Tamm Horsfall Glycoprotein and Uromodulin: It Is All about the Tubules!

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"The hyaline cast is made of Tamm Horsfall protein—the most abundant protein in the urine." This often-spoken statement is delivered universally in urinalysis lectures and represents the current knowledge of most students, physicians, and nephrologists regarding Tamm Horsfall glycoprotein—also known as uromodulin. This comment is almost always followed by the statement "next slide," indicating our lack of knowledge and understanding about the main function of this enigmatic protein. This limited knowledge also extends to the imbalance in our understanding of the renal tubulointerstitium compared with the glomerulus. Our primary marker of kidney function is creatinine, a marker of GFR, and our clinical understanding of glomerular diseases far surpasses that of tubular disorders.

Fortunately, our knowledge regarding uromodulin and tubulointerstitial kidney disease has significantly increased in the last decade. Two new publications (1,2) in this issue of the *Clinical Journal of the American Society of Nephrology* (*CJASN*) further add to this knowledge. In this editorial, we will review recent developments in this area and provide a context for the two new investigations.

Uromodulin synthesis occurs exclusively in the tubular epithelial cells in the thick ascending limb of Henle (3). It is a highly glycosylated molecule with a high number of cysteine residues. These cysteine residues crosslink to form the final filamentous gel–like structure of uromodulin (4). Uromodulin is translocated to the apical surface of the thick ascending limb, where it is bound to the cell membrane by a glycophosphatidylinositol anchor. Uromodulin faces the tubular lumen and remains anchored to the tubular cell until it undergoes enzymatic cleavage by an as yet unspecified enzyme (5). This then allows uromodulin to bind with other protein molecules in the lumen of the thick ascending limb, forming a protective coating that is eventually excreted in the urine.

A breakthrough in the understanding of uromodulin physiology was the identification of mutations in the *UMOD* gene encoding uromodulin as a cause of inherited kidney disease (6). Uromodulin kidney disease (UKD)—formerly known as medullary cystic kidney disease type 2 and familial juvenile hyperuricemic nephropathy—is inherited in an autosomal dominant manner. Clinical characteristics include early-onset gout in some family members, a bland urinary sediment, and slow progression of kidney failure, leading to ESRD in the fourth through seventh decades (7). Mutations in the *UMOD* gene result in improper crosslinking of the molecule and the inability of uromodulin to achieve its normal configuration. Abnormal uromodulin polymerizes within renal tubular cells (8,9), leading to accelerated tubular cell death, nephron loss, and progressive kidney disease.

The decreased urinary uric acid excretion in UKD was hypothesized to be caused by decreased production of normal uromodulin, but why would this be? Uromodulin is made in the thick ascending limb, and uric acid is reabsorbed proximally. Researchers then identified that uromodulin facilitates the intracellular trafficking of the furosemide–sensitive Na-K 2-Cl cotransporter (NKCC2) to the apical surface of the thick ascending limb (Figure 1) (10). With less uromodulin production, there is less expression of NKCC2 in the thick ascending limb, less sodium reabsorption, and more sodium excretion. In patients with UKD, mutant uromodulin leads to decreased NKCC2 apical expression, mild sodium wasting from the thick ascending limb (6), and a compensatory increase in sodium reabsorption proximally. Because proximal tubular urate reabsorption is linked to sodium reabsorption, patients have increased urate reabsorption and develop hyperuricemia.

In 2009, a genome–wide association study was performed by Köttgen *et al.* (11) to identify genes associated with GFR. Surprisingly, single-nucleotide polymorphisms (SNPs) in the *UMOD* gene were found to be associated with CKD in this large cohort of patients. This was subsequently confirmed in additional studies (12,13). SNPs are single-nucleotide changes that have occurred randomly over time in the human genome, with most SNPs occurring in noncoding regions. For instance, the *UMOD* promoter contains the SNP rs4293393. A thymine (T) nucleotide is present on approximately 83% of alleles of this SNP, and a cytosine nucleotide is present on 17% of the alleles in the general population. The T nucleotide is the risk variant associated with an increased risk of CKD.

Why was the *UMOD* SNP associated with CKD, and how did this relate to other recent findings regarding uromodulin? In 2013, Trudu *et al.* (14) elegantly pieced together these separate findings. Trudu *et al.* (14) showed that the rs4293393 T variant resulted in a 50% increase in urinary uromodulin excretion. Increased uromodulin production should be associated with increased apical expression of the NKCC2 transporter, leading to increased sodium reabsorption, hypertension,

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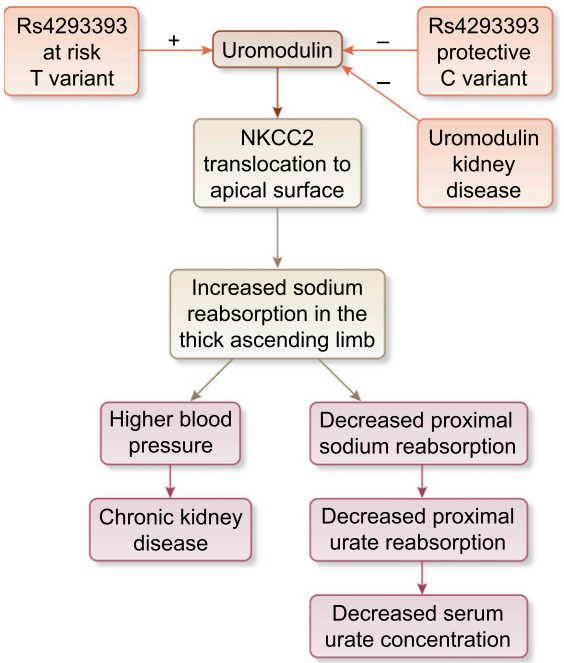

