Uromodulin: from physiology to rare and complex kidney disorders

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Abstract | Uromodulin (also known as Tamm-Horsfall protein) is exclusively produced in the kidney and is the most abundant protein in normal urine. The function of uromodulin remains elusive, but the available data suggest that this protein might regulate salt transport, protect against urinary tract infection and kidney stones, and have roles in kidney injury and innate immunity. Interest in uromodulin was boosted by genetic studies that reported involvement of the *UMOD* gene, which encodes uromodulin, in a spectrum of rare and common kidney diseases. Rare mutations in *UMOD* cause autosomal dominant tubulointerstitial kidney disease (ADTKD), which leads to chronic kidney disease (CKD). Moreover, genome-wide association studies have identified common variants in *UMOD* that are strongly associated with risk of CKD and also with hypertension and kidney stones in the general population. These findings have opened up a new field of kidney research. In this Review we summarize biochemical, physiological, genetic and pathological insights into the roles of uromodulin; the mechanisms by which *UMOD* mutations cause ADTKD, and the association of common *UMOD* variants with complex disorders.

In 1873, the Italian physician Carlo Rovida (University of Turin, Italy), described a substance that was produced by the tubular cells of the kidney. He noted that this substance, which he named cilindrina, formed hyaline casts in the tubular lumen[1]. Subsequently, in 1950, Igor Tamm and Frank Horsfall (Rockefeller Institute, USA) isolated an abundant mucoprotein from the urine of healthy individuals. This protein, which was named Tamm-Horsfall, precipitated in NaCl solutions and displayed potent inhibitory effects on viral haemagglutination[2]. During the next three decades, Tamm-Horsfall protein was extensively investigated, revealing a number of interesting biological properties. In particular, it was shown to be the most abundant protein in mammalian urine, with a mass of approximately 85 kDa, a high carbohydrate content (30%), a high number of cysteine residues, and a tendency to form large aggregates[3]. Tamm-Horsfall protein was also found to be produced by the cells lining the thick ascending limb (TAL) of the loop of Henle[4] and to be the major constituent of hyaline casts[5].

In 1985, Muchmore and Decker isolated and purified a 85-kDa glycoprotein from the urine of pregnant women[6]. This protein showed marked immunoregulatory activity *in vitro* with inhibition of human T-cell and monocyte activity at nanomolar concentrations. Based on these properties, it was named uromodulin. Two years later, using cDNA cloning and biochemical comparison, Pennica *et al.* demonstrated that uromodulin is identical to Tamm-Horsfall protein[7]. Testing of 150 different epithelial, connective, and haematopoietic tissues and cell lines indicated that uromodulin mRNA was only detected in the kidney. In addition to its immunosuppressive properties linked to the carbohydrate moiety, uromodulin was suggested to be potentially important for maintaining the water impermeability of the TAL. Autoantibodies against the protein were thought to be involved in renal tubular disorders and urinary tract infections (UTIs)[7].

Despite this long history and extensive biochemical studies, the physiological roles of uromodulin remained elusive for decades. Studies in uromodulin-knockout mice suggested a number of interesting possible functions, including regulation of salt transport and urinary concentration (based on potential interactions with transport systems operating in TAL cells); defense against UTIs via high-affinity binding to uropathogenic *Escherichia coli*, which prevented their internalization by the urothelium; protection against kidney stones by reducing the aggregation of calcium crystals; and a role in innate immunity by binding immunoglobulins and cytokines and/or activating monocytes and dendritic cells via Toll-like receptor 4 (TLR4)[8]. Interest in uromodulin was boosted by genetic studies that identified involvement of the *UMOD* gene in common and rare kidney diseases. Indeed, rare mutations in *UMOD* cause

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Key points

- Uromodulin — the most abundant urinary protein — is exclusively produced by renal epithelial cells; in the tubular lumen uromodulin forms high-molecular weight filaments that constitute the matrix of hyaline casts
- Important functions of uromodulin include regulation of ion transport in the thick ascending limb, immunomodulation and protection against urinary tract infections and kidney stones
- Levels of uromodulin in the urine and in the blood, where it is present in lower amounts, are valuable biomarkers for tubular mass and renal function
- Rare mutations in *UMOD* cause autosomal dominant tubulointerstitial kidney disease; these mutations lead to retention of mutant uromodulin in the endoplasmic reticulum of tubular cells, tubulointerstitial damage and decreased levels of urinary uromodulin
- Common variants in the *UMOD* promoter are associated with risk of chronic kidney disease (CKD) and hypertension; the unusually high prevalence of *UMOD* risk alleles suggests pathogen-driven selective pressure
- *UMOD* represents a paradigm as a continuum of genetic disease risk, from rare mutations in Mendelian disease to common variants associated with complex traits including CKD and hypertension

autosomal dominant tubulointerstitial kidney disease (ADTKD), which leads to chronic kidney disease (CKD) and renal failure[9,10]. In parallel, genome-wide association studies (GWAS) identified common *UMOD* variants that were strongly associated with renal function and risk of CKD in the general population[11,12]. These *UMOD* risk variants directly increase uromodulin expression and excretion in urine, leading to salt-sensitive hypertension and kidney damage[13,14].

In this Review, we illustrate how biochemical, physiological, genetic and clinical studies of uromodulin and *UMOD* have opened a new field of research, provided insights into a major protein exclusively made by the kidney, and demonstrated how a single gene may be involved in a continuum between rare and common kidney disorders.

Characteristics and regulation of uromodulin

Protein and domains

Uromodulin is synthesized in TAL cells as a precursor of 640 amino acids. The first 24 N-terminal amino acids constitute a signal peptide that directs the nascent protein into the endoplasmic reticulum (ER) and is then cleaved during maturation (FIG. 1a,b). The mature urinary protein is 616 amino acids in length and has a deduced molecular weight of 67 kDa. The amino acid composition displays a remarkably high number of cysteines (48 in the mature protein). Several conserved phosphorylation sites have been described but their physiological relevance is not yet known[7,15–17].

The predicted domains of uromodulin (FIG. 1b) include four epidermal growth factor (EGF)-like domains (of which EGF-II and EGF-III are predicted to be Ca^{2+} binding)[18], a cysteine-rich domain of unknown function (D8C), and a bipartite elastase-resistant C-terminal Zona Pellucida (ZP) domain[19]. EGF-like domains, which are likely important for protein–protein interactions, are found in a variety of secreted proteins and in the extracellular domain of membrane-bound proteins[20]. ZP domains are present in many extracellular eukaryotic proteins including uromodulin, egg coat proteins ZP1-3 and α and β-tectorins, and are essential for their assembly into extracellular polymers of supramolecular structure[19].

Intracellular protein maturation

The half-life of human uromodulin is estimated to be around 16 h[21]. Maturation of the protein along the secretory pathway is a rather slow process (8–12 h in stably transfected HeLa cells) that comprises membrane anchoring via glycosylphosphatidylinositol (GPI) and the engagement of all 48 cysteine residues in the formation of 24 intramolecular disulfide bonds inside the oxidizing milieu of the ER[7,22,23]. Correct tertiary folding is a prerequisite for ER sorting and subsequent extensive and complex glycan processing in the Golgi[24,25]. Accordingly, the membrane localization of uromodulin is markedly decreased when cells are cultured in reducing conditions that disrupt ER oxidative folding[26,27].

Glycosylation of uromodulin is particularly important for its function (discussed below). Eight potential Asn *N*-linked glycosylation sites have been identified in human uromodulin (Asn38, Asn76, Asn80, Asn232, Asn275, Asn322, Asn396 and Asn513), of which all but Asn38 are glycosylated[28]. Glycosylation accounts for 30% of the molecular mass of the protein[22]. The maturation of uromodulin in the Golgi involves the conversion of high-mannose residues to glycans, which are mainly of the polyantennary type and can be sialylated, fucosylated or sulfated (FIG. 1c,d). This maturation step is reflected by an increase in apparent molecular mass from ~85 kDa to 100 kDa in reducing conditions. One *N*-glycosylation site (Asn275) is not processed by the Golgi and retains the high-mannose chain[24,28]. Owing to the sialic acid residues, the isoelectrical point of uromodulin is ~3.5; therefore, it is present as a polyanionic protein in urine[29]. Notably, a special arrangement of terminal sugars in uromodulin forms a sequence known as the Sd^a antigen, which is a dominant blood group determinant that is present in 90% of the white population[30,31]. Evidence for *O*-glycosylation in uromodulin also exists; this type of glycosylation might be regulated by hormones[32].

A stretch of hydrophobic C-terminal amino acids (615–640 of the human protein) mediates GPI-anchoring of uromodulin to the membrane lipid bilayer immediately after co-translational insertion in the ER[17]. Owing to its GPI anchor, uromodulin is involved in highly ordered detergent-insoluble glycosphingolipid complexes (or lipid rafts) rich in cholesterol and sphingolipids. These microdomains organize the trafficking of selected membranes and associated proteins and serve as platforms for intracellular signalling[33]. The presence of uromodulin in these domains might convey some intracellular functions in TAL cells[34]. Whether GPI anchoring[35], in addition to *N*-glycosylation[36], contributes to polarized trafficking of uromodulin to the apical membrane is not yet clear[37].

Secretion and polymerization

Uromodulin is by far the most abundant protein in healthy urine, with mean daily secretion rates of 0.3 mg per 100 g body weight in rats and 50–150 mg per 24 h in humans[38–41]. Urinary uromodulin is a high molecular

weight polymer of approximately 7×10^6 Da, or multiples thereof[40]. The protein forms a porous, 3D matrix that is clearly visible on electron microscopy[41] (FIG. 1e,f).

The matrix structure formed by uromodulin consists of long protein filaments composed of smaller fibrils with a width of about 100 Å and an average length of 25,000 Å[41]. Each uromodulin filament consists of two protofilaments arranged in a right-handed double helix with an axial repeat of ~120 Å and a diameter of 90–140 Å[19]. The structural arrangements of uromodulin filaments depend on ionic conditions, with maximum compaction when NaCl and $CaCl_2$ concentrations approach those found in the pro-urine facing the TAL[42].

Similar to other ZP proteins, the presence of the ZP domain is essential and sufficient for uromodulin assembly[19]. The X-ray crystal structure of uromodulin, available for the C-terminal region encompassing the EGF-IV domain and ZP polymerization region (G295-Q610)[18], provided insight into the molecular mechanisms of uromodulin homopolymerization. This process relies on an extensive hydrophobic interface inside the ZP-N domain and a rigid interdomain linker between ZP-N and ZP-C and progresses through parallel β-sheet extension[18]. In addition, the interaction between uromodulin molecules involves intermolecular hydrogen bonds mediated by a conserved *N*-glycan at Asn396 (REF. 18).

During intracellular trafficking, uromodulin is kept in a polymerization-incompetent state by the hydrophobic interaction of two motifs, the internal (IHP) and external (EHP) hydrophobic patches. These motifs are located in the ZP linker region and between the proteolytic cleavage and GPI-anchoring sites, respectively. This intramolecular interaction is required for correct proteolytic cleavage at the apical membrane[37]. Apically targeted uromodulin is released into the urine by proteolytic cleavage at a conserved site (Phe587) directly C-terminal to the ZP domain[43]. The type II transmembrane serine protease hepsin is responsible for the physiological cleavage of uromodulin, releasing the EHP and thus permitting correct polymerization and release of the protein into the urine[44]. In mice lacking hepsin, uromodulin secretion is heavily reduced and the protein accumulates in the kidney[44]. Moreover, urinary uromodulin in these mice contains the EHP so is not polymeric.

