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Commentary

Albuminuria acts as a marker of progressive chronic kidney disease and as an indicator for initiation of hypertension treatment via modulation of the renin-angiotensin-aldosterone system with angiotensin receptor blockers or angiotensin-converting enzyme inhibitors. However, the true significance of albuminuria has yet to be fully defined. Is it merely a marker of underlying pathophysiology, or does it play a causal role in the progression of kidney disease? The answer remains under debate. In this issue of the *JCI*, Bedin et al. used next-generation sequencing data to identify patients with chronic proteinuria who had biallelic variants in the cubilin gene *(CUBN)*. Through investigation of these pathogenic mutations in *CUBN*, the authors have further illuminated the clinical implications of albuminuria.

Not all proteinuria is created equal

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Albuminuria acts as a marker of progressive chronic kidney disease and as an indicator for initiation of hypertension treatment via modulation of the renin-angiotensin-aldosterone system with angiotensin receptor blockers or angiotensin-converting enzyme inhibitors. However, the true significance of albuminuria has yet to be fully defined. Is it merely a marker of underlying pathophysiology, or does it play a causal role in the progression of kidney disease? The answer remains under debate. In this issue of the *JCI*, Bedin et al. used next-generation sequencing data to identify patients with chronic proteinuria who had biallelic variants in the cubilin gene *(CUBN)*. Through investigation of these pathogenic mutations in *CUBN*, the authors have further illuminated the clinical implications of albuminuria.

Biallelic mutations related to proteinuria

The kidneys filter approximately 180 liters of plasma daily to balance the excretion of water, acid, nitrogen, sodium, and potassium with varying environmental conditions. Larger proteins are retained in the plasma because of filtration barriers in the glomeruli, but the kidney tubules must still capture up to 2.5 g/d of low-molecular-weight (LMW) proteins that escape the glomerular barriers (1). Cubilin and megalin are located in the apical membrane of the proximal tubule and are responsible for receptor-mediated endocytosis of the filtered proteins that leak through the glomerular barriers. Biallelic mutations in megalin (*LRP2*) lead to Donnai-Barrow syndrome (DBS), characterized by LMW proteinuria and abnormal neurological development (2), whereas biallelic cubilin (*CUBN*) mutations can lead to another recessive disease, Imerslund-Gräsbeck syndrome (IGS), which is characterized by LMW proteinuria and megaloblastic anemia (3).

In this issue of the *JCI*, Bedin et al. (4) analyzed next-generation sequencing data from three different cohorts (n = 2216 patients) of patients with chronic proteinuria ranging from 0.5 to 2.5 g/d and identified 39 patients who had biallelic variants in the *CUBN* gene. Interestingly, the *CUBN* variants resulted in isolated proteinuria without the megaloblastic anemia typical of IGS, suggesting that there are separate binding sites in cubilin for vitamin B12–intrinsic factor (VitB12-IF) and albumin. Cubilin is an enormous protein, a 460-kDa peripheral membrane glycoprotein with 27 putative ligand-binding CUB domains (complement components C1r/C1s, UEGF, and bone morphogenic protein-1) (5). Although biochemical and structural methods have determined that the binding site for VitB12-IF is CUB6–8 (6), the manner in which cubilin binds to other proteins including albumin is still being investigated.

The patient cohorts in Bedin et al. offered an opportunity to genetically dissect cubilin-albumin binding, and the authors made the novel proposal that CUB13 or CUB26 are potential albumin-binding domains due to their proximity to Ca^{2+}-coordinating motifs (4). CUB13 and CUB26 have not yet been structurally characterized at high resolution, although recent low-resolution electron microscopy data suggest that these C-terminal domains should cluster at the distal end of a 700-Å-long tree-like structure (7) where they are readily exposed to the urinary filtrate and available to bind proteins. In addition to albumin, it is possible that these domains could bind other ligands of similar size and isoelectric point (pI) such as vitamin D–binding protein (DBP), which has a close evolutionary relationship to albumin.

Varying proteinuric phenotypes have been reported in the literature, depending on which domains of cubilin are affected. Previously, a p.P1297L point mutation within the VitB12-IF binding site was observed to lead to anemia without proteinuria. Additionally, numerous variants causing global cubilin dysfunction have been documented to cause IGS with both anemia and proteinuria that is inclusive of albumin as well as other cubilin ligands such as apoA-1, transferrin, and DBP (8). Now, the study by Bedin et al. extends the known proteinuric phenotypes resulting from *CUBN* variants to include isolated albumin-predominant proteinuria resulting from C-terminal mutations (4). Certainly, further analysis of urinary proteins from patients with *CUBN* variants is bound to delineate still more phenotypes of proteinuria.

The authors noted two interesting features of the patients in the studied cohorts: (a) despite experiencing chronic proteinuria, these patients retained normal estimated glomerular filtration rates (eGFRs), a standard assessment of renal function, which is consistent with prior studies reporting normal renal function in patients with IGS, and (b) the proteinuria they exhibited was albumin predominant, with, on average, more than 60% of the total proteinuria consisting of albumin (4).

