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Uromodulin: from monogenic to multifactorial diseases

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ABSTRACT

Uromodulin, the major protein secreted in normal urine, is exclusively produced in the thick ascending limb (TAL) cells of the kidney. The exact role uromodulin (UMOD) plays in renal physiology remains enigmatic. UMOD has been linked to water/electrolyte balance and to kidney innate immunity and it is believed to protect against urinary tract infections and renal stones. A renewed interest in UMOD has been triggered by the identification of UMOD mutations as cause of hereditary dominant renal diseases, now referred to as uromodulin-associated kidney diseases (UAKDs), presenting with tubulointerstitial fibrosis, defective urinary concentration, hyperuricaemia and gout, and progressive renal failure. In UAKDs, the key primary pathogenetic event is a delayed intracellular trafficking of mutant UMOD, causing its intracellular accumulation. In the last decade, multiple genome-wide association studies have identified common variants in the *UMOD* gene, causing independent susceptibility to chronic kidney disease (CKD) and hypertension, two complex traits representing major global health problems. The biological mechanism underlying the association between *UMOD* risk variants and susceptibility to CKD and hypertension was not understood until last year, when the link between UMOD and hypertension was found to be caused by overactivation of the TAL sodium–potassium–chloride co-transporter NKCC2, pointing to UMOD as a therapeutic target for lowering blood pressure and preserving renal function.

Keywords: chronic kidney disease, familial juvenile hyperuricaemic nephropathy, hypertension, medullary cystic kidney disease, uromodulin

INTRODUCTION

Uromodulin (UMOD), also known as Tamm–Horsfall protein, is a kidney-specific protein exclusively synthesized by epithelial cells lining the thick ascending limb (TAL) of the loop of Henle. UMOD is secreted by TAL cells in the urine at the average rate of 50 mg/24 h, and it represents the most abundant urinary protein under physiological conditions [1]. The process by which the protein achieves its final conformation in the endoplasmic reticulum (ER) is complex, resulting in very slow transport of UMOD. UMOD was first discovered in 1950 by Tamm and Horsfall, who isolated a mucoprotein from human urine inhibiting haemagglutination of viruses [2]. In 1985, Muchmore and Decker isolated a protein from the urine of pregnant women that they named UMOD, to underline its origin and its immunosuppressive activity [3]. In 1987, Pennica *et al.* determined the primary structure of UMOD, providing evidence that UMOD was identical to the Tamm–Horsfall protein [4]. In spite of more than 50 years of UMOD research, the role that UMOD plays in renal physiology has remained elusive, causing a gradual decline of research in UMOD. Recent discoveries have underscored the importance of UMOD, since *UMOD* mutations were found in hereditary dominant tubulointerstitial renal diseases [5–7]. Moreover, using genome-wide association studies (GWAS), common variants in the *UMOD* gene have been identified as a risk factor for complex traits, including chronic kidney disease (CKD) and hypertension [8, 9].

UMOD: STRUCTURE AND BIOLOGICAL FUNCTION

UMOD is encoded by the UMOD gene (11 exons), located on chromosome 16p12.3. The primary structure of UMOD includes an N-terminal signal peptide; three epidermal growth factor (EGF)-like domains, which mediate protein–protein interaction; a central domain of unknown function ('domain of 8 cysteines', D8C); a zona pellucida (ZP) domain, essential for protein polymerization; a glycosylphosphatidylinositol (GPI)-anchoring site. A key structural feature of UMOD is its high cysteine content, likely involved in forming disulphide bridges responsible for its complex 3D conformation. UMOD is synthesized on the rough ER of the TAL epithelial cells as an 84 kDa precursor that is converted to the mature glycosylated and GPI anchor-linked protein with an apparent molecular weight of 97 kDa. After transport to the apical plasma membrane, the protein is cleaved and released into the tubular fluid. In the urine, UMOD is found as a high-molecular-weight polymer. The rate-limiting step in UMOD maturation is the processing in the ER, likely because of the complex tertiary structure [1, 10].

GPI-anchoring, multidomain structure and the large quantities excreted in urine suggest that UMOD may have multiple physiological functions. In the tubule, UMOD polymerizes into complex, reversible, filamentous gel-like structures serving as a physical barrier to water permeability [10–13]. Such a barrier may play an important role in ion transport and maintenance of countercurrent gradient in the interstitium. Recent evidence from $Umod^{-/-}$ mice suggests that UMOD regulates the activity of the renal outer medullary potassium (ROMK) channel and of sodium–potassium–chloride (NKCC2) transporter, the two main ion transporters in the NaCl reabsorption by the TAL segment [14, 15].

