Clinical and genetic spectra of autosomal dominant tubulointerstitial kidney disease due to mutations in *UMOD* and *MUC1*

see commentary on page 549

Eric Olinger[1,2,16,23], Patrick Hofmann[1,3,23], Kendrah Kidd[4,20,23], Inès Dufour[1,9], Hendrica Belge[5], Céline Schaeffer[6], Anne Kipp[1], Olivier Bonny[7], Constantinos Deltas[8], Nathalie Demoulin[9,10], Thomas Fehr[1,11], Daniel G. Fuster[2], Daniel P. Gale[12], Eric Goffin[9,10], Kateřina Hodaňová[20], Uyen Huynh-Do[2], Andreas Kistler[13], Johann Morelle[9,10], Gregory Papagregoriou[8], Yves Pirson[9], Richard Sandford[14], John A. Sayer[15,16], Roser Torra[17], Christina Venzin[18], Reto Venzin[19], Bruno Vogt[2], Martina Živná[20], Anna Greka[21,22], Karin Dahan[5], Luca Rampoldi[6], Stanislav Kmoch[20], Anthony J. Bleyer Sr[4,20,24] and Olivier Devuyst[1,9,24]

[1]Institute of Physiology, University of Zurich, Zurich, Switzerland; [2]Department of Nephrology and Hypertension, Inselspital Bern University Hospital, Bern, Switzerland; [3]Department of Internal Medicine, Hospital Uster, Uster, Switzerland; [4]Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; [5]Center for Human Genetics, Institute of Pathology and Genetics, Gosselies, Belgium; [6]Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy; [7]Service of Nephrology, Lausanne University Hospital, Lausanne, Switzerland; [8]Molecular Medicine Research Center, Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus; [9]Division of Nephrology, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [10]Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium; [11]Department of Internal Medicine, Cantonal Hospital Graubuenden, Chur, Switzerland; [12]Department of Nephrology, University College of London, London, UK; [13]Department of Internal Medicine, Cantonal Hospital Frauenfeld, Frauenfeld, Switzerland; [14]Department of Medical Genetics, Cambridge Biomedical Campus, Cambridge, UK; [15]Renal Services, Newcastle upon Tyne Hospitals National Health Service Trust, Newcastle upon Tyne, UK; [16]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK; [17]Inherited Renal Disorders, Nephrology Department, Fundació Puigvert, Spanish Renal Research Network (REDinREN), Instituto de Investigaciones Biomédicas Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain; [18]Division of Nephrology, Department of Internal Medicine, Hospital Davos, Davos, Switzerland; [19]Division of Nephrology, Department of Internal Medicine, Cantonal Hospital Graubuenden, Chur, Switzerland; [20]Research Unit for Rare Diseases, Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic; [21]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA; and [22]Broad Institute of MIT and Harvard, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Autosomal dominant tubulointerstitial kidney disease (ADTKD) is an increasingly recognized cause of end-stage kidney disease, primarily due to mutations in *UMOD* and *MUC1*. The lack of clinical recognition and the small size of cohorts have slowed the understanding of disease ontology and development of diagnostic algorithms. We analyzed two registries from Europe and the United States to define genetic and clinical characteristics of ADTKD-*UMOD* and ADTKD-*MUC1* and develop a practical score to guide genetic testing. Our study encompassed 726 patients from 585 families with a presumptive diagnosis of ADTKD along with clinical, biochemical, genetic and radiologic data. Collectively, 106 different *UMOD* mutations were detected in 216/562 (38.4%) of families with ADTKD (303 patients), and 4 different *MUC1* mutations in 72/205 (35.1%) of the families that are *UMOD*-negative (83 patients). The median kidney survival was significantly shorter in patients with ADTKD-*MUC1* compared to ADTKD-*UMOD* (46 vs. 54 years, respectively), whereas the median gout-free survival was dramatically reduced in patients with ADTKD-*UMOD* compared to ADTKD-*MUC1* (30 vs. 67 years, respectively). In contrast to patients with ADTKD-*UMOD*, patients with ADTKD-*MUC1* had normal urinary excretion of uromodulin and distribution of uromodulin in tubular cells. A diagnostic algorithm based on a simple score coupled with urinary uromodulin measurements separated patients with ADTKD-*UMOD* from those with ADTKD-*MUC1* with a sensitivity of 94.1%, a specificity of 74.3% and a positive predictive value of 84.2% for a *UMOD* mutation. Thus, ADTKD-*UMOD* is more frequently diagnosed than ADTKD-*MUC1*, ADTKD subtypes present with distinct clinical features, and a simple score coupled with urine uromodulin measurements may help prioritizing genetic testing.

Kidney International (2020) **98,** 717–731; https://doi.org/10.1016/j.kint.2020.04.038

KEYWORDS: diagnostic score; dominant kidney disease; gout; mucin-1; uromodulin

Correspondence: *Eric Olinger or Olivier Devuyst, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. E-mail: eric.olinger@ncl.ac.uk or olivier.devuyst@uzh;ch. or Anthony J. Bleyer, Sr., Section on Nephrology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157, USA; ableyer@wakehealth.edu*

[23]EO, PH, and KK are co-first authors.

[24]AJB and OD are co-last authors.

Received 5 January 2020; revised 23 March 2020; accepted 2 April 2020; published online 22 May 2020

Autosomal dominant tubulointerstitial kidney disease (ADTKD) is characterized by tubular damage and interstitial fibrosis of the kidney in the absence of glomerular lesions. Affected individuals present with progressive chronic kidney disease (CKD), normal-to-mild proteinuria, and normal-sized kidneys, often with a positive family history.[1,2] The disease invariably progresses to end-stage kidney disease (ESKD). Dominant mutations in *UMOD* were first associated with ADTKD.[3,4] *UMOD* encodes uromodulin, a kidney-specific protein that is abundant in normal urine and plays multiple roles in the kidney.[4] Mutations in *MUC1* were subsequently identified as a cause for ADTKD.[5] *MUC1* encodes the glycoprotein mucin-1, which is important in epithelial barrier function and intracellular signaling.[6–8] Rare forms of ADTKD have also been associated with mutations in *HNF1B*, which encodes the transcription factor hepatocyte nuclear factor 1β[9,10]; *REN*, which encodes preprorenin, the precursor of renin[11]; and *SEC61A1*, which encodes the α1 subunit of the SEC61 complex that forms the core of the endoplasmic reticulum (ER) translocon.[12]

Due to the nonspecific nature of the clinical, biological, and pathological findings, ADTKD is underdiagnosed. In a recent study of whole exome sequencing in ~3000 patients with CKD, *UMOD* mutations were detected in 3% of patients with a monogenic cause of CKD, making it the sixth most common genetic diagnosis in CKD.[13] A single tertiary center survey in England estimated that up to 2% of patients with ESKD had ADTKD-*UMOD*, that is, the most common monogenic kidney disease after autosomal dominant polycystic kidney disease.[14] The prevalence of ADTKD-*MUC1* remains unclear, as mutations in *MUC1* are not detected by next-generation sequencing and require specialized genetic testing.[5,13] However, previous studies have identified ADTKD-*MUC1* and ADTKD-*UMOD* as the most common subtypes of ADTKD.[8,15] The pathophysiology of ADTKD-*UMOD* involves retention of mutant UMOD in the ER with ensuing ER stress ("gain of toxic function") and a cascade leading to inflammatory cell infiltrate, tubular dysfunction, and interstitial fibrosis.[16–18] ADTKD-*MUC1* is caused by mutations in the variable number of tandem repeat (VNTR) region of MUC1, leading to the formation of a frameshift, truncated protein (MUC1fs) that accumulates in intracellular vesicles and causes tubulointerstitial damage.[19]

To date, the largest clinical analysis of ADTKD-*UMOD* was performed in a cohort of French and Belgian patients with ADTKD-*UMOD* ($n = 70$ from 38 families), showing a median renal survival of 54 years and a 66% prevalence of gout.[20] The phenotype of ADTKD-*MUC1* was reported in a cohort of 95 patients from 24 families, with an age of onset of ESKD ranging from 16 to 80 years and a 24% prevalence of gout.[8] A Spanish cohort of 90 patients with ADTKD-*MUC1* (16 families) showed a trend toward earlier age at ESKD and a lower prevalence of gout compared with that of patients with ADTKD-*UMOD* ($n = 41$ from 9 families). The small size of these cohorts prevented the detection of significant differences between ADTKD subtypes.[15]

Because of the nonspecific presentation and relative rarity, a clinical characterization of ADTKD subtypes and practical tools to guide genetic testing for suspected ADTKD are missing. Here, we compared the phenotype of the ADTKD-*UMOD* and ADTKD-*MUC1* subgroups in 2 large cohorts from Europe (Belgo-Swiss ADTKD registry) and the United States (US ADTKD registry), representing the largest multicenter ADTKD cohort (726 patients from 585 families) to date. We observed distinct features among these ADTKD subtypes and established a simple score to orient diagnosis and prioritize genetic testing in ADTKD.

RESULTS

Clinical and genetic characteristics of patients with ADTKD

The International ADTKD Cohort included 726 patients from 585 families: 451 patients from 429 families from the US ADTKD registry and 275 patients from 156 families from the Belgo-Swiss ADTKD registry (Figure 1).[2] In the international cohort, 84% of patients presented with CKD and 43% had reached ESKD. Gout had an overall prevalence of 66% and a family history of either CKD and/or gout was reported in 92% of all cases (Table 1). The main differences between the Belgo-Swiss and US registries included age at presentation, which was older, and prevalence of ESKD, which was higher in the US registry, possibly due to a higher rate of patient self-referral when the disease became symptomatic.

Most patients (703 of 726), from 562 of 585 families, underwent mutational screening in the *UMOD* gene as a first diagnostic test. *UMOD* mutations were detected in 216 of 562 tested families (38.4%), corresponding to 303 of 703 tested patients (43.1%) (Figure 1). The *UMOD* mutation detection rate was 40.0% in the US registry and 34.6% in the Belgo-Swiss registry (Table 1). Next, mutations in *MUC1* were screened in 218 patients who were *UMOD*-negative, from 205 families that were *UMOD*-negative, mostly from the US registry. Of these, 83 patients from 72 families screened positive for *MUC1* mutations, yielding a proportion of 35.1% (72 of 205) of families with ADTKD-*MUC1* among families with *UMOD*-negative ADTKD. Of note, a subset of 23 patients from 23 families with ADTKD (most of them previously linked to chromosome 1q22) were first screened for *MUC1*, with a mutation in *MUC1* detected in 21 patients in this group (Figure 1). At the end of the screening process, 135 patients from 133 families were negative for mutations in both *UMOD* and *MUC1* (Figure 1). Based on these genetic results, the prevalence for ADTKD-*UMOD* is 37.1% [(216 positive) / (585 − 2) tested families] and for ADTKD-*MUC1* is 21.0% [(93 positive) / (585 − 141) tested families] among ADTKD families in this real-life cohort.

