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.

Kidney International (2011) **80,** 338–347; doi:10.1038/ki.2011.134; published online 8 June 2011

KEYWORDS: chronic kidney disease; Tamm–Horsfall protein; uromodulin

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

Received 12 January 2011; revised 2 March 2011; accepted 8 March 2011; published online 8 June 2011

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 immunosuppressive 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

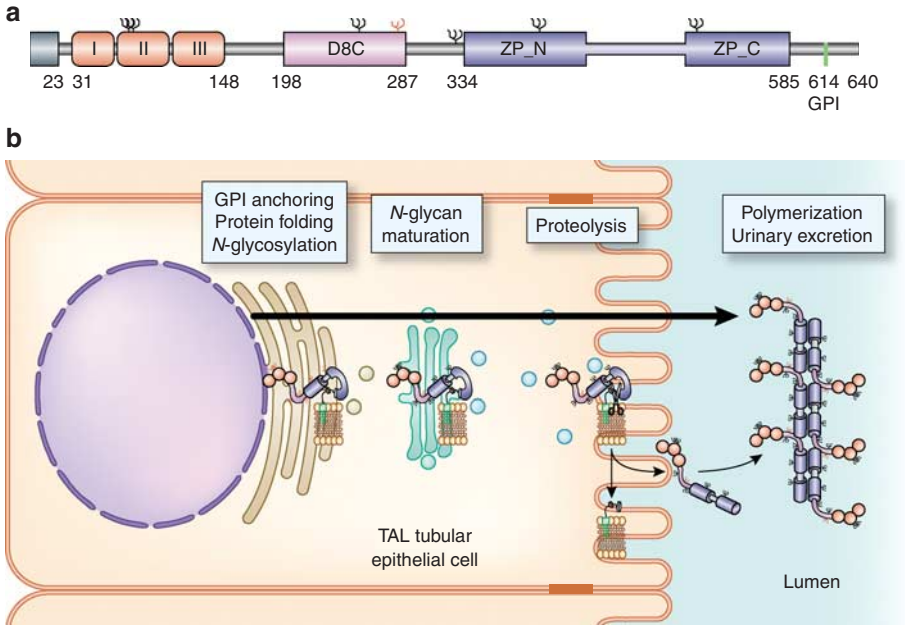

Figure 1 | Structure and maturation of uromodulin. (**a**) The predicted structure of uromodulin contains a leader peptide (predicted to be cleaved at residue 23), three epidermal growth factor (EGF)-like domains (EGF-II and EGF-III are calcium binding), a central domain of unknown function (named D8C 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 × 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 intracellular 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

