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The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulo-interstitial nephropathy to chronic kidney disease

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ABSTRACT

Uromodulin (Tamm-Horsfall protein) is the most abundant protein excreted in the urine under physiological conditions. It is exclusively produced in the kidney and secreted into the urine via proteolytic cleavage. Its biological function is still not fully understood. Uromodulin has been linked to water/electrolyte balance and to kidney innate immunity. Also, studies in knock-out mice demonstrated that it has a protective role against urinary tract infections and renal stone formation. Mutations in the gene encoding uromodulin lead to rare autosomal dominant diseases collectively referred to as uromodulin-associated kidney diseases. They are characterised by progressive tubulo-interstitial damage, impaired urinary concentrating ability, hyperuricaemia, renal cysts and progressive renal failure. Novel *in vivo* studies point at intracellular accumulation of mutant uromodulin as a key primary event in the disease pathogenesis. Recently, genome-wide association studies identified uromodulin as a risk factor for chronic kidney disease and hypertension and suggested that the level of uromodulin in the urine could represent a useful biomarker for the development of chronic kidney disease. In this review, we summarise these recent investigations, ranging from invalidation studies in mouse to Mendelian disorders and to genome-wide association, which led to a rediscovery of uromodulin and boosted the scientific and clinical interest for this ancient molecule.

KEYWORDS

Uromodulin, Tamm-Horsfall protein, Chronic kidney disease

Biology of uromodulin

In the early fifties, Igor Tamm and Frank Horsfall described a mucoprotein that could be purified from urine and inhibited viral haemagglutination (1). The protein, referred to as Tamm-Horsfall protein (THP), is the most abundant protein in urine under physiological conditions (2). In 1985, Muchmore and Decker isolated a protein from the urine of pregnant women that they called uromodulin in relation with its immunosuppressive activity documented *in vitro* (3). Two years later, THP and uromodulin were shown to be the same protein in a seminal work by Pennica et al. (4). The term uromodulin will be used hereafter in this review.

Protein sequence and domain composition

Uromodulin is synthesised as a 640 amino acids precursor. The protein enters the secretory pathway where it is GPI-anchored, glycosylated and sorted to the apical plasma membrane of epithelial cells. The rate-limiting step in uromodulin maturation is the processing in the endoplasmic reticulum (ER), likely because of the complex tertiary structure given by its high number of cysteine residues (48; 7% of amino acid content) all engaged in the formation of intra-molecular disulphide bonds (5). The molecular weight of uromodulin (105 kDa) is significantly contributed (30%) by N-glycosylation. Evidence for O-glycosylation has also been reported (6). The presence of such high glycan content is important for the chemico-physical properties and function of uromodulin (see below). The domain composition of uromodulin includes a leader peptide directing its entry in the secretory pathway, three EGF-like domains (EGF-II and EGF-III predicted to be calcium-binding), a central domain of unknown function (named D8C as it contains 8 conserved cysteines), a zona pellucida (ZP) domain and a glycosylphosphatidylinositol (GPI)-anchoring site (predicted at position 614) (Figure 1a). EGF-like domains are found in many secreted and extracellular proteins and are thought to play a role in processes as adhesion, coagulation, and receptor–ligand interaction.

In uromodulin, these modules are likely important for protein-protein interaction. The ZP domain is found in a variety of extracellular eukaryotic proteins, e.g. sperm receptors ZP1 and ZP3, tectorial membrane components alpha- and beta-tectorin, and is essential for protein polymerisation (7) (Figure 1b). Indeed, uromodulin is mainly present in the urine as a high molecular weight polymer (M_r 1-10x10^6 daltons) that appears at the electron microscopy analysis as a matrix composed by fibrils with a width of about 100 Å and an average length of 25,000 Å. Depending on the ionic conditions uromodulin matrices can form a gel-like structure that is water impermeable but allows ion movement (8).

Timing and tissue-specificity of expression

Uromodulin is a kidney-specific protein that is exclusively expressed by epithelial cells lining the thick ascending limb (TAL) of Henle's loop (9). It is mainly located at the apical plasma membrane (10) although localisation at the basolateral side of TAL cells has also been reported (11). A basolateral release of uromodulin is also suggested by studies on its trafficking in transfected polarised epithelial cells and by its presence at very low concentrations in the blood (12).

The presence of uromodulin protein and transcript is detected from embryonic day 16.5 in the developing mouse kidney (13, 14). In humans, the protein was detected from gestational week 16 in immuhistochemistry analysis and from week 20 in the amniotic fluid (15). Its expression steadily increases with time and maturation of TAL tubules till after birth. Uromodulin is produced at a very high rate by mature TAL cells being their most abundant transcript (16). The half-life of the protein is rather short (about 9 hours in rabbit and 16 hours in humans) (17), due to its high rate of secretion in the urine that ranges from 20 to 100 mg/day in humans under physiological conditions (18). Uromodulin is released from the apical plasma membrane of epithelial cells into the tubule lumen via a conserved proteolytic cleavage (19). Cleavage is necessary for protein polymerisation, as it releases an inhibitory motif that prevents premature protein assembly (20), similarly to what

described for zona pellucida protein ZP3 (21) (Figure 1b). Interestingly, data from our studies in transfected MDCK cells (20) and from urine peptidomes (22) suggest the presence of an alternative cleavage distal to the inhibitory motif releasing monomeric uromodulin. Little information is currently available on the presence of a specific protease(s) involved in uromodulin excretion in the urine and on how this is regulated.

