The rediscovery of uromodulin (Tamm–Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease

Luca Rampoldi[1], Francesco Scolari[2], Antonio Amoroso[3], GianMarco Ghiggeri[4] and Olivier Devuyst[5]

[1]Dulbecco Telethon Institute, Molecular Genetics of Renal Disorders Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy; [2]Division of Nephrology, Montichiari Hospital, Department of Experimental and Applied Medicine, University of Brescia, Brescia, Italy; [3]Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy; [4]Division of Nephrology, G Gaslini Institute, Genoa, Italy and [5]Institute of Physiology, Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

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 knockout 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 characterized by progressive tubulointerstitial damage, impaired urinary concentrating ability, hyperuricemia, 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 (CKD) and hypertension, and suggested that the level of uromodulin in the urine could represent a useful biomarker for the development of CKD. In this review, we summarize these recent investigations, ranging from invalidation studies in mouse to Mendelian disorders and genome-wide associations, which led to a rediscovery of uromodulin and boosted the scientific and clinical interest for this long discovered molecule.

Correspondence: *Luca Rampoldi, Dulbecco Telethon Institute, Molecular Genetics of Renal Disorders Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Via Olgettina 58, Milan 20132, Italy.*
E-mail: rampoldi.luca@hsr.it

BIOLOGY OF UROMODULIN

In the early fifties, Tamm and Horsfall[1] described a mucoprotein that could be purified from urine and inhibited viral hemagglutination. The protein, referred to as Tamm—Horsfall protein, is the most abundant protein in urine under physiological conditions.[2] In 1985, Muchmore and Decker[3] isolated a protein from the urine of pregnant women that they called uromodulin in relation with its immuno-suppressive activity documented *in vitro*. Two years later, Tamm–Horsfall protein 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 synthesized as a 640 amino-acid precursor. The protein enters the secretory pathway where it is glycosylphosphatidylinositol 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 intramolecular disulfide 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 epidermal growth factor (EGF)-like domains (EGF-II and EGF-III predicted to be calcium binding), a central domain of unknown function (named D8C as it contains eight conserved cysteines), a zona pellucida (ZP) domain, and a glycosylphosphatidylinositol-anchoring site (predicted at position 614; Figure 1a). EGF-like domains are found in many secreted and extracellular proteins, and are thought to have a role in processes of adhesion, coagulation, and
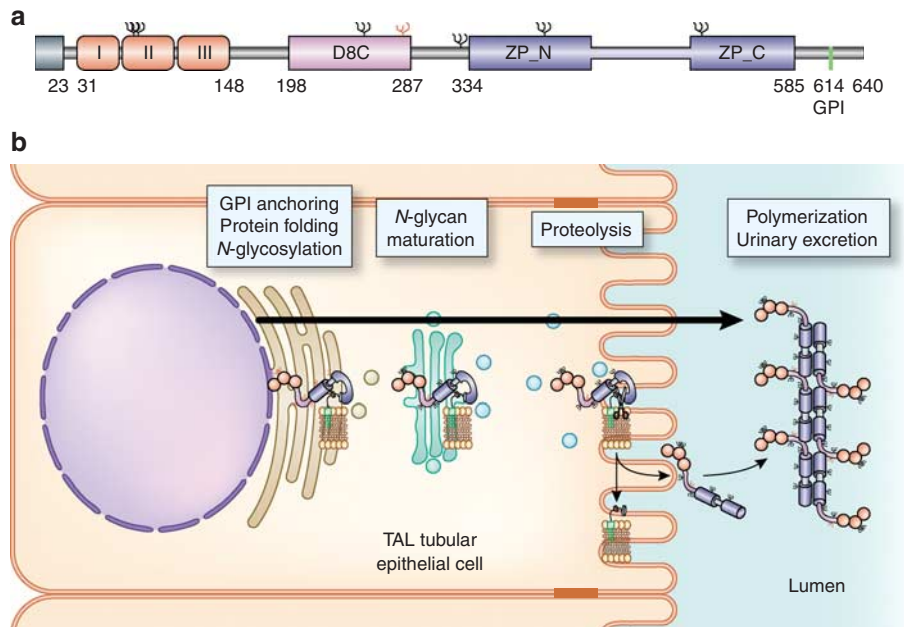
Figure 1 | Structure and maturation of uromodulin. (**a**) The predicted structure of uromodulin contains a leader peptide (predicted to be cleaved at residue 23), three epidermal growth factor (EGF)-like domains (EGF-II and EGF-III are calcium binding), a central domain of unknown function (named D8C as it contains eight 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 is shown in red. (**b**) Model of uromodulin maturation, excretion, and polymerization. Uromodulin is synthesized in thick ascending limb (TAL) tubular epithelial cells. It is co-translationally inserted in the endoplasmic reticulum where GPI anchoring, formation of intramolecular disulfide 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 polymerization-incompetent conformation kept by the interaction of two hydrophobic motifs (red), one within the ZP domain (internal hydrophobic patch) and one localized 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 polymerization-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]