Quantification

Methods for quantifying uromodulin in the urine and plasma include radioimmunoassays[39,45], ELISA[46] and high-performance liquid chromatography[47] coupled to mass spectrometry (MS)[48]. MS-based techniques are less liable to errors resulting from variable levels of protein glycosylation, aggregation or storage-mediated degradation than are immune-based approaches. However their applicability to high-throughput analysis is not established[48]. Uromodulin filaments are influenced by the ionic strength of the solution as well as by freezing and storage time[39,40,42]. Vortexing and centrifugation of urine samples both substantially alter the levels of detectable uromodulin[46]. Storage at −80 °C slows down degradation of uromodulin in urine samples[46]. Standard operating procedures are thus crucial for reliable quantification of urinary uromodulin in large-scale biomarker studies[46]. A sensitive ELISA has been established to assess levels of uromodulin in the circulation, which are ~1,000-fold lower than urinary levels[49].

Physiological regulators of uromodulin

Urinary excretion of uromodulin is subject to large fluctuations, both within and between individuals. Furthermore, considerable unexplained variation exists in urinary uromodulin levels between different isolated and non-isolated populations[14,45]. Little is known about the physiological regulators of uromodulin abundance in the kidney and urine. Consistent with uromodulin expression in the TAL, a nephron segment that is involved in NaCl reabsorption, modifications in dietary NaCl intake influence uromodulin expression in rats: high dietary salt intake increased *Umod* mRNA and protein levels in the kidney, but urinary levels were not investigated[50]. Several lines of evidence suggest that urinary excretion of uromodulin is salt sensitive and correlates positively with salt intake in humans[51,52].

The effect of the loop diuretic furosemide on uromodulin levels is complex. Furosemide infusion alone had no effect on uromodulin urinary excretion in rats[38], but increased *Umod* mRNA levels in rats on a high-salt diet[50], and increased uromodulin membrane expression and altered its glycosylation in arginine vasopressin (AVP)-infused rats[53]. Other potential regulators of uromodulin expression include protein intake (a high protein diet increased urinary uromodulin levels in rats[38]); antidiuretic hormone (AVP infusion reduced urinary uromodulin excretion and membrane expression in Brattleboro rats[38,53]); and thyroid hormones (hypothyroid rats have reduced levels of kidney and urinary uromodulin[54]).

Further work is needed to clarify the mechanisms that regulate uromodulin production in TAL cells and its release into the urine. The influence of kidney function and mass, electrolyte handling, acute kidney injury (AKI) and several disorders that affect the kidney (including diabetes mellitus and lupus nephritis) on uromodulin expression and excretion is discussed below.

The *UMOD* gene

UMOD is located on chromosome 16p12.3-16p13.11 and is composed of 11 exons (the first of which is non-coding) over a genomic region of about 20 kb[7,55]. The *UMOD* locus is remarkably conserved between human and mouse in terms of gene structure, tissue-specific expression, protein sequence, domain architecture and biochemical properties.

Expression and localization

Uromodulin transcripts are exclusively detected in the kidney[7,15,56] and *UMOD* is by far the most abundant transcript expressed in the human kidney[57]. Within the kidney, uromodulin is essentially distributed within the TAL segment (FIG. 1g,h). Deep RNA sequencing in microdissected rat nephron segments showed nearly 10-fold higher levels of uromodulin mRNA in the cortical TAL than in the medullary TAL and low levels in the distal convoluted tubule (DCT)[58]. Low but consistent

Umod expression in the DCT was also shown using serial analysis of gene expression in mouse nephron segments[59]. By contrast, uromodulin was not detected in the rat DCT nor in the tubular cells of the macula densa using radiolabelled mRNA *in situ* hybridization[60]. In the mouse and rat TAL, uromodulin is amongst the most abundant transcripts, corresponding to about 0.9–4.6% of the TAL cell poly-A^+ total transcriptome in the rat[58,59].

Immunoelectron and immunofluorescent microscopy analyses localized uromodulin along the entire TAL and in the early DCT in the mouse and human kidney[61,62]. Some caution is warranted regarding the presence of uromodulin in the DCT as the signal could reflect luminal uromodulin adhering on the apical region in segments downstream of its production. Furthermore, the low mRNA transcript levels described in the DCT could reflect tissue contamination by TAL segments.

Within TAL epithelial cells, uromodulin is mainly localized on the apical membrane[62] but has also been localized at the basolateral membrane[49,62], consistent with its presence at very low concentrations in the blood. Increased intracellular expression and relocalization of uromodulin to the basolateral membrane and interstitium has been observed in murine models of AKI[63].

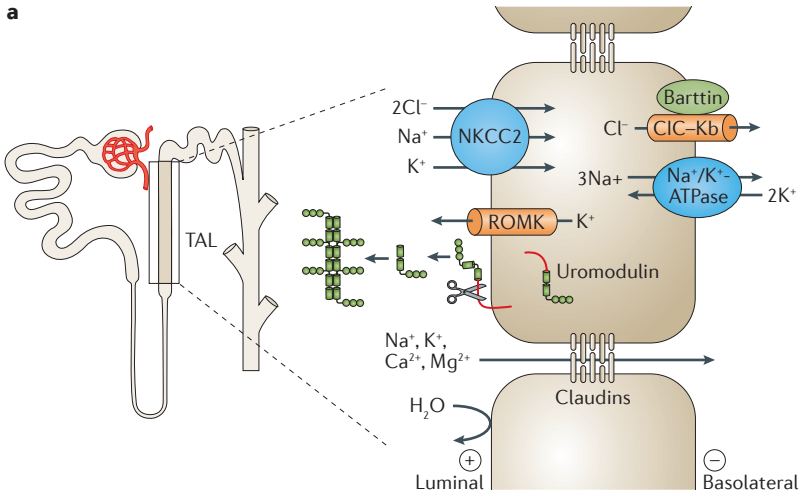
Expression during development

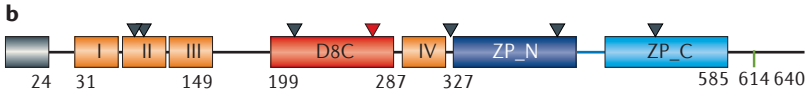
Uromodulin expression correlates with TAL maturation in developing kidneys[64]. In the mouse, uromodulin transcripts in the maturing loops of Henle are detected from embryonic day 15.5 (REF. 65), whereas in the hamster, positive immunofluorescent signals for uromodulin were observed late in gestation in the distal nephron segments, with a post-natal increase in signal intensity[66]. In humans, uromodulin has been detected (using immunohistochemistry) in the kidney between the 8th and 16th weeks of gestation, and was consistently detected in the amniotic fluid of healthy pregnancies starting from the 20th week of gestation[67]. Uromodulin expression steadily increases with maturation of the TAL segments, reaching a maximal level after birth[67]. As uromodulin levels correlate with gestational age, urinary uromodulin has been suggested as a biomarker for tubular development in premature neonates[68].

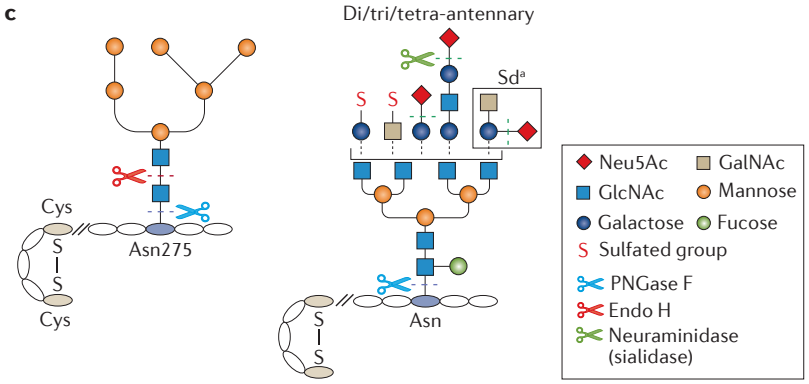
Regulation of uromodulin expression

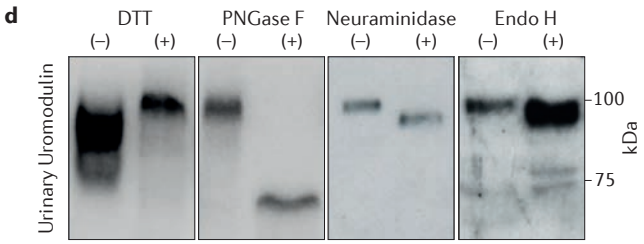
The regulation of uromodulin expression is poorly characterized. Studies in transgenic mice showed that the 3.0–3.7 kb 5′-flanking sequence from the promoter region of the uromodulin gene from different species (mouse, human and bovine) is sufficient to drive TAL-specific expression of a reporter gene or Cre recombinase, and to induce urinary excretion of a recombinant protein[56,69–71]. This finding suggests that this region contains all of the required *cis* elements that govern kidney and nephron-specific expression. In particular, the 5′ proximal flanking region of *UMOD* (~600 base pairs) is highly conserved between mice, rats, cattle and humans, and likely contains positive regulatory elements[56,69].