The biochemical properties of UMOD make it a candidate for being a host defence factor involved in clearing bacteria from the urinary tract. *In vitro* studies have shown that UMOD can specifically bind, via its high-mannose residues, to Type 1 fimbriated *Escherichia coli*, blocking the attachment of the *E. coli* cells to the uroplakins, the urothelial receptors for Type 1 fimbriae. These findings have been confirmed *in vivo*, since $UMOD^{-/-}$ mice revealed an increased susceptibility to urinary tract infection (UTI) when inoculated with type 1-fimbriated *E. coli* [16–18]. UMOD may also play a role in regulating stone formation. *In vitro* and *in vivo* studies in $UMOD^{-/-}$ mice suggest that UMOD is a potent inhibitor of the aggregation of calcium crystals. A direct binding between UMOD and calcium ions or calcium crystals might be crucial [19–22]. However, in humans, the protective role of UMOD against UTI and nephrolithiasis remains controversial, since individuals with reduced UMOD urinary levels, i.e. patients with *UMOD* mutation and tubulointerstitial nephritis (see below), do not show increased frequency of UTI or renal stone formation [5–7, 10].

Clinical and experimental studies indicate an involvement of UMOD in several forms of inflammatory kidney disease. UMOD has been suggested to play a key role in innate immunity of the kidney, triggering monocytes and granulocytes to produce inflammatory molecules. Moreover, intravenous challenge of animals with UMOD resulted in the induction of a tubulointerstitial nephritis, and anti-UMOD antibodies are consistently found in the peripheral blood of patients with UTI and acute/chronic pyelonephritis. Finally, abnormal deposition of UMOD and ensuing inflammatory reactions have been observed in cast nephropathy and urolithiasis [23–26]. However, the underlying mechanisms explaining how UMOD contributes to inflammatory reactions have remained obscure until recently, when it has been demonstrated that UMOD activates myeloid dendritic cells (DCs) via Toll-like receptor 4 (TLR4), triggering them to reach a fully mature phenotype. TLR4 knockout mice were found to be severely impaired in the UMOD-specific humoral immune responsiveness, suggesting that the TLR4 signalling pathway is essential for the UMOD-specific Ab response [27]. The immunostimulatory effects of UMOD by TLR4 could represent an important host defence mechanism employed in the human urinary tract system. In healthy mammals, it can be hypothesized that anti-UMOD antibodies or interstitial UMOD deposits are not produced because the exclusive localization of UMOD at the luminal surface of tubular cells keeps the protein from the adaptive and innate immunity machinery. This segregation could be abolished in kidney diseases by loss of cell integrity. Thus, UMOD may act as a danger-signalling molecule, which triggers an inflammatory response once the injury has damaged the nephron integrity, allowing UMOD to be released in the interstitial space. However, despite these *in vitro*/*in vivo* studies, the proinflammatory role of UMOD remains controversial. The presence of UMOD in a damaged renal area might be coincidental or reactive. In addition, UMOD knockout mice were recently shown to develop more functional/histologic renal damage and had delayed recovery after ischaemia-reperfusion injury, suggesting that UMOD may play a protective role in acute kidney injury by decreasing inflammation and enhancing recovery [28].

