys, may have causal Nek1 mutations.[14] Because deletions of each of these genes result in PKD, whether PC2, Nek1, and TAZ play interrelated roles with respect to development of polycystic kidneys is of great interest.

Previously, the Benjamin laboratory showed that phosphorylation of mouse TAZ on S306 (the equivalent of human S311) and S309 (the equivalent of human S314) is important for PC2 degradation by ubiquitination, and an altered PC1/PC2 ratio results in PKD.[9] The Guan laboratory showed that phosphorylation of human TAZ on S311 and S314 is necessary for TAZ destruction by ubiquitination.[11] Although TAZ is a transcriptional co-activator, phosphorylation on TAZ S309 has no effect on transcription of PC2 and Nek1.[6] Phosphorylation on S309 is reduced when a kinase-dead *Nek1* (*K33M*) gene is introduced, but the level of TAZ protein is unchanged. The catalytically dead construct results in increased PC2 compared with cells expressing the wild-type Nek1. Knockdown of *Nek1* with a short hairpin RNA or using kidney cells from the Kat^{2J} mutant mouse results in reduced TAZ protein levels and increased PC2 protein levels. TAZ regulates PC2 degradation through binding to $SCF^{\beta\text{-TrCP}}$ E3 ubiquitin ligase[8] and indirectly through its phosphorylation by Nek1. In *TAZ* knockout and knockdown cells, both Nek1 and PC2 protein levels increase. Ubiquitination of Nek1 and its loss are observed after overexpression of a *GST-TAZ* construct, whereas the addition of the proteosome inhibitor MG132 or the F-box deletion mutant $SCF^{\beta\text{-}Trcp\Delta F}$ prevents the loss of Nek1.

These results suggest an interesting negative feedback loop that regulates the levels of PC2. Nek1 phosphorylates TAZ S309, which allows formation of the TAZ-E3 ligase complex that ubiquitinates PC2 to promote its degradation. Phosphorylated TAZ then leads to ubiquitination of Nek1 and its degradation. Loss of Nek1 will result in less E3 ligase–activated TAZ that then will lead to an increase in PC2 and Nek1 levels.

TAZ plays roles in several pathways. In the Hippo pathway, it responds to signals for proliferation and cell death through *NF2*,

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MST1, *MST2*, *LATS1*, and *LATS2*, and mutations in these genes are often associated with a variety of human cancers. Phosphorylated TAZ that is retained in the cytoplasm by the action of LATS2 was thought to be inactive. However, cytoplasmic TAZ is implicated in regulation of PC2 as described already and in inhibiting the canonical Wnt signaling cascade.[15] The Wnt pathway is implicated in PKD,[16] but the role of cilia and Wnt signaling is still murky.[17] It remains hotly debated whether cilia and ciliary proteins affect (or are affected by) the canonical Wnt/β-catenin signaling pathway because results from different groups are contradictory.[18] Ocbina *et al.*[19] showed that mouse embryos lacking proteins of the anterograde ciliary transport (IFT) machinery show no change in Wnt target gene expression. However, several groups found that ciliary/basal body proteins may restrain Wnt signaling. Loss of the IFT/Golgi protein, Ift20, in the kidney causes PKD and results in an increase in nuclear β-catenin as well as increased expression of several Wnt target genes.[20] Chibby, a basal body protein, prevents nuclear entry of β-catenin and thus inhibits Wnt signaling as suggested for IFT20.[21]

Future work on TAZ and its causal relationship to PKD may want to consider the effects of the Wnt pathway on renal development and PC2 levels. Varelas *et al.*[15] showed that phosphorylation of TAZ S89 and its cytoplasmic localization result in its binding to Dishevelled (DVL2), which is likely to prevent DVL2 phosphorylation by CK1δ/ε, which prevents Wnt-induced transcriptional responses. TAZ would result in increased assembly of the destruction complex that contains Axin, adenomatous polyposis coli, CK1δ/ε, and GSK3. In the absence of TAZ, disassembly of this complex would occur. Thus, S89 phosphorylation could have two effects on the TAZ-Nek1 negative feedback loop. Reduced availability of CK1δ/ε may reduce the amount of S314 TAZ phosphorylation and TAZ binding to the E3 ligase that would lead to an increase in PC2 levels.[9]