Spectrum of *UMOD* and *MUC1* mutations

A total of 106 different *UMOD* mutations were detected in the 216 families with ADTKD-*UMOD* (Figure 2a; Supplementary Table S1). Variant calling was based on *in silico* prediction tools, previous reports, and/or family segregation analysis for undescribed variants. Missense mutations were by far the most

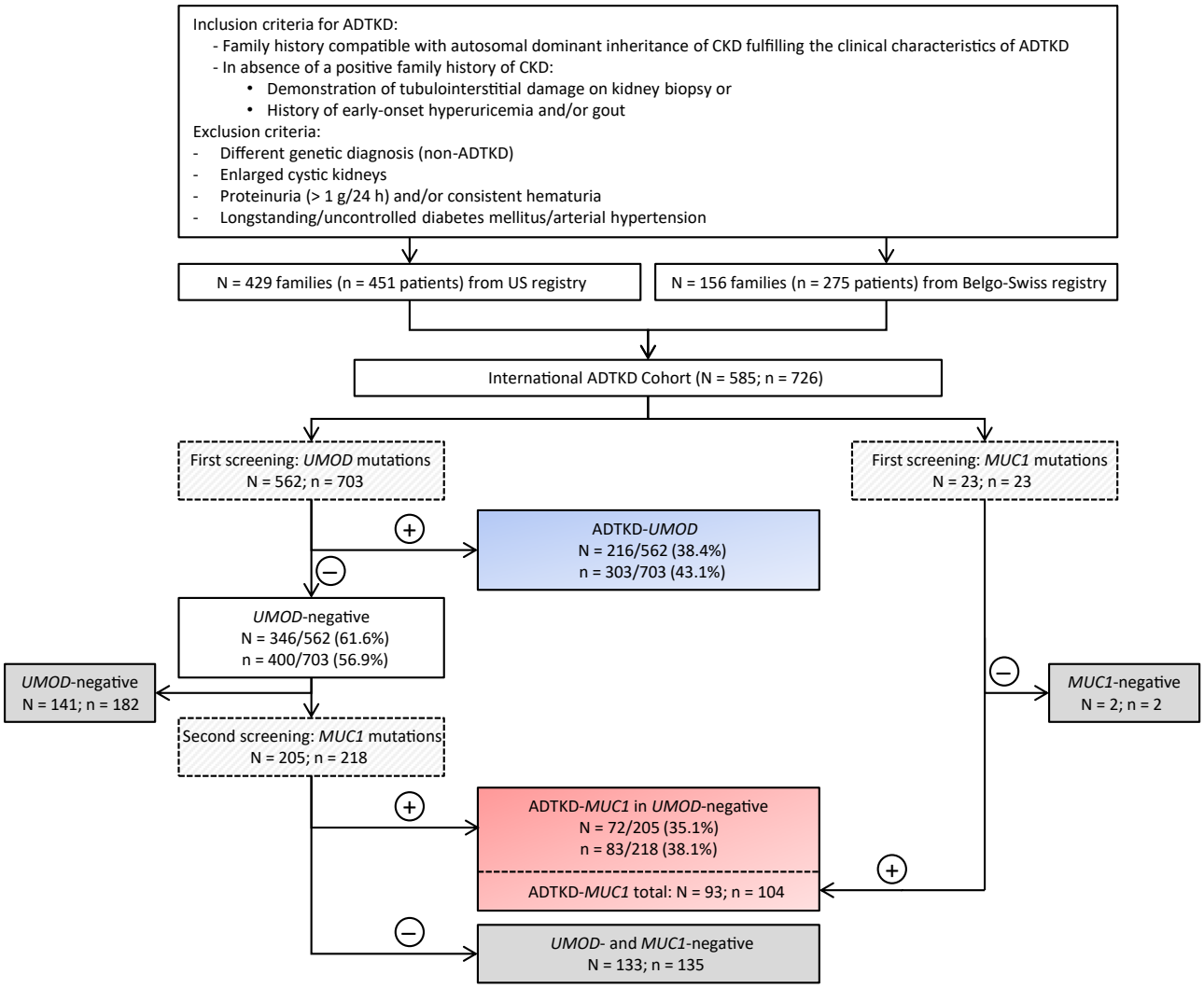

Figure 1 | Design and flowchart of mutation detection in the International ADTKD Cohort. Clinical characteristics of autosomal dominant tubulointerstitial kidney disease (ADTKD) are based on the Kidney Disease: Improving Global Outcomes consensus report[2]; see the Methods for more details. CKD, chronic kidney disease; n, number of patients; N, number of families.

common type of *UMOD* mutations (101 of 106, 95.3%). Four different deletions (H177-R185del, E188-L221del, K246-S252del, Y272del) and 1 indel (V93-G97del4ins) mutations were found. Of 106 mutations, 95 (89.6%) were clustered in exon 3 of the *UMOD* gene. Of the 101 missense mutations, 57 (56.4%) involved cysteine bonds, either by substituting a cysteine residue by another amino acid or by inserting a new cysteine (Figure 2b). Among the 17 mutations not described before (Supplementary Table S1), 6 involve a previously reported amino acid (N85S, C92G, C120R, C135W, V273L, C300S); 2 (Y272del, G201D) were validated in segregation analyses; and 1 (L284P) was clearly associated with ER retention in functional studies, similar to paradigm mutation C150S (Supplementary Figure S1), along with family history (3 generations with CKD and gout, bland urine sediment) and the absence of this substitution in the Genome Aggregation Database. Using *in silico* prediction tools, the remaining 8 mutations were predicted to be disease-causing (Supplementary Table S2).

We detected 2 families with genetically proven *de novo* *UMOD* mutations c.855C>A (p.A285E) and c.707C>T (p.P236L) and 1 family with clinically suspected neomutation c.707C>T (p.P236L). We did not detect *UMOD* mutations in the homozygous state.

Four different types of *MUC1* mutations (27dupC, 28dupA, 26_27insG, 23delinsAT) in the VNTR domain of *MUC1* were detected in this cohort (nomenclature based on the mutation position inside the canonical 60 nucleotide-long wild-type VNTR repeat as identified by *MUC1* VNTR sequencing[7]). Their localization inside the *MUC1* VNTR as well as their effect on the MUC1 structure are shown in Figure 2c. All these mutations are predicted to lead to the same frameshift and premature stop codon.[7] Among the 93 families with ADTKD-*MUC1*, 87 presented with a cytosine duplication (27dupC, 93.5%), 3 with an adenine duplication (28dupA, 3.2%), 2 with a guanine insertion (26_27insG, 2.2%), and 1 with a small indel (23delinsAT, 1.1%) (Figure 2d).

Table 1 | Clinical and genetic characteristics of patients with ADTKD

Characteristic	International ADTKD Cohort (N = 726)	Belgo-Swiss ADTKD registry (n = 275)	US ADTKD registry (n = 451)	n (BE-CH/US)
Number of families	585	156	429	
Sex, female	332/726 (46)	115/275 (42)	217/451 (48)	
Age at presentation (yr)	45 (31, 58)	34 (22, 49)	49 (37, 62)	174/377
Positive family history, gout and/or CKD	625/679 (92)	191/227 (84)	434/451 (96)	
eGFR at diagnosis (ml/min)	44.3 ± 30.0	45.1 ± 20.9	43.8 ± 34.3	137/229
CKD	492/586 (84)	205/258 (80)	287/328 (88)	
ESKD	216/503 (43)	70/258 (27)	146/245 (60)	
Age at ESKD (yr)	44 (32, 55)	44 (33, 56)	44 (32, 55)	245/146
Serum uric acid (μmol/l)	472 ± 141	479 ± 145	455 ± 128	173/74
Female	452 ± 149	457 ± 158	443 ± 129	67/33
Male	485 ± 134	494 ± 135	464 ± 129	106/41
Gout	305/461 (66)	130/218 (60)	175/243 (72)	
Female	98/256 (38)	40/91 (44)	58/165 (35)	
Male	207/305 (68)	90/127 (71)	117/178 (66)	
Age at gout onset (yr)	30 (20, 45)	31 (20, 47)	30 (21, 40)	235/160
Female	35 (22, 50)	40 (23, 56)	35 (22, 50)	98/55
Male	28 (20, 40)	30 (20, 41)	28 (20, 40)	135/105
UMOD mutations	216/562 (38.4)	54/156 (34.6)	162/406 (40.0)	

ADTKD, autosomal dominant tubulointerstitial kidney disease; BE-CH, Belgo-Swiss; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease.
Quantitative parameters are presented as medians (interquartile ranges) or means ± SD. Qualitative parameters are presented as fractions with percentages. n (BE-CH/US) denotes the number of patients from the Belgo-Swiss and US registries analyzed for the respective parameter.

Clinical characteristics of ADTKD-*UMOD* and ADTKD-*MUC1*

The size of the International ADTKD Cohort allowed us to analyze the clinical characteristics of ADTKD-*UMOD* and ADTKD-*MUC1* subtypes (Figure 3). Age at presentation (first patient contact) was earlier (median: 42 years [interquartile range (IQR): 27, 53] vs. 47 years [IQR: 37, 57], $P = 0.005$) and a positive family history of CKD and/or gout was more frequent (95% vs. 86%, $P = 0.007$) in patients with ADTKD-*UMOD* than in patients with ADTKD-*MUC1*. While the overall prevalence of CKD was significantly higher in patients with ADTKD-*UMOD*, ESKD was significantly more prevalent (44% vs. 58%, $P = 0.04$) and of earlier onset (median: 46 years [IQR: 39, 57] vs. 36 years [IQR: 30, 46], $P < 0.001$) in patients with ADTKD-*MUC1* (Figure 3b, upper panel). Conversely, the prevalence of gout was significantly higher (79% vs. 26%, $P < 0.001$) and gout onset was significantly earlier (median: 27 years [IQR: 19, 37] vs. 45 years [IQR: 29, 51], $P = 0.001$) in patients with ADTKD-*UMOD* (Figure 3b, lower panel). These findings were generally consistent in both sexes. In patients with ADTKD-*UMOD*, gout onset was significantly earlier in men than in women (median: 26 years [IQR: 18, 34] vs. 30 years [IQR: 21, 43], $P = 0.013$) (Figure 3a).

The key differences in terms of renal function and uric acid handling were substantiated by survival curves depicting freedom from ESKD and gout (Figure 4). Renal survival was significantly shorter in ADTKD-*MUC1* than in ADTKD-*UMOD* (median: 54 years, 95% confidence interval [CI]: 51.5–56.5 in ADTKD-*UMOD* vs. 46 years, 95% CI: 39.3–52.7 in ADTKD-*MUC1*, log rank test: $P = 0.013$) (Figure 4a). Conversely, gout-free survival was dramatically shorter in ADTKD-*UMOD* than in ADTKD-*MUC1* (median: 30 years, 95% CI: 27.3–32.7 in ADTKD-*UMOD* vs. 67 years, 95% CI: 57.9–76.1 in ADTKD-*MUC1*, log rank test: $P < 0.001$) (Figure 4b).

Among patients with ADTKD-*UMOD*, carriers of missense mutations involving cysteines (either by substituting a cysteine residue by another amino acid or by inserting a new cysteine) did not experience a worse prognosis in terms of onset of ESKD or age of gout onset when compared with patients with non-cysteine-involving ADTKD-*UMOD* (Supplementary Figure S2).

Comparing ADTKD-*UMOD* with ADTKD–NOS (not otherwise specified, i.e., no mutation detected) in the US ADTKD registry, we found that CKD (94.0% vs. 82.7%, $P < 0.001$) and ESKD (46.5% vs. 26.2%, $P < 0.001$) were more prevalent and the estimated glomerular filtration rate (eGFR) at diagnosis lower (34.7 ml/min vs. 48.1 ml/min, $P < 0.001$) in ADTKD-*UMOD* versus ADTKD–NOS, respectively. Similarly, CKD and ESKD were more prevalent in ADTKD-*MUC1* than in ADTKD–NOS (86.4% vs. 82.7%, $P < 0.001$ and 54.8% vs. 26.2%, $P < 0.001$, respectively) (Supplementary Table S3). These findings suggest a more severe kidney phenotype in ADTKD-*UMOD* and ADTKD-*MUC1* than in ADTKD cases without genetic diagnosis—a finding confirmed in the Belgo-Swiss registry.