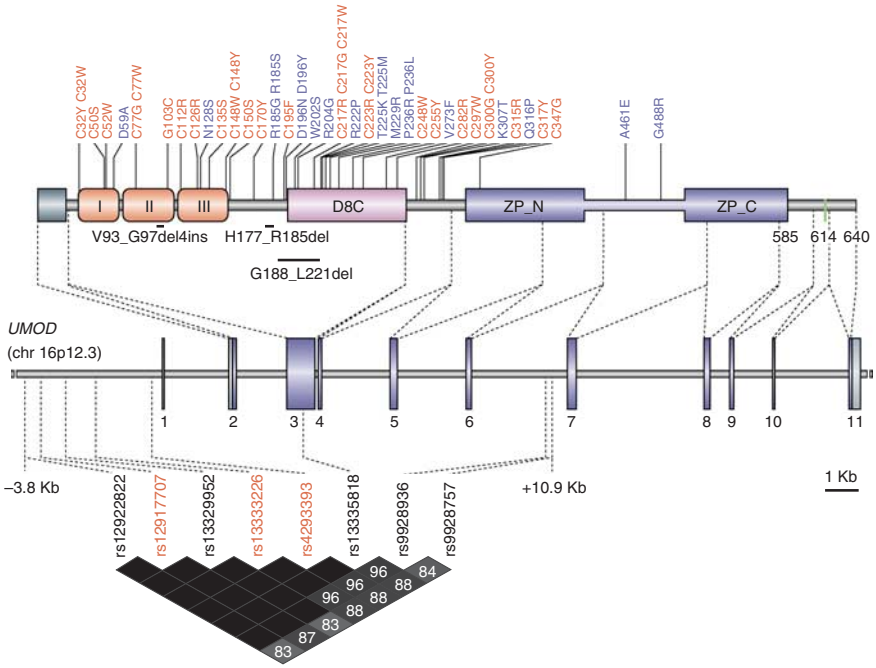

Figure 2 | Uromodulin genetic variants associated with chronic diseases of the kidney. Upper panel: all published uromodulin mutations in uromodulin-associated kidney disease patients are shown relative to their localization and effect in the protein sequence. A total of 51 mutations have been reported 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 ($Tg^{UmodC147W}$).[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 $Tg^{UmodC147W}$ mice was compared with expression-matched transgenic mice for wild-type protein (Tg^{Umodwt}). $Tg^{UmodC147W}$ 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, $Tg^{UmodC147W}$ 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. $Tg^{UmodC147W}$ 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 $Tg^{UmodC147W}$ 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 $Tg^{UmodC147W}$ 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

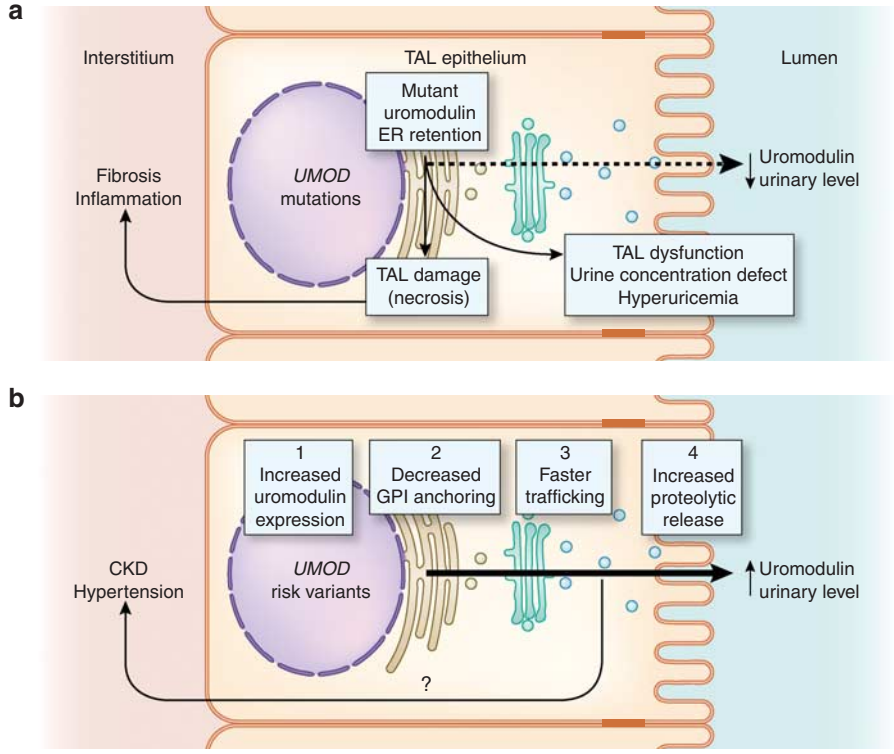

Figure 3 | Uromodulin and chronic diseases of the kidney. (**a**) In uromodulin-associated kidney disease, mutations in *UMOD* cause endoplasmic reticulum (ER) retention and aggregation of mutant protein. This leads to thick ascending limb (TAL) dysfunction and urinary concentrating defect and to a dramatic 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 $Tg^{UmodC147W}$ 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 dysfunction.[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.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by Telethon-Italy (TCR08006 to LR); the European Community's 7th Framework Program (HEALTH-F2-2007-201590, EUNEFRON program to OD and LR); Belgian agencies FNRS and FRSM (to OD); the 'Fondation Alphonse & Jean Forton' (to OD); a Concerted Research Action (05/10-328 to OD); an Inter-University Attraction Pole (IUAP P6/05 to OD); the DIANE Project (Communauté Française de Belgique; to OD); and the National Centre of Competence in Research 'Kidney.CH' (to OD). LR is an Associate Telethon Scientist.