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, such as sperm receptors ZP1 and ZP3 and tectorial membrane components α- and β-tectorin, and is essential for protein polymerization[7] (Figure 1b). Indeed, uromodulin is mainly present in the urine as a high-molecular-weight polymer (M_r $1–10 \times 10^6$ Da) 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 localization at the basolateral side of TAL cells has also been reported.[11] A basolateral release of uromodulin is suggested by studies on its trafficking in transfected polarized 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 by immunohistochemistry 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 the most abundant transcript in mature TAL cells where it is produced at a very high rate.[16] The half-life of the protein is rather short (about 9 h in rabbit and 16 h 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 polymerization, as it releases an inhibitory motif that prevents premature protein assembly,[20] similarly to what described for ZP protein ZP3 (ref. 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, which is the major

component of zymogen granule membranes of exocrine pancreas, and liver-specific ZP domain-containing protein. Glycoprotein 2 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), glycoprotein 2 protein is able to bind to *Escherichia 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* homologs in amphibian (*Xenopus tropicalis*) and fish (*Danio rerio*) genomes, with significant sequence similarity at the predicted protein level. The function of these homologs and their relevance for comparative physiology remain to be determined.

Biological function

The biological function of uromodulin is still rather elusive. Uromodulin has been hypothesized 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* knockout mice leads to significant upregulation of ROMK2, which 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 (Na-Cl cotransporter, α-epithelial Na channel) were also found to be significantly upregulated in uromodulin-deficient mice.[27]

Studies in *Umod* knockout mice showed that uromodulin has 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, for example, 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 has 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 knockout 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 knockout mice.[35] Although supported by *in vivo* evidence in *Umod* knockout mice, the relevance of uromodulin as a protective molecule against UTI and nephrolithiasis is still unclear. Indeed, individuals with extremely reduced uromodulin urinary level, as patients carrying *UMOD* mutations (see below), do not show increased rates of UTIs or renal stone formation.

Finally, uromodulin has also been suggested to have a role in innate immunity of the kidney. Several *in vitro* studies demonstrated that it can bind to immunity-related molecules, such as immunoglobulin G, complement 1q, and tumor necrosis factor-α.[36–38] Uromodulin can also act as a chemoattractant[39] and as a proinflammatory 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.[40] Administration of uromodulin induces tubulointerstitial nephritis in rabbits, rats, and mice.[41,42] In the mouse, this is accompanied by the production of anti-uromodulin antibodies, which is dependent on toll-like receptor 4 function.[40] Taken together, these data suggest that uromodulin may act as a danger-signaling 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 proinflammatory role of uromodulin remains controversial and has been recently challenged by El-Achkar *et al.*,[44] who showed that mice lacking uromodulin develop more functional and histological renal damage after ischemia–reperfusion injury compared with wild-type animals.