Alterations in GSK3 levels may also influence cilia. In the unicellular alga *Chlamydomonas*, inhibition of GSK3 results in elongated flagella.[22] Less GSK3 could result in longer cilia, and more GSK3 could result in short cilia; each could alter PC2/PC1 ratios. TAZ, through its many partners, is a key regulator; the Hippo pathway and the canonical Wnt pathway can alter its phosphorylation. Through these pathways, PC2 levels can be altered in response to a variety of signals.

Goldilocks made her decisions by trial and error. It seems likely that a kidney cell uses many more metrics for making decisions about how much PC2 is around. To get it just right, the cells must carefully modulate the level using a growing number of feedback loops.

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DISCLOSURES

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See related article, "Nek1 and TAZ Interact to Maintain Normal Levels of Polycystin 2," on pages 832–837.

Third-Hit Signaling in Renal Cyst Formation

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During the past decade, primary cilia and the associated centrosomes have moved to center stage in investigations to understand the molecular mechanisms that lead to renal cyst growth in polycystic kidney disease (PKD) and other so-called ciliopathies.[1–3] Renal tubule epithelial cells possess exactly one primary cilium that protrudes into the tubule lumen. These mechanosensors bend in response to intralumenal fluid flow and trigger a calcium signal. Numerous cilia-associated proteins have been identified, and mutations in many of them lead to proliferation of tubule epithelial cells and renal cystic disease.[4] These moieties include the polycystins, which are affected in autosomal dominant PKD (ADPKD).

The consensus among many investigators has been that the loss of function of renal cilia somehow leads to aberrant proliferation of tubule cells. However, it is unknown what the actual purpose of renal cilia is and why flow sensing of fluid movement should have anything to do with the regulation of proliferation in the essentially nonproliferative adult kidney.

Several groups around the same time made a surprising observation using inducible-gene null mouse models; the elimination of polycystins in mature kidneys—or even of primary cilia altogether—had no apparent immediate consequence on the kidneys for months. Whereas disruption of polycystins or cilia in embryonic or early postnatal mice led to rapid, massive renal cyst growth, the same disruption in fully grown kidneys led to cyst growth only after a lag of several months.[5–9] Therefore, polycystins and primary cilia seem to regulate proliferation and cyst growth in the developing and growing kidney but are dispensable for the minute-to-minute operation of healthy adult kidneys.

How, then, does one explain the renal cyst growth in ADPKD that is thought to involve numerous somatic *second-hit* mutations that presumably occur during adulthood in individual tubule cells? This loss of heterozygosity mechanism involves the inherited *first-hit* germline mutation in a polycystin gene, followed by later somatic *second-hit* mutations in the remaining polycystin allele, leading to the growth of genotypically heterogeneous clonal cysts. If polycystins and cilia were indeed dispensable in adult kidneys, then a second-hit mutation should be inconsequential.

Recent results from several groups, including an article in this issue of *JASN*,[10] provide important insights to explain these puzzling findings. The bottom line is that the simple loss of polycystins or cilia in mature kidneys indeed does not always lead to immediate renal cyst formation. Although gene dose or epistasis may play a role,[11–13] another event—which has logically been termed a *third hit*[3]—may need to occur, which then leads to proliferation and cyst growth. Ischemic[7,14,15] and nephrotoxic injury[16] have been identified recently as important stress events providing a *third hit*.