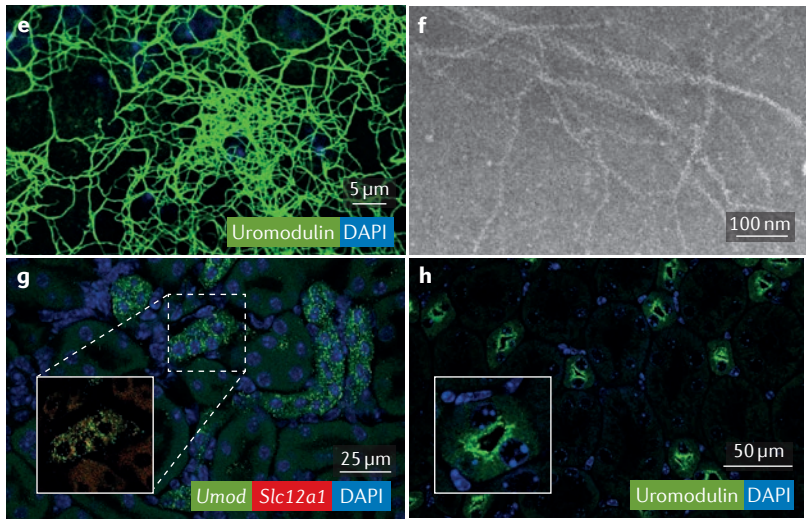
Figure 1 | **Segmental expression and biochemical characteristics of uromodulin. a** | Uromodulin is produced by the cells that line the thick ascending limb (TAL). This tubular segment is involved in the reabsorption of NaCl, which is mediated by the apical Na^+-K^+-$2Cl^-$ cotransporter NKCC2 and the basolateral exporters Na^+/K^+-ATPase and the ClC–Kb/Barttin complex. The apical recycling of K^+ via the potassium channel ROMK generates a lumen-positive transepithelial voltage, which is the driving force for paracellular reabsorption of monovalent and divalent cations. Uromodulin is cleaved at the apical membrane by the serine protease hepsin and released in the urine where it forms macromolecular polymers. **b** | The predicted structure of uromodulin contains a leader peptide (grey); four EGF-like domains (orange) of which EGF-II and EGF-III are predicted to be Ca^{2+} binding; a cysteine-rich D8C domain (red); a bipartite C-terminal Zona Pellucida domain (ZP_N and ZP_C, blue) connected by a rigid interdomain linker; and a glycosylphosphatidylinositol-anchoring site at position 614 (green). The seven *N*-glycosylation sites are indicated by small triangles; the high-mannose chain on residue Asn275 is shown in red. **c** | Schematic representation of *N*-linked glucomoieties carried by human urinary uromodulin. Most *N*-glycosylation sites carry glycans of the di-antennary, tri-antennary or mainly the tetra-antennary type that can be sialylated or sulfated at their terminal residues (various possibilities for nonreducing terminal units are indicated[28,31]). Fucosylation is often present at the reducing end. The GalNAcβ1,4(Neu5Acα2,3)Galβ1,4GlcNAc sequence, known as the Sd^a antigen is highlighted[30]. The Asn275 retains a high-mannose configuration (mainly $Man_6GlcNac_2$)[28]. Putative cleavage sites of various glycosidases are shown and a disulfide bridge is indicated between two cysteine residues. **d** | Biochemical properties of human urinary uromodulin as assessed by shifts in electrophoretic mobility. Reduction of disulfide bridges by dithiothreitol (DTT) leads to a narrower uromodulin band with slower migration (at ~100 kDa). The shift in electrophoretic mobility is compatible with the high number of disulfide bridges in the protein. Removal of all *N*-linked glycans, of sialylated residues and of *N*-linked high-mannose moieties by peptide-*N*-glycosidase F (PNGase F), neuraminidase and endoglycosidase H (Endo H), respectively, reveals the relative contribution of these glycans to human urinary uromodulin. The shift from 100 kDa to ~70 kDa after PNGase F treatment confirms that ~30% of the molecular weight of uromodulin is constituted by *N*-linked glycans. Membranes were blotted with polyclonal sheep anti-uromodulin antibodies. **e** | Immunofluorescence staining showing a dense network of uromodulin filaments (green) covering a monolayer of primary TAL cells obtained from microdissected mouse kidney. These primary cells are polarized and retain endogenous expression of uromodulin[186]. Nuclei are counterstained with DAPI. **f** | Negative stain transmission electron microscope image of uromodulin polymers secreted by primary mouse TAL cells. The helical arrangement is suggested by regular protrusions at an interval of ~100Å. Image courtesy of Gregor Weiss, ETH Zurich. **g** | *In situ* hybridization (RNAscope®) for *Umod* mRNA and *Slc12a1* mRNA (inset) in mouse kidney cortex. Several tubule profiles show abundant co-expression of *Umod* mRNA and *Slc12a1* mRNA, which encodes NKCC2 in the TAL (inset). Nuclei are counterstained with DAPI. **h** | Immunofluorescence staining for uromodulin in the mouse kidney, showing an enhanced signal at the apical membrane of TAL cells (inset). Nuclei are counterstained with DAPI. Part **a** reproduced with permission from Elsevier © Devuyst, O. & Bochud, M. *Kidney Int.* **88**, 944–646 (2015).











In silico phylogenetic footprinting of upstream regions of *UMOD* from 16 different primate and rodent species identified several conserved binding motifs[72]. This finding provided insight into the evolutionary dynamics of the *UMOD* promoter across primates and identified transcription factors expressed in the kidney that could be relevant for regulation of uromodulin expression. For example, hepatic nuclear factor 1β (HNF1B) physically interacts with two distinct *Umod* chromatin DNA target sites (at positions −1.1 kb and −0.58 kb relative to the putative transcriptional start site) and positively regulates the expression of uromodulin[73]. In mice lacking *Hnf1b,* the expression of *Umod* is dramatically downregulated by around ninefold[73].

UMOD contains a strikingly high proportion of asymmetrically distributed (more in the 5' exons) CpG dinucleotides[7]. Interestingly, no CpG-rich regions are present in the *UMOD* promoter, but a CpG island of 731 base pairs spans almost the entire length of exon 3 (REF. 74). As cytosines of CpG are the predominant site of DNA methylation, this finding suggests epigenetic regulation of *UMOD* expression.

Urinary uromodulin levels show substantial heritability, ranging from 17% to 28% in various cohorts[14]. A GWAS reported that common genetic variants located in a large linkage disequilibrium block encompassing the *UMOD* promoter region are associated with urinary levels of uromodulin in the general population[14]. Furthermore, an independent intronic variant of *PDILT*, which flanks *UMOD*, was shown to influence urinary uromodulin levels[14].

Evolution

UMOD is evolutionarily conserved (FIG. 2) and homologues are present in fish, amphibians, reptiles, birds and mammals[75]. Uromodulin protein has been detected in the kidneys of all placental mammals tested to date, but in no other class of vertebrates[76]. Consistent with this finding, uromodulin immunoreactivity has been found in the kidneys of mammals but not in birds or reptiles[77]. The distal tubules of the kidneys of some amphibians as well as the superficial layers of skin, mucosa and gills of some amphibians and fish reacted with an anti-uromodulin antibody[76]. However the function of such homologues and their relevance for comparative physiology remains to be determined. A relatively high degree of cross-species amino acid sequence conservation has been reported between placental mammals, including 95% similarity with 77% identity between rat and human[78] and 79% similarity with 70% identity between mouse and human[16].

The ancestral *UMOD* gene likely underwent independent duplication events, as suggested by the presence of three *UMOD*-like genes in *Xenopus* and by high similarity between *UMOD* and the flanking gene *GP2* (which encodes pancreatic glycoprotein-2) in mammalian genomes (FIG. 2). Glycoprotein-2 and uromodulin share 86% similarity and 53% identity in their C-terminal regions and both are attached with a GPI anchor and apically secreted in the extracellular compartment where they form homopolymers[78]. Whether

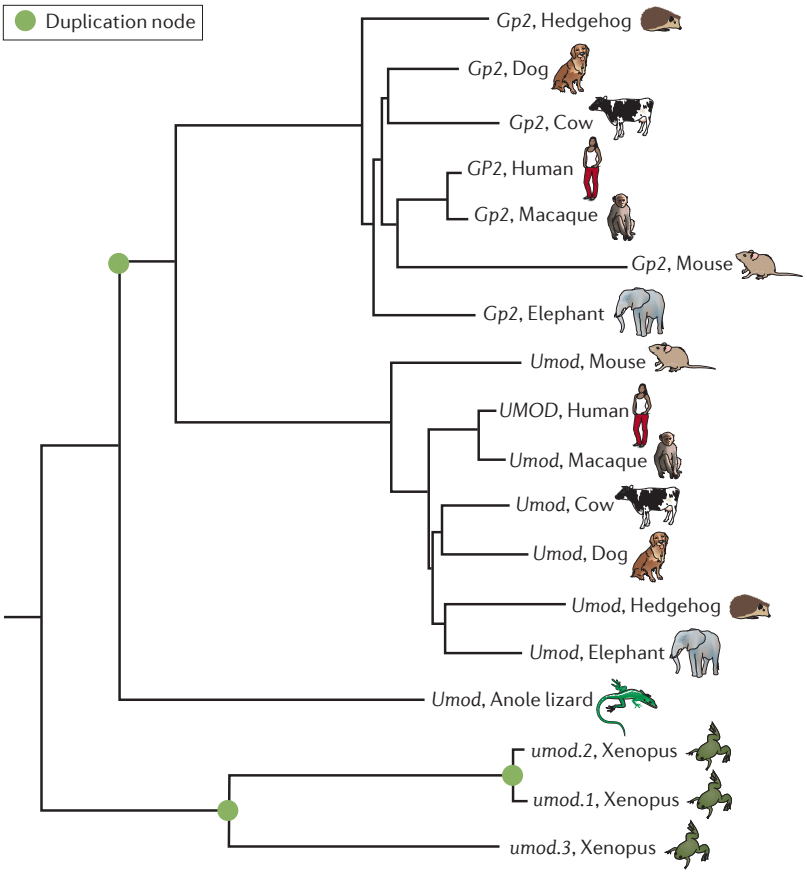


Figure 2 | **Evolution of the *UMOD* gene.** Phylogenetic tree showing the inferred evolutionary relationships between *UMOD* homologues and the flanking *GP2* gene in different organisms. *Xenopus* has three *UMOD*-like genes. This tree was derived and modified using Ensembl comparative genomics data (http://www.ensembl.org/).

uromodulin and GP-2 share functional homology in the urine and in the intestinal tract, respectively, is unknown.

Roles of uromodulin

Despite insights from biochemical and *in vitro* studies, the physiological roles of uromodulin remained elusive for a long time. During the past decade, studies in uromodulin-knockout mice (TABLE 1) have provided key insights into the roles of uromodulin. Despite the potential difficulties in extrapolating some of these data to humans, these studies support a major multifaceted role of the protein (FIG. 3).

Water homeostasis

Uromodulin is a phylogenetically conserved marker of the TAL, a segment that is critical for urine concentration in mammals[79,80]. The observation that uromodulin forms a hydrophobic, gel-like structure led to the early suggestion that this protein might act like a seal and contribute to the water impermeability of the TAL[42]. Uromodulin was subsequently shown to regulate the activity of the Na^+-K^+-$2Cl^-$ cotransporter NKCC2 and the potassium channel ROMK[81,82], thus influencing the tonicity of the medulla and urinary concentrating capacity. Furthermore, *Umod*-knockout mice show impaired urinary concentration ability after water deprivation[83], supporting a critical role of uromodulin in TAL function.

Salt handling and blood pressure regulation

Genetic deletion of uromodulin does not affect kidney morphology[83], but data suggest that the protein does influence renal electrolyte handling by acting on the TAL and downstream segments. Uromodulin increases the activity of NKCC2 under baseline conditions and after several stimuli (including AVP)[81], and also increases the surface expression of ROMK[82]. In *Umod*-knockout mice, increased luminal NaCl load to the macula densa (resulting from impaired NKCC2 and ROMK function) led to reduced renin biosynthesis and a tubuloglomerular feedback loop with decreased creatinine clearance[83]. Likewise, several transporters operating in segments distal to the TAL, including NCC and ENaCα, are upregulated in *Umod*-knockout mice[81,83]. Similar manifestations of tubular dysfunction and renal losses of Na^+ and K^+ were confirmed in *Umod*-knockout mice with a different genetic background[84].

Dietary studies substantiate the link between uromodulin and salt intake. Increases in dietary salt increase the expression of uromodulin in rat kidney[50]. In humans, high salt intake results in increased urinary uromodulin excretion, whereas the reverse is seen with low salt intake[51]. Direct evidence also exists for a role of uromodulin in the salt-sensitivity of blood pressure. Mice that overexpressed uromodulin showed salt-sensitive hypertension[13], whereas *Umod*-knockout mice had lower blood pressure than controls and were resistant to salt-induced hypertension[84]. The fact that mice overexpressing uromodulin showed an increased response to furosemide[13], contrasting with the blunted response observed in *Umod*-knockout mice[81], highlights the link between uromodulin expression and NKCC2 activity in the TAL. The mechanism by which uromodulin regulates NKCC2 phosphorylation could involve signalling through tumour necrosis factor (TNF) as well as regulation of SPAK and OSR1 kinases[13,84].