UROMODULIN-ASSOCIATED KIDNEY DISEASES

Mutations in the *UMOD* gene cause medullary cystic kidney disease type 2 (MIM 603860) and familial juvenile hyperuricemic nephropathy (MIM 162000) that are autosomal dominant tubulointerstitial kidney diseases. Being allelic disorders, medullary cystic kidney disease type 2 and familial juvenile hyperuricemic nephropathy are collectively referred to as uromodulin-associated kidney disease (UAKD).[45] UAKD is a rare disorder. About 50 mutations have been reported so far (see below), and its prevalence is estimated to be 1/100,000 (http://www.orphanet.org). The earliest symptom in UAKD patients is often hyperuricemia that results from reduced fractional excretion of uric acid, is present in ~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 interfamilial variability has been observed. At the histological analysis, UAKD is characterized by diffuse tubulointerstitial fibrosis with moderate inflammatory cell infiltrate and

tubular atrophy.[47,48] Renal cysts (generally measuring 0.5–3 cm in diameter) are sometimes detected, mainly at the corticomedullary junction.[47,49,50] There is no specific therapy other than correction of water and electrolyte imbalance that may occur. Hyperuricemia can be effectively treated with allopurinol[51] or uricosuric drugs such as benzbromarone.[52] 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 (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 three affected individuals from a Spanish consanguineous family.[55] Homozygous individuals display a more severe phenotype in comparison with 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 intracel-

lular aggregates in the cells lining the TAL.[47,48,56,57] These inclusions colocalize with 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 UAKD patients.[47,48,57,60] The reduction was irrespective of renal function, suggesting a possible dominant-negative effect on the trafficking of wild-type protein.[47,60]

Similar clinical findings, that is, tubulointerstitial nephritis and hyperuricemia, can be found associated with mutations in the gene encoding the transcription factor hepatocyte nuclear factor-1β (*TCF2*; MIM 137920)[61] and in the *REN* gene encoding renin (MIM 613093).[62] Moreover, two additional loci have been mapped on chromosome 1q21 (medullary cystic kidney disease type 1)[63] and p22.1–p21 (familial juvenile hyperuricemic nephropathy 3).[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 ∼50% (28/51) of the cases one of the conserved
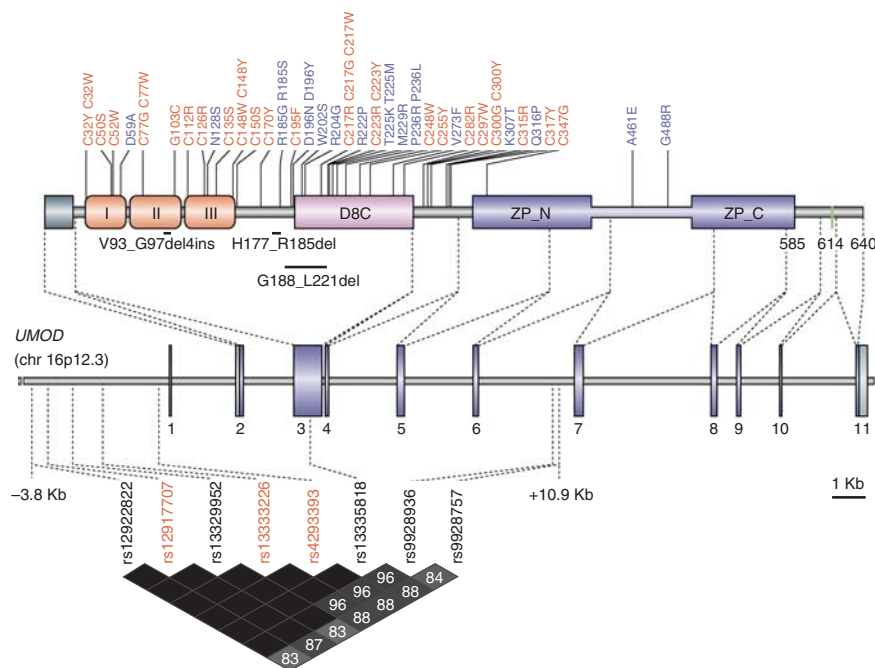

