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Clinical and Genetic Spectra of Autosomal Dominant Tubulointerstitial Kidney Disease due to Mutations in *UMOD* and *MUC1*

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US ADTKD Registry

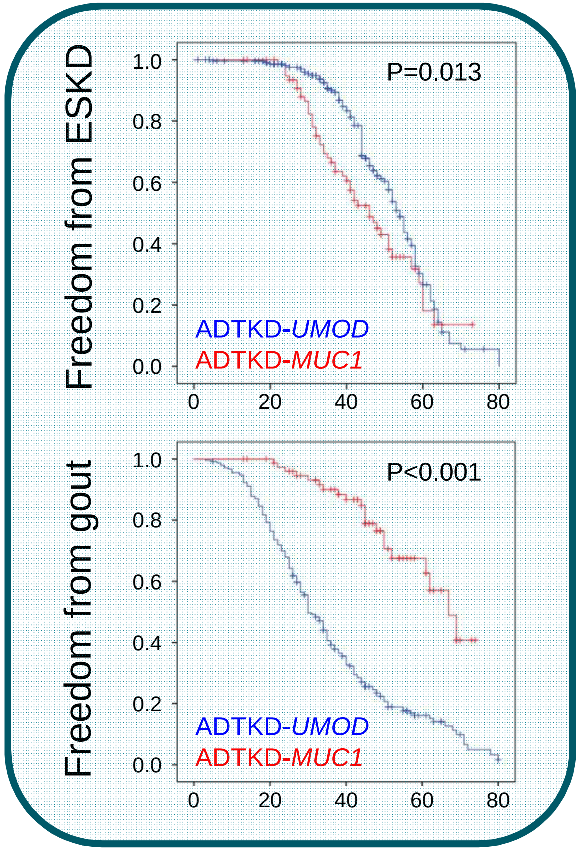
Belgo-Swiss ADTKD Registry

International ADTKD Cohort

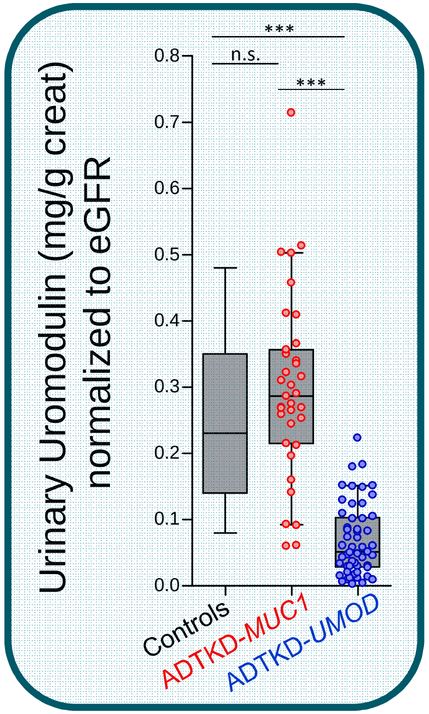
N=585 families
n=726 patients

ADTKD-*UMOD:* N=216
ADTKD-*MUC1:* N=93

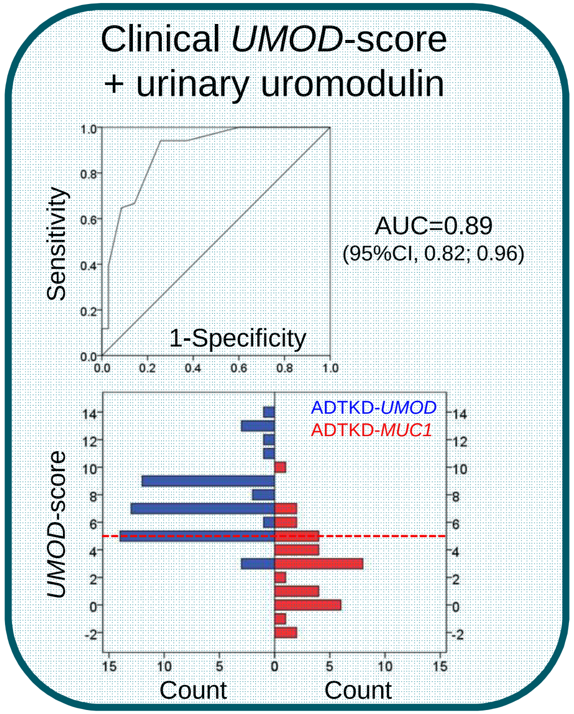
Clinical Characterization



Biological Characterization



Diagnostic Score



Largest international retrospective ADTKD cohort study:

- **Detailed clinical and genetic phenotyping of ADTKD-*UMOD* & ADTKD-*MUC1***
- **Uromodulin biology is not altered in ADTKD-*MUC1***
- **Clinical and biochemical *UMOD*-score discriminates between most common ADTKD subtypes**

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Clinical and Genetic Spectra of Autosomal Dominant Tubulointerstitial Kidney Disease due to Mutations in *UMOD* and *MUC1*

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Abstract

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. To expand on this, 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: Uromodulin, Mucin-1, Diagnostic score, Dominant kidney disease, Gout

Introduction

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 for the transcription factor hepatocyte nuclear factor 1β (HNF1β) [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 non-specific nature of the clinical, biological and pathological findings, ADTKD is underdiagnosed. In a recent study of whole exome sequencing in ~3000 CKD patients, *UMOD* mutations were detected in 3% of patients with a monogenic cause of CKD, making it the 6th 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*, i.e. the most common monogenic kidney disease after autosomal dominant polycystic kidney disease (ADPKD) [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 [15,16]. The pathophysiology of ADTKD-*UMOD* involves retention of mutant uromodulin in the endoplasmic reticulum (ER) with ensuing ER stress ("gain of toxic function") and a cascade leading to inflammatory cell infiltrate, tubular dysfunction and interstitial fibrosis [17-19]. ADTKD-*MUC1* is caused by mutations in the variable number of tandem repeat (VNTR) region of mucin-1, leading to the formation of a frameshift, truncated protein (MUC1fs) that accumulates in intracellular vesicles and cause tubulointerstitial damage [20].

To date, the largest clinical analysis of ADTKD-*UMOD* was performed in a cohort of French and Belgian ADTKD-*UMOD* patients (n=70 from 38 families), showing a median renal survival of 54 years and a 66% prevalence of gout [21]. The phenotype of ADTKD-*MUC1* patients 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 ADTKD-*MUC1* patients (16 families) showed a trend towards earlier age at ESKD and a lower prevalence of gout compared to ADTKD-*UMOD* patients (n=41 from 9 families). The small size of these cohorts prevented the detection of significant differences between ADTKD subtypes [16].

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 two large cohorts from Europe (Belgo-Swiss ADTKD Registry) and the US (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 ADTKD patients

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). 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 (703/726), from 562/585 families, underwent mutational screening in the *UMOD* gene as a first diagnostic test. *UMOD* mutations were detected in 216 out of 562 tested families (38.4%), corresponding to 303 out 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 *UMOD*-negative patients, from 205 *UMOD*-negative families, mostly from the US Registry. Of these, 83 patients from 72 families screened positive for *MUC1* mutations, yielding a proportion of 35.1% (72/205) families with ADTKD-*MUC1* among *UMOD*-negative ADTKD families. Of note, a subset of 23 patients from 23 ADTKD families (most of them previously linked to chromosome 1q22) were first screened for *MUC1*, with a mutation in *MUC1* detected in 21 of 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 ADTKD-*UMOD* families (Figure 2A; 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 type of *UMOD* mutations (101/106, 95.3%). Four different deletions (H177-R185del, E188-L221del, K246-S252del, Y272del) and one insertion-deletion (V93-G97del4ins) mutations were found. 95/106 (89.6%) mutations were clustered in exon 3 of the *UMOD* gene. 57/101 (56.4%) of all missense mutations 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 (Table S1), 6 involve a previously reported amino acid (N85S, C92G, C120R, C135W, V273L, C300S); two (Y272del, G201D) were validated in segregation analyses; and one (L284P) was clearly associated with ER retention in functional studies, similar to paradigm mutation C150S (Figure S1), along with family history (Three generations with CKD and gout, bland urine sediment) and the absence of this substitution in gnomAD. The remaining eight mutations were predicted disease causing using *in silico* prediction tools (Table S2).