UMOD-ASSOCIATED KIDNEY DISEASE

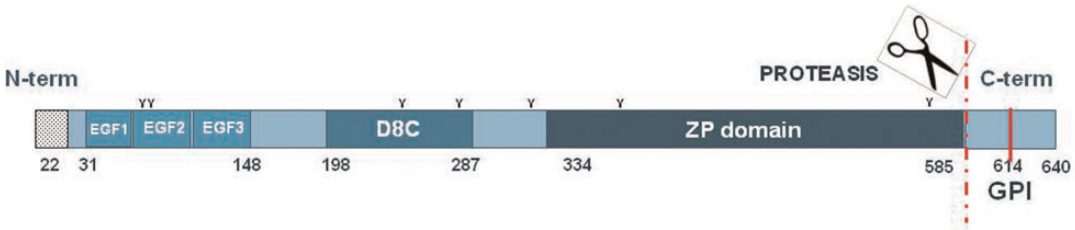
The importance of UMOD in renal diseases had not been fully appreciated until mutations of UMOD were discovered in a group of hereditary autosomal-dominant tubulointerstitial diseases, encompassing medullary cystic kidney disease type II (MCKD2; MIM 603860), familial juvenile hyperuricaemic nephropathy (FJHN; MIM 603860) and glomerulocystic kidney disease (GCKD; MIM 609886) [5–7, 10]. MCKD2 is a tubulointerstitial nephritis developing during adulthood. The earliest symptom is hyperuricaemia and gout, developing after adolescence. End-stage renal disease (ESRD) is reached in late adulthood. Renal imaging may reveal corticomedullary cysts. Histology shows interstitial fibrosis and tubular atrophy [29–31]. The phenotypic features of FJHN overlap those of MCKD2, being characterized by hyperuricaemia, precocious gout and progressive tubulointerstitial nephropathy [7, 32]. ESRD typically ensues in young adulthood. Renal biopsy findings are non-specific, with interstitial fibrosis and tubular

atrophy. As a rule, cysts are not present. Based on the striking clinico-pathological resemblance and a strong linkage to the same chromosomal interval, Dahan *et al.* suggested a possible allelism between MCKD2 and FJHN [33]. In 2002, Hart *et al.* provided evidence that MCKD2 and FJHN arise from mutation of the *UMOD* gene and are allelic disorders [5]. Since then, FJHN and MCKD2 are collectively referred to UMOD-associated kidney disease (UAKD). Mutations in the *UMOD* gene were also reported in two families affected by a variant of GCKD (MIM 609886), resembling the UAKD phenotype [6, 34]. Histology is similar to other UAKD except for the presence of cystic dilatation of the Bowman's space. GCKD is genetically heterogeneous, because it can be also found with mutation in the hepatocyte nuclear factor 1-β gene (*HNF 1-β*) [35].

UAKD is a rare disease. However, the true prevalence of UADK is difficult to determine, because the condition is frequently underdiagnosed. The findings of slowly progressive renal failure, non-significant urinalyses and unremarkable renal ultrasounds make the correct diagnosis elusive. Families with UAKD have been reported from Europe, USA, Asia and Africa. A nationwide epidemiologic survey of UAKD conducted in Austria revealed a prevalence of 1.7 cases per million population and 1 case per 1000 renal replacement therapy patients. No other systematic study of the epidemiology of UAKD is available. However, a prevalence of UADK of 1.52 and of 0.7 patients per million population has been calculated in the Czech Republic and in France (and Belgium), respectively [36]. In families presenting with symptoms fulfilling diagnostic criteria of FJHN/MCKD2, UMOD mutations can be detected in 12–31% [37].

The renal clinical phenotype caused by *UMOD* mutation is characterized by dominant inheritance, CKD due to chronic tubulointerstitial nephritis, hyperuricaemia, gout and, inconstantly, renal cysts [5–7, 10]. More than 100 mutations in the *UMOD* gene have been described so far; the majority of reported *UMOD* mutations cluster in exons 4 and 5, resulting in the replacement of cysteine residues and leading to misfolding of the UMOD molecule [5–7, 10, 38] (Figure 1). The clinical phenotype of UAKD and genotype–phenotype correlations have been examined in two large cohorts of patients. In a French study, 37 *UMOD* mutations were identified in 109 patients from 45 families. The majority of patients had hyperuricaemia; gout was present in 75% of men and 50% of women. The median age at first gouty episode was 21 years. Cysts were detected in 34% of patients. The median renal survival was 54 years. Phenotype was not accurately predictive of *UMOD* mutation and a high intrafamilial variability of renal survival was observed [37]. In a second series of 202 patients from 74 families with 59 different UMOD mutations, median ages at onset of hyperuricaemia, gout and ESRD were 24, 40 and 56 years, respectively. Men developed gout and ESRD significantly earlier than did women. The location of the mutation appeared to affect the progression of renal disease, because the median age at ESRD development was lowest in patients with mutations in the EGF2 and EGF3 domains [39].