In these latter experiments, polycystin 1 or a protein required for cilia formation, Kif3a, was eliminated in adult animals by inducible gene knockout. Subsequent renal injury led to cyst growth instead of the normal tissue regeneration and resolution of injury. Collectively, these findings suggest that polycystin 1 and cilia may not have major functions in the healthy adult kidney but are required to orchestrate the orderly execution of tissue regeneration in response to renal injury. They seem to be especially involved in the inhibition of proliferation once tubules have been repaired because tubule cells seem to keep going to form cysts in the absence of cilia or polycystin. Therefore, PKD could be regarded as a disease facilitated by unexpected or inappropriate continuous activation of an innate renal epithelial repair program. This notion is consistent with the fact that renal repair and PKD exhibit numerous similarities with regard to the renal activation of signaling pathways (mammalian target of rapamycin [mTOR]), protein expression (kidney injury molecule 1), and tissue abnormalities (fibrogenesis).[17]

The article in this issue of *JASN* adds another important piece to the puzzle.[10] Similar to the previous work described, the investigators eliminated renal cilia in adult mice by gene knockout of the intraflagellar transport protein polaris. It was previously shown that this loss does not result in renal cyst formation until approximately 6 months later.[5]

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One week after the polaris gene was eliminated, unilateral nephrectomy was performed. Normally, this leads to compensatory hypertrophy of the remaining kidney involving an increase in the size of the tubule epithelial cells but very little cell proliferation. In kidneys lacking cilia, however, this treatment led to induction of proliferation and massive cystic disease by 3 months.[10]

These new results suggest that compensatory hypertrophy is another, *third hit* leading to renal cyst growth in addition to ischemic and nephrotoxic injury. Interestingly, the mTOR pathway is activated in renal epithelial cells in all of these conditions: After injury, during hypertrophy, and in virtually all forms of PKD.[17–19] Treatment with the mTOR inhibitor rapamycin strongly inhibits compensatory hypertrophy and proliferation during injury repair and in PKD.[17,18] Furthermore, primary cilia and fluid flow downregulate mTOR activity in renal epithelial cells.[20]

A possible model emerging from these studies is that cilia and polycystins are required to *turn off* hypertrophic and proliferative signaling in renal epithelial cells after they have completed their response to stress-related insults. In the same way, cilia and polycystins may be required to *turn off* proliferation after renal maturation is complete, around day P13 in the mouse. Once cilia and polycystins have done their job, they seem no longer required to suppress proliferation on a day-to-day basis as long as the kidney does not experience any new stress.

Tubule epithelial cells lacking functional cilia as a result of genetic disease or manipulation seem hypersensitive to growth factor signaling that occurs after nephrectomy or injury. Many open questions remain about the molecular mechanisms that connect primary cilia and the regulation of proliferation. In particular, what is the role of polycystins, mechanosensation, calcium signaling, growth factors, and the immune system?

The induction of cyst growth in hypertrophic and injured kidneys could explain the rapid decline in renal function in the late stages of ADPKD. Progressive cyst growth leads to increased injury of normal tissue and increased functional impairment to which the kidneys may attempt to respond with more hypertrophy and repair, but this should make matters only worse because it should induce accelerated cyst growth until renal destruction spirals out of control. Although there is some evidence that family history predicts renal dysfunction from cystic disease,[21] this notion of a *third hit* could also explain the enormous heterogeneity of phenotypes even within the same family with ADPKD. Untoward environmental factors may lead to subclinical renal injuries that trigger bursts of cyst growth that accelerate disease progression.

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DISCLOSURES

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See related article, "Loss of Primary Cilia Upregulates Renal Hypertrophic Signaling and Promotes Cystogenesis," on pages 839–848.

Cystatin C Is More than GFR, and This May Be a Good Thing

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For measuring GFR, an exogenous marker is injected into the patient with timed marker levels assayed in the urine and plasma (urinary clearance) or plasma alone (plasma clearance). The essential properties of the exogenous marker are that it is metabolically inert and cleared exclusively by glomerular filtration, but cost, time, and discomfort make measured GFR impractical in most clinical settings. Instead, endogenous markers have been widely used. Unlike exogenous markers, non-GFR determinants of endogenous markers exist but are sometimes difficult to interpret.