UMOD biology in ADTKD-*UMOD* and ADTKD-*MUC1*

Given the colocalization of MUC1 with UMOD in the kidney tubule[6] and the fact that MUC1fs accumulates in several tissues without causing extrarenal manifestations,[7] we tested the hypothesis that MUC1fs might interact with UMOD processing in the thick ascending limb (TAL) in ADTKD-*MUC1*. We used a validated enzyme-linked immunosorbent assay[21] to assess the

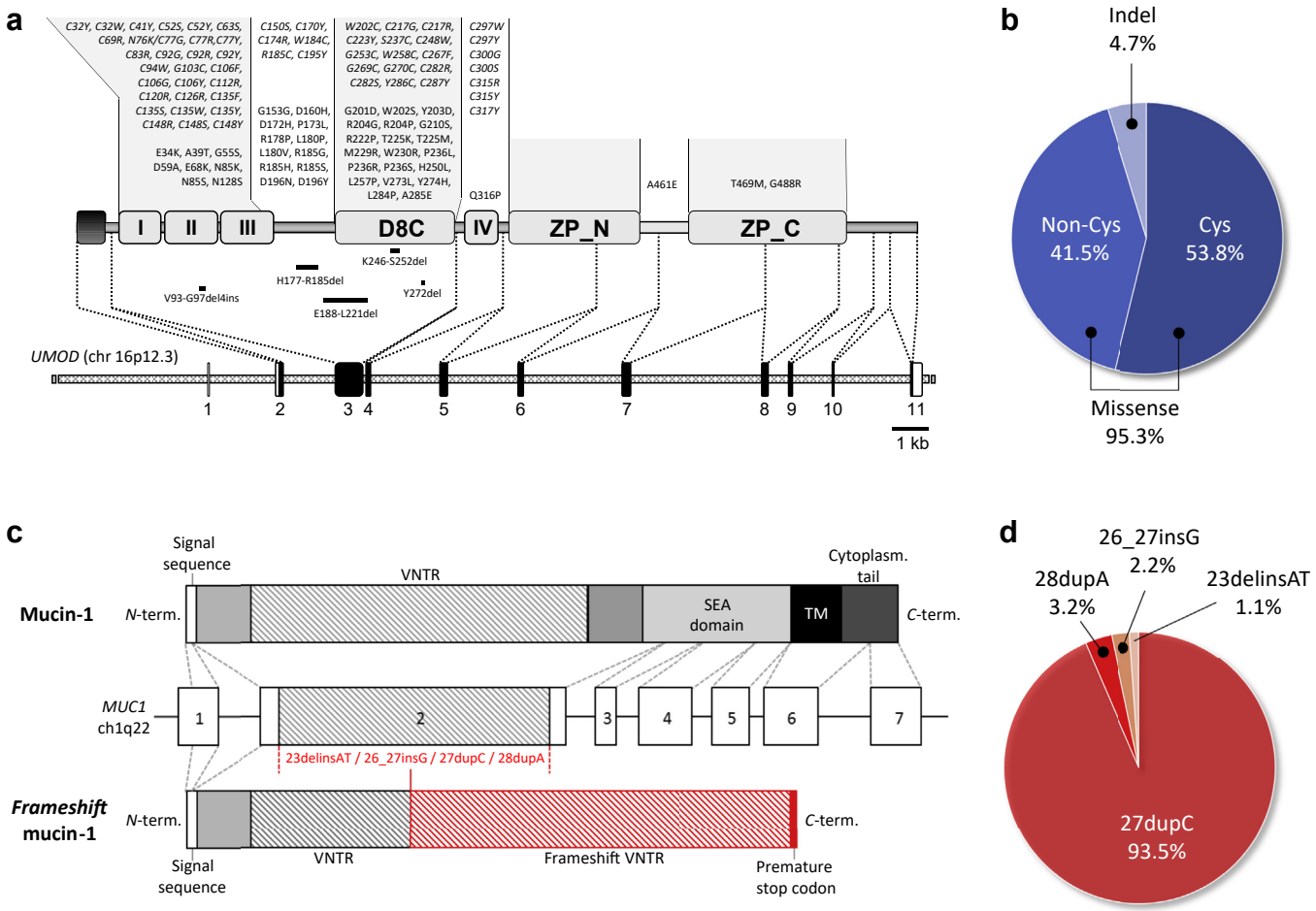

Figure 2 | Spectrum of mutations in *UMOD* and *MUC1*. (**a**) *UMOD* gene and protein domain structure with the 106 *UMOD* mutations reported in the international cohort depicted relative to domain localization. Mutations involving cysteine residues are indicated in italics, on top of each box. (**b**) The prevalence of different *UMOD* mutations: missense mutations (101 of 106; 95.3%), affecting cysteine ([Cys]; 57 of 106; 53.8%) or noncysteine (44 of 106; 41.5%) amino acids and indels (5 of 106; 4.7%). (**c**) *MUC1* gene exon-intron structure (middle panel) and normal protein structure (above) with the 4 detected mutations (in red) in the variable number tandem repeat (VNTR) domain and the consequence on protein structure (below). (**d**) Prevalence of identified *MUC1* mutations in reported families with autosomal dominant tubulointerstitial kidney disease (ADTKD)–*MUC1*. SEA, self-cleavage module; term, terminal; TM, transmembrane domain.

levels of urinary UMOD in a population-based cohort (Cohorte Lausannoise), confirming the positive correlation between urinary UMOD (mg/g creatinine) and eGFR between 15 and 90 ml/min per 1.73 m^2 (test for linear trend: $P = 0.001$) (Supplementary Figure S3A), as previously described.[22] Normalizing urinary UMOD for eGFR (by dividing UMOD concentrations by urinary creatinine and by eGFR) mitigated the linear dependency (test for linear trend: $P = 0.54$) (Supplementary Figure S3B), allowing a more robust comparison of urinary UMOD levels between patients and controls. We next measured urinary UMOD levels in patients with ADTKD-*MUC1* and ADTKD-*UMOD* compared with levels in controls ($n = 180$) from the population-based cohort strictly matched for eGFR (45–60 ml/min per 1.73 m^2). In contrast to patients with ADTKD-*UMOD*, who showed strongly reduced urinary UMOD levels (median: 2.8 vs. 14.7 mg/g creatinine, $P < 0.0001$), patients with ADTKD-*MUC1* showed urinary levels of UMOD similar to those of controls (median: 15.7 vs. 14.7 mg/g creatinine, $P = 0.99$) (Figure 5a, left panel). Normalizing urinary UMOD levels to eGFR [(mg/g creatinine)/eGFR] confirmed strongly reduced levels in patients with ADTKD-*UMOD* versus in controls ($n = 2717$) with eGFR spanning 15 to 90 ml/min per 1.73 m^2 {[(0.05 vs. 0.23 mg/g creatinine)/eGFR], $P < 0.0001$, respectively}, in contrast with unchanged levels in patients with ADTKD-*MUC1* versus in controls {[(0.29 vs. 0.23 mg/g creatinine)/eGFR], $P = 0.29$, respectively} (Figure 5a, right panel).

Next, we performed immunofluorescence staining for UMOD on kidney biopsies from healthy individuals (normal human kidney), from 2 patients with ADTKD-*UMOD*, and from 2 patients with ADTKD-*MUC1*. While we were able to see the characteristic intracellular UMOD deposits in the patients with ADTKD-*UMOD*, UMOD staining was largely confined to the apical membrane in patients with ADTKD-*MUC1*, similar to the pattern observed in normal kidney (Figure 5b). The accumulation of mutant UMOD in the TAL

a

	ADTKD-*UMOD* (n = 303)	ADTKD-*MUC1* (n = 104)	n (*UMOD*/*MUC1*)	*P* value
Number of families	216	93		
Sex (%)			257/80	1.0
- Female	130 (51)	40 (50)		
- Male	127 (49)	40 (50)		
Age at presentation (yr)	42 (27, 53)	47 (37, 57)	218/78	**0.005**
Positive family history, gout and/or CKD (%)	243/257 (95)	69/80 (86)		**0.007**
eGFR at presentation (ml/min)	39.2 ± 20.3	50 ± 51.9	136/52	0.157
CKD (%)	231/257 (90)	53/80 (66)		**< 0.001**
ESKD (%)	112/257 (44)	46/80 (58)		**0.04**
- Age at ESKD (yr)	46 (39, 57)	36 (30, 46)	224/80	**< 0.0001**
- Female	44 (40, 55)	34 (28, 46)	117/40	**0.002**
- Male	50 (39, 58)	39 (32, 47)	107/40	**0.007**
				#0.349
				$0.46
Serum uric acid (µmol/l)	497.9 ± 136.6	443.6 ± 121.7	110/14	0.159
- Female	478.7 ± 133.2	418.7 ± 136.1	53/5	0.341
- Male	515.7 ± 138.5	457.4 ± 119.2	57/9	0.237
				#0.158
				$0.590
Gout (%)	202/257 (79)	21/80 (26)		**< 0.001**
- Female	96/130 (74)	4/40 (10)		**< 0.001**
- Male	106/127 (83)	17/40 (43)		**< 0.001**
				#0.069
				$0.001
Age at gout onset (yr)	27 (19, 37)	45 (29, 51)	199/18	**0.001**
- Female	30 (21, 43)	28 (21, 41)	93/4	0.828
- Male	26 (18, 34)	45 (33, 54)	106/14	**< 0.001**
				#0.013
				$0.10

b

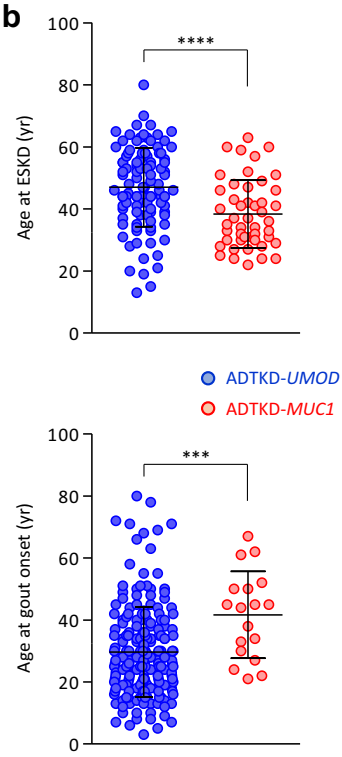

Figure 3 | Clinical characteristics of autosomal dominant tubulointerstitial kidney disease (ADTKD)–*UMOD* and ADTKD-*MUC1*. (**a**) Quantitative parameters are presented as medians and interquartile ranges or means ± SD. Qualitative parameters are presented as fractions with percentages. χ^2 test for categorial variables and Mann-Whitney *U* test and unpaired *t* test for quantitative parameters were used. #,$Sex comparison within ADTKD-*UMOD* and ADTKD-*MUC1*, respectively. The *n* (*UMOD*/*MUC1*) column denotes the number of patients with ADTKD-*UMOD* and ADTKD-*MUC1* analyzed for the respective parameter. (**b**) Scatter plots for age at end-stage kidney disease (ESKD) and onset of gout for patients with ADTKD-*UMOD* and ADTKD-*MUC1*. Bars indicate means ± SD. ***$P < 0.001$, ****$P < 0.0001$. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

cells from patients with ADTKD-*UMOD* induced ER stress, as shown by colocalization with the unfolded protein response regulator glucose-regulated protein 78 ([GRP78], also known as binding Ig protein). Conversely, GRP78 could not be detected in the TALs of patients with ADTKD-*MUC1* (Figure 5b; Supplementary Figure S4).