We detected two families with genetically proven *de novo UMOD* mutations c.855C>A (p.A285E) and c.707C>T (p.P236L) and one 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 sequencing [7]). Their localization inside the *MUC1* VNTR as well as their effect on the mucin-1 protein structure are shown in Figure 2C. All these mutations are predicted to lead to the same frame-shift and premature stop codon [7]. Among the 93 ADTKD-*MUC1* families, 87 presented with a cytosine duplication (27dupC, 93.5%), three with an adenine duplication (28dupA, 3.2%) and two with a guanine insertion (26_27insG, 2.2%) and one 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 [IQR 27; 53] vs. 47 years [IQR 37; 57], p=0.005) and a positive family history of CKD and/or gout more frequent (95% vs. 86%, p=0.007) in ADTKD-*UMOD* compared to ADTKD-*MUC1* patients. While the overall prevalence of CKD was significantly higher in ADTKD-*UMOD* patients, 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 ADTKD-*MUC1* patients (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 ADTKD-*UMOD* patients (Figure 3B lower panel). These findings were generally consistent in both genders. In ADTKD-

UMOD patients, gout onset was significantly earlier in men compared to 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* compared to ADTKD-*UMOD* (Median: 54 years, 95% 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* compared to 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 ADTKD-*UMOD* patients, 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 non-cysteine-involving ADTKD-*UMOD* patients (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 eGFR at diagnosis lower (34.7ml/min vs. 48.1ml/min, p<0.001) in ADTKD-*UMOD* vs. ADTKD-NOS, respectively. Similarly, CKD and ESKD were more prevalent in ADTKD-*MUC1* compared to ADTKD-NOS (86.4% vs. 82.7%, p<0.001 and 54.8% vs. 26.2%, p<0.001, respectively) (Table S3). These findings suggest a more severe kidney phenotype in ADTKD-*UMOD* and ADTKD-*MUC1* compared to ADTKD cases without genetic diagnosis – a finding confirmed in the Belgo-Swiss Registry (see below).

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

Given the colocalization of mucin-1 with uromodulin 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 uromodulin processing in the TAL in ADTKD-*MUC1*. We used a validated ELISA [22] to assess the levels of urinary uromodulin in a population-based cohort (Cohorte Lausannoise), confirming the positive correlation between urinary uromodulin (mg/g creatinine) and eGFR between 15 and 90mL/min/1.73m^2 (Figure S3A, test for linear trend, p: 0.001), as previously described [23]. Normalizing urinary uromodulin for eGFR (in addition to urinary creatinine) mitigated the linear dependency (Figure S3B, test for linear trend, p: 0.54), allowing a more robust comparison of urinary uromodulin levels between patients and controls. We next measured urinary uromodulin levels in ADTKD-*MUC1* and ADTKD-*UMOD* patients, compared to controls (n=180) from the population-based cohort strictly matched for eGFR (45-60mL/min/1.73m^2). In contrast to ADTKD-*UMOD* patients, who showed strongly reduced urinary uromodulin levels (Median: 2.8 vs. 14.7mg/g creatinine, p<0.0001), ADTKD-*MUC1* patients showed urinary levels of uromodulin similar to controls (Median: 15.7 vs. 14.7mg/g creatinine, p=0.99) (Figure 5A left panel). Normalizing urinary uromodulin levels to eGFR (mg/g creatinine/eGFR) confirmed strongly reduced levels in ADTKD-*UMOD* vs. 2717 controls with eGFR spanning 15-90 mL/min/1.73m^2 (0.05 vs. 0.23mg/g creatinine/eGFR, p<0.0001, respectively), in contrast with unchanged levels in ADTKD-*MUC1* vs. controls (0.29 vs. 0.23mg/g creatinine/eGFR, p=0.29, respectively) (Figure 5A right panel).

Next, we performed immunofluorescence staining for uromodulin on kidney biopsies from healthy individuals (NHK, normal human kidney), from two ADTKD-*UMOD* patients and from two ADTKD-*MUC1* patients. While we were able to see the characteristic intracellular uromodulin deposits in the ADTKD-*UMOD* patients, uromodulin staining was largely confined to the apical membrane in ADTKD-*MUC1* patients, similar to the pattern observed in normal kidney (Figure 5B). The accumulation of mutant uromodulin in the TAL cells from ADTKD-*UMOD* patients induced ER stress, as shown by colocalization with the unfolded protein response (UPR) regulator GRP78 (also known as Binding immunoglobulin protein, BiP). Conversely, GRP78 could not be detected in the TALs of ADTKD-*MUC1* patients (Figure 5B; 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 *UMOD*-positive families (n=132 patients) and 102 *UMOD*-negative families (n=143 patients) (Figure 1; Figure S5), we designed a clinical score to estimate the probability of ADTKD-*UMOD*. Clinical characteristics in ADTKD patients with/without *UMOD* mutations guided the scoring system (Figure S6). Compared to *UMOD*-negative patients, 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±153.4μmol/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 ADTKD-*UMOD* compared to *UMOD*-negative patients (36% vs 57%, p=0.001) (Figure S6).