Estimated GFR (eGFR) based on serum creatinine ($eGFR_{Cr}$) uses age, gender, and race in the estimating equation to model the non-GFR determinants of serum creatinine (largely muscle mass).[1,2] However, these demographics do not fully account for non-GFR determinants of creatinine. In particular, GFR is higher at the same serum creatinine level in healthy individuals with higher muscle mass than those with chronic kidney disease (CKD) and lower muscle mass.[3,4] Cystatin C is another endogenous marker cleared by filtration, and its serum levels are more highly correlated with GFR than serum creatinine.[5] The non-GFR determinants of cystatin C are less defined and more curious; cystatin C is a 14-kD protease inhibitor with anti-atherosclerotic activity in animal models[6,7] and is a strong predictor of mortality or cardiovascular disease (CVD) among individuals with normal $eGFR_{Cr}$[8,9] or CKD.[10–12]

In this issue of *JASN*, Mathisen *et al.*[13] provide novel insight into the non-GFR determinants of both serum creatinine and cystatin C. They found that higher serum creatinine levels (or lower $eGFR_{Cr}$) had a residual association with higher diastolic BP, not smoking, and increased physical activity. Nonsmokers and physically active individuals may be expected to have higher muscle mass, resulting in higher serum creatinine levels, potentially explaining this association. They also found that higher cystatin C levels (or lower $eGFR_{cysC}$) had a residual association with being a smoker, decreased physical activity, higher triglycerides, higher LDL cholesterol, lower HDL cholesterol, and obesity. This residual association with smoking and obesity has been previously reported, but previous studies adjusted for urinary creatinine clearance instead of measured GFR (mGFR).[14] Mathisen *et al.*[13] also found increased Framingham risk scores with lower $eGFR_{cysC}$ but not with lower mGFR or lower $eGFR_{Cr}$. These findings argue that $eGFR_{cysC}$ is a better predictor of CVD than GFR because the non-GFR determinants of cystatin C, possibly its anti-atherosclerotic activity, also reflect cardiovascular risk.

One potential objection to their conclusion is that eGFR may have a residual association with cardiovascular risk factors because mGFR is imprecise. In other words, could eGFR capture a residual association that reflects the true GFR signal missed as a result of error with mGFR? The study by Mathisen *et al.*[13] suggests this hypothesis is unlikely. The residual associations with $eGFR_{Cr}$ were different and sometimes in the opposite direction of residual associations with $eGFR_{cysC}$. If residual associations with eGFR could be fully explained by imprecision of mGFR, then residual associations with $eGFR_{Cr}$ should be similar to residual associations with $eGFR_{cysC}$. Furthermore, a sensitivity analysis was performed assuming 30% of the variance in direct GFR measurement was error, but the residual associations with eGFR remained.

How should these findings influence clinical practice? A new cystatin C equation that uses obesity, smoking, and serum lipids to improve the estimation of GFR could be developed. However, such an equation would have substantial drawbacks. Obesity, smoking, and lipids are only correlates of the non-GFR determinants of cystatin C and do not fully capture all of the non-GFR determinants of cystatin C. Incorporating the variables obesity, smoking, and lipids into a new $eGFR_{cysC}$ may lead to a more accurate estimate of GFR, but, paradoxically, the new $eGFR_{cysC}$ would be less predictive of CVD than cystatin C alone. In particular, individuals who are obese, smoke, or have dyslipidemia would have their eGFR increased by the new cys-

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tatin C equation. In fact, by controlling for these cardiovascular risk factors, the new $eGFR_{cysC}$ may be *less* predictive of CVD than GFR! Indeed, the variables used to model non-GFR determinants of a marker fundamentally influences how eGFR predicts outcomes. For example, the use of age to model the non-GFR determinants of serum creatinine inflates mortality risk estimates with $eGFR_{Cr}$ because age itself is a potent predictor of mortality.[15]

The study by Mathisen *et al.*[13] has a few limitations worth noting. Patients who reported renal disease, diabetes, or CVD were excluded. Similar studies of less select samples are needed. All analyses were adjusted for age and gender. However, the residual associations between eGFR and cardiovascular risk factors with adjustment only for mGFR should have been provided for three reasons. First, age and gender are variables used to calculate $eGFR_{Cr}$. Second, age and gender may correlate with the non-GFR determinants of cystatin C. Third, there has been no adjustment for age and gender with the use of eGFR to define CKD.