Evolutionary conservation

The sequence and domain composition of uromodulin is very similar to the one of glycoprotein 2 (GP2), that is the major component of zymogen granule membranes of exocrine pancreas, and liver-specific ZP domain-containing protein (LZP). *GP2* and *UMOD* genes lie adjacent on chromosome 16p12.3 suggesting that they could have evolved through duplication-divergence of a common ancestral gene. As uromodulin (see below), GP2 protein is able to bind to *E. coli* of the fimbriated type I suggesting that the two proteins exert similar protective functions in the urinary and digestive systems (23).

Uromodulin is present in the kidney of all mammals. Immunoreactive protein in the layers of the skin of several amphibians and fishes, and in the distal tubules of the kidney of some amphibians was reported (24). Comparative genomics analysis reveals putative *UMOD* homologues in amphibian (*X. tropicalis*) and fish (*D. rerio*) genomes with significant sequence similarity at the predicted protein level. The function of these homologues and their relevance for comparative physiology remain to be determined.

Biological function

The biological function of uromodulin is still rather elusive. Uromodulin has been hypothesised to have a role in water/electrolyte balance in the TAL. This hypothesis is based on its gelification and physico-chemical properties (8) and on the evidence that its expression is increased by a high-salt

diet and by chronic administration of the loop diuretic furosemide (25). More direct evidence comes from a recent work by Renigunta et al. showing that expression of uromodulin significantly increases the activity of ROMK2 channel through direct interaction and positive regulation of its delivery to the plasma membrane. Lack of uromodulin in *Umod* knock-out mice leads to significant upregulation of ROMK2 that results from a reduction of the channel amount at the plasma membrane and its increase in the vesicular pool (26). However, the specificity of this effect requires further investigation as ion transporters of downstream segments (NCC, alpha-ENaC) were also found to be significantly upregulated in uromodulin deficient mice (27).

Studies in *Umod* knock-out mice showed that uromodulin plays a defensive role against urinary tract infection (UTI) (28, 29). This function is due to its ability to bind to pathogens of the urinary tract, e.g. type 1-fimbriated *E. coli*, competing with their binding to uroplakins on the urothelium (30) and is mediated by its high-mannose moiety. Indeed, one the seven sites of glycosylation (Asn 274) retains a high-mannose chain (Figure 1a), a feature that is conserved throughout evolution and that likely depends on the protein primary structure and folding (18). Uromodulin also plays a role in preventing the formation of kidney stones. Several *in vitro* studies (31) complemented by *in vivo* investigations on a rat nephrolithiasis model (32) showed that uromodulin reduces the aggregation of calcium crystals. Moreover, lack of uromodulin in knock-out mice leads to the formation of calcium crystals in the kidneys and progressive renal calcification (33, 34). Uromodulin exerts its protective function acting synergistically with osteopontin, as shown in double knock-out mice (35). Although supported by *in vivo* evidence in *Umod* knock-out mice, the relevance of uromodulin as a protective molecule against UTI and nephrolithiasis is still unclear, as individuals with extremely reduced uromodulin urinary level, as patients carrying *UMOD* mutations (see below), do not show increased rates of urinary tract infections or renal stone formation.

Finally, uromodulin has also been suggested to play a role in innate immunity of the kidney. Several *in vitro* studies demonstrated that it can bind to immunity related molecules as IgG,

complement 1q and TNF-alpha (36-38). Uromodulin can also act as a chemoattractant (39) and as a pro-inflammatory molecule, able to interact with and activate components of the immune system, including monocytes, neutrophils (39) and myeloid dendritic cells via Toll-like receptor 4 (TLR4) (40). Administration of uromodulin induces tubulo-interstitial nephritis in rabbits, rats and mice (41, 42). In the mouse, this is accompanied by the production of anti-uromodulin antibodies that is dependent on TLR4 function (40). Taken together, these data suggest that uromodulin may act as a danger-signalling molecule, able to elicit an inflammatory response following conditions that damage the nephron integrity and lead to uromodulin release in the interstitial space. This hypothesis is supported by the evidence of interstitial uromodulin release associated with inflammatory cell infiltrate as well as of increased uromodulin-specific autoantibodies in several inflammatory disorders and infections of the urinary tract (43). However, the pro-inflammatory role of uromodulin remains controversial and has been recently challenged by El-Achkar et al. who showed that mice lacking uromodulin develop more functional and histological renal damage after ischemia-reperfusion injury compared with wild type animals, suggesting that uromodulin has a protective role against inflammation and necrosis induced by ischemic damage (44).