Propensity to kidney stones

Uromodulin polymerizes in the lumen of the distal nephron, where it may modulate the propensity to form kidney stones. The kidney is continuously exposed to crystallization because of urine supersaturation with crystal-forming compounds such as calcium oxalate (CaOx) or calcium phosphate (CaP). Supersaturation starts in the TAL where uromodulin appears in the urine[85].

Similar to many other inhibitors of crystal aggregation, uromodulin is negatively charged in urine[29]. Sialylated, negatively-charged glycans in uromodulin inhibit crystal aggregation of CaOx and CaP *in vitro*[86]. The uromodulin content of sialic acid was reduced in rats that developed nephrocalcinosis compared to controls[87]. Likewise, uromodulin purified from human urine that is enzymatically desialylated or deglycosylated can no longer inhibit CaOx and CaP crystallization[88]. The biological relevance of these properties has been

demonstrated in *Umod*-knockout mice, which develop spontaneous kidney calcifications and are excessively susceptible to calcium crystal formation under high calcium and high oxalate conditions. This phenotype is consistent, with 85–100% of *Umod*-knockout mice developing intrarenal crystals by the age of 15 months[89]. Crystals are composed of CaOx and CaP and are located in the collecting duct lumen and in the interstitial space in the deep medulla and papilla. Ureteral obstructive stones with hydronephrosis are observed in older mice[89,90]. The urine of *Umod*-knockout mice is supersaturated with CaP and has reduced levels of citrate[89].

Table 1 | **Characteristics and phenotypes of genetically modified *Umod* mouse models**

Type	Strain	Phenotype	Refs
Knockout (homologous recombination: disruption of exon 2)	129/Sv	• Normal kidney structure, absence of fibrotic or cystic changes • Increased susceptibility to bladder colonization and upper urinary tract infection by type 1-fimbriated *E. coli* • Higher susceptibility to lower and upper urinary tract infections by other bacteria	83,95, 181,207
		• Salt loss, impaired urinary concentration, decreased creatinine clearance (salt-sensitive) • Functional defect in NKCC2 and ROMK (accumulation in subapical vesicles) • Compensatory upregulation of distal sodium transporters and channels • Decreased systolic blood pressure at baseline and resistance to NaCl-induced hypertension	81–84
Knockout (homologous recombination: deletion of promoter region and first four exons)	129/SvEv	• Normal histology, absence of interstitial fibrosis • Reduced glomerular filtration • Urinary loss of Na^+, Cl^-, K^+ and P; increased excretion of calcium oxalate; reduced excretion of citrate • Formation of renal calcium crystals (spontaneous and after administration of ethylene glycol and vitamin D3); pelvic and ureteral stones; secondary hydronephrosis	89,90, 208
		• Predisposition to bladder infections by type 1-fimbriated *E. coli*	94
		• Increased renal inflammation and tubular necrosis with exaggerated rise in serum creatinine after ischaemia–reperfusion injury	63,102, 103
		• Enhanced bone marrow granulopoiesis, splenic enlargement with macrophage infiltration • Increased levels of circulating inflammatory cytokines	104,209
Transgenic wild-type (dose-dependent increase of uromodulin expression and urinary secretion)	FVB/N	• Increased blood pressure (salt-sensitive owing to activation of NKCC2); enhanced response to furosemide • Normal renal function (measured using FITC–sinistrin clearance) • Focal renal damage, biomarkers of tubule damage (NGAL, Kim-1)	13
Transgenic C147W mutant (corresponding to human C148W mutation)	FVB/N	• ER accumulation of mutant uromodulin; TAL damage • Decreased urinary uromodulin levels • Defective urinary concentration, hypercalciuria • Mild renal failure (increased BUN and creatinine) • Interstitial fibrosis and inflammatory cell infiltrate, tubular atrophy, cortico-medullary tubular cysts, thickened and/or multilayered tubular basal membrane; hyperplastic ER on electron microscopy	176
ENU mutagenesis: A227T (recessive)	C3H	• Renal failure, polyuria, defective urinary concentration • Increased excretion of Ca^{2+} and Mg^{2+} • Decreased uromodulin excretion • Intracellular accumulation of uromodulin, hyperplastic ER in TAL cells	177
ENU mutagenesis: C93F (dominant)	C3H	• Renal failure, polyuria, defective urinary concentration • Increased FE of Na^+, K^+, Ca^{2+}, Mg^{2+}; decreased FE of uric acid • Decreased urinary uromodulin levels • Accumulation of immature uromodulin in the ER • Tubulointerstitial lesions, fibrosis and cysts	210
		• Markers of ER stress and unfolded protein response • Reduced mitochondrial and peroxisomal proteins • Increased mitophagy • Altered cellular energy homeostasis, induction of hypoxia-inducible genes	179
Knock-in C125R (corresponding to C126R in humans)	C57Bl/6J	• Defective uric acid excretion, renal failure, urinary concentrating defect • Impaired uromodulin trafficking, maturation and secretion • Renal fibrosis, interstitial immune cell infiltration, ER stress, upregulation of the unfolded protein response	180

ENU, *N*-ethyl-N-nitrosourea; ER, endoplasmic reticulum; FE, fractional excretion; TAL, thick ascending limb.

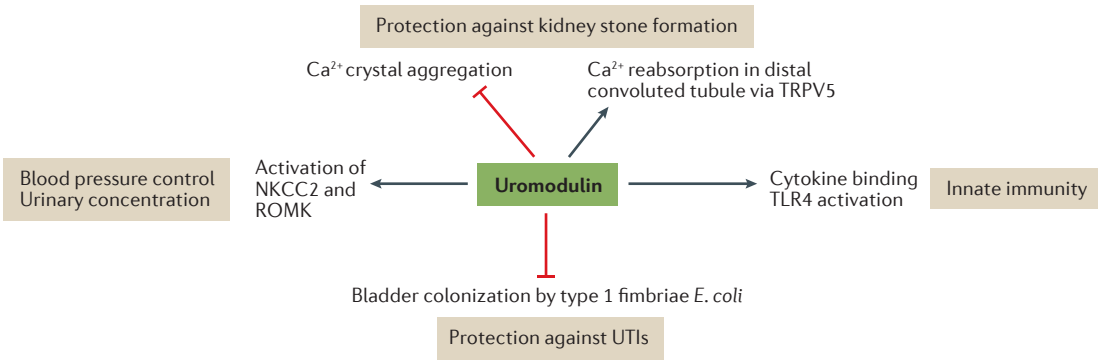


Figure 3 | **Proposed physiological roles of uromodulin.** Studies primarily using *Umod*-knockout mice have shown that uromodulin has roles in blood pressure control, urine concentration, activation of the innate immune system and protection against kidney stone formation and urinary tract infections (UTIs). NKCC2, Na^+-K^+-$2Cl^-$ cotransporter NKCC2; ROMK, potassium channel ROMK; TLR4, Toll-like receptor 4; TRPV5, transient receptor potential cation channel subfamily V member 5.

In addition to its potential role as a direct inhibitor of crystallization, the capacity of uromodulin to modulate the activity of the TAL might impact on urinary concentration and on the paracellular handling of Ca^{2+} and, therefore, influence the urinary concentration of CaOx and CaP[91]. Uromodulin also stabilizes the calcium channel transient receptor potential cation channel subfamily V member 5 (TRPV5) in the DCT, thus decreasing calcium excretion[92]. Genetic evidence obtained in Icelandic and Dutch cohorts supports a protective role of uromodulin against kidney stones[93].

Protection against UTIs

Umod-knockout mice are more prone to type-1 fimbriated *E. coli* bladder colonization than are their wild-type littermates[94,95]. This observation is explained by the fact that the *N*-linked high mannose chains of uromodulin mimic the mannosylated uroplakin receptors on the urothelium, thus competing for the binding of *E. coli* type 1 lectin FimH, impeding their epithelial adherence and virulence[86,94,96]. A prospective cohort study of elderly community-dwelling individuals found that those with urinary uromodulin concentrations in the highest quartile had a lower risk of UTI events than those in the lowest quartile, independent of classical UTI risk factors[97]. The protective effect of uromodulin against UTIs was substantiated in a Swiss population-based cohort; in both men and women, levels of urinary leukocytes (used as a surrogate marker for UTI) inversely correlated with urinary uromodulin levels[98]. After adjusting for urinary creatinine, age, and sex, urinary uromodulin levels were negatively associated with the presence of urinary nitrites, which are also a marker of UTI.

Immunomodulation and protection against AKI

Uromodulin inhibits viral haemagglutination[2] and suppresses antigen-mediated T-cell proliferation and monocyte function *in vitro*[6]. In the urine, uromodulin can bind several immunoregulatory proteins including IgG[99], recombinant IL-1 and TNF, making it a unique renal ligand for lymphokines[15]. The immunomodulatory properties of uromodulin and its binding to IL-1 and TNF are mediated by *N*-linked carbohydrate sequences, in particular sialic acid glycomoieties[15,100,101].

Uromodulin might participate in crosstalk between tubular segments and protect against acute ischaemic tubular injury. In an ischaemia-reperfusion mouse model, uromodulin accumulated after injury and was redirected toward the basolateral membrane of TAL cells, which is in direct contact with cells lining the proximal S3 segment[63]. *Umod*-knockout mice showed delayed renal recovery after ischaemic injury[63], which could be explained by the capacity of uromodulin to trap damaging proinflammatory and chemoattractant molecules[63], to modulate neutrophilic invasion[102] and/or to regulate the expression pattern of S3-expressed TLR4 (REF. 103). The basolateral relocalization of uromodulin was associated with increased serum levels of the protein, which could also modulate the immune response[63]. Notably, *Umod*-knockout mice have increased bone marrow granulopoiesis and systemic neutrophilia, which likely result from activation of the IL-23/IL-17 axis in proximal tubular epithelial cells[104]. The physiological relevance of this tubular crosstalk remains unclear.

Uromodulin aggregates can act as damage-associated molecular patterns and activate the innate immune system by binding to TLR4, triggering NF-κB activation[105] and the NACHT, LRR and PYD domains-containing protein 3 inflammasome[106]. In addition, uromodulin can bind human polymorphonuclear leukocytes through sialic-specific cell surface receptors[107], regulating chemotaxis, phagocytosis and apoptosis[108] and facilitating neutrophil transepithelial migration[109]. The *in vivo* relevance of the proinflammatory properties of uromodulin remains to be deciphered.

Cast nephropathy

Uromodulin is the matrix component of hyaline casts and of proteinaceous casts associated with low-molecular-weight proteinuria[5,110]. These proteinaceous casts might cause renal failure, as exemplified by cast nephropathy in multiple myeloma (known as myeloma kidney). In this disorder, monoclonal free light chains massively produced by plasma cell clones are freely

filtered, saturate the reabsorption capacity of the proximal tubule and are excreted in the urine, resulting in Bence Jones proteinuria.

Free light chains interact with uromodulin and precipitate to form casts in the distal nephron, causing obstruction and tubulointerstitial damage[111,112]. In rats, distal casts could be reproduced by injecting purified nephrotoxic human free light chains[113]. The sialic acid moiety of uromodulin is considered to be important for the interaction, because administration of colchicine prevented cast formation by decreasing uromodulin excretion and its sialylation[114]. A binding site for the third complementary-determining region (CDR3) of free κ and λ light chains has been identified on uromodulin (eight amino acids in the D8C domain). This site contains one cysteine bridge and a histidine residue, which explain the sensitivity of co-aggregation to both reducing agents and pH[115,116]. In a rodent model, cast nephropathy could be prevented by injecting a cyclized competitor peptide based on the critical CDR3 region[113].