Establishment of a clinical *UMOD*-score in the Belgo-Swiss ADTKD registry

Based on the Belgo-Swiss ADTKD registry with detailed phenotyping, including 54 families that are *UMOD*-positive ($n = 132$ patients) and 102 families that are *UMOD*-negative ($n = 143$ patients) (Figure 1; Supplementary Figure S5), we designed a clinical score to estimate the probability of ADTKD-*UMOD*. Clinical characteristics in patients with ADTKD with or without *UMOD* mutations guided the scoring system (Supplementary Figure S6). Compared with patients who are *UMOD*-negative, patients with a *UMOD* mutation had a more frequent family history of CKD and/or gout (90% vs. 76%, $P < 0.001$); a higher prevalence of CKD (83% vs. 75%, $P = 0.03$) and ESKD (33% vs. 20%, $P = 0.02$), with earlier onset of CKD (median: 32 years vs. 42 years, $P = 0.002$) and ESKD (median: 42 years vs. 48 years, $P = 0.007$); a higher level of serum uric acid (mean: 507.0 ± 131 vs. 454.5 ± 153.4 µmol/l, $P = 0.017$); and an earlier onset of gout (median: 24 years vs. 33 years, $P = 0.001$). Of note, the prevalence of renal cysts, as detected by sonography and/or computed tomography or magnetic resonance imaging, was lower in patients with ADTKD-*UMOD* compared with in patients who were *UMOD*-negative (36% vs. 57%, $P = 0.001$) (Supplementary Figure S6).

The weighted *UMOD*-score was developed on 8 items using these discriminative clinical, biochemical, histological, and imaging characteristics of ADTKD-*UMOD* (Figure 6a). The maximal item value of +3 points was attributed to gout before 30 years and uricemia >500 µmol/l, which are the most specific discriminants (Supplementary Figure S6). Because the prevalence of CKD and autosomal dominant inheritance was higher in ADTKD-*UMOD*, these criteria were weighted with +2 points. Clinical findings suggesting an alternative diagnosis (e.g., proteinuria, uncontrolled hypertension) were attributed negative points. Values for each available item are added to obtain a final additive score for each patient. The clinical *UMOD*-score was applied on patients with ADTKD from the Belgo-Swiss registry, for which information for at least 5 of the 8 items were present ($n = 211$ patients: 106 *UMOD*-positive and 105 *UMOD*-negative). The receiver-operating characteristics curve, with *UMOD*

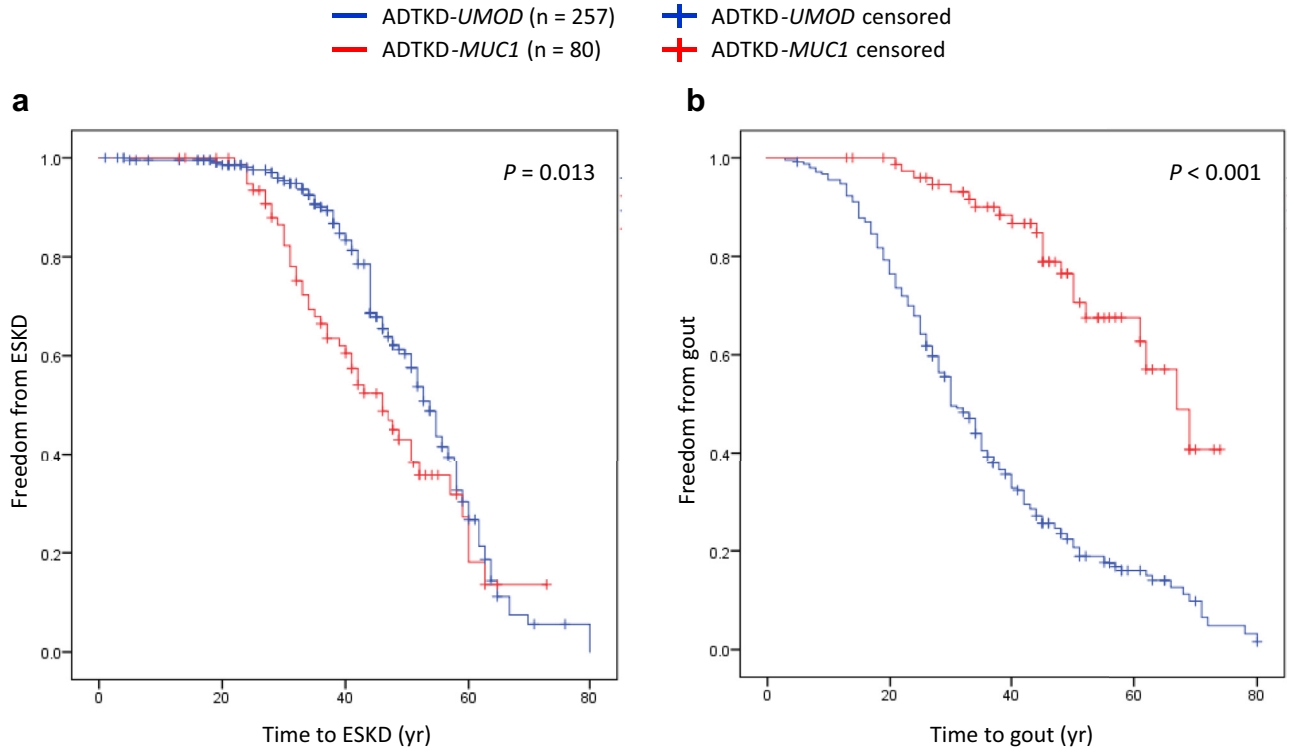

Figure 4| Freedom from end-stage kidney disease (ESKD) and gout in autosomal dominant tubulointerstitial kidney disease (ADTKD)–*UMOD* and ADTKD-*MUC1*. (**a**) Kaplan-Meier curve of renal survival in patients with ADTKD-*UMOD* and ADTKD-*MUC1*. Median renal survival was 54 years (95% confidence interval [CI]: 51.5–56.5) in ADTKD-*UMOD* and 46 years (95% CI: 39.3–52.7) in ADTKD-*MUC1*. (**b**) Kaplan-Meier gout-free survival curve in patients with ADTKD-*UMOD* and ADTKD-*MUC1*. Median gout-free survival was 30 years (95% CI: 27.3–32.7) in ADTKD-*UMOD* and 67 years (95% CI: 57.9–76.1) in ADTKD-*MUC1*. Log rank test was used. Censored: event of interest has not occurred during the follow-up time.

mutation status as the dependent variable yielded an area under the curve (AUC) of 0.72 (95% CI: 0.66–0.79, $P < 0.001$) (Figure 6b). The *UMOD*-score cut-off of ≥5 was selected, yielding a sensitivity of 98.1% and specificity of 41.4% for positive *UMOD* mutation testing, corresponding to a negative predictive value (NPV) of 94.3% and a positive predictive value (PPV) of 59.1% (Figure 6c; Supplementary Table S4). This cut-off also proved to be optimal for group discrimination corresponding to a Youden index (sensitivity + specificity − 1) of 0.395 (Supplementary Table S4).

UMOD-score and urine UMOD levels to guide genetic testing in ADTKD

The score was validated in patients that were *UMOD*-positive ($n = 124$) and *UMOD*-negative ($n = 183$) from the US ADTKD registry, yielding similarly high sensitivity and low specificity for *UMOD* mutations using a cut-off of ≥5 (sensitivity: 97.6%, specificity: 16.4%, NPV: 91.0%, PPV: 44.2%, data not shown), altogether making ADTKD-*UMOD* very unlikely for score results <5. We tested how the clinical score separated the 2 most common etiologies of ADTKD in a subset of patients with ADTKD-*UMOD* ($n = 125$) and ADTKD-*MUC1* ($n = 80$) from the US registry for which at least 5 of the 8 clinical items and/or urinary UMOD levels were available. The clinical *UMOD*-score alone separated the 2 entities with an AUC of 0.69 (95% CI: 0.62–0.77, $P = 0.037$) (Figure 7a, left panel). However, the specificity for *UMOD* increased considerably with higher *UMOD*-score values (for instance, a score ≥8 had a sensitivity of 48.8%, a specificity of 83.7%, an NPV of 50.8% and a PPV of 81.3% for *UMOD* mutation) (Supplementary Table S5). Only a few patients, mostly those with ADTKD-*MUC1*, had score results of <5 (Figure 7a, right panel).

We next investigated whether addition of urinary UMOD levels to the clinical score improved its ability to discriminate ADTKD-*UMOD* from ADTKD-*MUC1*. Based on the normalized urinary UMOD values in the reference population [(mg/g creatinine)/eGFR] (Figure 5a, right panel), we assigned, respectively, +1 and +3 points for urinary UMOD values between the median and 25th percentile [(0.14–0.23 mg/g creatinine)/eGFR] and below the 25th percentile. Similarly, we assigned, respectively, −1 and −3 points for urinary UMOD values between the median and 75th percentile [(0.23–0.35 mg/g creatinine)/eGFR] and above the 75th percentile. Applied to a cohort of 51 patients with ADTKD-*UMOD* and 35 patients with ADTKD-*MUC1* for which urinary UMOD data were available, this combined clinical and biochemical score separated ADTKD-*UMOD* from ADTKD-*MUC1* with an improved AUC of 0.89 (95%