Uromodulin-associated kidney diseases

Mutations in the *UMOD* gene cause medullary cystic kidney disease type 2 (MCKD2; MIM 603860) and familial juvenile hyperuricemic nephropathy (FJHN; MIM 162000) that are autosomal dominant tubulointerstitial kidney diseases. Being allelic disorders, MCKD2 and FJHN are collectively referred to as uromodulin-associated kidney disease (UAKD) (45). UAKD is a rare disorder. About fifty mutations have been reported so far (see below) and its prevalence is estimated to be 1/100,000 (www.orphanet.org). The earliest symptom in UAKD patients is often hyperuricemia that results from reduced fractional excretion of uric acid, is present in about 80% of

patients and is frequently associated with gout (45, 46). Mild urine concentrating ability is an almost constant finding, sometimes resulting in polyuria and polydipsia (47). Chronic renal failure generally occurs between the second and fourth decade of life, although a significant intra- and inter-familial variability has been observed. At the histological analysis, UAKD is characterised by diffuse tubulointerstitial fibrosis with moderate inflammatory cell infiltrate and tubular atrophy (47, 48). Renal cysts (generally measuring 0,5 to 3 cm in diameter) are sometimes detected, mainly at the cortico-medullary junction (47, 49, 50). There is no specific therapy other than correction of water and electrolyte imbalance that may occur. Hyperuricaemia can be effectively treated with allopurinol (51) or uricosuric drugs as benzbromarone (52). Dialysis followed by renal transplantation is the preferred approach for renal failure. Few follow-up data are available on transplanted patients and suggest that renal transplant can effectively cure UAKD (53).

Mutations in the *UMOD* gene were also reported in two families affected by a variant of glomerulocystic kidney disease (GCKD) (MIM 609886), resembling UAKD phenotype (48, 54). Patients showed marked dilation of Bowman's space in most glomeruli that was associated with hyperuricemia, severe impairment of urine concentrating ability and no evidence of diabetes. Interestingly, homozygosity for a *UMOD* mutation has been reported in 3 affected individuals from a Spanish consanguineous family (55). Homozygous individuals display a more severe phenotype in comparison to heterozygous members of the same family in terms of earlier onset of hyperuricemia and faster progression to end-stage renal disease.

The analysis of uromodulin in renal biopsies and urine samples from patients with UAKD revealed some key findings. Immunohistochemistry and immunofluorescence analysis showed the presence of large uromodulin intracellular aggregates in the cells lining the TAL (47, 48, 56, 57). These inclusions co-localise with endoplasmic reticulum (ER) markers, suggesting that mutations affect protein delivery to the plasma membrane (57, 58). These findings are consistent with the presence of fibrillar or amorphous material within expanded stacks of the ER and of hyperplastic bundles of

the ER at the ultrastructural analysis (49, 57, 59). Defective transport of mutant protein was also demonstrated by a significant decrease of the urinary excretion of uromodulin UAKD patients (47, 48, 57, 60). The extent of the reduction was not entirely due to the progressive loss of renal function, suggesting a possible dominant negative effect on the trafficking of wild type protein.

Similar clinical findings, i.e. tubulo-interstitial nephritis and hyperuricaemia, can be found associated with mutations in the gene encoding the transcription factor hepatocyte nuclear factor (HNF)-1beta (*TCF2*) (MIM 137920) (61) and in the *REN* gene encoding renin (MIM 613093) (62). Moreover, two additional loci have been mapped on chromosome 1q21 (MCKD1) (63) and p22.1-p21 (FJHN3) (64).

In vitro studies

To date 51 *UMOD* mutations have been published (Figure 2). All but three (in-frame deletions) are missense changes that affect in about 50% (28/51) of the cases one of the conserved cysteine residues. *UMOD* mutations are clustered (94%) in exons 3 and 4 encoding for the N-terminal half of the protein; only three mutations have been reported so far in exons 5 (C347G) and 7 (A461E, G488R) and affect residues within the ZP domain (65-67).

The majority of reported *UMOD* mutations are predicted to cause protein misfolding, either by directly affecting the disulphide bond pattern or by destabilising the structure of EGF-like domains. Consistent with an effect of mutations on protein folding, we demonstrated in different cellular models that mutant uromodulin isoforms are defective in trafficking to the plasma membrane and are retained in the ER (48, 68). The results of our *in vitro* studies on *UMOD* mutations were confirmed by other reports on different mutation panels and different cell lines (12, 57, 66), clearly showing a common effect for all *UMOD* mutations. Overall, these data indicate ER retention of mutant uromodulin as a key step in the pathogenesis of UAKD and point at these diseases as an

additional member of the known ER storage diseases (59). Interestingly, *in vitro* studies suggest the presence of two classes of *UMOD* mutations, according to the extent of mutant protein retention in the ER. At the moment, no clear genotype-phenotype could be established due to the small number of affected families and/or incomplete or heterogeneous available clinical data.

Although the analyses of *in vitro* models identified a common effect of all *UMOD* mutations, they did not allow the identification of potential pathogenetic events downstream of mutant uromodulin ER retention. Mutant uromodulin expression in cellular models was reported to lead to cell apoptosis that could be rescued by treatment with colchicine and sodium 4-Phenylbutyrate (69). However, evidence for a proteotoxic effect of mutant uromodulin expression has not been observed when using different kidney cell lines under basal or stress conditions ((12), our unpublished results). As the cellular models so far reported were ineffective in reproducing key cellular hallmarks of the disease, i.e. mutant uromodulin aggregation, ER membrane expansion and dilation, and were developed in cell lines not expressing endogenous uromodulin, it cannot be excluded that a fully differentiated TAL cell is needed to properly assess *in vitro* the effect of mutant protein expression.