Several physiological aspects of UAKD, especially the putative link between UMOD-mutated protein, renal salt wasting and uric acid handling remain enigmatic. Immunohistochemistry analysis of renal biopsies of UAKD patients showed the presence of large UMOD intracellular aggregates, colocalizing with ER markers, in the cells lining the TAL [6, 7]. Different cellular models revealed that mutant UMOD isoforms are defective in trafficking to the plasma membrane, being retained in the ER [6, 7, 40]. A recent transgenic UAKD mouse model recapitulated most of the UAKD features, confirming that the key primary event is ER accumulation of mutant UMOD in the TAL cells, which precedes a progressive renal damage, characterized by tubulointerstitial fibrosis with inflammatory cell infiltration and tubule dilation [41]. Thus, UMOD mutations affect biosynthesis of the protein, leading to an aberrant intracellular trafficking, ER storage, abnormal UMOD expression in the kidney and decreased urinary UMOD excretion. As opposed to mice expressing mutant UMOD, mice lacking



PROTEIN	EGF1	EGF2	EGF3		D8C		Zona-Pellucida		TOTAL
CODING EXONS	Exon 4					Exon 5	Exons 6-8	Exon 9	
% OF MUTATIONS	82%					11,2%	6%	0,8%	100%
NUMBER OF MUTATIONS	11	12	16	19	35	12	7	1	113

FIGURE 1: Structure of UMOD protein and summary of the published mutations associated with UADK. Upper panel: schematic representation of the structure of the UMOD protein, showing a leader peptide, three epidermal growth factor EGF-like domains, a central domain named D8C, a ZP domain and a glycosylphosphatidylinositol GPI-anchoring site. Glycosylation sites are represented as Y. Lower panel: summary of all UMOD mutations found with their corresponding functional domain and exon. A total of 113 mutations [5, 6, 42, 43, 44] have been reported to date. Most of UMOD mutations are clustered in exons 4 (>80%) and 5 (>11%).

UMOD do not recapitulate the biochemical, clinical and histological features of UAKD in humans, suggesting a gain-of-function effect of UMOD mutations.

However, the potential pathogenetic events downstream of mutant UMOD ER retention are not clear. We can hypothesize that ER accumulation of mutant UMOD may lead to the functional and structural injury of the TAL [30]. Loss of the integrity of the TAL would decrease the concentrating ability of the loop of Henle, resulting in decreased urinary concentration. The decreased ability to reabsorb sodium in the TAL would be balanced by an increase in reabsorption of sodium in the proximal tubule and secondarily of uric acid, resulting in hyperuricaemia. Moreover, UMOD retention may initiate an inflammatory process, likely triggered by ER stress pathways activated in the TAL cells, resulting in progressive interstitial fibrosis and tissue scarring. Finally, the ER UMOD retention reduces the amount of UMOD reaching the apical membrane, affecting the trafficking of the wild-type protein. A recent *in vitro* and *in vivo* study demonstrated that the mutant UMOD can partially escape the ER quality control, being trafficked to the plasma membrane, and secreted [42]. In the urine, mutant UMOD, which has a higher propensity to aggregation than the wild-type protein, tends to form large extracellular aggregates that interfere with wild-type protein polymerization, causing a detrimental effect, suggesting that the proteotoxic effect of the mutant UMOD could be exerted through both intra- and extracellular gain-of-function mechanisms. Finally, more recently, in a mouse model of UADK, activation of NF-kB pathway in TAL of Henle's loop cells was demonstrated, identifying a novel disease mechanism of UAKD [43].