Figure 2 | Uromodulin genetic variants associated with chronic diseases of the kidney. Upper panel: all published uromodulin mutations in uromodulin-associated kidney disease patients are shown relative to their localization and effect in the protein sequence. A total of 51 mutations have been reported to date: 29 (57%) affect or introduce cysteine residues directly altering the disulfide bond pattern; 19 (37%) are missense changes affecting residues other than cysteine; and 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 single-nucleotide polymorphisms that were identified in different genome-wide association studies is shown (red). These variants are within the same linkage disequilibrium (LD) block spanning *UMOD* promoter to exon 7, as shown by the LD plot (r^2 values, adapted from Haploview 4.2 output, data from HapMap CEU, release no. 28). chr, chromosome; *UMOD*, uromodulin; ZP, zona pellucida.

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 disulfide bond pattern or by destabilizing 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 because of 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, which 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, that is, 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* knockout mouse models have been reported and extensively characterized. As previously mentioned, these animals are more susceptible to UTIs 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 3 years of age.[70] *Umod* knockout mice show mild urinary concentrating defect after water deprivation test, which 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 support 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 ethylnitrosourea mutagenesis. The *Umod*[A227T] mice show some features of UAKD, such as the presence of uromodulin aggregates in TAL cells, urine concentration ability defect, and reduced fractional excretion of uric acid. However, they lack inflammation and fibrosis and have additional metabolic alterations.[72]

We recently generated and characterized an *in vivo* model of UAKD, that is 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 characterized *in vitro*.[48] The phenotype in Tg[*Umod*C147W] mice was compared with expression-matched transgenic mice for wild-type protein (Tg[*Umod*wt]). Tg[*Umod*C147W] mice specifically show progressive signs of renal damage, that is, tubulointerstitial 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 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-week-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 (C3HeB/FeJ versus FVB/N), 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 findings 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* knockout mouse.[26] The loss of functional TAL segment could explain the urinary concentrating defect in UAKD and lead to hyperuricemia. The latter could be due to
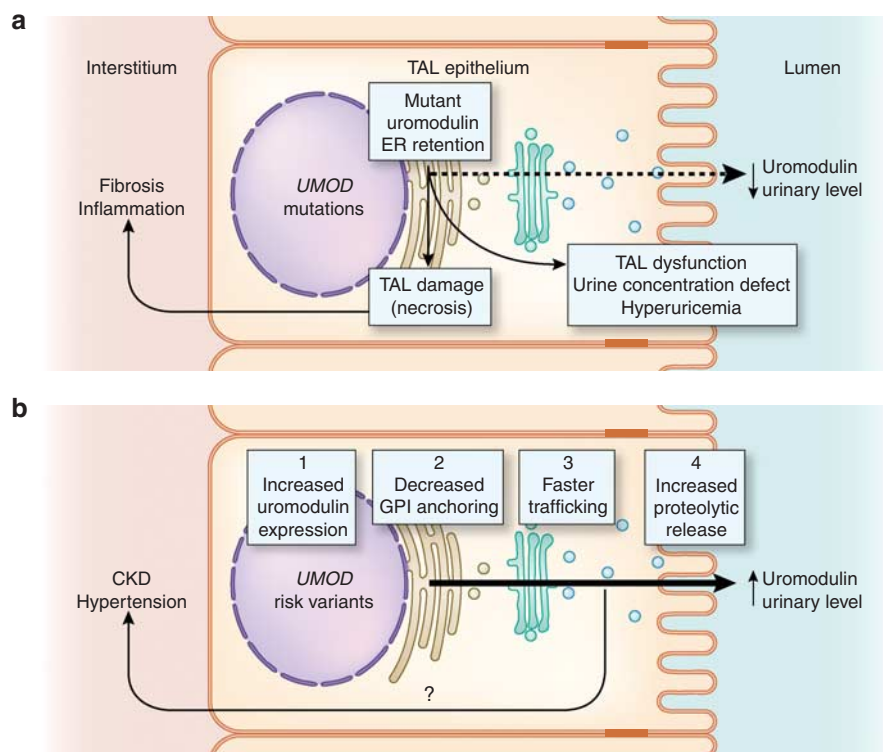
Figure 3 | Uromodulin and chronic diseases of the kidney. (a) In uromodulin-associated kidney disease, mutations in *UMOD* cause endoplasmic reticulum (ER) retention and aggregation of mutant protein. This leads to thick ascending limb (TAL) dysfunction and urinary concentrating defect and to a dramatic 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, for example, 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) Genome-wide association studies identified *UMOD* variants that are associated with estimated glomerular filtration rate and increased risk of chronic kidney disease (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 among *UMOD* variants, increased urinary uromodulin, and development of CKD, hypertension, or cardiovascular disease is presently unknown. GPI, glycosylphosphatidylinositol.