In vivo models

Two *Umod* knock-out mouse models have been reported and extensively characterised. As previously mentioned, these animals are more susceptible to urinary tract infections and more prone to renal stone formation and nephrocalcinosis. However, animals lacking uromodulin show few if any signs of UAKD, consistent with a gain-of-function effect of *UMOD* mutations. Indeed, no interstitial fibrosis, inflammatory infiltrate and renal cysts were observed up to three years of age (70). *Umod* knock-out mice show mild urinary concentrating defect after water deprivation test that could be due to a defect in the TAL reabsorptive capacity (27). Recent data showing reduced amount of ROMK2 channel at the plasma membrane in these animals supports this hypothesis (26).

Noteworthy, no renal phenotype was described in a transgenic mouse model expressing C148W human mutant uromodulin, likely because of low expression of the transgene (71). A mouse expressing uromodulin variant A227T was obtained by ENU mutagenesis. *Umod*^{A227T} mice show some features of UAKD as the presence of uromodulin aggregates in TAL cells, urine concentration ability defect and reduced fractional excretion of uric acid but lack any of the histopathological signs of the disease and have additional metabolic alterations (72).

We recently generated and characterised an *in vivo* model of UAKD, i.e. a transgenic mouse expressing mutant uromodulin (Tg$^{Umod C147W}$) (58). The mutation introduced in the murine protein (C147W) corresponds to the human mutation C148W that we previously identified in UAKD patients and extensively characterised *in vitro* (48). The phenotype in Tg$^{Umod C147W}$ mice was compared to expression-matched transgenic mice for wild type protein (Tg$^{Umod wt}$). Tg$^{Umod C147W}$ mice specifically show progressive signs of renal damage, i.e. tubulo-interstitial fibrosis with inflammatory cell infiltration and tubule dilation. Interestingly, necrotic cells but no apoptosis were detected in distal tubules. Similarly to UAKD patients, Tg$^{Umod C147W}$ mice show urinary concentrating defect of renal origin that is present in young animals (12 weeks of age) and precedes renal failure. ER retention of mutant uromodulin precedes all other features starting at 1 week of age (our unpublished results) and progressing to the formation of massive intracellular aggregates and hyperplasia of ER membranes in 24 weeks-old kidneys. Tg$^{Umod C147W}$ mice hence recapitulate most of the disease features with the exception of hyperuricemia, likely because mice express urate oxidase, an enzyme that catalyses urate to allantoin conversion and that is absent in primates. We believe that the different phenotype in *Umod*^{A227T} and Tg$^{Umod C147W}$ mouse models could be ascribed to the different genetic backgrounds in the two models (C3H *versus* FVB), to the different expression level of total uromodulin or to the fact that the A227T variant was not associated with UAKD in patients.

On the bases of current knowledge, we envisage a model of UAKD pathogenesis in which the key primary event is ER accumulation of mutant uromodulin in the TAL cells that could have both a gain-of-function and a loss-of-function effect (Figure 3a). On the one hand, it leads to TAL functional and structural injury, as suggested by distal tubule cell necrosis in $Tg^{Umod C147W}$ mice. On the other hand, it reduces the amount of uromodulin entering the secretory pathway and reaching the apical membrane, also affecting the trafficking of the wild-type protein. This could affect the efficient delivery of ion transporters in the TAL segment, as suggested by reduced amount of ROMK at the apical plasma membrane in the *Umod* knock-out mouse (26). Loss of functional TAL segment could be at the bases of the urinary concentrating defect in UAKD and lead to hyperuricaemia, likely as a secondary effect of increased compensatory sodium uptake in the proximal tubule (59), through a mechanism similar to the one leading to hyperuricaemia following chronic loop diuretic administration (73). The inflammatory process in the kidney of $Tg^{Umod C147W}$ mice could be triggered by ER stress pathways activated in TAL cells and/or by TAL cell necrosis eventually resulting in progressive interstitial fibrosis and tissue scarring, a final common pathway for many acute and chronic kidney injuries. Although we did not detect significant evidence of interstitial uromodulin we cannot exclude the possibility that inflammation could be enhanced by basolateral release of mutant uromodulin. An increase of uromodulin in the serum, possibly as a consequence of its interstitial release, was indeed reported in some UAKD patients (12) and could lead to the induction of pro-inflammatory cytokines (74). Renal cysts in UAKD could be a consequence of progressive TAL cellular damage and secondary proliferation. Recent findings showing the presence of uromodulin in primary cilia and a significant reduction of ciliary uromodulin in UAKD patients suggest the possibility that cysts could be related to cilia dysfunction (75). However, this seems unlikely taking into account the lack of renal cysts in *Umod* knock-out mice and recent evidence that strong *Umod* gene down-regulation is not sufficient to induce cystogenesis following HNF-1beta inactivation in the mouse (76).

Further studies will be needed in order to address this and other key open questions that would have relevance for potential therapeutic strategies, as the identification of the stress pathways that are activated by mutant uromodulin ER retention, the molecular basis of inflammation and fibrosis and the contribution of each factor in disease progression.