Given this residual association of cardiovascular risk factors with cystatin C levels, it might seem that cystatin C should not be used as a kidney function test. Perhaps the focus with cystatin C should be to improve prediction of clinical outcomes instead of optimizing the estimation of GFR. To the extent that cystatin C helps identify patients at higher risk for kidney failure, mortality, and CVD not detected by serum creatinine, it is useful. If the incremental improvement in risk prediction with cystatin C is due in part to its non-GFR determinants, then one might argue that cystatin C is not a *pure* kidney marker. However, much of the variation in GFR itself is not due to parenchymal injury in the kidney. Indeed, there is no association between mGFR and nephrosclerosis on renal biopsy among normal adults after controlling for age.[16]

Variation in GFR can be due to nonrenal factors such as dietary protein intake, volume status, hemodynamics, or even the indexing of GFR to body surface area.[17] Perhaps the most useful application of cystatin C is as a confirmatory test for individuals with an $eGFR_{Cr}$ <60 ml/min per 1.73 m^2, where cystatin C identifies the subset with nearly all of the increased risk for kidney failure, cardiovascular events, and mortality.[18] If cystatin C can find high-risk patients for whom targeted management is beneficial, then it is clinically useful. This should be the focus instead of a more exact GFR estimate.

DISCLOSURES

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See related article, "Esimated GFR Associates with Cardiovascular Risk Factors Independently of Measured GFR," on pages 927–937.

Statin Use Associates with Less Acute Kidney Injury after Major Elective Surgery

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Hepatic hydroxymethyl glutaryl–CoA reductase inhibitors, known as statins, are among the most commonly prescribed drugs in the world. Scientists studying microorganism host defense first identified statins in the 1970s. They were eventually shown in large randomized trials, such as the Scandinavian Simvastatin Survival Study and the West of Scotland Coronary Prevention Study, to confer substantial clinical benefits over placebo in individuals with hypercholesterolemia. Statins are now a cornerstone for both primary and secondary prevention of coronary heart disease. A number of noncardiovascular benefits—such as in dementia, sepsis, and cancer—have also been proposed, largely on the basis of observational data.

In this issue of *JASN*, Molnar *et al.*[1] report the results of an observational study on the association between statin use and decreased incidence of perioperative acute kidney injury (AKI) and mortality. The population-based cohort contained data on 213,347 Ontario Drug Benefits Plan recipients who were aged ≥66 years and underwent elective cardiac, thoracic, vascular, abdominal, or retroperitoneal surgery between 1995 and 2008. As anticipated, those who received statins tended to have more comorbid atherosclerotic disease, hypertension, diabetes, and congestive heart failure; to be treated with a greater number of total and cardiovascular-related medications; to have undergone more extensive cardiovascular diagnostic evaluations and procedures; and more likely to be undergoing cardiac and vascular surgery. On this basis, unadjusted analyses demonstrated an increased risk in perioperative AKI and dialysis use among statin users. However, upon multivariable and propensity score adjustment, statin use was associated with a 14 to 17% reduction in these outcomes. Curiously, unadjusted mortality was 27% lower in the statin group despite greater comorbid disease burden; mortality risk remained 15 to 21% lower after statistical adjustment. Analyses that appropriately accounted for healthier adherer bias and dose-response trends yielded corroborative findings. The population-based cohort design promotes generalizability, although, in fairness, only to elderly patients undergoing elective surgery.

As with all research on humans, internal validity of findings is contingent on accurate characterization of events and conditions. Absent available laboratory data, AKI was characterized solely on the basis of diagnostic codes. Considering the cohort's era, the majority of hospitalizations would have been coded using the *International Classification of Diseases, Ninth Revision* classification system, which has only 28.3% sensitivity for AKI.[2] Moreover, chronic kidney disease (CKD)—arguably the most important covariate—was assessed with only 22.9% sensitivity and 87.5% specificity (Appendix D-2).[1] Thus, both the outcome and a critical covariate had substantial error rates.