a compensatory sodium uptake in the proximal tubule,[59] by a mechanism similar to the one causing hyperuricemia during 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 proinflammatory 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 dysfunc-

tion.[75] However, this seems unlikely taking into account the lack of renal cysts in *Umod* knockout mice and recent evidence that strong *Umod* gene downregulation is not sufficient to induce cystogenesis following hepatocyte nuclear factor-1β inactivation in mouse.[76]

Further studies will be needed to address this and other open questions that would have relevance for potential therapeutic strategies, such 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 pathological 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 analyzed 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, the urinary excretion of shorter uromodulin fragments was 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 (for review, see ref. 83). 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 ∼20,000 participants of European ancestry from unselected, population-based cohorts from the CHARGE Consortium, identified a top single-nucleotide polymorphism (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. Further prospective data from the ARIC Study ($n = 952$ cases) showed that the T allele of rs12917707 was associated with a lower relative hazard of incident CKD over ∼15 years of follow-up (hazard ratio = 0.81, 95% confidence interval = 0.72–0.92).[84] The strong association of rs12917707 with CKD was supported by a subsequent analysis performed in 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 eGFRcrea, and lower risk of developing CKD, consistent with a protective effect.[84,85] As expected, the variants described above explain a small percentage (typically, <1%) of the variance in eGFRcrea. Of note, there was no significant association of rs12917707 with hyperuricemia and gout.[84]

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

Top SNP	Position[a] (bp)	Trait	Consortium[b]	Discovery cohort (*n*)	Replication (*n*)	Reference
rs12917707	−3403	CKD[c]	CHARGE	19,877	21,466	Kottgen *et al.*[84]
rs12917707	−3403	CKD[c]	CKDGen	62,237	NA	Kottgen *et al.*[85]
rs12917707	−3403	eGFRcrea	CHARGE	18,127	21,466	Kottgen *et al.*[84]
rs12917707	−3403	eGFRcys	CHARGE	12,266	NA	Kottgen *et al.*[84]
rs12917707	−3403	eGFRcrea	CKDGen	67,093	NA	Kottgen *et al.*[85]
rs12917707	−3403	eGFRcys	CKDGen	20,957	NA	Kottgen *et al.*[85]
rs4293393	−300	sCreat	EUROSPAN[d]	4006	NA	Pattaro *et al.*[87]
rs12917707	−3403	sCreat	Nine studies[e]	23,090	16,626	Chambers *et al.*[88]
rs4293393	−300	CKD	Iceland	2903 versus 35,818[f]	300 versus 2964[f]	Gudbjartsson *et al.*[86]
rs4293393	−300	sCreat	Iceland	22,256	2379	Gudbjartsson *et al.*[86]
rs4293393	−300	sUrate	Iceland	6583	NA	Gudbjartsson *et al.*[86]
rs4293393	−300	sUrea	Iceland	4084	NA	Gudbjartsson *et al.*[86]
rs4293393	−300	Kidney stones	Iceland	1689 versus 37,076[f]	1972 versus 6125[f,g]	Gudbjartsson *et al.*[86]
rs13333226	−1867	Hypertension[h]	Global BPgen	1621 versus 1699[f]	19845 versus 1654[f]	Padmanabhan *et al.*[90]