Uromodulin involvement in other pathological conditions of the kidney

Several studies reported association of uromodulin abnormalities with pathologic conditions of the kidney. This includes accumulation of uromodulin in cast nephropathy and presence of protein deposits in the renal interstitium in reflux nephropathy, rejecting renal allografts and interstitial diseases (77-80). These deposits are sometimes associated with inflammatory infiltrate. Additionally, uromodulin urinary excretion is positively correlated with the estimated glomerular filtration rate (eGFR) and is reduced in several conditions affecting kidney function and/or integrity including glomerulonephritis, diabetes nephropathy, lupus nephritis, tubulointerstitial nephropathy and polycystic kidney disease (81). The use of urinary uromodulin as a diagnostic marker of renal disease has been recently questioned in an analysis of uromodulin urinary levels in 77 patients with chronic kidney disease (CKD) showing that 22% of the analysed patients have normal urinary levels of uromodulin (74). Qualitative changes in uromodulin processing have also been reported. The presence of shorter abnormally processed urinary uromodulin fragments was observed in Fabry disease patients (82). Interestingly, shorter uromodulin fragments were significantly decreased following enzyme replacement therapy.

Uromodulin in genome-wide association studies

Genome-wide association studies (GWAS) have successfully identified genomic loci containing

susceptibility variants associated with the risk of complex traits and markers of renal function ((83), for review). In particular, common variants in the *UMOD* gene have been associated with the risk of CKD, eGFR and other complex traits such as kidney stones and hypertension (Table 1, Figure 2).

The first GWAS on CKD, conducted in ~20000 participants of European ancestry from unselected, population-based cohorts from the CHARGE consortium, identified a top SNP (rs12917707) located 3.4 kb upstream of *UMOD* associated with the risk of CKD. The minor T allele of rs12917707 was associated with a 20% reduction in the risk of CKD, and the association was independent of major kidney disease risk factors including older age, male gender, and presence of hypertension or diabetes. Furthermore, prospective information from the ARIC study (n=952 cases) demonstrated that the T allele of rs12917707 was associated with a lower relative hazard of incident CKD over ~15 years of follow-up (HR 0.81, 95% CI= 0.72-0.92) (84). The strong association of rs12917707 with CKD was supported by a subsequent analysis performed in the larger cohort of the CKDGen consortium (85). It is important to note that the rs12917707 variant of *UMOD* was also associated with two indices of renal function, eGFRcrea and eGFRcys, based on serum creatinine and serum cystatin C respectively (84, 85). The rs12917707 variant of *UMOD* was associated with both higher eGFRcys and eGFRcreat and lower risk of developing CKD, consistent with a protective effect (84, 85). As expected, the variants described above explain a small percentage (typically, less than 1%) of the variance in eGFRcrea. Of note, there was no significant association of rs12917707 with hyperuricemia and gout (84).

An independent replication of the findings of Köttgen et al. was provided by Gudbjartsson et al. (86) who found that the SNP rs4293393, located 300 bp upstream of *UMOD*, is associated with increased risk of CKD and elevated serum creatinine (sCreat) in a large Icelandic population. The rs4293393 variant was also associated with increased serum levels of uric acid and increased risk of gout, contrasting with a *lower* risk of formation of calcium-containing kidney stones in the Icelandic population. The association of the rs4293393 and rs12917707 variants with serum

creatinine levels was confirmed in a meta-analysis of five European isolates (EUROSPAN) (87) and in the large European cohort reported by Chambers et al. (88). Of note, the rs4293393 variant is in perfect linkage disequilibrium (LD) in the HapMap CEU (89) with the rs12917707 variant (D' = 1; $r^2 = 1$) (Figure 2).

Recently, the Global BPGen consortium used an extreme case-control design to identify a locus in the 5' region of *UMOD* (rs13333226) associated with hypertension (90). The minor G allele of rs13333226 was associated with a lower risk of hypertension (OR: 0.6; 95% CI= 0.5-0.73), with each copy of the G allele being associated with 0.49 mmHg lower SBP and 0.30 mmHg DBP. The minor allele of rs13333226 was also associated with eGFR, but adjustment for this variable in a subset of 13466 individuals confirmed the association of rs13333226 with lower risk for hypertension. The rs13333226 variant of *UMOD* was also associated with long-term cardiovascular outcomes among the 26654 subjects from the Swedish population-based MDC study: each copy of the G allele associated with a 7.7% reduction in risk of cardiovascular disease after adjusting for age, sex, BMI and smoking status followed-up for 12 years. The rs13333226 variant is in complete LD with rs4293393 and rs12917707 variants (D' = 1, $r^2 = 1$, HapMap CEU) (Figure 2).