UAKD belongs to a group of renal hereditary disorders, linked by common findings of tubulointerstitial disease and dominant inheritance, for which Ekici *et al.* recently proposed the unifying term of autosomal dominant tubulointerstitial kidney disease (ADTKD) [44]. According to these authors, the ADTKD family of renal diseases should include, in addition to *UADK*, MCKD1 (MIM 174000), FJHN2 (MIM 613092) and *HNF1-β*-associated renal diseases. Medullary cystic kidney disease type 1 (MCKD1) is caused by mutation in the variable number tandem repeat (VNTR) region of MUC1 gene, which encodes the mucoprotein mucin-1, a transmembrane protein expressed on the apical borders of secretory epithelial cells [45]. To date, all disease-causing mutations add one cytosine to a tract of seven cytosine nucleotides, resulting in a frameshift mutation causing truncation of the VNTR. The abnormal gene product appears to be improperly processed and deposited in the cytoplasm of the cells from the Henle's loop, distal convoluted tubule and collecting duct, where it is thought to induce cellular death resulting in a slowly progressive kidney disease. Because of the complex structure of the VNTR region of the MUC1 gene, this unusual type of mutation is recalcitrant to detection by both Sanger and massively parallel sequencing and requires specific genotyping assays to be detected. For this reason, the disease-causing gene of MCKD1 has been only recently identified, 15 years after the first mapping of the locus on chromosome 1q21 [46]. Although MUC1 is widely expressed (renal distal tubular cells, skin, breast, lung, gastrointestinal tract and salivary glands), MCKD1 has a very limited clinical presentation, and there is no evidence of clinical alterations of organs or tissues beside the kidney. Individuals with the MUC1 mutation show an adult onset of slowly progressive renal failure, usually in the absence of gout. Urinalysis reveals a bland sediment and minimal or no proteinuria. The age of onset of end-stage kidney disease is highly variable, ranging from the third to seventh decade of life [45, 47]. FJHN2 is a very rare disease, described in only four families and defined by the presence of a mutation in REN, the gene encoding renin. For this, it is also known as REN-associated kidney disease. Heterozygous REN mutations segregate with a phenotype of early onset hypoproliferative anaemia, low blood pressures (BPs), mild hyperkalaemia, low plasma renin activity, hyperuricaemia and gout and progressive kidney failure [48]. In humans, mutations of the HNF 1-β gene can be associated with a complex and heterogenous renal phenotype. Usually, mutations of the HNF 1-β gene produce maturity-onset diabetes of the young, Type 5 (MODY5) associated with renal cystic dysplasia of variable severity. The acronym RCAD (renal cysts and diabetes) has been coined to describe this syndrome. In addition to cysts, other kidney abnormalities have been observed in MODY5, such as renal agenesis or hypoplasia. Infrequently, however, mutations in the HNF1-β gene have been reported with a renal phenotype of chronic tubulointerstitial nephritis, particularly in adults [35].

In conclusion, it is well established that the renal phenotype in adults with UMOD, MUC1, REN and HNF1-β mutations is clearly one of chronic tubulointerstitial nephritis. However, this group of inherited tubulointerstitial nephritis is clinically heterogeneous, particularly showing distinct extrarenal features. HNF1-β nephropathy is also characterized by a wide clinical heterogeneity of the renal phenotype, with a large spectrum of renal morphologic, structural and parenchymal manifestations. Moreover, while there has been significant advancement in the understanding of the pathophysiology of UADK over the recent past, the pathophysiology of the renal findings observed in MCKD1, REN disease and HNF1-β nephropathy is largely unknown. Finally, it is of note that the nomenclature for UAKD and related conditions is very confusing, and other terms in the past have been commonly used for dominant tubulointerstitial diseases, including MCKD, FJHN and dominant nephronophthisis. All together, these considerations suggest a cautious approach when proposing a new terminology for these disorders. Additional clinical data and pathogenetic knowledge are needed before accepting this new nomenclature for dominant tubulointerstitial diseases, although this unifying terminology might facilitate the clinical recognition of the diseases. The existence of families with tubulointerstitial nephritis and with gene locus outside of the known genes (on chromosome p22.1–p21; FJHN3) [49] seems to further support this prudent approach.

UMOD AS A RISK FACTOR FOR HYPERTENSION AND CKD

The scientific interest on UMOD has been further boosted in recent years by the seminal work by Köttgen *et al.* [50] that

identified common variants in the *UMOD* gene associated with estimated glomerular filtration rate (eGFR) and increased risk of CKD through GWAS in population-based cohorts, mostly of European ancestry. In this study, the authors reported that the minor T allele for the lead SNP rs12917707, located in the gene promoter, is associated with a 20% reduction of the risk of CKD and with higher eGFR. The genetic association was independent of major kidney disease risk factors such as the presence of hypertension or diabetes. These findings were later replicated in a similar study in a large Icelandic population [51] and by other studies in European isolates [52] and in a large European cohort [53]. In these works, a second *UMOD* variant, rs4293393, was associated with the risk of CKD and renal function. This variant is also located in the *UMOD* gene promoter and it is in full linkage disequilibrium (LD) with rs12917707. Interestingly, another SNP, rs13333226, mapping in the *UMOD* promoter within the same LD block was reported to be associated with increased risk of hypertension and cardiovascular disease in a large European case–control study [54]. As for SNPs associated with CKD and renal function, the minor G allele of rs13333226 was associated with a protective effect. Additional studies have identified genetic association of *UMOD* promoter gene variants with ESRD [55, 56], type 2 diabetic nephropathy [57] uric acid and increased risk of gout and renal stones [51], though in the latter case with an opposite effect, i.e. risk variants for CKD were protective. Of note, in two studies the association of UMOD variants with increased risk of CKD was clearly age-dependent, UMOD being the only gene for which the effect was significantly greater in individuals >65 years of age [51, 58].