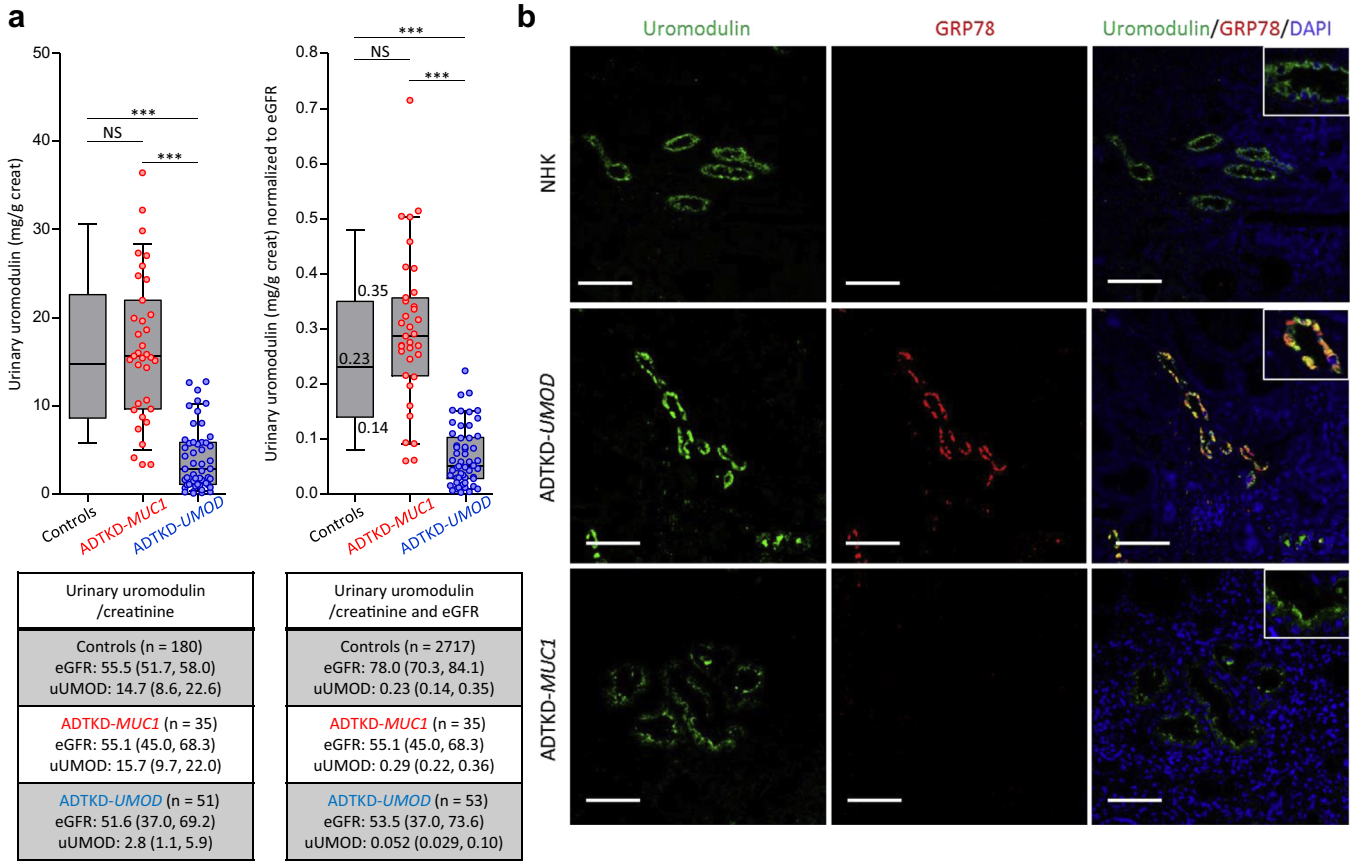

Figure 5 | UMOD processing in autosomal dominant tubulointerstitial kidney disease (ADTKD)–*UMOD* and ADTKD-*MUC1*. (**a**) Urinary UMOD excretion normalized to urinary creatinine (creat) (left panel) and normalized to urinary creatinine and estimated glomerular filtration rate (eGFR) (right panel) in patients with ADTKD-*MUC1*and ADTKD-*UMOD* and a reference population (controls). Median, 25th percentile, and 75th percentile values in the reference population are indicated (right panel). Numerical values (medians and interquartile ranges) for urinary UMOD (uUMOD), eGFR, and sample size are below the graph. Outlier removed with GraphPad (*ROUT Q* = 1%), 1-way analysis of variance $P < 0.0001$ for both graphs, and Tukey's multiple comparison test was applied with not significant (NS) and ***$P < 0.0001$. (**b**) Immunofluorescence staining for UMOD (green) and glucose-regulated protein 78 (GRP78; red) in ADTKD-*MUC1*, ADTKD-*UMOD*, and normal human kidney (NHK) biopsy. Bars = 50 μm. DAPI, 4′,6-diamidino-2-phenylindole. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

CI: 0.82–0.96, $P < 0.001$). The cut-off value of ≥5 still appears as the optimal cut-off value to discriminate ADTKD-*UMOD* from ADTKD-*MUC1* (Youden index: 0.684) with a sensitivity of 94.1%, a specificity of 74.3%, an NPV of 89.7%, and a PPV of 84.2% for a *UMOD* mutation (Figure 7b; Supplementary Table S5). Based on the clinical and biochemical *UMOD*-score, we suggest a diagnostic algorithm to guide genetic testing in ADTKD (Figure 8).[1,23,24]

DISCUSSION

This international cohort study represents the largest dataset of patients with ADTKD-*UMOD* and ADTKD-*MUC1* reported to date, providing new insights into the phenotype and disease progression of the main subtypes of ADTKD. Because of the autosomal dominant inheritance and regional familial clustering, considerable differences in the prevalence of ADTKD subgroups are mentioned in national cohorts.[2,15,20] In the International ADTKD Cohort, ADTKD-*UMOD* represents the most frequent subtype of ADTKD with an estimated prevalence of 37.1%, followed by ADTKD-*MUC1* in 35.1% of families that are *UMOD*-negative, and an estimated overall prevalence of 21.0%. Of note, a systematic effort to screen for mutations in *HNF1B, REN, DNAJB11,* and *SEC61A1* is ongoing in the 133 families that are *UMOD*- negative and *MUC1*-negative and for mutations in *MUC1* in the 141 families that are *UMOD*-negative in the registry.

Based on the large sample size, we observed distinct features in the clinical presentation of ADTKD-*UMOD* and ADTKD-*MUC1*, with relevance for clinical practice and patient counselling. Kidney disease appears more severe in patients with ADTKD-*MUC1*, with a higher prevalence of ESKD (58% vs. 44% in ADTKD-*UMOD*, $P = 0.04$), an earlier onset of ESKD (36 years vs. 46 years in ADTKD-*UMOD*, $P < 0.001$), and a shorter median renal survival (46 years vs. 54 years in ADTKD-*UMOD*, $P = 0.013$). Previous studies reported an older age at ESKD (mean: 44.9 years) in patients with ADTKD-*MUC1*,[8] which could be explained by inclusion

a

Clinical *UMOD*-score		
Characteristics	Item	Value
Family history of CKD or early gout (<40 yr) compatible with autosomal dominant inheritance		+ 2
CKD of unknown origin[a]		+ 2
Age at gout onset	<30 yr	+ 3
	>30 yr	+ 1
Serum uric acid	>500 µmol/l (>8.41 mg/dl)	+ 3
	<500 µmol/l (<8.41 mg/dl)	+ 1
Histological findings compatible with ADTKD[b]		+ 2
Proteinuria/hematuria[c]		− 1
Diabetes/uncontrolled hypertension[d]		− 1
Renal cysts/enlarged kidneys[e]		− 1

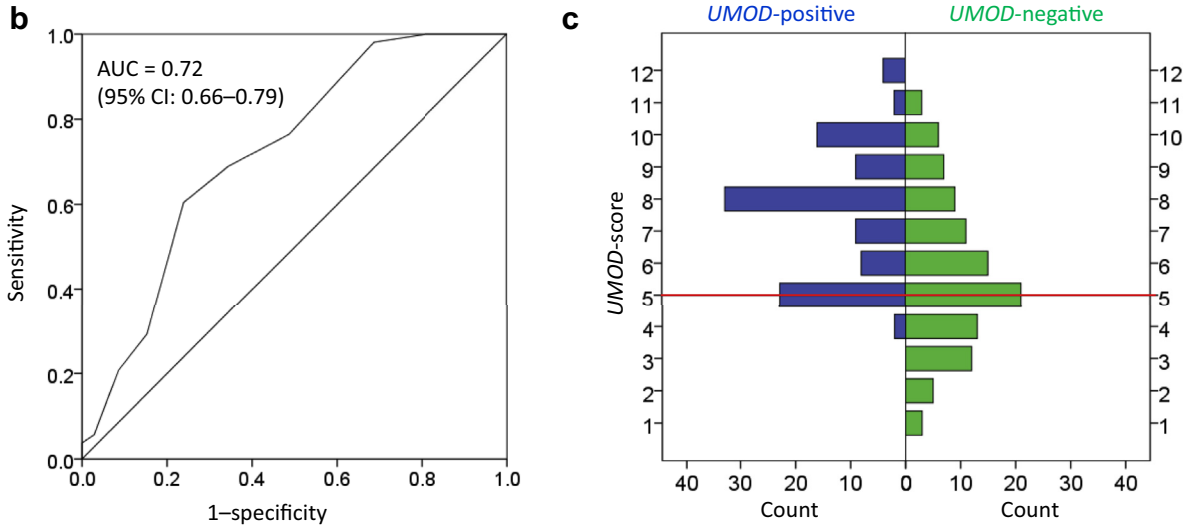

Figure 6 | Clinical *UMOD*-score and performance in the Belgo-Swiss ADTKD registry. (**a**) Clinical *UMOD*-score based on clinical, biochemical, histological, and imaging data. Attributed points for specific characteristics are shown on the right. [a]After routine work-up including urinary sediment and urinalysis and kidney imaging. [b]Interstitial fibrosis, tubular atrophy, thickening and lamellation of tubular basement membranes, tubular dilatation (microcysts), negative immunofluorescence for complement, and Igs. [c]Proteinuria >300 mg/dl, persistent hematuria (both eumorphic and dysmorphic) in repeated urinalysis. [d]Hemoglobin A1c >10% or repeated blood pressure measurements > 160/100 mm Hg and/or corresponding clinical findings of hypertensive cardiopathy and/or nephropathy. [e]At least 1 cyst at any location diagnosed by ultrasonography, computed tomography scan, or magnetic resonance imaging. Example: 35-year-old patient, gout onset at 32 years (+1); serum uric acid 550 µmol/l (+3); estimated glomerular filtration rate 55 ml/min per 1.73 m^2, bland urine analysis and sediment, kidneys without cysts and normal size on magnetic resonance imaging, no diabetes or hypertension (+2 for chronic kidney disease [CKD] of unknown origin); and family history of CKD documented on 3 generations (+2) yields a total clinical *UMOD*-score of 8 points. (**b**) Receiver-operating characteristics curve of the clinical *UMOD*-score in the Belgo-Swiss registry ($n = 211$ patients with autosomal dominant tubulointerstitial kidney disease [ADTKD] with available data) are as follows: area under the curve (AUC): 0.72; 95% confidence interval (CI): 0.66–0.79; $P < 0.001$; the cut-off value of ≥5 has a sensitivity of 98.1% and specificity of 41.4% for *UMOD* mutation; negative predictive value: 94.3%; positive predictive value: 59.1%. (**c**) Histogram of clinical *UMOD*-score results in patient who are *UMOD*-positive ($n = 106$) and *UMOD*-negative ($n = 105$). The red horizontal line indicates the cut-off value of 5.

of historically affected patients (clinically affected relatives of genetically diagnosed patients), whereas we only included individuals with an established genetic diagnosis. The heterogeneity of ADTKD-*MUC1* in terms of CKD and/or renal disease progression is intriguing and suggests considerable modifier effects.

Gout has been classically described in patients with *UMOD* mutations. Indeed, our data suggest that gout is strikingly more prevalent and of significantly earlier onset in ADTKD-*UMOD* than in ADTKD-*MUC1*. Defective urinary concentration resulting in polydipsia and polyuria has been described in patients with ADTKD-*UMOD*, most likely because of impaired activity of TAL-based Na^+-K^+-$2Cl^-$-cotransporter.[15,17] Plasma volume contraction and compensatory higher reabsorption activity of the proximal tubule including upregulation of Na^+-coupled urate transporters most likely explain the hyperuricemia phenotype in ADTKD-*UMOD*.[25,26] A similar mechanism was shown in aged *Umod* knockout mice that displayed reduced activity of the Na^+-K^+-$2Cl^-$-cotransporter.[26] Even though ADTKD-*MUC1* presumably originates from the distal tubule, gout was considerably less prevalent in this disorder.