Typically, GWAS yield loci that are statistically associated with a quantitative trait or a disease state. In an effort to evaluate the functional link between variants in *UMOD*, the level of uromodulin in the urine and the risk of developing CKD, Köttgen et al. performed a nested case-control study (n=200) of incident CKD (followed-up for 9.9 years) within the Framingham Heart Study (91). They showed that baseline urinary uromodulin levels were 51% higher in CKD than controls and that the protective C allele of rs4293393 was associated with *lower* urinary uromodulin levels and higher eGFR, in a dose-dependent manner (91). The perfectly correlated minor G allele of rs13333226 (which is protective against hypertension) was associated with lower urinary excretion of uromodulin in a subset of participants of the population-based HERCULES study (n=110) and hypertensive individuals from the BRIGHT study (n=256), with a potential relation

with lower fractional excretion of sodium and lower endogenous lithium clearance (90). The minor T allele of rs12917707 that is protective for CKD, was also shown to be associated with lower urinary uromodulin among coronary artery disease (CAD) patients (n=120) from the Heart and Soul Study (92). However, in these patients uromodulin urinary levels were not associated with CKD, suggesting that different mechanisms could be responsible for kidney function decline in patients with and without CAD. With the limitation of small sample size and complexity of the factors (age, renal function, diet, drugs, etc.) influencing the excretion of uromodulin, these studies (*i*) point to the potential of uromodulin as a biomarker for CKD; and (*ii*) suggest that higher urinary excretion of uromodulin may be deleterious and precede the development of CKD and/or hypertension (Figure 3b).

Future studies should confirm the potential association between *UMOD* variants and blood pressure (as a continuous trait) and investigate the causality of the variants described above, which are all in strong LD in the 5' region of *UMOD*, or determine causal variants by resequencing. They should also characterise the factors influencing the urinary excretion of uromodulin in large cohorts, as well as the function, regulation and functional interactions of uromodulin in the epithelial cells lining the TAL in the human kidney (93). The transgenic and knock-out mouse models described above will undoubtedly be useful to analyse the role of uromodulin and distinct between primary effects or functional adaptations caused by its deletion or overexpression in the kidney.

Conclusions and perspectives

In the past years uromodulin has been rediscovered thanks to key findings from multidisciplinary studies that ranged from *in vitro* studies, genetic analysis in Mendelian disorders, characterisation of different mouse models and genome-wide association studies. This has led to a scientific Renaissance of the field that will boost future investigations aimed at understanding the precise role

of uromodulin in the TAL and its potential link with ion transport and innate immunity of the kidney, the molecular mechanisms that regulate its expression and secretion, the significance of its basolateral and urinary release and its use as a biomarker. Moreover, studies on uromodulin-associated kidney diseases will help understanding the pathophysiology of the TAL segment and will take advantage of pre-clinical models to identify potential therapeutic strategies. Finally, the association of uromodulin with CKD and hypertension will need further investigation to clarify the biological effect of the identified risk variants and to assess for the presence of additional linked variants that may have a causal role.

DISCLOSURE

None to declare.

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TITLES AND LEGENDS

Figure 1. (**a**) The predicted structure of uromodulin contains a leader peptide (predicted to be cleaved at residue 23), three EGF-like domains (EGF-II and EGF-III are calcium-binding), a central domain of unknown function (named D8C as it contains 8 conserved cysteines), a zona pellucida (ZP) domain and a glycosylphosphatidylinositol (GPI)-anchoring site (predicted at position 614). The seven N-glycosylation sites are indicated. The high-mannose chain on residue Asn 274 in shown in red. (**b**) Model of uromodulin maturation, excretion and polymerisation. Uromodulin is synthesised in TAL tubular epithelial cells. It is co-translationally inserted in the ER where GPI-anchoring, formation of intramolecular disulphide bonds and N-glycosylation take place. In the Golgi, all glycan chains are modified with the exception of the one on Asn 274 that retains a high-mannose moiety. Uromodulin reaches the plasma membrane in a polymerisation-incompetent conformation kept by the interaction of two hydrophobic motifs (red), one within the ZP domain (Internal Hydrophobic Patch) and one localised between the ZP domain and the GPI-anchoring site (External Hydrophobic Patch). Proteolytic cleavage by a yet to be identified protease (scissors) releases the hydrophobic interaction generating a polymerisation-competent monomer that is assembled into polymeric filaments. The orientation of uromodulin monomers within a filament is hypothetical and deduced from structural data on ZP3 protein (94).

Figure 2. Summary of uromodulin genetic variants associated with chronic diseases of the kidney. Upper panel: all published uromodulin mutations in UAKD patients are shown relative to their localisation and effect in the protein sequence. Fifty-one mutations have been reported to date: 29 (57%) affect or introduce cysteine residues directly altering the disulphide bonds pattern; 19 (37%) are missense changes affecting residues other than cysteine; 3 (6%) are in-frame deletions. Middle panel: exon/intron structure of the human *UMOD* gene. Coding parts are shown in blue. Most of *UMOD* mutations are clustered in exons 3 and 4. Lower panel: the position of top SNPs that were

identified in different GWAS is shown (red). These variants are within the same linkage disequilibrium block spanning *UMOD* promoter to exon 7, as shown by the linkage disequilibrium plot (r^2 values, adapted from Haploview 4.2 output, data from HapMap CEU, release #28).

Figure 3. Uromodulin and chronic diseases of the kidney. (**a**) In UAKD, mutations in *UMOD* cause ER retention and aggregation of mutant protein. This leads to TAL dysfunction and urinary concentrating defect and to a dramatic decrease of uromodulin urinary levels, suggesting a dominant negative effect on the transport of wild type uromodulin to the plasma membrane. This effect could also affect the delivery of other proteins, e.g. ion transporters, on the plasma membrane contributing to TAL dysfunction. Through a still unknown mechanism, intracellular retention eventually results in TAL cell damage and necrosis leading to fibrosis and inflammatory cell infiltrate. Inflammation could be enhanced by basolateral release of uromodulin. (**b**) GWAS studies identified *UMOD* variants that are associated with eGFR and increased risk of CKD, hypertension and cardiovascular disease. These variants are associated with increased urinary uromodulin levels that could be due to increased uromodulin expression, decreased membrane-anchoring efficiency, faster protein sorting to the apical membrane or increased proteolytic release. The causal relationship between *UMOD* variants, increased urinary uromodulin and development of CKD, hypertension or cardiovascular disease is presently unknown.