Figure 1. | Uromodulin facilitates intracellular translocation of the furosemide–sensitive Na-K 2-Cl cotransporter (NKCC2) to the apical surface of the thick ascending limb, with multiple potential downhill consequences.

and CKD (Figure 1). Trudu *et al.* (14) provided convincing evidence in this regard. When they upregulated uromodulin production in transgenic mice, the mice developed higher BP that was sodium sensitive. These mice also had increased renal tubulointerstitial fibrosis. Similar to transgenic mice with increased uromodulin production, Trudu *et al.* (14) showed that humans with one or two of the rs4293393 T–variant alleles produced increased amounts of uromodulin, more commonly suffered from sodium-sensitive hypertension, and were more likely to have tubulointerstitial fibrosis in postmortem studies. This important study informed us of not only the role of uromodulin but also, the importance of sodium in hypertension and CKD (14).

Factors associated with uromodulin excretion are evaluated in two studies in this issue of the *CJASN* (1,2). Each study was a large cross–sectional survey in which urinary samples were obtained, and the urinary uromodulin concentration was determined. The Canadian study by Troyanov *et al.* (2) included 943 participants randomly selected from 20,000 individuals in the CARTaGENE Study, Quebec's population-based biobank for public health and personalized genomics. A strength of this investigation was the ability to perform genetic analysis of the uromodulin SNP rs4293393. The Swiss study by Pruijm *et al.* (1) included 817 individuals from the Swiss Kidney Project on Genes in Hypertension Study and 5706 adults from the Cohorte Laussanoice. This study included information about urinary markers but not genetic polymorphisms.

What did we learn from these studies? As suggested by Pruijm *et al.* (1) and Troyanov *et al.* (2), urinary uromodulin seems to be a biomarker of tubular mass. In the Swiss study by Pruijm *et al.* (1), kidney mass determined by ultrasound was positively correlated with an increase in urinary uromodulin. Increasing age was associated with decreasing uromodulin excretion. For eGFR<90 ml/min per 1.73 m^2, there was a positive correlation between eGFR and urinary uromodulin. A relationship between eGFR and urinary uromodulin was also seen in the Canadian cohort. These studies indicate that the amount of tubular function is associated with uromodulin excretion.

Both studies also showed a decline in urinary uromodulin excretion with diabetes mellitus or elevated hemoglobin A1c (1,2). Prior studies have investigated the relationship between urinary uromodulin and diabetes (15), but none have had the population size present in these studies. The results suggest that diabetes may be associated with direct tubular toxicity on renal tubular cells. This finding is notable and consistent with recent studies of patients with advanced CKD and diabetes who do not have significant proteinuria/glomerular dysfunction (16).

Urinary electrolytes were also associated with changes in uromodulin excretion in both studies. In the CARTaGENE cohort, the fractional excretion of uric acid showed the strongest relationship with urinary uromodulin excretion (2) Increased uromodulin production would be expected to increase the amount of NKCC2 present at the apical surface of the thick ascending limb, leading to volume expansion, decreased proximal reabsorption of sodium and uric acid, and consequently, a higher fraction excretion of uric acid. In the Swiss cohort, there was a positive association with sodium, chloride, potassium, osmolality, and urinary volume (1). Because uromodulin functions to coat the apical membrane of the thick ascending limb, it would seem logical that factors increasing urinary flow would require increased uromodulin excretion to continuously coat the luminal surface of the thick ascending limb.

There were several other interesting findings from the Canadian study (2). Consistent with prior studies, uromodulin excretion was significantly higher in individuals with the rs4293393T variant. In addition, the uricosuric medications fenofibrate and losartan were associated with decreased uromodulin excretion. This finding was surprising, because other medications prescribed for similar indications were not associated with a decline in uromodulin excretion. Is it possible that somehow tubular uric acid regulates uromodulin excretion? The cause of this finding remains to be determined.

These studies help us understand the relationship between uromodulin excretion and excretion of urinary solutes, eGFR, and clinical conditions (1,2). The correlation between eGFR, kidney mass, and uromodulin excretion rate suggests that urinary uromodulin might be a biomarker for renal tubular mass and function. In addition, urinary uromodulin could be a marker of the effect of diabetes mellitus and hyperglycemia on renal tubular function. Unfortunately, urinary volume and electrolyte excretion all affect urinary uromodulin excretion as well, which could obscure the relationship between urinary uromodulin and tubular function. Additional investigations will continue to unravel the mystery of uromodulin and increase our knowledge of the importance of this molecule, hopefully leading to the increasing use of urinary uromodulin measurements in both clinical and epidemiologic settings.

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Disclosures

None.

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See related articles, "Associations of Urinary Uromodulin with Clinical Characteristics and Markers of Tubular Function in the General Population," and "Clinical, Genetic, and Urinary Factors Associated with Uromodulin Excretion," on pages 70–80 and 62–69, respectively.