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

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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.


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

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 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. The disease invariably progresses to end-stage kidney disease (ESKD). Dominant mutations in UMOD were first associated with ADTKD. UMOD encodes uromodulin, a kidney-specific protein that is abundant in normal urine and plays multiple roles in the kidney. Mutations in MUC1 were subsequently identified as a cause for ADTKD. MUC1 encodes the glycoprotein mucin-1, which is important in epithelial barrier function and intracellular signaling.

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. 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. The prevalence of ADTKD-MUC1 remains unclear, as mutations in MUC1 are not detected by next-generation sequencing and require specialized genetic testing. However, previous studies have identified ADTKD-MUC1 and ADTKD-UMOD as the most common subtypes of ADTKD. 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, tubulointerstitial fibrosis, and interstitial fibrosis. 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.

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. 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. 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.

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). 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 common genetic diagnosis in CKD. 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. The prevalence of ADTKD-MUC1 remains unclear, as mutations in MUC1 are not detected by next-generation sequencing and require specialized genetic testing. However, previous studies have identified ADTKD-MUC1 and ADTKD-UMOD as the most common subtypes of ADTKD. 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, tubulointerstitial fibrosis, and interstitial fibrosis. 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.

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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 neo-mutation 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 sequencing⁷). 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).
**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 tubule⁶ 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 assay⁸ to assess the

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**Table 1 | Clinical and genetic characteristics of patients with ADTKD**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>International ADTKD Cohort (N = 726)</th>
<th>Belgo-Swiss ADTKD registry (n = 275)</th>
<th>US ADTKD registry (n = 451)</th>
<th>n (BE-CH/US)</th>
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</thead>
<tbody>
<tr>
<td>Number of families</td>
<td>585</td>
<td>156</td>
<td>429</td>
<td></td>
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<tr>
<td>Sex, female</td>
<td>332/726 (46)</td>
<td>115/275 (42)</td>
<td>217/451 (48)</td>
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<tr>
<td>Age at presentation (yr)</td>
<td>45 (31, 58)</td>
<td>34 (22, 49)</td>
<td>49 (37, 62)</td>
<td>174/377</td>
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<tr>
<td>Positive family history, gout and/or CKD</td>
<td>625/679 (92)</td>
<td>191/227 (84)</td>
<td>434/451 (96)</td>
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<tr>
<td>eGFR at diagnosis (ml/min)</td>
<td>44.3 ± 30.0</td>
<td>45.1 ± 20.9</td>
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<td>CKD</td>
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<td>205/258 (80)</td>
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<td>216/603 (43)</td>
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<tr>
<td>Age at ESKD (yr)</td>
<td>44 (32, 55)</td>
<td>44 (33, 56)</td>
<td>44 (32, 55)</td>
<td>245/146</td>
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<tr>
<td>Serum uric acid (µmol/l)</td>
<td>472 ± 141</td>
<td>479 ± 145</td>
<td>455 ± 128</td>
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<td>452 ± 149</td>
<td>457 ± 158</td>
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<td>494 ± 135</td>
<td>464 ± 129</td>
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<td>Gout</td>
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<tr>
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<td>207/305 (68)</td>
<td>90/127 (71)</td>
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<tr>
<td>Age at gout onset (yr)</td>
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<td>31 (20, 47)</td>
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<tr>
<td>Female</td>
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<td>98/55</td>
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<tr>
<td>Male</td>
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<td>30 (20, 41)</td>
<td>28 (20, 40)</td>
<td>135/105</td>
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<td>UMOD mutations</td>
<td>216/562 (38.4)</td>
<td>54/156 (34.6)</td>
<td>162/406 (40.0)</td>
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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.
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 m² (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 m²). 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 m² (\([0.05 \text{ vs. } 0.23 \text{ mg/g creatinine}]/\text{eGFR}\), \( P < 0.0001 \)), in contrast with unchanged levels in patients with ADTKD-MUC1 versus in controls (\([0.29 \text{ vs. } 0.23 \text{ mg/g creatinine}]/\text{eGFR}\), \( P = 0.29 \)) (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

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**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.
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