REFERENCES

1. Tamm I, Horsfall FL. Characterisation and separation of an inhibitor of viral hemagglutination present in urine. *Proc Soc Exp Biol Med* 1950; **74**: 108–114.
2. Kumar S, Muchmore A. Tamm-Horsfall protein—uromodulin (1950–1990). *Kidney Int* 1990; **37**: 1395–1401.
3. Muchmore AV, Decker JM. Uromodulin: a unique 85-kilodalton immunosuppressive glycoprotein isolated from urine of pregnant women. *Science* 1985; **229**: 479–481.
4. Pennica D, Kohr WJ, Kuang WJ *et al.* Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein. *Science* 1987; **236**: 83–88.
5. Serafini-Cessi F, Malagolini N, Hoops TC *et al.* Biosynthesis and oligosaccharide processing of human Tamm-Horsfall glycoprotein permanently expressed in HeLa cells. *Biochem Biophys Res Commun* 1993; **194**: 784–790.
6. Easton RL, Patankar MS, Clark GF *et al.* Pregnancy-associated changes in the glycosylation of Tamm-Horsfall glycoprotein. Expression of sialyl Lewis(x) sequences on core 2 type O-glycans derived from uromodulin. *J Biol Chem* 2000; **275**: 21928–21938.
7. Jovine L, Qi H, Williams Z *et al.* The ZP domain is a conserved module for polymerization of extracellular proteins. *Nat Cell Biol* 2002; **4**: 457–461.
8. Wiggins RC. Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under different physicochemical conditions. *Clin Chim Acta* 1987; **162**: 329–340.
9. Bachmann S, Metzger R, Bunnemann B. Tamm-Horsfall protein-mRNA synthesis is localized to the thick ascending limb of Henle's loop in rat kidney. *Histochemistry* 1990; **94**: 517–523.
10. Bachmann S, Koeppen-Hagemann I, Kriz W. Ultrastructural localization of Tamm-Horsfall glycoprotein (THP) in rat kidney as revealed by protein A-gold immunocytochemistry. *Histochemistry* 1985; **83**: 531–538.
11. Peach RJ, Day WA, Ellingsen PJ *et al.* Ultrastructural localization of Tamm-Horsfall protein in human kidney using immunogold electron microscopy. *Histochem J* 1988; **20**: 156–164.
12. Jennings P, Aydin S, Kotanko P *et al.* Membrane targeting and secretion of mutant uromodulin in familial juvenile hyperuricemic nephropathy. *J Am Soc Nephrol* 2007; **18**: 264–273.
13. Chen YT, Kobayashi A, Kwan KM *et al.* Gene expression profiles in developing nephrons using Lim1 metanephric mesenchyme-specific conditional mutant mice. *BMC Nephrol* 2006; **7**: 1.
14. Jouret F, Debaix H, Devuyst O. A catalog of gene expression in the developing kidney. *Kidney Int* 2004; **66**: 867–868.
15. Zimmerhackl LB, Rostasy K, Wiegele G *et al.* Tamm-Horsfall protein as a marker of tubular maturation. *Pediatr Nephrol* 1996; **10**: 448–452.
16. Chabardes-Garonne D, Mejean A, Aude JC *et al.* A panoramic view of gene expression in the human kidney. *Proc Natl Acad Sci USA* 2003; **100**: 13710–13715.
17. Grant AM, Neuberger A. The turnover rate of rabbit urinary Tamm-Horsfall glycoprotein. *Biochem J* 1973; **136**: 659–668.
18. Serafini-Cessi F, Malagolini N, Cavallone D. Tamm-Horsfall glycoprotein: biology and clinical relevance. *Am J Kidney Dis* 2003; **42**: 658–676.