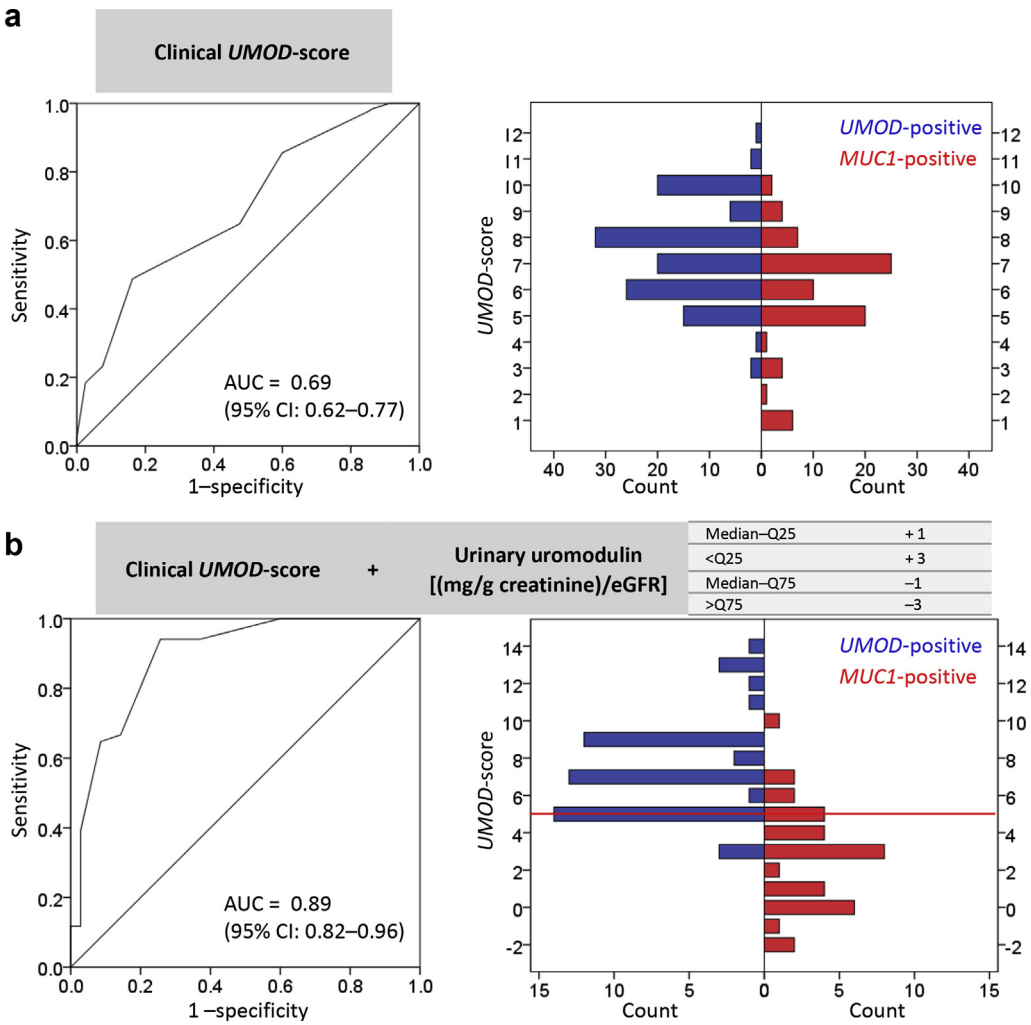

Figure 7 | *UMOD*-score comparing autosomal dominant tubulointerstitial kidney disease (ADTKD)–*UMOD* versus ADTKD-*MUC1* in the US ADTKD registry. (**a**, left panel) Receiver-operating characteristics curve of the clinical *UMOD*-score in the US registry ($n = 205$ patients with ADTKD-*UMOD* and ADTKD-*MUC1* with available data) are as follows: area under the curve (AUC): 0.69; 95% confidence interval (CI): 0.62–0.77; $P < 0.037$. A cut-off value of ≥8 has a sensitivity of 48.8% and specificity of 83.7% for *UMOD* mutations, while a cut-off value of ≥5 has a sensitivity of 97.6% and specificity of 15.0% for *UMOD* mutations. (**a**, right panel) Histogram of clinical *UMOD*-score results in patients with ADTKD-*UMOD* ($n = 125$) and ADTKD-*MUC1* ($n = 80$). (**b**, left panel) Receiver-operating characteristics curve of the clinical *UMOD*-score including urine UMOD levels in the US registry ($n = 86$ patients with ADTKD-*UMOD* and ADTKD-*MUC1* with available urinary UMOD data) are as follows: AUC: 0.89; 95% CI: 0.82–0.96; $P < 0.001$. The cut-off value of ≥5 has the highest Youden index for discrimination (0.684) and has a sensitivity of 94.1% and specificity of 74.3% for *UMOD* mutation; negative predictive value: 89.7%; positive predictive value: 84.2%. (**b**, right panel) Histogram of clinical + urinary *UMOD*-score results in patients with ADTKD-*UMOD* ($n = 51$) and ADTKD-*MUC1* ($n = 35$). The red horizontal line indicates the cut-off value of 5. Q, quartile.

We investigated 2 cardinal biological features described in ADTKD-*UMOD* with likely pathophysiological relevance: aberration in UMOD export mechanisms and induction of ER stress. Based on the observation that MUC1 is expressed in the distal kidney tubule including the TAL where it colocalizes with UMOD[6] and on the observation that MUC1fs is accumulating in other MUC1-expressing tissues (skin, breast, lung, colon) without causing extrarenal manifestations,[7] one could hypothesize that MUC1fs might interact with UMOD in TAL. Yet, in contrast to ADTKD-*UMOD*, we found no difference in the urinary level of UMOD between patients with ADTKD-*MUC1* and the normal population. Furthermore, analysis of *MUC1*-mutant kidney biopsies revealed a normal distribution of UMOD in TAL cells, without evidence for ER stress (GRP78 expression), which is a hallmark of ADTKD-*UMOD*. These novel findings suggest that the processing of UMOD is not altered in ADTKD-*MUC1* and that ER stress is not a main finding in ADTKD-*MUC1*. Along the same line, a recent study found entrapment of MUC1fs in vesicles of the early secretory pathway in models of ADTKD-*MUC1*.[19]

Previous reports described intracellular accumulation of UMOD in kidney biopsies from patients with ADTKD-*UMOD*.[1,2] However, such staining is not available in a large number of patients, preventing us from speculating on its value in clinical decision making. In our experience, the

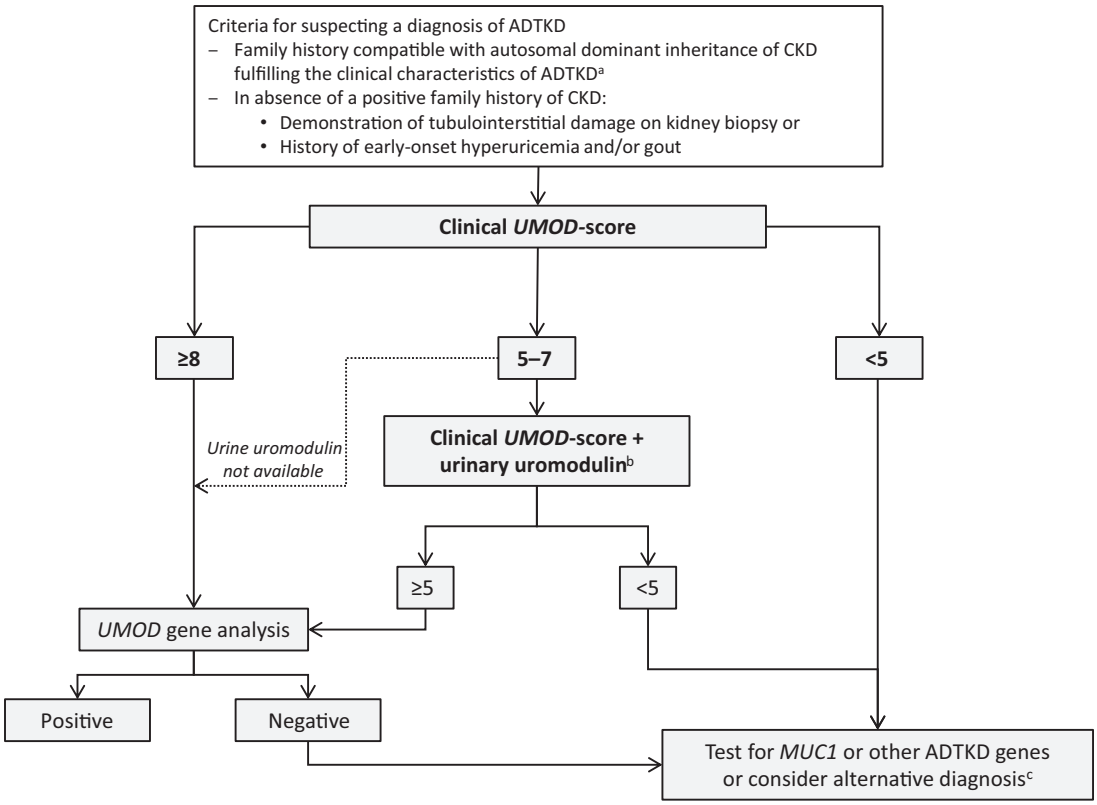

Figure 8 | Diagnostic algorithm for suspected autosomal dominant tubulointerstitial kidney disease (ADTKD) based on clinical *UMOD*-score and urinary UMOD levels. [a]Progressive loss of renal function, bland urinary sediment, normal-to-mild albuminuria and/or proteinuria, normal-sized kidneys on ultrasound, and no consumption of drugs linked to tubulointerstitial nephritis. [b]Assessed by validated enzyme-linked immunosorbent assay and normalized to urinary creatinine and estimated glomerular filtration rate. Obtained values should be interpreted against family members who are *UMOD*-negative or reference populations.[23,24] See Results and Discussion sections for more details. [c]For diagnostic algorithm including other ADTKD genes, refer to Devuyst *et al.*[1] Alternative diagnoses include nephronophthisis (autosomal recessive), autosomal dominant polycystic kidney disease (large cystic kidneys), autosomal dominant glomerulopathies (proteinuria and/or hematuria), other causes of tubulointerstitial kidney disease (autoimmune, tubulointerstitial nephritis, and uveitis syndrome) including drugs and toxins (nonsteriodal anti-inflammatory drugs, aristolochic acid, calcineurin inhibitors, lithium). CKD, chronic kidney disease.

UMOD staining is operator-dependent, requiring rigorous positive and negative controls, and it might depend on the underlying *UMOD* mutation. Furthermore, the availability of kidney biopsies is restricted. The assessment of urinary UMOD levels in patients at time of diagnosis and during disease progression might offer a noninvasive diagnostic tool and biomarker in ADTKD-*UMOD*. Because urinary UMOD levels show a positive correlation with eGFR (for eGFR <90 ml/min per 1.73 m^2) and tubular mass,[23,24] they need to be normalized for residual eGFR and interpreted against matched controls. Based on data from a large control cohort, we show here that urinary UMOD (in mg/g creatinine to account for urine concentration) normalized for eGFR can be applied in the clinical setting of ADTKD.

A recent study based on exome sequencing reported mutations in *UMOD* accounting for ~3% of all patients with a genetic finding in this cohort.[13] However, considerable hurdles in the diagnostic approach of ADTKD subtypes persist. These include but are not limited to (i) limited availability of *MUC1* testing due to technical challenges, (ii) lack of validated diagnostic or genetic algorithm due to unappreciated clinical differences between ADTKD subtypes, and (iii) missing disease biomarkers due to small and scattered disease cohorts. For everyday practice and cost-effectiveness, practical tools such as scoring systems are very useful to guide genetic testing.[1] The Belgo-Swiss registry was instrumental in delineating a clinical *UMOD*-score because it revealed key discriminatory clinical features, including positive family history of CKD and/or gout, age at presentation, prevalence of kidney disease and progression to ESKD, and history of gout. Of interest, renal cysts are less common in patients with ADTKD-*UMOD*, which is in line with previous studies.[8,15,20] The delineated clinical *UMOD*-score showed an excellent NPV for *UMOD* mutations (cut-off ≥5) in the Belgo-Swiss (NPV: 94.3%) and US (NPV: 91.0%) registries. As ADTKD-*UMOD* and ADTKD-*MUC1* present considerable clinical overlap, we were not surprised that the clinical *UMOD*-score separated modestly between these 2 entities (AUC: 0.69). Yet, higher *UMOD*-score values showed a solid specificity for *UMOD* mutations (e.g., cut-off ≥8: specificity of 83.7% and

PPV of 81.3% for an *UMOD* mutation). Adding urinary UMOD measurements, a pathophysiological biomarker for ADTKD-*UMOD*, considerably increased the discriminating power of the score (AUC: 0.89) with a PPV of 84.2% for an *UMOD* mutation (cut-off ≥5 points). Because the progression of kidney disease and the prevalence and onset of gout seem dependent on the underlying genetic diagnosis, a genetic diagnosis is recommended as it might impact on the management of patients with ADTKD (e.g., follow-up, scheduling of renal transplantation, and gout-preventive strategies). Furthermore, targeted therapies might be in reach at least for ADTKD-*MUC1*.