<table>
<thead>
<tr>
<th>Number of families</th>
<th>ADTKD-UMOD (n = 303)</th>
<th>ADTKD-MUC1 (n = 104)</th>
<th>n (UMOD/MUC1)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Female</td>
<td>130 (51)</td>
<td>40 (50)</td>
<td>257/80</td>
<td>1.0</td>
</tr>
<tr>
<td>- Male</td>
<td>127 (49)</td>
<td>40 (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at presentation (yr)</td>
<td>42 (27, 53)</td>
<td>47 (37, 57)</td>
<td>218/78</td>
<td>0.005</td>
</tr>
<tr>
<td>Positive family history, gout and/or CKD (%)</td>
<td>93 (90)</td>
<td>69/80 (86)</td>
<td>0.007</td>
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</tr>
<tr>
<td>eGFR at presentation (ml/min)</td>
<td>39 ± 29.3</td>
<td>50 ± 51.9</td>
<td>136/52</td>
<td>0.157</td>
</tr>
<tr>
<td>CKD (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Age at CKD (yr)</td>
<td>112 (257)</td>
<td>46 (58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>44 (39, 55)</td>
<td>34 (28, 46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum uric acid (µmol/l)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Female</td>
<td>497.9 ± 136.6</td>
<td>443.6 ± 121.7</td>
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<tr>
<td>- Male</td>
<td>478.7 ± 133.2</td>
<td>418.7 ± 136.1</td>
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<tr>
<td>Gout (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>- Female</td>
<td>202/257 (79)</td>
<td>21/80 (26)</td>
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<td>- Male</td>
<td>96 (130)</td>
<td>4 (40)</td>
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<tr>
<td>Age at gout onset (yr)</td>
<td>27 (19, 37)</td>
<td>45 (29, 51)</td>
<td>199/18</td>
<td>0.001</td>
</tr>
<tr>
<td>- Female</td>
<td>30 (21, 43)</td>
<td>28 (21, 41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>26 (18, 34)</td>
<td>45 (33, 54)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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. χ² test for categorial variables and Mann-Whitney U test and unpaired t test for quantitative parameters were used. (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.
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)/2 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 $\geq 5$ (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 $\geq 8$ 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%
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
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⁻/C₀⁻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⁻/C₀⁻cotransporter.26 Even though ADTKD-MUC1 presumably originates from the distal tubule, gout was considerably less prevalent in this disorder.

![Figure 6](https://example.com/figure6.png)

**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 analysis and kidney imaging. b Interstitial fibrosis, tubular atrophy, thickening and lamellation of tubular basement membranes, tubular dilatation (microcysts), negative immunofluorescence for complement, and lgs. 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 m², 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.
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<sup>6</sup> and on the observation that MUC1fs is accumulating in other MUC1-expressing tissues (skin, breast, lung, colon) without causing extrarenal manifestations,<sup>7</sup> 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.<sup>19</sup>

Previous reports described intracellular accumulation of UMOD in kidney biopsies from patients with ADTKD-UMOD.<sup>1,2</sup> 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

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**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.
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 m²) and tubular mass, 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.

Figure 8 | Diagnostic algorithm for suspected autosomal dominant tubulointerstitial kidney disease (ADTKD) based on clinical UMOD-score and urinary UMOD levels. *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. †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. See Results and Discussion sections for more details. ‡For diagnostic algorithm including other ADTKD genes, refer to Devuyst et al. 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 (nonsteroidal anti-inflammatory drugs, aristolochic acid, calcineurin inhibitors, lithium). CKD, chronic kidney disease.

A recent study based on exome sequencing reported mutations in UMOD accounting for ~3% of all patients with a genetic finding in this cohort. 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. 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.

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 consensus2 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 (Supplementary Appendix S1). 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).

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 hypertension, proteinuria (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 (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 Harvard30 and the First Faculty of Medicine, Charles University. 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/), 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 Synchrop 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 generated by site-directed 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’) gatgcactgtgctctcggggctcg, reverse (5’→3’) caggagcgcggggactacagtcgcac and for mutation L248P: forward (5’→3’) cccgagtgctacccgggtactgcaca, reverse (5’→3’) tgtgcattgcgggctcgcacagtggg.

**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% CO2. 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 glycercophosphat and protease inhibitor cocktail [Sigma-Aldrich, St. Louis, MO]) for 1 hour at 4 °C followed by 10 minutes of centrifugation at 17,000g. 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 were used 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; A2282; 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 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).\textsuperscript{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,\textsuperscript{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,\textsuperscript{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 \( \beta \) score.\textsuperscript{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 \( \chi^2 \) 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.

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**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 noncystine 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**