19. Santambrogio S, Cattaneo A, Bernascone I *et al.* Urinary uromodulin carries an intact ZP domain generated by a conserved C-terminal proteolytic cleavage. *Biochem Biophys Res Commun* 2008; **370**: 410–413.
20. Schaeffer C, Santambrogio S, Perucca S *et al.* Analysis of uromodulin polymerization provides new insights into the mechanisms regulating ZP domain-mediated protein assembly. *Mol Biol Cell* 2009; **20**: 589–599.
21. Jovine L, Qi H, Williams Z *et al.* A duplicated motif controls assembly of zona pellucida domain proteins. *Proc Natl Acad Sci USA* 2004; **101**: 5922–5927.
22. Kistler AD, Mischak H, Poster D *et al.* Identification of a unique urinary biomarker profile in patients with autosomal dominant polycystic kidney disease. *Kidney Int* 2009; **76**: 89–96.
23. Yu S, Lowe AW. The pancreatic zymogen granule membrane protein, GP2, binds Escherichia coli Type 1 fimbriae. *BMC Gastroenterol* 2009; **9**: 58.
24. Howie AJ, Lote CJ, Cunningham AA *et al.* Distribution of immunoreactive Tamm-Horsfall protein in various species in the vertebrate classes. *Cell Tissue Res* 1993; **274**: 15–19.
25. Ying WZ, Sanders PW. Dietary salt regulates expression of Tamm-Horsfall glycoprotein in rats. *Kidney Int* 1998; **54**: 1150–1156.
26. Renigunta A, Renigunta V, Saritas T *et al.* Tamm-Horsfall glycoprotein interacts with renal outer medullary potassium channel ROMK2 and regulates its function. *J Biol Chem* 2010.
27. Bachmann S, Mutig K, Bates J *et al.* Renal effects of Tamm-Horsfall protein (uromodulin) deficiency in mice. *Am J Physiol Renal Physiol* 2005; **288**: F559–F567.
28. Bates JM, Raffi HM, Prasadan K *et al.* Tamm-Horsfall protein knockout mice are more prone to urinary tract infection: rapid communication. *Kidney Int* 2004; **65**: 791–797.
29. Mo L, Zhu XH, Huang HY *et al.* Ablation of the Tamm-Horsfall protein gene increases susceptibility of mice to bladder colonization by type 1-fimbriated *Escherichia coli*. *Am J Physiol Renal Physiol* 2004; **286**: F795–F802.
30. Pak J, Pu Y, Zhang ZT *et al.* Tamm-Horsfall protein binds to type 1 fimbriated Escherichia coli and prevents E. coli from binding to uroplakin Ia and Ib receptors. *J Biol Chem* 2001; **276**: 9924–9930.
31. Chen WC, Lin HS, Tsai FJ *et al.* Effects of Tamm-Horsfall protein and albumin on the inhibition of free radicals. *Urol Int* 2001; **67**: 305–309.
32. Miyake O, Yoshioka T, Yoshimura K *et al.* Expression of Tamm-Horsfall protein in stone-forming rat models. *Br J Urol* 1998; **81**: 14–19.
33. Mo L, Huang HY, Zhu XH *et al.* Tamm-Horsfall protein is a critical renal defense factor protecting against calcium oxalate crystal formation. *Kidney Int* 2004; **66**: 1159–1166.
34. Liu Y, Mo L, Goldfarb DS *et al.* Progressive renal papillary calcification and ureteral stone formation in mice deficient for Tamm-Horsfall protein. *Am J Physiol Renal Physiol* 2010; **299**: F469–F478.
35. Mo L, Liaw L, Evan AP *et al.* Renal calcinosis and stone formation in mice lacking osteopontin, Tamm-Horsfall protein, or both. *Am J Physiol Renal Physiol* 2007; **293**: F1935–F1943.