The limits of this study include the retrospective "real-life" cohort design of consecutively recruited patients, with inherent difficulties such as limited access to full clinical information, missing DNA samples for further genetic testing, and lack of strict inclusion and exclusion criteria. We included all genetically resolved cases of a given family, potentially introducing the risk for selection bias. However, we estimate that this represents a negligible risk as in general only 1 to 2 patients were included per family and considerable intrafamilial clinical variability exists in ADTKD.[8,20] Because kidney biopsies are rarely performed in these diseases and yield nonspecific findings (e.g., interstitial fibrosis, tubular atrophy), we did not include histopathology information in the analysis. A survey of histopathology results from the Belgo-Swiss registry showed that interstitial fibrosis with tubular atrophy (in ∼60% of available pathology reports) and interstitial nephritis (in ∼40% of available pathology reports) were the preponderant histological findings in patients with ADTKD-*UMOD* and those who were *UMOD*-negative. A more detailed histological description of biopsies performed in ADTKD-*UMOD* and ADTKD-*MUC1* warrants a dedicated analysis.

It should be pointed out that systematic screening for *UMOD* mutations in all 10 coding exons has only been performed in a subset of patients with ADTKD. Based on previous screens and whole exome sequencing, we estimate that very few *UMOD* mutations outside exons 3 and 4 might have been missed in ADTKD-*UMOD*.[13,15] Furthermore, large deletions or insertions in *UMOD* are not detected by direct sequencing methods. With the availability of gene panel testing and next-generation sequencing approaches, the utility of a clinical score in directing targeted gene testing will probably decrease. However, at the current stage, *MUC1* mutations are missed by next-generation sequencing and availability of specialized testing is limited. To the best of our knowledge, clinical-grade genetic testing for *MUC1* is only performed by the Broad Institute. For these reasons, we estimate that simple clinical and biochemical tools to estimate pretest probability impacts on diagnostic work-up and potentially reduces the costs associated with unjustified genotyping.

In conclusion, this large international retrospective cohort study provides a detailed phenotype analysis of patients with ADTKD-*UMOD* and ADTKD-*MUC1*. The clinical hallmarks of the 2 most common ADTKD subtypes are hyperuricemia and early gout in ADTKD-*UMOD* and a heterogeneous, but generally more severe kidney disease in ADTKD-*MUC1*. The clinical *UMOD*-score is a sensitive and, coupled to urinary UMOD levels, potentially specific tool to select patients for genetic *UMOD* testing. These results should help clinicians to improve diagnostic rates, clinical management, and patient counselling in ADTKD.

METHODS

International ADTKD Cohort

The International ADTKD Cohort consists of patients from the Belgo-Swiss ADTKD registry and the US ADTKD registry. The inclusion criteria were those defined by the Kidney Disease: Improving Global Outcomes consensus[2] and included the following in any combination: a family history compatible with autosomal dominant inheritance of CKD with features of ADTKD, including progressive loss of kidney function, bland urinary sediment, absent-to-mild albuminuria and/or proteinuria, normal-sized or small-sized kidneys on ultrasound; and/or (in absence of a positive family history of CKD) a history of early-onset hyperuricemia and/or gout and/or the presence of interstitial fibrosis and/or tubular atrophy on kidney biopsy. Exclusion criteria included the following: a different genetic diagnosis (non-ADTKD), the presence of enlarged cystic kidneys, proteinuria (>1 g/24 h) and/or consistent hematuria, long-standing or uncontrolled diabetes mellitus or arterial hypertension, and the consumption of drugs linked to tubulointerstitial nephritis. Only patients screened for mutations of *UMOD* and/or *MUC1* were included in the cohort. Anonymized demographics and clinical and genetic information were recorded in a database. This study was approved by the institutional review board of Wake Forest School of Medicine), the Université catholique de Louvain (UCL) Medical School, and the European Community's Seventh Framework Programme "European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics).

Belgo-Swiss ADTKD registry. The Belgo-Swiss ADTKD registry includes patients referred to UCLouvain and University of Zurich (UZH) by clinical partners mostly from Europe (Supplementary Appendix S1). In 2019, the registry included 275 patients who had been enrolled since 2003. The clinical data included a family pedigree, onset and evolution of kidney function decline, onset of hyperuricemia and/or gout (age of gout onset was defined as the patient's age at the first episode of gouty arthritis) and fractional excretion of uric acid, imaging and histopathology data (where available), and information on potential extrarenal manifestations (e.g., pancreatic enzymes, liver function tests). ESKD was defined as eGFR<10 ml/min or initiation of renal replacement therapy (dialysis or kidney transplantation).

US ADTKD registry. The US ADTKD registry includes families with tubulointerstitial kidney disease referred to Wake Forest School of Medicine since 1999 (Supplementary Appendix S1). Information collected included demographics, pedigree, age of ESKD, laboratory values, and ultrasound results.

Genetic testing

Informed written consent was obtained from all patients. Genomic DNA was isolated from peripheral blood leukocytes using standard procedures and DNA was stored at 4 °C.

Direct sequencing of *UMOD* exons was initially performed by Sanger sequencing, as previously described.[27] More recently, the

UMOD gene is analyzed by massive parallel sequencing using a tubulopathy gene panel designed by the work package tubulopathies of the European Consortium EURenOmics.[28,29] Mutational analysis was carried out in exons 3 and 4 for all enrolled patients and in all 10 coding exons for a subset of patients.

MUC1 genotyping was performed using a *MUC1* VNTR sequencing approach coupled with a spectrometry-based probe extension assay as previously described.[7,30] *MUC1* testing was provided by the Broad Institute of MIT and Harvard [30] and the First Faculty of Medicine, Charles University.[7] Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_003361.3). Alamut Visual software (Interactive Biosoftware, Rouen, France; www.interactivebiosoftware.com) was used to assist in determining variant pathogenicity. Identified variants were successively checked against relevant databases, such as Clinvar (National Center for Biotechnology Information, Bethesda, MD; https://www.ncbi.nlm.nih.gov/clinvar/), Human Gene Mutation Database (Institute of Medical Genetics in Cardiff, Cardiff, UK; http://www.hgmd.cf.ac.uk/ac/index.php), VarSome (Saphetor, Lausanne, Switzerland; https://varsome.com/), and local databases to assess for previous publication.

Variants were considered disease-causing based on previous reports, family segregation analysis, or prediction algorithms (Sorting Intolerant from Tolerant [SIFT], Align GVD, mutation taster, and Polymorphism Phenotyping v2 [PolyPhen-2]) for pathogenicity.

The variants were classified according to the guidelines published by the American College of Medical Genetics in 2015.[31] Variants of interest were verified by Sanger sequencing.

Measurements of urinary levels of UMOD

A validated enzyme-linked immunosorbent assay method was used to measure urinary UMOD levels (second morning urine sample) from 86 patients with ADTKD.[21] Urinary creatinine was measured using a Synchron DXC800 analyzer (Beckman Coulter, Fullerton, CA). The reference samples ($n = 2717$) were obtained from the Cohorte Lausannoise, a population-based study including 6000 people 35 to 75 years of age from the city of Lausanne, Switzerland.[22] eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation. Informed consent was obtained from all participating individuals.

UMOD expression constructs

cDNA of human wild-type UMOD was cloned in pcDNA 3.1(+) (Thermo Fisher Scientific, Waltham, MA) and a hemagglutinin tag was inserted after the leader peptide in between T26 and S27 in the protein sequence.[32] The C150S and L284P mutant isoforms were obtained by mutagenesis using the QuikChange Lightning mutagenesis kit (Agilent, Santa Clara, CA) following the manufacturer's instructions. Primers were designed using the software QuikChange Primer Design Program (Agilent). Primers used for mutation C150S: forward (5′→3′) gatggcactgtgagtcctccccgggctcctg, reverse (5′→3′) caggagcccggggaggactcacagtgccac and for mutation L248P: forward (5′→3′) cccgagtgtcacccggcgtactgcaca, reverse (5′→3′) tgtgcagtacgccgggtgacactcggg.

Cell culture conditions

HEK293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 200 U/ml penicillin, 200 µg/ml streptomycin, and 2 mmol/l glutamine at 37 °C, 5% CO_2. HEK293 cells were transfected using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's protocol and analyzed 24 hours after transfection.

Western blot

Cells were lysed in octylglucoside lysis buffer (50 mmol/l Tris-HCl, pH 7.4, 150 mmol/l NaCl, 60 mmol/l octyl β-D-glucopyranoside, 10 mmol/l NaF, 0.5 mmol/l sodium orthovanadate, 1 mmol/l glycerophosphate and protease inhibitor cocktail [Sigma-Aldrich, St. Louis, MO]) for 1 hour at 4 °C followed by 10 minutes of centrifugation at 17,000*g*. Soluble fractions were quantified by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). Western blot experiments were performed as described in Schaeffer *et al.*[32] Antibodies used were mouse purified anti-HA.11 Epitope Tag antibody (dilution 1:1000; 901502; BioLegend, San Diego, CA) and mouse monoclonal anti-β-actin (dilution 1:20,000; A2228; Sigma-Aldrich).

Immunofluorescence

Kidney biopsies. Immunodetection of UMOD and GRP78 was performed on 5-µm-thick kidney sections obtained from nephrectomy samples of patients with ADTKD-*UMOD* (female, 41 years old, ESKD; male, 42 years old, ESKD) and ADTKD-*MUC1* (female, 60 years old, ESKD; male, 47 years old, ESKD). Slides were deparaffinized in xylene and rehydrated in a graded ethanol series. Antigen retrieval was carried out for 10 minutes with citrate buffer (pH 6.0) at 98 °C. After 20 minutes in blocking solution, slides were incubated overnight with GRP78 primary antibody (1:300; ab21685; Abcam, Cambridge, UK), followed by incubation with AlexaFluor555-conjugated goat anti-rabbit antibody for 45 minutes (1:200; Invitrogen, Thermo Fisher Scientific). The slides were probed with sheep anti- UMOD primary antibody (1:800; K90071C; Meridian Life Science Inc., Memphis, TN), followed by AlexaFluor488-conjugated donkey anti-sheep (1:200; Invitrogen). Coverslips were mounted with Prolong gold antifade reagent with 4′,6-diamidino-2-phenylindole (Invitrogen) and analyzed under a Zeiss LSM 510 Meta Confocal microscope (Carl Zeiss, Jena, Germany) with high numerical aperture lenses (Plan-Neofluar 20×/0.5). The use of these samples has been approved by the Université catholique de Louvain Ethical Review Board.[33]

HEK293 cells. Cells grown on coverslip were fixed in 4% paraformaldehyde for 15 minutes, permeabilized 10 minutes with 0.5% Triton, and blocked 30 minutes with 10% donkey serum. Cells were labelled for 1 hour, 30 minutes at room temperature with a mouse purified anti-HA.11 Epitope Tag antibody (dilution 1:500; 901502; BioLegend) and a rabbit polyclonal anti-calreticulin (dilution 1:500; C4606; Sigma-Aldrich) followed by incubation for 1 hour with the appropriate AlexaFluor conjugated secondary antibodies (dilution 1:500; Thermo Fisher Scientific). Cells were stained with 4′,6-diamidino-2-phenylindole and mounted using fluorescence mounting medium (Dako; Agilent). All pictures were taken with an UltraVIEW ERS spinning disk confocal microscope (UltraVIEW ERS-Imaging Suite Software; Zeiss 63X/1.4; PerkinElmer Life and Analytical Sciences, Boston, MA). All images were imported in Photoshop CS (Adobe Systems, Mountain View, CA) and adjusted for brightness and contrast.