Uromodulin as a biomarker

In contrast to glomerular biomarkers, markers of tubular function are critically lacking in clinical practice. Uromodulin is a kidney-specific protein that is abundantly produced in a defined tubular segment that is crucial for electrolyte handling. The association of *UMOD* with rare disorders and more common conditions, as well as the availability of validated ELISA methods for measuring urinary and serum uromodulin levels support the use of uromodulin as a biomarker of tubular function in healthy individuals and in patients with kidney disease.

Urinary uromodulin

Congenital disorders. As uromodulin is produced by the human fetus and cannot cross the placental barrier, this protein has been suggested as a biomarker of *in utero* tubular development[67] and for the detection or prediction of AKI in newborns[117]. Urinary uromodulin levels are strongly reduced in children with antenatal Bartter syndrome, contrasting with intermediate or normal values in patients with post-natal Bartter syndrome[118].

Tubular function and estimated glomerular filtration rate (eGFR). Early studies based on radioimmunoassays found reduced uromodulin excretion in patients with tubular damage or autosomal dominant polycystic kidney disease (ADPKD)[119,120]. Studies based on mass-spectrometry or ELISA identified uromodulin as a potential biomarker in Fabry nephropathy[121], active lupus nephritis with tubulointerstitial inflammation[122] and in ADPKD[123]. The observation of reduced levels of uromodulin in rats with streptozotocin-induced diabetes[124] has led to evaluation of the protein as a biomarker for tubular dysfunction in diabetes mellitus. Although results in the early phases of diabetic nephropathy are conflicting, long-lasting diabetic kidney injury is associated with reduced urinary levels of uromodulin[125,126].

The availability of a well-validated ELISA has enabled measurement of urinary uromodulin levels in population-based cohorts[14,46]. Positive associations exist between uromodulin levels and urinary Na^+, Cl^-, and K^+ excretion as well as urine osmolality. 24-h uromodulin excretion positively associates with kidney length and volume (measured using ultrasound) and with urine volume, and negatively associates with age and diabetes status. In multivariate analyses, spot uromodulin concentration and 24-h uromodulin excretion linearly and positively associated with eGFR[127]. Of note, the fractional excretions of urate and Na^+ positively correlated with uromodulin, likely linked to the extracellular volume status. The presence of glycosuria and the use of uricosuric drugs, which both increase the fraction excretion of urate, were independently associated with a lower uromodulin excretion[128]. Variants in the TAL genes *KCNJ1*, *CAB39* and *SORL1* have also been associated with urinary uromodulin concentration[14]. Taken together, these studies validated urinary uromodulin as a biomarker for tubular mass and tubular function in the general population as well as in disease subgroups. Expression studies on kidney tubules from patients with CKD and control individuals showed that uromodulin mRNA levels strongly correlate with eGFR[129].

The predictive value of urinary uromodulin levels was tested by Garimella *et al.*, who measured urinary uromodulin levels in 192 elderly (mean age 78 years) participants of the Cardiovascular Health Study over 9 years[130]. Each standard deviation increase in urinary uromodulin was associated with a 23% lower odds of eGFR decline and a 10% lower risk of mortality. Thus, in elderly individuals, low urinary uromodulin levels may identify those at risk of progressive kidney disease and mortality beyond established markers.

Acute kidney injury. The dynamic regulation of uromodulin expression in models of AKI suggests that the protein might be a useful biomarker in this setting[63,131]. Moreover, clinical data suggest that urinary uromodulin could be used as a predictive marker for AKI after cardiac surgery. Lower pre-operative urinary uromodulin levels were associated with higher risk of post-operative AKI, suggesting that either low urinary uromodulin is a marker of poorer tubular health at baseline or the protein protects against AKI[103,132].

Kidney stones. As discussed above, evidence suggests that uromodulin might protect against intrarenal calcium crystallization and kidney stone formation in humans. The nephrolithiasis phenotype in *Umod*-knockout mice varies with gene dosage[89], suggesting that an insufficient level of uromodulin in urine might underlie recurrent kidney stone formation. This hypothesis was substantiated by a study that showed reduced urinary uromodulin levels in 35 patients with recurrent CaOx stones compared with healthy individuals[133]. This result was only partially replicated, however, in a larger cohort ($n = 100$) of patients with recurrent CaOx and CaP stones[134]. These discrepancies, and the potential effects of dietary differences on kidney stone propensity, require clarification in larger cohorts. Similarly, the question of whether uromodulin from stone-forming patients is qualitatively different

from that of individuals who do not form stones, for instance with respect to sialic acid content, has not been resolved[135,136].

Circulating uromodulin

The possibility that a fraction of uromodulin is released into the circulation has been supported by immunolocalization studies showing the presence of uromodulin at the basal plasma membrane of TAL cells[49,62]. Using radioimmunoassays, low levels of serum uromodulin (70–540 ng/ml) have been detected in the general population[137]. Serum uromodulin was not detectable in anephric patients, whereas in patients with end-stage renal disease (ESRD) the levels were very low but increased after kidney transplantation[137]. Plasma uromodulin concentrations correlate with urinary uromodulin excretion but circulating levels of uromodulin are substantially lower than urinary levels (ng/ml versus μg/ml, respectively)[120].

Plasma uromodulin levels correlate with creatinine clearance in patients with CKD[120] and could potentially be used to improve the identification of early CKD[138]. Positive correlations between serum uromodulin concentration and eGFR have been reported in patients with CKD stages 1–5 (REFS 49,139) and in healthy people aged ≥60 years[140]. The increase in serum uromodulin levels that occurs after renal transplantation is proportional to the recovery of graft function[49], and serum uromodulin levels predicted kidney graft loss equivalently to creatinine, eGFR, blood urea nitrogen and cystatin C levels in a cohort of 91 patients[141]. By contrast, Prajczer *et al.* reported a negative correlation between eGFR and serum uromodulin levels in 77 patients with CKD[142]. Caution is warranted in interpreting this result, however, as all of the study participants had low serum uromodulin levels and the levels were similar in the CKD and healthy control groups.

The potential value of serum uromodulin as a biomarker of renal disease has also been tested in the paediatric population. Serum uromodulin levels increase with age in healthy infants and children, and correlate with eGFR in children with CKD[143]. Serum uromodulin levels were lower in 168 children with renal diseases than in controls[144]. However, high serum levels of uromodulin were found in patients with vesicoureteral reflux, including those with reduced creatinine clearance[144], as well as in cases of urinary tract obstruction[145].

In a large cohort of patients undergoing coronary angiography ($n = 3057$), higher serum uromodulin levels were associated with a favourable metabolic profile, a lower prevalence of comorbidities, and a lower risk of 10-year mortality, independently of other cardiovascular risk factors including eGFR[146]. The fact that higher uromodulin levels were associated with reduced mortality even after adjustment for eGFR supports extrarenal, beneficial roles of the protein, for example in innate immunity.

Antibodies to uromodulin

Anti-uromodulin antibodies have been detected in patients with a number of infectious, functional or malignant diseases of the urinary tract[147–150]. These antibodies are considered to be a marker of misrouting of uromodulin to the plasma. They are associated with nephrotoxicity, as suggested by the formation of uromodulin-IgG–C3 immune complexes in rats immunized against uromodulin, and by the correlation between levels of anti-uromodulin antibodies and severity of tubulointerstitial lesions[151]. Tubulointerstitial nephritis has been reported in rabbits immunized with homologous urine or uromodulin[152] and in mice immunized with rat uromodulin[153]. Interpretation of human studies is hampered by the effect of age, variable immunoglobulin classes, and possible sequelae of infection[147,154]. The presence of crossreactive serum proteins[155] and the low specificity of direct ELISA approaches[156] are also limiting factors. As low levels of uromodulin are present in blood, autoantibodies to uromodulin are consistently detected in healthy people[157].

Mendelian disorders

Mutations in *UMOD* cause autosomal dominant kidney disorders, medullary cystic kidney disease type 2 (MCKD2; MIM 603860) and familial juvenile hyperuricemic nephropathy (FJHN; MIM 162000), which are collectively referred to as uromodulin-associated kidney disease. A similar clinical phenotype can also be associated with mutations in *MUC1* (located on chromosome 1q21), which are responsible for MCKD type 1, *HNF1B* (located on chromosome 17q12) and more rarely in *REN* (which encodes renin and is located on chromosome 1q32) and *SEC61A1* (the α subunit of the SEC61 translocon)[158–161]. Evidence for further genetic heterogeneity of autosomal dominant kidney disorders has also been reported[162]. To overcome potential confusion with the multiplicity of the disease names and the fact that cysts are not pathognomonic in these disorders, the term ADTKD appended by the causal gene name (for example ADTKD-*UMOD*) has been implemented[163].

Clinical and pathological findings

ADTKD-*UMOD* is a rare disorder. In Austria, the reported prevalence of 1.67 cases per million population[164] is likely an underestimate owing to the use of various nomenclatures and the lack of shared diagnostic algorithms. The fact that *UMOD* mutations were detected in five individuals from a cohort of 911 kidney transplant recipients suggests that the disease is more common than expected[165].

Most clinical laboratory and histological findings in ADTKD are nonspecific[163]. However, ADTKD-*UMOD* is typically characterized by decreased fractional excretion of urate, which causes hyperuricaemia and gout[166]. Hyperuricaemia is present in about 80% of patients and is often the first symptom of the disease, starting before the onset of CKD. Gout is also often associated with ADTKD and has a highly variable age of onset, sometimes occurring as early as in the teenage years, especially in males. Neither hyperuricaemia nor gout are specific for ADTKD. A mild defect of urine concentrating ability is frequently reported[166]. The urinary sediment is typically normal, with mild or absent proteinuria. The penetrance of ADTKD-*UMOD* seems

to be close to 100%, with a marked inter-familial and intra-familial variability in age of onset and disease severity. Individuals with *UMOD* mutations reportedly reach ESRD between the ages of 25 and 70 years or older, whereas the onset of gout occurs between the ages of 3 and 51 years[166,167].

In ADTKD, kidney size is initially normal but declines with disease progression. Renal cysts of varying number and size can occur, generally in advanced CKD, but their frequency is no higher than in other 'non-cystic' kidney diseases[166,168]. Glomerular cysts have been reported in a few cases[26,169]. Patients typically have no history of arterial hypertension preceding the onset of impaired kidney function.

At renal histology, ADTKD-*UMOD* is characterized by diffuse tubulointerstitial damage with moderate inflammatory cell infiltrate, interstitial fibrosis, thickening and lamellation of the tubular basement membrane and tubular atrophy[170]. Tubular dilatation and microcysts have also been reported[170]. Immunofluorescence for complement and immunoglobulins is negative. The analysis of uromodulin localization in patient renal biopsy samples typically shows the presence of large intracellular deposits[10,26] that co-localize with ER markers in TAL cells[171]. Accumulation of mutant uromodulin in the ER is confirmed by the presence of fibrillar or amorphous material in expanded ER cisternae on electron microscope analysis[172].

A decrease in urinary uromodulin levels is a hallmark of ADTKD-*UMOD* that reflects impaired trafficking of the mutant protein[10,173]. A direct comparison of heterozygote and homozygote *UMOD* mutation carriers within the same family revealed that urinary uromodulin levels were reduced in a gene-dosage dependent manner[174].