Abbreviations: CKD, chronic kidney disease; eGFRcrea, estimated glomerular filtration rate based on serum creatinine; eGFRcys, estimated glomerular filtration rate based on serum cystatin C; GWAS, genome-wide association study; LD, linkage disequilibrium; NA, not available; sCreat, serum creatinine; SNP, single-nucleotide polymorphism; sUrate, serum urate; *UMOD*, uromodulin.
[a]Position relative to *UMOD* transcription start site (UCSC Genome Browser, GRCh37).
[b]All cohorts are from European descent.
[c]CKD definition: eGFR < 60 ml/min per 1.73 m^2 using the Modification of Diet in Renal Disease study equation.
[d]EUROSPAN is a combination of five European genetic isolates.
[e]European participants from the following studies: LOLIPOP, CoLaus, SardiNIA, TwinsUK, BRIGHT, Fenland, NFBC1966, NESDA, and InChianti.
[f]Cases versus controls.
[g]Combined replication on Icelandic and Dutch subjects.
[h]Hypertension defined as at least two consecutive blood pressure measurements of ⩾160 mm Hg systolic and ⩾100 mm Hg diastolic pressure, with diagnosis made before age 63 years.
The frequency of the minor allele for rs12917707, rs13333226, and rs4293393 is ∼0.18. All the indicated SNPs are in complete LD ($D'=1$, $r^2=1$; HapMap CEU, release no. 28).

An independent replication of the findings of Köttgen et al.[84] was provided by Gudbjartsson et al.,[86] who showed that the single-nucleotide polymorphism rs4293393, located 300 bp upstream of *UMOD*, is associated with increased risk of CKD and elevated serum creatinine in a large Icelandic population. The rs4293393 variant was also associated with increased serum levels of uric acid and increased risk of gout, and 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 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 (odds ratio = 0.6; 95% confidence interval = 0.5–0.73), with each copy of the G allele being associated with 0.49 mm Hg lower systolic blood pressure and 0.30 mm Hg lower diastolic blood pressure. The minor allele of rs13333226 was also associated with eGFR, but adjustment for this variable in a subset of 13,466 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 26,654 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, body mass index, and smoking status followed up for 12 years. The rs13333226 variant is in complete linkage disequilibrium 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 among variants in *UMOD*, the level of uromodulin in the urine, and the risk of developing CKD, Köttgen et al.[91] performed a nested case–control study ($n = 200$) of incident CKD (followed up for 9.9 years) within the Framingham Heart Study. 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, which is protective for CKD, was also shown to be associated with lower urinary uromodulin among coronary artery disease patients ($n = 120$) from the Heart and Soul Study.[92] However, in these patients, the urinary levels of uromodulin were not associated with CKD, suggesting that different mechanisms could be responsible for kidney function decline in patients with and without coronary artery disease. 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 linkage disequilibrium in the 5′ region of *UMOD*, or determine causal variants by resequencing. They should also characterize 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 knockout mouse models described above will undoubtedly be useful to analyze the role of uromodulin and distinct between primary effects or functional adaptations caused by its deletion or overexpression in the kidney.

CONCLUSIONS AND PERSPECTIVES

The last ten years have witnessed a rediscovery of uromodulin, thanks to key findings from *in vivo* investigations, genetic analysis in Mendelian disorders, characterization of different mouse models, and GWAS. This has led to a scientific renaissance of the field that will boost future studies on 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 UAKD will help understanding the pathophysiology of the TAL segment and will take advantage of preclinical 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 evaluate linked variants that may have a causal role.

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