The impact of albuminuria on renal function

Albuminuria is a predictor of negative clinical outcomes including the progression of renal disease, but the degree to which albuminuria can be used independently as a surrogate therapeutic target is a matter of

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ongoing discussion (9–12). Furthermore, whether albuminuria causes progressive chronic kidney disease (CKD) also remains unknown. Causality has been suggested by reductions in albuminuria associated with reduced CKD progression to end-stage renal disease (ESRD) in several different clinical trials that evaluated the treatment of CKD patients with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) (13–16). Notably, the Renoprotection of Optimal Antiproteinuric Doses (ROAD) trial showed a reduction in CKD progression in nondiabetic proteinuric patients when renin-angiotensin-aldosterone system (RAAS) blockade was titrated to albuminuria targets (17), but the significance of those results has been disputed (10). So far, a reduction in albuminuria beyond that achieved by ACEI/ARB monotherapy has yet to show an effect on hard outcomes (18–22), raising the possibility that RAAS inhibition itself, rather than the associated reductions in albuminuria, mediates the clinical benefits.

There are reasonable grounds to posit a role for albuminuria in the pathogenesis of proteinuric CKD. In vitro evidence has shown that protein overload in proximal tubule cells activates NF-κB signaling, upregulates inflammation via monocyte chemotactic protein 1, and induces vasoconstriction via endothelin 1 (23), which together can contribute to the development of tubulointerstitial disease. The in vivo evidence, however, does not fully support the hypotheses generated from in vitro experiments. Humans with loss of function of megalin or cubilin have up to 2.5 g/d of proteinuria (1), implying that the proximal tubule is chronically exposed to gram quantities of albumin that must be efficiently endocytosed by megalin-cubilin. This suggests at least that chronic albumin exposure in the proximal tubule is insufficient for the development of tubulointerstitial disease.

The proteinuric consequences of cubilin mutations have been well documented (8), but Bedin et al. (4) went further to query the impact of albuminuria on renal function. Their 39 patients were primarily children, but the age range encompassed newborns, teenagers, seven patients aged 30 to 40 years, and one aged 66 years, all of whom exhibited normal eGFRs of greater than 80 mL/min. Although the authors could not document an extended longitudinal follow-up of renal function measurements in their patients, their observation of normal or near-normal eGFRs in patients with decades of exposure to albuminuria is nonetheless intriguing. To further investigate their findings, the authors conducted a meta-analysis of cohorts from the CKDGen Consortium using *CUBN* variants previously associated with albuminuria in GWAS studies (24). Through this cross-sectional population-based approach and using an additive model to test for associations, the authors found an association of four different *CUBN* variants with both albuminuria and a small but statistically significant increase in eGFR. Thus, in these patients with *CUBN* loss of function and isolated chronic proteinuria, the consequence of long-term albumin exposure to the kidney tubules may be benign. The authors reanalyzed their data for one of the variants under a recessive model and found a stronger association with albuminuria in homozygotes. It will be informative to reanalyze all these GWAS data under a recessive model to confirm an association with more severe proteinuria in individuals carrying two of these risk variants. In addition, the availability of large data sets such as those from the UK Biobank will enable detection of associations of these variants with phenotypes beyond albuminuria.

Clinical implications

That the proteinuria in the Bedin et al. patient cohorts was albumin predominant yields important conclusions for practicing nephrologists: all albuminuria is not of glomerular origin, and isolated albuminuria may not be toxic to kidney function (4). Albuminuria has long been recognized as a hallmark of glomerular disease and indicative of a compromised filtration barrier that has lost size and charge selectivity. Although another genetic form of tubular proteinuria, Dent disease, results in kidney failure, interpretation is confounded by concomitant hypercalciuria and nephrocalcinosis. Here, Bedin et al. show that albuminuria can be associated with normal renal function in the context of *CUBN* loss of function (4). Identifying genetic causes of renal disease that are intractable to medical therapy is a growing area of clinical practice that is essential for avoiding unnecessary therapeutic or diagnostic interventions. For instance, seven of the patients in the Bedin et al. cohorts were treated with ACEI/ARB therapy without benefit, and others underwent renal biopsies that were nondiagnostic (4). The importance of genetic screening to help exclude unnecessary therapy is even more pointed in situations in which steroid treatment is being considered, such as in cases of focal sclerosing glomerulosclerosis (or in presumed cases of minimal change disease in children.

Ultimately, more longitudinal data will be required to definitively establish that long-term, isolated albumin-predominant proteinuria is truly benign. The diversity of proteinuria phenotypes that can occur in human disease and their subsequent, variable clinical consequences still remain to be fully appreciated. For instance, Bedin et al. draw a distinction between the albumin-predominant proteinuria resulting from *CUBN* mutations and the proteinuria seen in *LRP2* mutations that features a higher proportion of α1- and β2-microglobulins as well as a possible association with reduced eGFR (4, 25). This phenotypic distinction is striking, given that megalin and cubilin associate in the apical membrane of the proximal tubule and are coreceptors for a large repertoire of proteins, including albumin. That loss of function of one or the other of these coreceptors results in different patterns of proteinuria with differing clinical consequences is a remarkable fact that highlights how much there is yet to discover about proteinuria and its physiological consequences.

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