An often-repeated mantra in epidemiologic research is that nondifferential misclassification biases toward the null hypothesis. In other words, if information on AKI or CKD or other covariates were inaccurate but randomly so, then a study would tend to find no association even if an association existed and would therefore not account for the protective association seen in this study. However, this is an oversimplification for at least two reasons. First, if errors in diagnostic codes were correlated—that is, errors in AKI codes were more common in those with errors in CKD codes, such as might occur for patients with limited medical follow-up—then measures of association could be biased in either direction.[3] Second, we previously showed that AKI diagnostic codes in fact suffer from relevant nondifferential misclassification: codes have higher sensitivity in men than in women, in the elderly, and in those who die in-hospital.[2] To the extent that statin use in the Ontario cohort differed by race, gender, and mortality, misclassification bias cannot be ignored.

As with any observational study, the potential for causal inference must also be interpreted in light of the underlying biological basis and literature precedent. Studies of animal models demonstrated that statin use is associated with a decreased risk for ischemia-reperfusion kidney injury. Proposed mechanisms include favorable effects on oxidative metabolism involving heme oxygenase 1, NF-κB, activator protein 1, mevalonate, and nitric oxide.[4–7] However, animal data do not invariably translate to clinical practice. For example, N-acetyl cysteine showed similar promise in preclinical models yet demonstrated little to no clinical efficacy in preventing perioperative AKI.[8–10]

In addition, any cogent attempt to rationalize the study's findings as causal should account for the graded trend toward incrementally better outcomes among patients exposed to statins for *shorter* periods (adjusted odds ratios 0.86, 0.77, and 0.61 for statin use >90, 30 to 90, and <30 days, respectively),

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which does not follow intuitively on the basis of the proposed mechanism and may instead signal residual confounding.

The plausibility of the magnitude of the observed benefits also needs to be considered in light of previous studies. Molnar *et al.*[1] report a striking 21% lower odds of death, which is comparable to—or greater than—effects sizes seen in placebo-controlled trials of statins in patients at cardiovascular risk; that is, those with hypercholesterolemia *and* previous coronary heart disease. It is hard to imagine that the mortality benefit of statins in this population-based study could equal or exceed that seen in populations with high cardiovascular risk.

Notwithstanding these issues, let us consider the therapeutic implications of the study's findings assuming that estimates are both unbiased and causal. Perioperative AKI was observed in 1.9% of patients. Using the most favorable estimate for the effect of statins (odds ratio 0.84), 1000 patients would need to be treated to avert three instances of AKI. This number treated would avert fewer than one episode of dialysis-requiring AKI. At the same time, estimates from the literature suggest that such treatment would result in approximately four cases of aminotransferase elevation,[11] as well as a heightened risk for myalgias, myopathy, and rhabdomyolysis.[12] Furthermore, informed assessment of the net health benefits of perioperative statin renal prophylaxis would require simultaneous consideration of permanent renal injury; that is, residual CKD or ESRD and sequelae thereof. Although the article does not address this specifically, absence of association between statin use and reduced dialysis dependence 90 to 120 days postoperatively may be cause for pessimism vis-à-vis long-term renal benefit.

Alternatively, it is possible that perioperative renal prophylaxis with statins could be targeted to patients at high risk for perioperative AKI, for whom the risk-benefit ratio might be more favorable. However, the data at hand cannot provide supportive evidence for such a paradigm given that subgroup analyses restricted to those at highest risk– for example, patients with diabetes and those with pre-existing CKD– were not performed.