36. Rhodes DC, Hinsman EJ, Rhodes JA. Tamm-Horsfall glycoprotein binds IgG with high affinity. *Kidney Int* 1993; **44**: 1014–1021.
37. Rhodes DC. Binding of Tamm-Horsfall protein to complement 1q measured by ELISA and resonant mirror biosensor techniques under various ionic-strength conditions. *Immunol Cell Biol* 2000; **78**: 474–482.
38. Hession C, Decker JM, Sherblom AP *et al.* Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. *Science* 1987; **237**: 1479–1484.
39. Schmid M, Prajczer S, Gruber LN *et al.* Uromodulin facilitates neutrophil migration across renal epithelial monolayers. *Cell Physiol Biochem* 2010; **26**: 311–318.
40. Saemann MD, Weichhart T, Zeyda M *et al.* Tamm-Horsfall glycoprotein links innate immune cell activation with adaptive immunity via a Toll-like receptor-4-dependent mechanism. *J Clin Invest* 2005; **115**: 468–475.
41. Mayrer AR, Kashgarian M, Ruddle NH *et al.* Tubulointerstitial nephritis and immunologic responses to Tamm-Horsfall protein in rabbits challenged with homologous urine or Tamm-Horsfall protein. *J Immunol* 1982; **128**: 2634–2642.
42. Hoyer JR. Tubulointerstitial immune complex nephritis in rats immunized with Tamm-Horsfall protein. *Kidney Int* 1980; **17**: 284–292.
43. Saemann MD, Weichhart T, Horl WH *et al.* Tamm-Horsfall protein: a multilayered defence molecule against urinary tract infection. *Eur J Clin Invest* 2005; **35**: 227–235.
44. El-Achkar TM, Wu XR, Rauchman M *et al.* Tamm-Horsfall protein protects the kidney from ischemic injury by decreasing inflammation and altering TLR4 expression. *Am J Physiol Renal Physiol* 2008; **295**: F534–F544.
45. Hart TC, Gorry MC, Hart PS *et al.* Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. *J Med Genet* 2002; **39**: 882–892.
46. Bleyer AJ, Hart TC. Familial juvenile hyperuricaemic nephropathy. *QJM* 2003; **96**: 867–868.
47. Dahan K, Devuyst O, Smaers M *et al.* A cluster of mutations in the UMOD gene causes familial juvenile hyperuricemic nephropathy with abnormal expression of uromodulin. *J Am Soc Nephrol* 2003; **14**: 2883–2893.
48. Rampoldi L, Caridi G, Santon D *et al.* Allelism of MCKD, FJHN and GCKD caused by impairment of uromodulin export dynamics. *Hum Mol Genet* 2003; **12**: 3369–3384.
49. Nasr SH, Lucia JP, Galgano SJ *et al.* Uromodulin storage disease. *Kidney Int* 2008; **73**: 971–976.
50. Scolari F, Ghiggeri GM, Casari G *et al.* Autosomal dominant medullary cystic disease: a disorder with variable clinical pictures and exclusion of linkage with the NPH1 locus. *Nephrol Dial Transplant* 1998; **13**: 2536–2546.
51. Bleyer AJ, Zivna M, Kmoch S. Uromodulin-associated kidney disease. *Nephron Clin Pract* 2011; **118**: c31–c36.
52. Lhotta K, Gruber J, Sgonc R *et al.* Apoptosis of tubular epithelial cells in familial juvenile gouty nephropathy. *Nephron* 1998; **79**: 340–344.
53. Labriola L, in Dahan K, Pirson Y. Outcome of kidney transplantation in familial juvenile hyperuricaemic nephropathy. *Nephrol Dial Transplant* 2007; **22**: 3070–3073.