Table 1. *UMOD* variants and traits identified by GWAS

Top SNP	Position[a]	Trait	Consortium[d]	Discovery cohort (n)	Replication (n)	Reference
rs12917707	-3403 bp	CKD[b]	CHARGE	19877	21466	(84)
rs12917707	-3403 bp	CKD[b]	CKDGen	62237	NA	(85)
rs12917707	-3403 bp	eGFRcrea	CHARGE	18127	21466	(84)
rs12917707	-3403 bp	eGFRcys	CHARGE	12266	NA	(84)
rs12917707	-3403 bp	eGFRcrea	CKDGen	67093	NA	(85)
rs12917707	-3403 bp	eGFRcys	CKDGen	20957	NA	(85)
rs4293393	-300 bp	sCreat	EUROSPAN[e]	4006	NA	(87)
rs12917707	-3403 bp	sCreat	Nine studies[f]	23090	16626	(88)
rs4293393	-300 bp	CKD	Iceland	2903 *vs.* 35818[g]	300 *vs.* 2964[g]	(86)
rs4293393	-300 bp	sCreat	Iceland	22256	2,379	(86)
rs4293393	-300 bp	sUrate	Iceland	6583	NA	(86)
rs4293393	-300 bp	sUrea	Iceland	4084	NA	(86)
rs4293393	-300 bp	Kidney stones	Iceland	1689 *vs.* 37076[g]	1972 *vs.* 6125[g,h]	(86)
rs13333226	-1867 bp	Hypertension[c]	Global BPgen	1621 *vs.* 1699[g]	19845 *vs.* 1654[g]	(90)

Footnote: The frequency of the minor allele for rs12917707, rs13333226 and rs4293393 is ~ 0.18. All the indicated SNPs are in complete linkage disequilibrium (D'=1, r^2=1; HapMap CEU, release #28).

[a]Position relative to *UMOD* transcription start site (UCSC Genome Browser, GRCh37); [b]CKD definition: eGFR< 60mL/min/1.73m2 using the MDRD equation; [c]Hypertension defined as at least two consecutive blood pressure measurements of ≥160 mmHg systolic and ≥100 mmHg diastolic , with diagnosis made before age 63 years; [d]All cohorts are from European descent; [e]EUROSPAN is a combination of five European genetic isolates; [f]European participants from the following studies: LOLIPOP, CoLaus, SardiNIA, TwinsUK, BRIGHT, Fenland, NFBC1966, NESDA, InChianti; [g]cases *vs.* controls; [h]combined replication on Icelandic and Dutch subjects.

Abbreviations: eGFRcrea: estimated GFR based on serum creatinine; eGFRcys, estimated GFR based on serum cystatin C; sCreat, serum creatinine; CKD, chronic kidney disease; NA, not available.

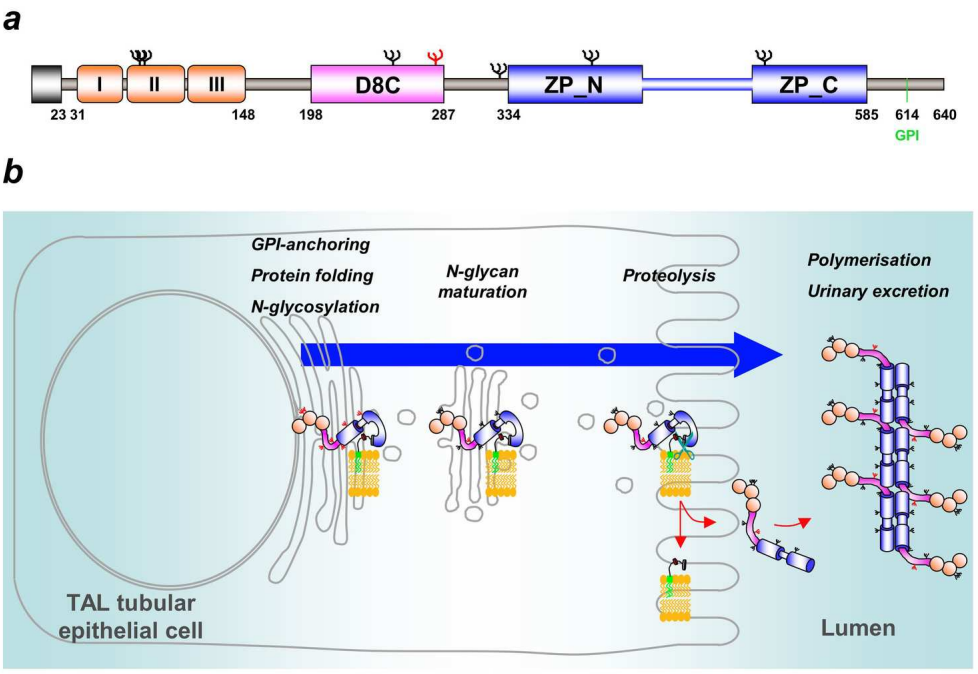


Figure 1

63x46mm (600 x 600 DPI)