As the authors note, sample size requirements likely preclude the possibility that a dedicated, hard end point, randomized trial will ever be conducted. Considering residual uncertainties, we wonder whether a smaller randomized trial using kidney injury biomarker surrogate end points might be warranted. Would the finding of lower kidney injury biomarker levels postoperatively in patients randomly assigned to statins *versus* placebo provide sufficient evidence to justify widespread use of statins overall or in high-risk patients? Possibly, but consider the instructive story of torcetrapib, which increased HDL levels but also increased the risk for death in the Investigation of Lipid Level Management to Understand its Impact in Atherosclerosis Events (ILLUMINATE) trial.[13] Approval of torcetrapib on the basis of improvement in a biologically plausible but untested surrogate end point would have been a catastrophic failure.[14] Although the U.S. Food and Drug Administration and other international regulatory agencies have qualified several kidney injury biomarkers for preclinical nephrotoxicity monitoring,[15] it remains unclear whether and in which contexts novel biomarkers may be acceptable surrogate end points for AKI trials. At the very least, follow-up observational studies using cohorts with available laboratory data are needed before clinical adoption.

To summarize, Molnar *et al.*[1] have produced a careful analysis of a large data set suggesting an association between statin use and lower risk for postoperative AKI. Unfortunately, potentially insurmountable limitations related to confounding by indication and differential misclassification of exposure and outcome status render the findings insufficiently persuasive to warrant adoption into clinical practice despite the rigor of the authors' analytic approach. Barring corroborative findings in other studies, this analysis may be relegated to be yet another footnote in the long, sad saga of failed AKI therapeutics.

DISCLOSURES

None.

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New Insights to Fibroblast Growth Factor 23 in Kidney Transplant

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Some abnormalities in mineral metabolism are evident even at very early stages of chronic kidney disease (CKD) and are important determinants of subsequent bone and cardiovascular disease.[1,2] A decade ago, fibroblast growth factor 23 (FGF23) was recognized by a few as the protein responsible for several rare inherited and acquired syndromes of osteomalacia and rickets.[3,4] Only recently have studies demonstrated the significance of FGF23 in mineral metabolism in the larger population of patients with CKD and ESRD.[5–7] In 2007, Fliser *et al.*[8] reported that higher FGF23 levels were strongly associated with progression of non diabetic CKD. The next year, Gutierrez *et al.*[9] published that incident hemodialysis patients with higher FGF23 levels were at substantially greater risk for all-cause mortality. These and other studies generated considerable interest in the role of FGF23 in mineral metabolism homeostasis in CKD.

Recent reports extend these findings by showing that FGF23 levels are elevated at very early stages of CKD,[2] and the associations of FGF23 with all-cause mortality or cardiovascular disease extend to patients with stages 3 to 4 CKD,[10] and even to individuals with ostensibly normal kidney function.[11] In this issue of *JASN*, Wolf *et al.*[12] report that higher FGF23 levels associate with the composite outcome of all-cause mortality or kidney allograft loss among 984 stable transplant recipients. Most of the participants were several years after transplantation. This finding is significant for several reasons. First, in conjunction with other literature, this study demonstrates that higher FGF23 levels identify patients at increased risk for adverse outcomes across the spectrum of CKD. Second, because most kidney transplant recipients have survived an extended period on dialysis before receiving an allograft and often have a high burden of vascular disease, the study demonstrates that FGF23 continues to provide risk information in this late stage of disease. Last, given the pattern of other mineral metabolism abnormalities observed in kidney transplant recipients, the study provides new insights into potential mechanisms, as described further next.

With these discoveries come new challenges. Among the most pressing is a better understanding of mechanisms responsible for the link of FGF23 with adverse outcomes.[7] Several possibilities require special consideration. A main biological function of FGF23 is to increase urine phosphorus excretion.[4,13] A wealth of data spanning from the laboratory to population-based studies implicates hyperphosphatemia as a key factor inducing and promoting arterial calcification.[14–16] Thus, perhaps high FGF23 levels are linked with mortality through alterations in phosphorus homeostasis. Several studies have investigated this possibility. Consistently and observed again in the article by Wolf *et al.* in this issue, statistical adjustment for serum phosphorus levels measured concurrently with FGF23 does not attenuate its relationship with outcomes.[8–12] However, phosphorus may remain an important intermediary nonetheless. Contemporary clinical laboratories precisely measure serum phosphorus levels, typically with coefficients of variation $<3\%$. However, there is considerable biological variability in serum phosphorus levels within individuals over time.[17] This is analogous to serum glucose, for which one can precisely determine the blood level at a given moment, but it gives a mere snapshot of average glucose levels over time. Thus,

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one possibility is that higher FGF23 levels increase in response to higher phosphorus levels[18] and may serve as a more accurate indicator of time-averaged serum phosphorus exposure than serum phosphorus levels themselves, analogous to a hemoglobin A1c as an indicator of time-averaged glucose levels. Future studies with repeated measurements of serum phosphorus over time are required to investigate this possibility. However, the study by Wolf *et al.* in this issue provides some early insights arguing against this hypothesis.