54. Lens XM, Banet JF, Outeda P *et al.* A novel pattern of mutation in uromodulin disorders: autosomal dominant medullary cystic kidney disease type 2, familial juvenile hyperuricemic nephropathy, and autosomal dominant glomerulocystic kidney disease. *Am J Kidney Dis* 2005; **46**: 52–57.
55. Rezende-Lima W, Parreira KS, Garcia-Gonzalez M *et al.* Homozygosity for uromodulin disorders: FJHN and MCKD-type 2. *Kidney Int* 2004; **66**: 558–563.
56. Bleyer AJ, Hart TC, Willingham MC *et al.* Clinico-pathologic findings in medullary cystic kidney disease type 2. *Pediatr Nephrol* 2005; **20**: 824–827.
57. Vylet'al P, Kublova M, Kalbacova M *et al.* Alterations of uromodulin biology: a common denominator of the genetically heterogeneous FJHN/MCKD syndrome. *Kidney Int* 2006; **70**: 1155–1169.
58. Bernascone I, Janas S, Ikehata M *et al.* A transgenic mouse model for uromodulin-associated kidney diseases shows specific tubulo-interstitial damage, urinary concentrating defect and renal failure. *Hum Mol Genet* 2010; **19**: 2998–3010.
59. Scolari F, Caridi G, Rampoldi L *et al.* Uromodulin storage diseases: clinical aspects and mechanisms. *Am J Kidney Dis* 2004; **44**: 987–999.
60. Bleyer AJ, Hart TC, Shihabi Z *et al.* Mutations in the uromodulin gene decrease urinary excretion of Tamm-Horsfall protein. *Kidney Int* 2004; **66**: 974–977.
61. Bingham C, Ellard S, van't Hoff WG *et al.* Atypical familial juvenile hyperuricemic nephropathy associated with a hepatocyte nuclear factor-1beta gene mutation. *Kidney Int* 2003; **63**: 1645–1651.
62. Zivna M, Hulkova H, Matignon M *et al.* Dominant renin gene mutations associated with early-onset hyperuricemia, anemia, and chronic kidney failure. *Am J Hum Genet* 2009; **85**: 204–213.
63. Christodoulou K, Tsingis M, Stavrou C *et al.* Chromosome 1 localization of a gene for autosomal dominant medullary cystic kidney disease. *Hum Mol Genet* 1998; **7**: 905–911.
64. Piret SE, Danoy P, Dahan K *et al.* Genome-wide study of familial juvenile hyperuricaemic (gouty) nephropathy (FJHN) indicates a new locus, FJHN3, linked to chromosome 2p22.1-p21. *Hum Genet* 2011; **129**: 51–58.
65. Tinschert S, Ruf N, Bernascone I *et al.* Functional consequences of a novel uromodulin mutation in a family with familial juvenile hyperuricaemic nephropathy. *Nephrol Dial Transplant* 2004; **19**: 3150–3154.
66. Williams SE, Reed AA, Galvanovskis J *et al.* Uromodulin mutations causing familial juvenile hyperuricaemic nephropathy lead to protein maturation defects and retention in the endoplasmic reticulum. *Hum Mol Genet* 2009; **18**: 2963–2974.
67. Lee DH, Kim JK, Oh SE *et al.* A case of familial juvenile hyperuricemic nephropathy with novel uromodulin gene mutation, a novel heterozygous missense mutation in Korea. *J Korean Med Sci* 2010; **25**: 1680–1682.
68. Bernascone I, Vavassori S, Di Pentima A *et al.* Defective intracellular trafficking of uromodulin mutant isoforms. *Traffic* 2006; **7**: 1567–1579.
69. Choi SW, Ryu OH, Choi SJ *et al.* Mutant tamm-horsfall glycoprotein accumulation in endoplasmic reticulum induces apoptosis reversed by colchicine and sodium 4-phenylbutyrate. *J Am Soc Nephrol* 2005; **16**: 3006–3014.
70. Raffi HS, Bates Jr JM, Laszik Z *et al.* Tamm-Horsfall protein acts as a general host-defense factor against bacterial cystitis. *Am J Nephrol* 2005; **25**: 570–578.
71. Takiue Y, Hosoyamada M, Yokoo T *et al.* Production and characterization of transgenic mice harboring mutant human UMOD gene. *Nucleosides Nucleotides Nucleic Acids* 2008; **27**: 596–600.
72. Kemter E, Rathkolb B, Rozman J *et al.* Novel missense mutation of uromodulin in mice causes renal dysfunction with alterations in urea handling, energy, and bone metabolism. *Am J Physiol Renal Physiol* 2009; **297**: F1391–F1398.