Pathophysiology of ADTKD-UMOD

To date, 125 *UMOD* mutations have been reported (FIG. 4). All but five (in-frame deletions) are missense changes that are clustered in the N-terminal half of the protein (95% localize in *UMOD* gene exons 3 and 4). In about 60% of cases (78 of 125), mutations either replace or delete one of the 48 conserved cysteine residues or insert a cysteine. As all cysteines are involved in forming intramolecular disulfide bonds, these *UMOD* mutations likely lead to protein misfolding, either by disrupting a native disulfide bond or by destabilizing protein structure.

The effect of *UMOD* mutations on protein folding has been confirmed by studies in cellular models that demonstrated impaired trafficking of mutant uromodulin to the plasma membrane and reduced secretion into the culture medium owing to ER retention[26] (FIG. 5). This effect has been shown for several *UMOD* mutations expressed in different cell systems[27,171,175], demonstrating that ADTKD-*UMOD* is an ER storage disease. Age at ESRD does not seem to correlate with mutation type (cysteine versus polar residue substitution)[166], but rather with mutation position, being earlier in patients carrying mutations in the cbEGF-2 or cbEGF-3 domain compared with carriers of more distal mutations[167].

Mouse models that express mutant uromodulin have been described (TABLE 1). Bernascone *et al.* generated and characterized the first transgenic mouse model ($Tg^{UmodC147W}$) that expresses mutant murine uromodulin C147W, homologous to patient mutation C148W[176]. These mice recapitulate the main features of the human disease and reproduce the main cellular effects of *UMOD* mutations — ER retention and aggregation of mutant protein, associated with decreased urinary excretion[176]. These effects lead to progressive structural and functional damage of the TAL segment, resulting in a urinary concentrating defect and progressive tubulointerstitial damage with inflammatory cell infiltrate and fibrosis, tubular atrophy, cysts in the cortico-medullary area, and mild renal failure.

A similar phenotype has been described in two other mouse models, $Umod^{A227T}$ and $Umod^{C93F}$, which express uromodulin variants A227T and C93F, respectively[177,178]. These variants have similar effects to human *UMOD* mutations as they lead to ER retention of mutant protein. These models also show additional defects in energy, lipid and bone metabolism with unclear clinical relevance. Comparative analysis of $Umod^{A227T}$ and $Umod^{C93F}$ mice demonstrated that the onset and speed of progression of the renal disease depends on the degree of the trafficking defect[178]. Proteomic analysis of kidney samples suggested induction of the unfolded protein response (UPR), as well as secondary mitochondrial dysfunction[179]. Subsequently, a knockin mouse model harbouring the C125R mutation showed impaired uromodulin trafficking causing ER stress, upregulation of the UPR pathway and tubulointerstitial lesions driving progressive renal failure[180].

Taken together, these mouse studies clearly establish a main gain-of-function effect of uromodulin mutations, which is supported by the lack of histological damage in *Umod*-knockout mice[181]. The importance of dosage and extent of ER retention of mutant uromodulin is highlighted by the extremely rare case of a patient from a consanguineous family carrying a homozygous *UMOD* mutation (p.C120Y)[174]. Comparison of heterozygote and homozygote mutation carriers revealed a gene-dosage effect, with unprecedented low levels of uromodulin and aberrant uromodulin fragments in the urine of the homozygote proband. Despite an amplified biological effect of the homozygous mutation, the proband did not show a strikingly more severe clinical evolution, contrasting with an earlier report of three homozygous carriers in a large consanguineous pedigree[182].

A key open question for ADTKD-*UMOD* relates to the pathogenic events that are triggered by ER accumulation of mutant uromodulin, leading to interstitial fibrosis and CKD. *In vitro* studies showed that expression of mutant uromodulin leads to increased apoptosis[183,184] that could be rescued by treatment with the chemical chaperone sodium 4-phenylbutyrate. These findings could not, however, be confirmed in *in vivo* models of ADTKD-*UMOD*[176,185]. A differentiated primary TAL cell system that could enable the identification of primary pathogenic events in mouse models of ADTKD-*UMOD* has now been developed[186].

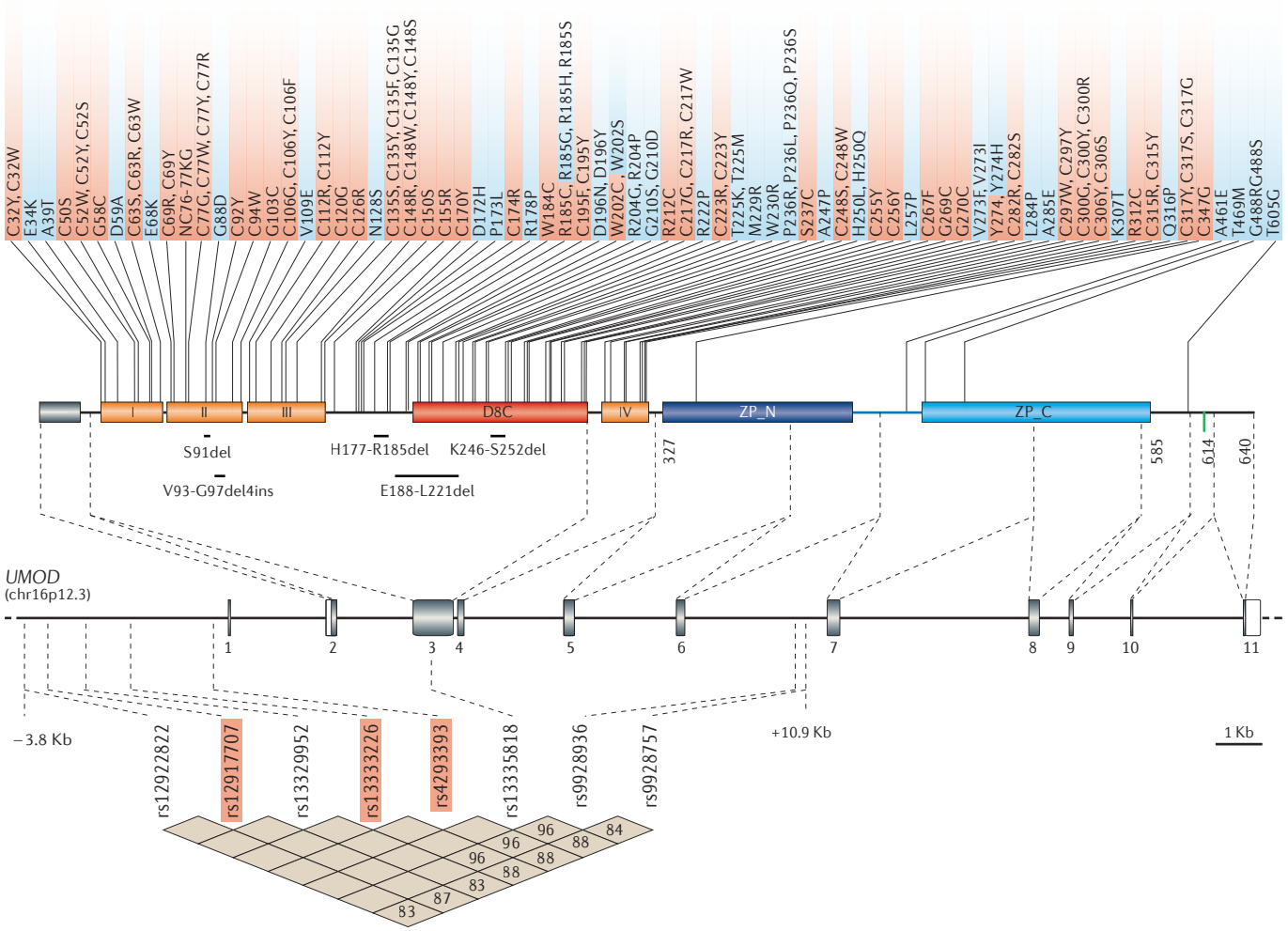


Figure 4 | **Genetic variants of uromodulin that are associated with kidney diseases.** The upper panel shows the localization and effect on the protein sequence of all reported *UMOD* mutations. To date, 125 mutations have been reported of which 78 (62%) affect or introduce cysteine residues, 42 (34%) are missense changes that affect residues other than cysteine and 5 (4%) are in-frame deletions. The middle panel shows the exon/intron structure of the human *UMOD* gene. The coding parts are shaded grey. Most of the *UMOD* mutations are clustered in exons 3 and 4. The lower panel shows the positions of the top single nucleotide polymorphisms (boxed in red) that have been identified in genome-wide association studies. These variants are within the same linkage disequilibrium block spanning the *UMOD* promoter to intron 6, as shown by the linkage disequilibrium plot (r^2 values, data obtained from 1,000 Genomes phase 3, Ensembl human genome GRCh38).

UMOD locus and complex disorders

Associations with CKD and kidney function

The importance of the *UMOD* locus for kidney-related traits and disorders has been substantiated by GWAS in large population-based and disease-based cohorts (TABLE 2). In a pioneering European population-based study, the frequent single-nucleotide polymorphism (SNP) rs12917707 (average minor allele frequency 0.18) located in the promoter region of *UMOD* was strongly associated with creatinine-based eGFR (eGFRcrea) and risk of CKD, independent of hypertension and diabetes[11]. The minor T allele at rs12917707 was associated with a reduction in risk of CKD (OR 0.8) and an allele-dependent increase in eGFRcrea[11].

A genome-wide significant association between rs4293393 (in complete linkage disequilibrium with rs12917707) and several indices of renal function was shown in an Icelandic cohort[93]. The major T allele at rs4293393, which has a population frequency of 80%, was associated with an increased risk of CKD (OR 1.25) and increased serum levels of creatinine and urea. Interestingly, the associations of this allele with serum creatinine and with CKD became stronger with age and number of comorbidities, suggesting that this variant affects age-related kidney adaptation and vulnerability to risk factors[93].