Generation and validation of the ADTKD *UMOD*-score

The weighted *UMOD*-score was based on ADTKD criteria, specific clinical characteristics of ADTKD-*UMOD* (i.e., early gout onset and hyperuricemia), and parameters that are negatively associated with ADTKD (i.e., providing alternative explanation for CKD: proteinuria and/or hematuria, diabetes and/or uncontrolled hypertension, renal

cysts and/or enlarged kidneys).[2,15,20] For weighting the items of the score, we used integer values between −1 and +3. A score of +2 was given for the general ADTKD criteria,[2] +1 or +3 for the *UMOD*-specific clinical and laboratory findings, and −1 for each negatively associated item. The score was first tested in the Belgo-Swiss ADTKD registry and validated in the US ADTKD registry. To discriminate ADTKD-*UMOD* from ADTKD-*MUC1*, we defined a normal range of urinary UMOD [(mg/g creatinine)/eGFR] using 2717 urine samples from the general population. Based on the pathophysiology of ADTKD-*UMOD*, on previous reports,[34] as well as on our findings (Figure 5a), we assigned, respectively, +1 and +3 points for urinary UMOD values between the median and 25th percentile and below the 25th percentile of normal urinary UMOD levels. Similarly, we assigned, respectively, −1 and −3 points for urinary UMOD values between the median and 75th percentile and above the 75th percentile of normal urinary UMOD levels. Conceptualization of the score was based on the previously published hepatocyte nuclear factor 1β score.[35]

Statistical analysis

Quantitative parameters are presented as median and IQR (25th, 75th percentiles) (for scale variables) or means ± SD (for continuous variables), and qualitative parameters are presented as fractions with percentages. Categorical variables were compared using the χ^2 test. Continuous variables were compared using the Mann–Whitney U test or unpaired *t* test. Analysis of variance testing with Tukey's multiple comparison test was used to compare urinary UMOD levels. Kaplan-Meier curves were generated to display ESKD-free and gout-free survival. Patients who had not reached ESKD or developed gout at the end of the study (outcome of interest not occurred during follow-up time) were considered censored individuals. Censoring time was defined as age at last follow-up. A log-rank test was used for comparison of survival curves. The performance of the *UMOD*-score was assessed by calculating the AUC of the receiver-operating characteristic curve. The Youden index was used to define the optimal discriminatory cut-off point for the *UMOD*-score. Statistical analysis was performed using SPSS Statistics (IBM Corp., Armonk, NY). $P < 0.05$ was considered statistically significant, 2-sided tests were used.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

EO is supported by the Fonds National de la Recherche Luxembourg (grant 6903109), the University Research Priority Program "Integrative Human Physiology, ZIHP" of the University of Zurich, and an Early Postdoc Mobility-Stipendium of the Swiss National Science Foundation (P2ZHP3_195181). LR is supported by the Italian Society of Nephrology (SIN) under the "Adotta un progetto di ricerca" program, Telethon-Italy (grant GGP14263) and the Italian Ministry of Health (grant RF-2010-2319394). KK and AJB are supported by National Institutes of Health–National Institute of Diabetes and Digestive and Kidney Diseases (grant R21 DK106584). MŽ, KH, and SK were supported by the Ministry of Health of the Czech Republic (grant NV17-29786A), the Ministry of Education of the Czech Republic (grant LTAUSA19068), and by Charles University in Prague (institutional programs UNCE/MED/007 and PROGRES-Q26/LF1). They thank the National Center for Medical Genomics (grant LM2015091) for help with *UMOD* and *MUC1* sequencing. OD is supported by the European Community's Seventh Framework Programme (grant 305608), the European Reference Network for Rare Kidney Diseases (project ° 739532), the Swiss National Science Foundation's National Center of Competence in Research Kidney Control of Homeostasis program, and the Swiss National Science Foundation (grant 310030-189044). OD and OB were supported by the Gebert-Rüf Foundation for research on ADTKD-*UMOD*. JAS is supported by Kidney Research UK and the Northern Counties Kidney Research Fund. RT is funded by Instituto de Salud Carlos III: Redes Temáticas de Investigación Cooperativa Red de Investigación Renal (grant RD16/0009) and Fondo de Investigación Sanitarias Fondo Europeo de Desarollo Regional (grant PI15/01824, PI18/00362). JM is supported by the Fonds de la Recherche Scientifique–Communauté Française de Belgique. DGF was supported by the Swiss National Centre of Competence in Research TransCure, the Swiss National Centre of Competence in Research Kidney Control of Homeostasis program, and the Swiss National Science Foundation (grants 31003A_152829 and 33IC30_166785/1). Genetic testing for *MUC1* is supported by the Slim Initiative for Genomic Medicine in the Americas, a collaboration of the Broad Institute with the Carlos Slim Foundation.

We thank all participating patients and families. The Cohorte Lausannoise is acknowledged for providing reference urine samples and eGFR information. We are grateful to Maegan Harden and her team on the Broad Genomics Platform for expert assistance with genetic testing for *MUC1*.

Parts of these data were presented as a poster during the 2017 American Society of Nephrology Kidney Week (October 31–November 5, 2017, New Orleans, Louisiana).

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Appendix S1. Referring Physicians.
Figure S1. The L284P uromodulin shows trafficking defect.
Figure S2. Freedom from ESKD and gout in ADTKD-*UMOD* according to noncysteine versus cysteine mutations.
Figure S3. Association between urinary uromodulin and glomerular filtration in the general population.
Figure S4. Uromodulin processing in ADTKD-*UMOD* and ADTKD-*MUC1*.
Figure S5. Design and flowchart of the Belgo-Swiss ADTKD registry.
Figure S6. Clinical characteristics of *UMOD*-positive and *UMOD*-negative patients in the Belgo-Swiss ADTKD registry.
Table S1. List of the 106 *UMOD* mutations in the Belgo-Swiss and US ADTKD registries.
Table S2. Allele frequency and pathogenicity scores for novel *UMOD* variants.
Table S3. Clinical characteristics of ADTKD patients in the US ADTKD registry according to genetic diagnosis.
Table S4. Coordinates of the receiver-operating characteristics (ROC) curve of the clinical *UMOD*-score in the Belgo-Swiss ADTKD registry (*UMOD*-positive and *UMOD*-negative patients) ($n = 211$ patients with available data).
Table S5. Coordinates of the receiver-operating characteristics (ROC) curve of the *UMOD*-score in the US ADTKD registry (patients with ADTKD-*UMOD* and ADTKD-*MUC1*) without and with urinary uromodulin ($n = 205$ and $n = 86$ with urinary uromodulin).

REFERENCES

1. Devuyst O, Olinger E, Weber S, et al. Autosomal dominant tubulointerstitial kidney disease. *Nat Rev Dis Primers*. 2019;5:60.
2. Eckardt KU, Alper SL, Antignac C, et al. Autosomal dominant tubulointerstitial kidney disease: diagnosis, classification, and management—a KDIGO consensus report. *Kidney Int*. 2015;88:676–683.
3. 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.
4. Devuyst O, Olinger E, Rampoldi L. Uromodulin: from physiology to rare and complex kidney disorders. *Nat Rev Nephrol*. 2017;13:525–544.
5. Kirby A, Gnirke A, Jaffe DB, et al. Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in MUC1 missed by massively parallel sequencing. *Nat Genet*. 2013;45:299–303.

6. Knaup KX, Hackenbeck T, Popp B, et al. Biallelic expression of mucin-1 in autosomal dominant tubulointerstitial kidney disease: implications for nongenetic disease recognition. *J Am Soc Nephrol*. 2018;29:2298–2309.
7. Zivna M, Kidd K, Pristoupilova A, et al. Noninvasive immunohistochemical diagnosis and novel MUC1 mutations causing autosomal dominant tubulointerstitial kidney disease. *J Am Soc Nephrol*. 2018;29:2418–2431.
8. Bleyer AJ, Kmoch S, Antignac C, et al. Variable clinical presentation of an MUC1 mutation causing medullary cystic kidney disease type 1. *Clin J Am Soc Nephrol*. 2014;9:527–535.
9. 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.
10. Faguer S, Decramer S, Chassaing N, et al. Diagnosis, management, and prognosis of HNF1B nephropathy in adulthood. *Kidney Int*. 2011;80:768–776.
11. 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.
12. Bolar NA, Golzio C, Zivna M, et al. Heterozygous loss-of-function SEC61A1 mutations cause autosomal-dominant tubulo-interstitial and glomerulocystic kidney disease with anemia. *Am J Hum Genet*. 2016;99:174–187.
13. Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med*. 2019;380:142–151.
14. Gast C, Marinaki A, Arenas-Hernandez M, et al. Autosomal dominant tubulointerstitial kidney disease-UMOD is the most frequent non polycystic genetic kidney disease. *BMC Nephrol*. 2018;19:301.
15. Ayasreh N, Bullich G, Miquel R, et al. Autosomal dominant tubulointerstitial kidney disease: clinical presentation of patients with ADTKD-UMOD and ADTKD-MUC1. *Am J Kidney Dis*. 2018;72:411–418.
16. Trudu M, Schaeffer C, Riba M, et al. Early involvement of cellular stress and inflammatory signals in the pathogenesis of tubulointerstitial kidney disease due to UMOD mutations. *Sci Rep*. 2017;7:7383.
17. 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.
18. Johnson BG, Dang LT, Marsh G, et al. Uromodulin p.Cys147Trp mutation drives kidney disease by activating ER stress and apoptosis. *J Clin Invest*. 2017;127:3954–3969.
19. Dvela-Levitt M, Kost-Alimova M, Emani M, et al. Small molecule targets TMED9 and promotes lysosomal degradation to reverse proteinopathy. *Cell*. 2019;178:521–535.e523.
20. Bollée G, Dahan K, Flamant M, et al. Phenotype and outcome in hereditary tubulointerstitial nephritis secondary to UMOD mutations. *Clin J Am Soc Nephrol*. 2011;6:2429–2438.
21. Youhanna S, Weber J, Beaujean V, et al. Determination of uromodulin in human urine: influence of storage and processing. *Nephrol Dial Transplant*. 2014;29:136–145.
22. Pruijm M, Ponte B, Ackermann D, et al. Associations of urinary uromodulin with clinical characteristics and markers of tubular function in the general population. *Clin J Am Soc Nephrol*. 2016;11:70–80.
23. Troyanov S, Delmas-Frenette C, Bollée G, et al. Clinical, genetic, and urinary factors associated with uromodulin excretion. *Clin J Am Soc Nephrol*. 2016;11:62–69.
24. Olden M, Corre T, Hayward C, et al. Common variants in UMOD associate with urinary uromodulin levels: a meta-analysis. *J Am Soc Nephrol*. 2014;25:1869–1882.
25. Scolari F, Caridi G, Rampoldi L, et al. Uromodulin storage diseases: clinical aspects and mechanisms. *Am J Kidney Dis*. 2004;44:987–999.
26. Liu Y, Goldfarb DS, El-Achkar TM, et al. Tamm-Horsfall protein/uromodulin deficiency elicits tubular compensatory responses leading to hypertension and hyperuricemia. *Am J Physiol Renal Physiol*. 2018;314:F1062–F1076.
27. Dahan K. 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.
28. Ashton EJ, Legrand A, Benoit V, et al. Simultaneous sequencing of 37 genes identified causative mutations in the majority of children with renal tubulopathies. *Kidney Int*. 2018;93:961–967.
29. Hureaux M, Ashton E, Dahan K, et al. High-throughput sequencing contributes to the diagnosis of tubulopathies and familial hypercalcemia hypocalciuria in adults. *Kidney Int*. 2019;96:1408–1416.
30. Blumenstiel B, DeFelice M, Birsoy O, et al. Development and validation of a mass spectrometry-based assay for the molecular diagnosis of mucin-1 kidney disease. *J Mol Diagn*. 2016;18:566–571.
31. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424.
32. 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.
33. 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.
34. 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.
35. Faguer S, Chassaing N, Bandin F, et al. The HNF1B score is a simple tool to select patients for HNF1B gene analysis. *Kidney Int*. 2014;86:1007–1015.