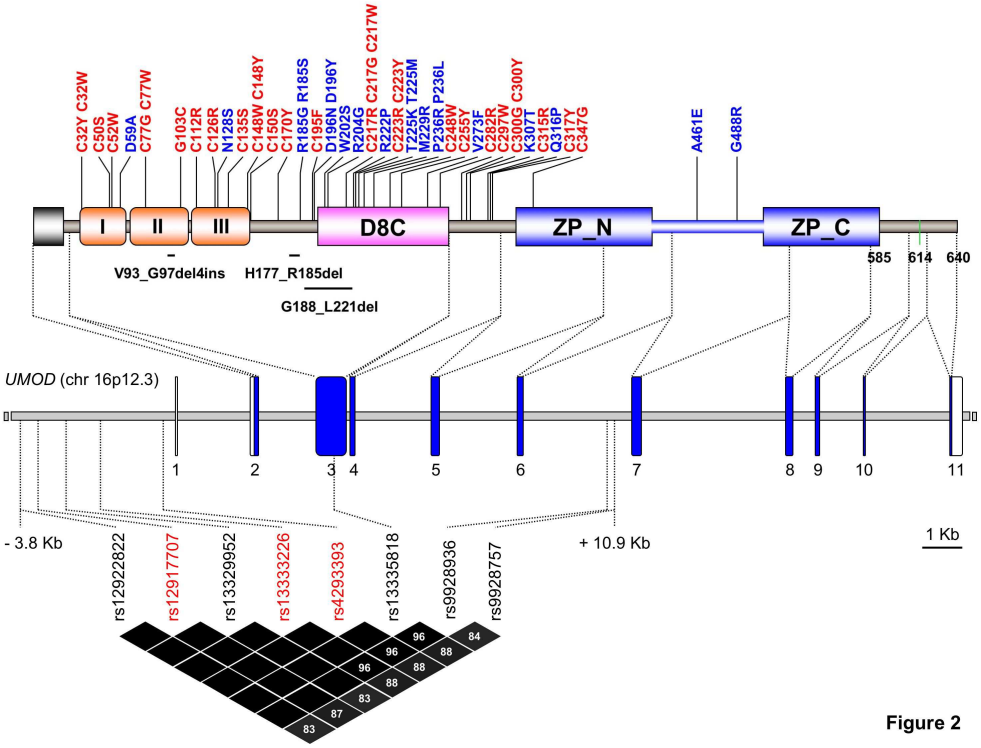
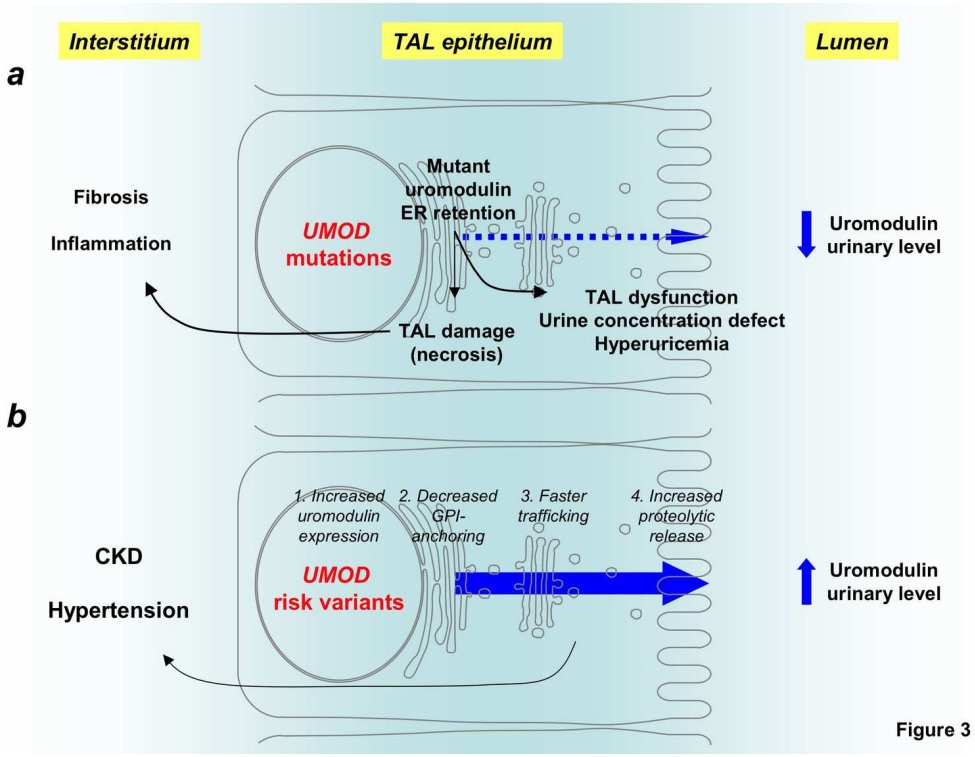


Figure 2

177x133mm (428 x 428 DPI)



64x48mm (600 x 600 DPI)