The association of *UMOD* promoter variants with renal traits has now been confirmed in GWAS of European[187–189], Icelandic[190], African[191] and East-Asian[192] populations. Pattaro *et al.* reported that the strength of the association between *UMOD* variants and eGFR is greatest in older individuals (age >65 years) and in those with hypertension and/or diabetes mellitus[189]. The largest nephrology meta-analysis of GWAS conducted to date, which included data from 133,413 individuals, confirmed that *UMOD* promoter variants show the

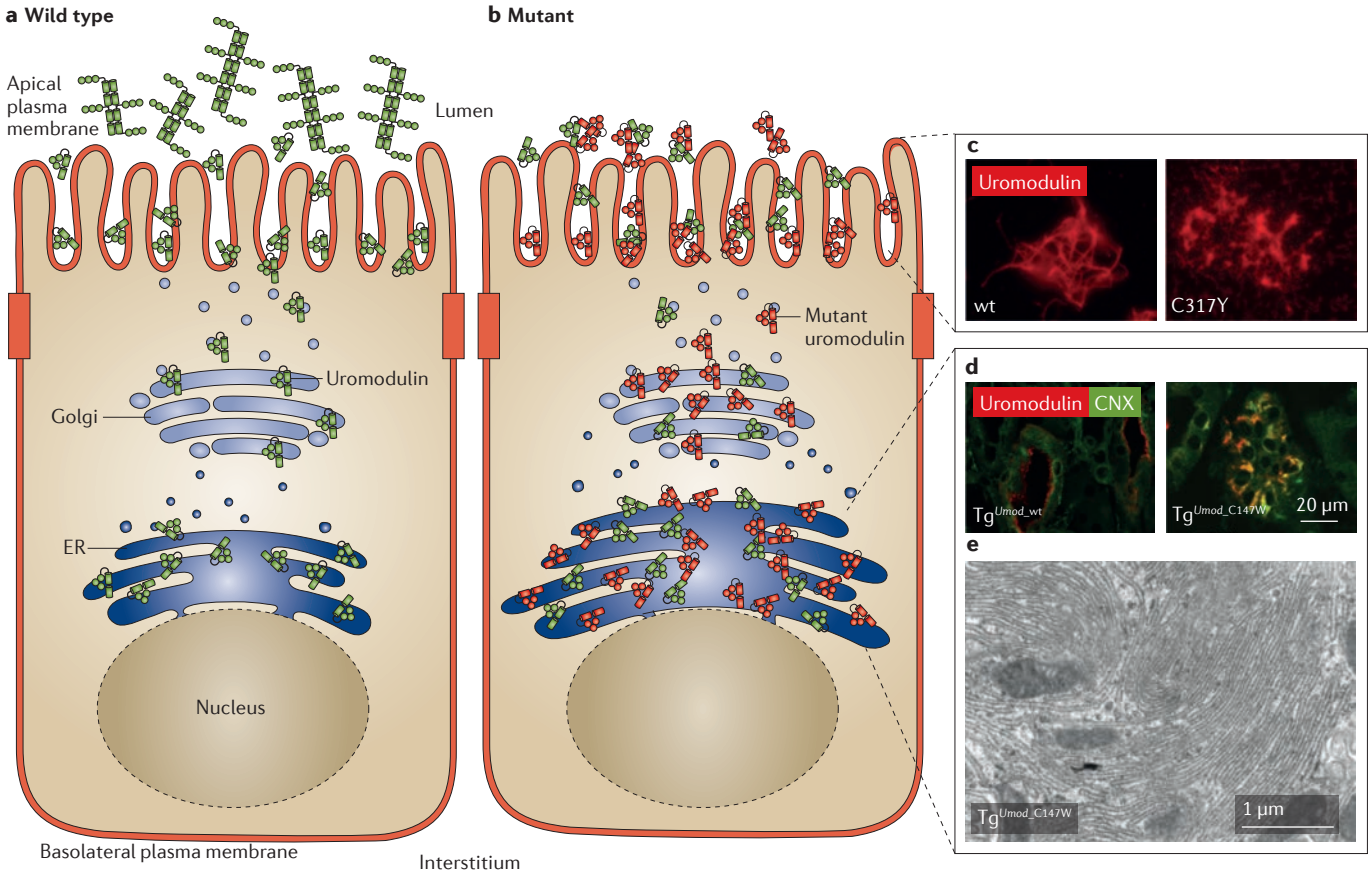


Figure 5 | **Pathophysiology of autosomal dominant tubulointerstitial kidney disease (ADTKD)-*UMOD*. a** | In physiological conditions, uromodulin is synthesized in the cells lining the thick ascending limb (TAL). The protein enters the secretory pathway and is directed to the apical membrane. Cleavage mediated by hepsin[44] activates the uromodulin polymerization Zona Pellucida domain, enabling the formation of extracellular protein filaments. **b** | Mutations in *UMOD* that are associated with ADTKD cause endoplasmic reticulum (ER) retention and aggregation of mutant uromodulin. Mutant uromodulin that escapes ER quality control mechanisms reaches the apical membrane where it forms extracellular aggregates, rather than organized polymers. **c** | Immunofluorescence images showing extracellular uromodulin secreted by Madin–Darby canine kidney cells that stably express wild type (wt) or mutant (C317Y) uromodulin isoforms. **d** | Immunofluorescence images showing the localization of uromodulin in the TAL segments of 12 week-old transgenic mice expressing wt (Tg^{Umodwt}) or mutant ($Tg^{UmodC147W}$) uromodulin. Colocalization with calnexin (CNX) indicates ER accumulation of uromodulin in the mutant mice. **e** | Transmission electron microscopic image of kidneys from 24-week-old $Tg^{UmodC147W}$ mice showing hyperplasia of ER membranes in a TAL cell.

strongest genome-wide associations with eGFRcrea and CKD[12]. The highest effect size on eGFR was seen in individuals with diabetes. Of note, the 53 loci that associated with eGFR and/or CKD with genome-wide significance explained only 3.22% of the variance in eGFRcrea in the population analyzed[12].

Importantly, the *UMOD* rs12917707 variant is also associated with incidence of CKD in healthy individuals with European ancestry, and was nominally associated with ESRD in a case-control study[193]. The association of *UMOD* promoter variants with incident CKD risk in the general population of European descent was confirmed in a subsequent study[194].

A Swedish case-controlled candidate-gene study that included ~5,000 unrelated patients with type 2 diabetes mellitus (T2DM), of whom ~1,000 had nephropathy, showed that *UMOD* rs13333226 was independently associated with risk of developing diabetic nephropathy as well as with blood pressure control and eGFR[195]. Similarly, *UMOD* promoter variants were associated with eGFR, CKD progression and albuminuria in a UK T2DM cohort[196]. The lack of such an association in African Americans suggests that the *UMOD* effect in T2DM kidney disease might be population specific[197].

Taken together, these association studies establish the *UMOD* locus as the main genetic risk factor associated with renal traits in the population. All *UMOD* variants associated with renal traits are common, but the associations are characterized by fairly small effect sizes so are inappropriate for CKD risk prediction at an individual level[198].

Associations with cardiovascular events

A study with an extreme case-control design showed associations of the *UMOD* locus (rs13333226) with the risk of hypertension and with incident cardiovascular

disease events, independently of major risk factors including eGFR[52]. The available evidence suggests that the effect of the variant is mediated by renal sodium handling[13,52].

Associations with uric acid levels

Consistent with the link between mutations in *UMOD* and hyperuricaemia (as seen in ADTKD-*UMOD*), rs4293393 was significantly associated with serum uric

Table 2 | ***UMOD* variants and traits identified by GWAS and association studies**

Trait	Top variant	Study sample (replication cohort)	Ethnicity	Ref.
GWAS studies				
CKD	rs4293393	2,903 (300)	Icelandic	93
	rs4293393, rs12917707	194,286	Icelandic	190
CKD, eGFRcrea	rs12917707	19,877 (21,466)	European	11
	rs12917707	74,354 (56,246)	European	189
	rs77924615*	71,638	African American, Hispanic, European and East Asian	211
CKD, eGFRcrea, eGFRcys	rs12917707	67,093 (22,982)	European	187
	rs13329952	133,413 (42,166)	European	12
eGFR change‡	rs12917707	45,530 (18,028)	European	194
eGFRcrea	rs4293393	8,110 (4,358)	African American§	191
eGFRcrea, serum creatinine	rs11864909	51,327 (19,822)	East Asian	192
	rs11864909‖	194,286	Icelandic	190
Hypertension	rs13333226	1,621 cases versus 1,699 controls (validation stages 1 and 2 in 19,845 cases versus 16,541 controls)¶	European	52
Serum creatinine	rs12917707	23,812 (16,626)	European	188
	rs4293393	22,256 (2,379)	Icelandic	93
	rs77924615*	194,286	Icelandic	190
Serum uromodulin	rs12917707	2,984	European	146
Urinary uromodulin (indexed to urinary creatinine and not indexed)	rs12917707	10,884	European	14
Candidate gene association studies				
Diabetic nephropathy, eGFRcrea, SBP	rs13333226	880 cases versus 4,008 controls	Scandinavian	195
eGFR in patients with T2DM, time to stage 3B CKD	rs12917707	3,028	European	196
End-stage renal disease	rs12917707	3,775 cases versus 4,577 controls	European	193
Incident CKD	rs12917707	26,308	European	193
Kidney stones	rs4293393	3,617 cases versus 43,201 controls	Icelandic and Dutch	93
Plasma uric acid	rs4293393	6,583	Icelandic	93
	rs13333226, rs6497476, rs4293393	1,000 (642)	Chinese (European and Hispanic)	199
SBP and DBP (continuous measurement)	rs13333226	79,133	European	52

CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; eGFRcrea, eGFR based on serum creatinine; eGFRcys, eGFR based on serum cystatin C; GWAS, genome-wide association study; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus. CKD is defined as eGFRcrea <60 ml/min/1.73 m^2. Stage 3B CKD is defined as eGFRcrea <45 ml/min/1.73 m^2. **UMOD/PDILT* variant. ‡Annual decline in eGFR (ml/min/1.73 m^2 per year). §Liu *et al.*[191] tested 24 known renal susceptibility loci identified in European ancestry consortia. The *UMOD* variant reached nominal significance for eGFRcrea in African Americans, and was replicated. ‖*PDILT* variant. ¶Extreme case-control design. The frequency of the minor allele for rs12917707, rs13333226, rs13329952, rs4293393 and rs77924615 is ~0.20–0.22 (Europeans, Ensembl human genome GRCh38). The single nucleotide polymorphisms rs4293393, rs13333226, rs12917707 and rs13329952 are in complete linkage disequilibrium (D' = 1, r^2 = 1; 1,000 Genomes phase 3, Ensembl human genome GRCh38).

Table 3 | **The rs4293393 promoter variant of the *UMOD* gene**

rs4293393	Frequency*	*UMOD* expression	Urinary uromodulin levels	GWAS: CKD, hypertension	GWAS: kidney stones	Potential role for UTI	Potential selective pressure
Ancestral T allele	0.80	High	High	Risk‡	Protective	Protective	Positive or neutral
Derived C allele	0.20	Low	Low	Protective‡	Risk	Deleterious	Negative or neutral

CKD, chronic kidney disease; GWAS, genome-wide association study; UTI, urinary tract infection. *1,000 Genomes latest release. ‡Association with renal function is significantly higher in individuals aged over 65 years[189]. Modified with permission from the American Society of Nephrology © Ghirotto, S. *et al. J. Am. Soc. Nephrol.* 27, 2983–2996 (2016).

acid levels in an Icelandic cohort[93]. *UMOD* promoter variants also associated with plasma uric acid levels in a Chinese community-based cohort; this association partially explains the high heritability of uric acid handling reported in twin studies[199].

Associations with kidney stones

In an Icelandic study that included 1,689 patients with kidney stones and 37,076 population controls, the rs4293393 T allele was significantly associated with reduced risk of kidney stones (OR 0.88)[93]. This association was replicated in a cohort that combined two additional sample sets from Iceland (1,271 patients and 3,177 controls) and the Netherlands (701 patients and 2,948 controls)[93].

Associations with uromodulin

UMOD promoter variants were associated with urinary levels of uromodulin in a meta-analysis of 10,884 individuals of European descent[14] and with serum uromodulin levels in a smaller cohort of 2,826 individuals[146].

Biological relevance of UMOD variants

The case of *UMOD* promoter variants is a rare example in which the gap between GWAS data, biological function and pathophysiological mechanisms has been, at least partly, bridged[200,201] (TABLE 3). The first insight came with the discovery that rs4293393 was associated with urinary levels of uromodulin in a nested case-control study[202]. An analysis of the genetic factors that influence urinary uromodulin excretion in multiple cohorts showed that the *UMOD* promoter variant rs12917707 strongly associated with urinary levels of uromodulin, with each risk allele for CKD increasing the urinary excretion of uromodulin in a dose-dependent fashion[14]. In fact, the GG (risk) carriers had twofold higher indexed uromodulin levels than TT (protective) carriers. The influence of rs4293393 on urinary uromodulin levels was confirmed in a multivariable adjusted correlation study in a Canadian population[128]. As all promoter variants are in perfect linkage disequilibrium (FIG. 4), the *UMOD* promoter haplotype associated with higher uromodulin excretion, lower eGFR, higher risk of CKD and hypertension and lower risk of kidney stones is referred to as the risk haplotype (TABLE 3).