Kidney transplant recipients frequently develop posttransplantation hypophosphatemia. Although this abnormality often wanes with time, a subgroup remains persistently hypophosphatemic.[19] In the study by Wolf *et al.* did not provide repeated measures of serum phosphorus, subgroup analysis demonstrated that higher FGF23 levels remain strongly associated with death or allograft loss in patients with serum phosphorus in the lowest tertile (<2.9 mg/dl) at study enrollment.[12] Although not definitive, these data suggest that viewing FGF23 as a marker of time-averaged serum phosphorus may be too simplistic.

FGF23 also inhibits conversion of calcidiol to calcitriol. Calcitriol deficiency may also activate the renin-angiotensin-aldosterone axis,[20] affect inflammatory stress and glycemia, and low levels associate with adverse outcomes.[21] Thus, perhaps high FGF23 levels lead to low calcitriol, which in turn may lead to adverse outcomes. This hypothesis has not been fully investigated.[10] Calcitriol levels are found at approximately 1000-fold lower levels in circulation than calcidiol, making them difficult to measure precisely.[21] Moreover, calcitriol has a short serum half-life.[22] Thus, similar to serum phosphorus levels, future studies may require multiple measures of calcitriol and use more precise measurement techniques to evaluate whether it may represent a causal intermediary between FGF23 and adverse outcomes.

When FGF23 binds its receptor, it requires the co-factor klotho to exert its effect on target cells.[4] Klotho is expressed in the kidney, parathyroid gland, and choroid plexus but can be cleaved from the cell surface and released into blood, and biological significance of soluble klotho is uncertain. A recent study demonstrated that plasma klotho levels were low in a rat CKD model and that klotho inhibited phosphorus uptake and mineralization of rat vascular smooth muscle cells *in vitro*.[23] Whether klotho levels are low in humans with CKD and how klotho interacts with vascular smooth muscles to induce these changes are unknown. Nonetheless, if similar effects hold in humans, one can speculate about feedback mechanisms stimulating higher FGF23 to compensate for low klotho levels in patients with CKD. Perhaps those with the lowest soluble klotho have the greatest burden of arterial calcium deposition and higher adverse event rates, the higher FGF23 may be serving as a marker of klotho activity within vascular tissues. Thus, whether high FGF23 levels may serve as a marker of soluble klotho or may interact with klotho in vascular tissues are important topics for future research.

Additional challenges include identification of methods to safely and reliably lower FGF23 levels. Preliminary studies suggest that non–calcium-based oral phosphorus binders and cinacalcet may lower FGF23 levels in patients with ESRD.[4,24,25] Short-term studies evaluating oral phosphorus binders in stages 3 to 4 CKD provide conflicting results regarding changes in FGF23 concentrations,[26,27] and short-term dietary phosphorus loading or restriction in patients with CKD have not resulted in significant changes in FGF23.[6,27] To date, all studies in CKD have evaluated short-term interventions, and future studies with longer follow-up may provide different results. Nontraditional methods that alter phosphorus levels in individuals with or without CKD should also be investigated in regard to their effect on FGF23.[28–32]

In summary, FGF23 has emerged as a robust risk marker for death, cardiovascular events, and kidney disease progression across the spectrum of CKD. Moving forward, the new challenge will be to elucidate mechanisms responsible for these associations, to identify safe and effective methods to alter serum FGF23 levels, and ultimately to determine whether such interventions translate into demonstrable improvements in health outcomes in our patients.

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DISCLOSURES

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