In order to understand the functional link of *UMOD* variants with the risk of developing CKD, Köttgen *et al.* carried out a case–control study of incident CKD and could show that urinary UMOD levels were significantly higher in individuals carrying *UMOD* risk variants in a dose-dependent fashion [59]. These data suggested that *UMOD* variants identified through GWAS could have a direct effect on UMOD urinary excretion and that this effect could be linked with the development of CKD and hypertension. The same group also published sequencing data of the *UMOD* gene that seemed to exclude the involvement of other *UMOD* variants in LD with the lead ones from GWAS [60]. The role of *UMOD* variants in regulating UMOD excretion has been recently confirmed in a large meta-analysis on 10 884 individuals of European descent from three genetic isolates and three urban cohorts [61]. Through a genome-wide approach, Olden *et al.* identified a very significant association (P = 7.85E−73) of UMOD urinary levels with SNP rs12917707, located in the *UMOD* gene promoter. Each copy of the G allele, associated with increased risk of CKD in the study by Köttgen *et al.* [50], was associated with higher levels of UMOD in all six cohorts analysed and with lower levels of GFR in the CKDGen Consortium participants. The biological bases of UMOD association have been uncovered by the recent work of Trudu *et al.* [9]. In this study, the authors first demonstrated that *UMOD* risk variants in the gene promoter are associated with increased gene expression, both *in vitro* and *in vivo*. This effect was also confirmed at the urinary protein level through the quantification of UMOD excretion in a large population-based cohort. The effect of *UMOD* variants on gene expression was modelled *in vivo* through the use of transgenic mice that overexpress UMOD relative to control mice at levels comparable with the expression difference observed between individuals homozygous for either the risk or protective UMOD variants. Over-expression of UMOD leads to salt-sensitive hypertension in UMOD transgenic mice, establishing a causal relationship between the effect of UMOD risk variants on gene expression and increased BP. The authors demonstrated that this effect is essentially due to over-activation of the TAL sodium–potassium–chloride co-transporter NKCC2. Indeed, acute treatment of transgenic mice with furosemide, a well-known diuretic that specifically blocks NKCC2, was associated with higher natriuretic response and drop in BP relative to control mice. Interestingly, this mechanism seems to play a role in human hypertension, as furosemide was effective in reducing BP only in hypertensive patients homozygous for *UMOD* risk variants. The role of UMOD in regulating BP is also supported by the work by Graham *et al.* [62]. These authors demonstrated that mice lacking UMOD have reduced baseline BP that is not increased by high-salt diet, as opposed to control mice. Such a phenotype is consistent and complementary to the one reported on *Umod* overexpressing mice, linking UMOD expression, salt intake and BP regulation. Data collected in TAL primary cells suggest that the link between salt intake and UMOD could be explained by its negative modulation of the action of tumour necrosis factor alpha that induces lower NKCC2 expression. Overall, these interesting studies expand our current knowledge of the role of UMOD suggesting that this 'old' molecule plays fundamental functions in the kidney and that the variation of its levels is associated with common diseases as hypertension and CKD. Although the GWAS on UMOD urinary level by Olden *et al.* clearly point to UMOD variants as major determinants of its urinary levels, the same studies also identified a second associated variant within the PDILT gene, located near UMOD, whose association seems unlikely to be driven by LD. Moreover, variants in other genes expressed in the TAL, as KCNJ1 (encoding ROMK), SORL1 and CAB39 were shown to be associated with urinary UMOD levels in candidate-based analysis. These results suggest the presence of uncharacterized regulatory networks that deserve further investigation [62]. More studies are warranted to clarify the role of UMOD in hypertension, CKD and possibly other common human diseases (e.g. UTI, nephrolithiasis) and to gain further insight into its complex biological functions.

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CONFLICT OF INTEREST STATEMENT

None declared.

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