The studies of Trudu *et al.* demonstrated that the promoter variants rs12917707 and rs4293393 affect uromodulin transcription *in vitro* and *in vivo*; nephrectomy samples from homozygote carriers of the risk allele had twofold higher *UMOD* transcript levels than homozygote carriers of the protective allele, paralleled by higher levels of uromodulin in urine[13]. As rs4293393 maps to a highly conserved promoter region that acts as a glucocorticoid responsive element, the protective allele could potentially disrupt binding at this site and explain altered transcription levels.

To further mimic the human situation, Trudu *et al.* generated a mouse line (TABLE 1) that overexpresses wild-type uromodulin (twofold increase in transcription) and used control or transgenic *Umod* mice to model carriers of the protective or risk haplotypes, respectively[13]. The transgenic *Umod* mice displayed salt-sensitive hypertension with normal renal function. However, the kidneys of aged transgenic *Umod* mice showed focal lesions and increased expression of damage markers including lipocalin-2, Kim-1 and the chemokines CCl2 and CCl5, which were absent in control mice. Similar focal lesions were seen in kidney biopsy samples from individuals aged >65 years, suggesting that they represent renal ageing. Importantly, the area of these lesions was increased in individuals homozygous for *UMOD* risk variants relative to those homozygous for protective variants. These results indicate that increased expression of uromodulin does not lead to renal failure *per se*, but rather support a second hit model in which high uromodulin production over time predisposes to kidney damage induced by additional comorbidities[13].

Studies that have addressed the biological effect of the *UMOD* variants are distinct from those that have investigated the potential biomarker value of uromodulin levels in urine and blood. Urinary and serum uromodulin levels are (in a very small part) determined by genotype at the *UMOD* locus — and the haplotype that is associated with higher expression is also associated with increased risk of CKD[11,14]. On the other hand, clinical and biological studies have shown that urinary and serum levels of uromodulin reflect functioning nephron mass. For example, urinary uromodulin levels were independently associated with eGFR, indexes of renal tubular function, and kidney size[127]. Higher levels of uromodulin in urine or in serum, which reflect higher renal functional mass, are hence expected to be associated with a lower risk of kidney disease[130,132]. Thus, although urinary and serum levels of uromodulin directly correlate with renal function, the effect of *UMOD* variants, besides being age-dependent, contributes to a small part of the overall risk of CKD, which is determined by many other genetic and environmental factors.

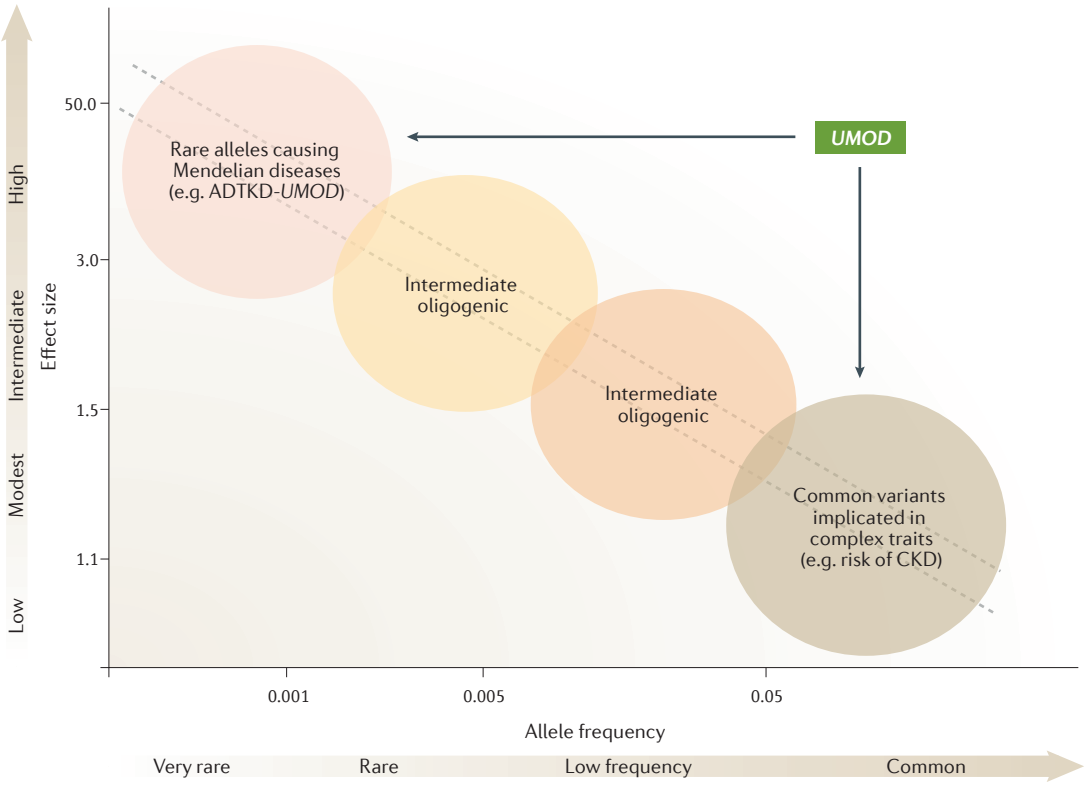


Figure 6 | **Involvement of *UMOD* in a spectrum of diseases.** The genetic spectrum of disease is characterized by an inverse correlation between the effect size and the allele frequency in the population. Dominant mutations in *UMOD* are involved in rare Mendelian diseases, such as autosomal dominant tubulointerstitial kidney disease (ADTKD)-*UMOD*, whereas common variants (in the promoter region) are associated with the risk of chronic kidney disease (CKD) and other complex disorders in the general population. Of note, the common variants of *UMOD* have strong biological activity, as they double the production of uromodulin in the kidney and its excretion in the urine. Modified with permission from Macmillan Publishers Limited, part of Springer Nature © Manolio, T. A. *et al. Nature*, **461**, 747–753 (2009).

In transgenic *Umod* mice, blood pressure is increased as a result of uromodulin-mediated hyperactivation (increased phosphorylation) of NKCC2 in the TAL, confirmed by an increased response to furosemide *in vivo*[13]. The gain-of-function mechanism associated with uromodulin expression completes the paradigm linking renal tubular salt transport with blood pressure regulation[203]. This mechanism could be relevant to human hypertension and raises possibilities for a personalized medical approach. For example, furosemide could be particularly indicated in patients with hypertension who harbour *UMOD* promoter risk variants[13].

UMOD variants: an evolutionary perspective

The data summarized above demonstrate that common variants located in the promoter of *UMOD* are consistently associated with eGFR, risk of developing CKD and also with hypertension in the general population (TABLE 3). These risk variants directly increase the expression of uromodulin and cause salt-sensitive hypertension and kidney damage in mice and humans[13]. Importantly, the frequency of the *UMOD* risk variants is unusually high, ranging from 70–80% in Africans and Europeans to 92–95% in East Asians. This observation, combined with the strong biological readout, suggests some sort of selection pressure related to the biological role of uromodulin in the urine.

An investigation of the effect of selective pressure on the frequency of the lead *UMOD* variant rs4293393 showed that the risk allele T at rs4293393 is the ancestral allele, as it is the only one detected in chimpanzees, non-human primates and anatomically modern humans[98]. Examination of global distribution patterns suggest that the latitudinal cline observed for rs4293393 alleles (highest frequencies of the risk variants outside of Africa) is different from the ancestral susceptibility model that is applicable when salt-retention is the primary motor for evolutionary selection (for example for variants in the salt-retaining genes *CYP3A5* and *AGT*)[204,205]. Instead, the derived, protective allele of *UMOD* rose in frequency fairly late, approximately 15,000 years ago, under neutral selective pressure. The persistently high prevalence of the ancestral allele must, therefore, reflect some evolutionary advantage, following the model of selection on standing variation[98].

As the ancestral allele increases urinary levels of uromodulin, this variant was hypothesized to have a role in protection against UTIs. A study that used data from the Human Genome Diversity Project showed that

the frequencies of the rs4293393 ancestral allele significantly correlated with pathogen diversity (bacteria and helminths) but not with other environmental variables including climate and diet[98]. The ancestral allele also correlated with the prevalence of antibiotic resistance in Gram-negative uropathogens (that is areas with a higher prevalence of UTIs). The protective effect of uromodulin against UTIs was substantiated by significant, inverse correlations between the levels of uromodulin in urine and markers of UTIs (for example urinary leukocytes and nitrites) in the population. Taken together, these data suggest that the ancestral *UMOD* allele has been kept at a high frequency due to its protective effect against UTIs, which mainly affect young women and have important consequences in terms of fitness and reproduction. The salt-retaining effect of this variant could also confer an advantage in cases of severe infection (e.g. sepsis), particularly in children. The fact that the risk of CKD conferred by the ancestral variants of *UMOD* has been shown to be age-dependent[93,189] implies that this risk is unlikely to have a role in evolutionary selection. Of note, the *UMOD* situation differs from that of selection at the *APOL1* locus, where the derived allele rather than the ancestral allele confers a selective advantage against a pathogen (*Trypanosoma brucei*, which causes sleeping sickness)[206].

Conclusions and perspectives

The past 15 years have seen a tremendous increase in our knowledge of the multifaceted role of uromodulin in the kidney (FIG. 3), reflecting its localization, abundant expression, and biochemical properties that sustain various types of biological interactions. These findings have led to a paradigm of *UMOD* as a continuum of genetic kidney disease risk, from rare mutations causing ADTKD to common regulatory variants involved in the risk of CKD (FIG. 6). The *UMOD* locus has by far the largest effect size in terms of genetic risk among all known loci associated with eGFR and/or CKD and has also been associated with complex traits such as hypertension and kidney stones. The unusually high prevalence of the *UMOD* risk allele points to a selective pressure linking pathogens and risk of kidney disease. In turn, this new knowledge has generated many fascinating questions concerning the biology of the protein and its role in disease.

Fundamental questions that remain to be resolved include the mechanisms that sustain production of uromodulin in epithelial cells and the regulators involved, the apical versus basolateral sorting, the structure of the protein, and the coupling between secretion and transport activities in the TAL. More insights are needed into the intracellular and extracellular roles of uromodulin as well as the intriguing possibility of a role in the blood.

With respect to disease, the link between mutant uromodulin, UPR, ER stress and tubulointerstitial damage in ADTKD, and the potential role of basolateral, interstitial uromodulin as a danger signal remain to be deciphered. The potential relationships between uromodulin and the products of other genes involved in ADTKD (such as *MUC1*) also need to be investigated as do the mechanisms that link uromodulin with the risk of CKD, hypertension and kidney stones. Major translational perspectives include the value of uromodulin as a biomarker in CKD and beyond, as well as the potential to define drugable targets in the uromodulin pathway, with therapeutic opportunities for rare (ADTKD) and common disorders (kidney ageing, CKD, hypertension, UTIs, and cast nephropathy).

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Competing interests statement
The authors declare no competing interests.

Author contributions
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