73. Jackson EK. Diuretics. In: Hardman JG, Limbird LE, Gilman AG (eds). *Goodman & Gilman's the Pharmacological Basis of Therapeutics*. McGraw-Hill: New York, 2001, pp 757–787.
74. Prajczer S, Heidenreich U, Pfaller W *et al.* Evidence for a role of uromodulin in chronic kidney disease progression. *Nephrol Dial Transplant* 2010; **25**: 1896–1903.
75. Zaucke F, Boehnlein JM, Steffens S *et al.* Uromodulin is expressed in renal primary cilia and UMOD mutations result in decreased ciliary uromodulin expression. *Hum Mol Genet* 2010.
76. Verdeguer F, Le Corre S, Fischer E *et al.* A mitotic transcriptional switch in polycystic kidney disease. *Nat Med* 2010; **16**: 106–110.
77. Resnick JS, Sisson S, Vernier RL. Tamm-Horsfall protein. Abnormal localization in renal disease. *Lab Invest* 1978; **38**: 550–555.
78. Zager RA, Cotran RS, Hoyer JR. Pathologic localization of Tamm-Horsfall protein in interstitial deposits in renal disease. *Lab Invest* 1978; **38**: 52–57.
79. Cotran RS, Galvanek E. Immunopathology of human tubulo-interstitial diseases: localization of immunoglobulins complement and Tamm-Horsfall protein. *Contrib Nephrol* 1979; **16**: 126–131.
80. Cohen AH, Border WA, Rajfer J *et al.* Interstitial Tamm-Horsfall protein in rejecting renal allografts. Identification and morphologic pattern of injury. *Lab Invest* 1984; **50**: 519–525.
81. Vyletal P, Bleyer AJ, Kmoch S. Uromodulin biology and pathophysiology—an update. *Kidney Blood Press Res* 2010; **33**: 456–475.
82. Vylet'al P, Hulkova H, Zivna M *et al.* Abnormal expression and processing of uromodulin in Fabry disease reflects tubular cell storage alteration and is reversible by enzyme replacement therapy. *J Inherit Metab Dis* 2008; **31**: 508–517.
83. Kottgen A. Genome-wide association studies in nephrology research. *Am J Kidney Dis* 2010; **56**: 743–758.
84. Kottgen A, Glazer NL, Dehghan A *et al.* Multiple loci associated with indices of renal function and chronic kidney disease. *Nat Genet* 2009; **41**: 712–717.
85. Kottgen A, Pattaro C, Boger CA *et al.* New loci associated with kidney function and chronic kidney disease. *Nat Genet* 2010; **42**: 376–384.
86. Gudbjartsson DF, Holm H, Indridason OS *et al.* Association of variants at UMOD with chronic kidney disease and kidney stones-role of age and comorbid diseases. *PLoS Genet* 2010; **6**: e1001039.
87. Pattaro C, De Grandi A, Vitart V *et al.* A meta-analysis of genome-wide data from five European isolates reveals an association of COL22A1, SYT1, and GABRR2 with serum creatinine level. *BMC Med Genet* 2010; **11**: 41.
88. Chambers JC, Zhang W, Lord GM *et al.* Genetic loci influencing kidney function and chronic kidney disease. *Nat Genet* 2010; **42**: 373–375.
89. Consortium TIH. A haplotype map of the human genome. *Nature* 2005; **437**: 1299–1320.
90. Padmanabhan S, Melander O, Johnson T *et al.* Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. *PLoS Genet* 2010; **6**: e1001177.
91. Kottgen A, Hwang SJ, Larson MG *et al.* Uromodulin levels associate with a common UMOD variant and risk for incident CKD. *J Am Soc Nephrol* 2010; **21**: 337–344.
92. Shlipak MG, Li Y, Fox C *et al.* Uromodulin concentrations are not associated with incident CKD among persons with coronary artery disease. *BMC Nephrol* 2011; **12**: 2.
93. Belge H, Gailly P, Schwaller B *et al.* Renal expression of parvalbumin is critical for NaCl handling and response to diuretics. *Proc Natl Acad Sci USA* 2007; **104**: 14849–14854.
94. Han L, Monne M, Okumura H *et al.* Insights into egg coat assembly and egg-sperm interaction from the X-ray structure of full-length ZP3. *Cell* 2010; **143**: 404–415.