The weighted *UMOD*-score was developed on eight 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 >500µmol/L - the most specific discriminants (Figure S6). Since 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 (eg. proteinuria, uncontrolled hypertension) were attributed negative points. Values for each available item are added in order to obtain a final additive score for each patient. The clinical *UMOD* score was applied on ADTKD patients from the Belgo-Swiss Registry, for which information for at least 5/8 items were present (n=211: 106 *UMOD*-positive and 105 *UMOD*-negative patients). The receiver operating characteristics (ROC) curve, with *UMOD* 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; Table S4). This cut-off also proved to be optimal for group discrimination corresponding to a Youden index (sensitivity+specificity-1) of 0.395 (Table S4).

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

The score was validated in *UMOD*-positive (n=124) and *UMOD*-negative (n=183) patients 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 two most common etiologies of ADTKD in a subset of ADTKD-*UMOD* (n=125) and ADTKD-*MUC1* (n=80) patients from the US Registry for which at least 5/8 clinical item and/or urinary uromodulin levels were available. The clinical *UMOD*-score alone separated the two 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 score ≥8 had a sensitivity of 48.8%, a specificity of 83.7%, a NPV of 50.8% and a PPV of 81.3% for an *UMOD* mutation) (Table S5). Only a few, mostly ADTKD-*MUC1* patients had score results of <5 (Figure 7A right panel).

We next investigated whether addition of urinary uromodulin levels to the clinical score improved its ability to discriminate ADTKD-*UMOD* from ADTKD-*MUC1*. Based on the normalized urinary uromodulin values in the reference population (mg/g creatinine/eGFR) (Figure 5A right panel), we assigned respectively +1 and +3 points for urinary uromodulin 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 uromodulin 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 ADTKD-*UMOD* and 35 ADTKD-*MUC1* patients for which urinary uromodulin 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% and specificity of 74.3% and a NPV 89.7%, PPV 84.2% for a *UMOD* mutation (Table S5 and Figure 7B). Based on the clinical and biochemical *UMOD* score, we suggest a diagnostic algorithm to guide genetic testing in ADTKD (Figure 8).

Discussion

This international cohort study represents the largest dataset of ADTKD-*UMOD* and ADTKD-*MUC1* patients 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 [2,16,21]. In this 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 *UMOD*-negative families 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 *UMOD*- and *MUC1*-negative families; and for mutations in *MUC1* in the 141 *UMOD*-negative families 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 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 ADTKD-*MUC1* patients [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 compared to ADTKD-*MUC1*. Defective urinary concentration resulting in polydipsia and polyuria has been described in ADTKD-*UMOD* patients, most likely because of impaired activity of TAL-based Na^+-K^+-$2Cl^-$-cotransporter NKCC2 [16,18]. 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* [24,25]. A similar mechanism was shown in aged *Umod* KO mice that displayed reduced activity of NKCC2 [25]. Even though ADTKD-*MUC1* presumably originates from the distal tubule, gout was considerably less prevalent in this disorder.

We investigated two cardinal biological features described in ADTKD-*UMOD* with likely pathophysiological relevance: aberration in uromodulin export mechanisms and induction of ER stress. Based on the observation that mucin-1 is expressed in the distal kidney tubule including the TAL where it colocalizes with uromodulin [6] and on the observation that MUC1fs is accumulating in other mucin-1-expressing tissues (skin, breast, lung, colon) without causing extrarenal manifestations [7], one could hypothesize that MUC1fs might interact with uromodulin in TAL. Yet, in contrast to ADTKD-*UMOD*, we found no difference in the urinary level of uromodulin between ADTKD-*MUC1* patients and the normal population. Furthermore, analysis of *MUC1*-mutant kidney biopsies revealed a normal distribution of uromodulin in TAL cells, without evidence for ER stress (GRP78 expression) - a hallmark of ADTKD-*UMOD*. These novel findings suggest that the processing of uromodulin is not altered in ADTKD-*MUC1* and that ER stress is not a main finding in ADTKD-*MUC1*. In line, a recent study found entrapment of MUC1fs in vesicles of the early secretory pathway in models of ADTKD-*MUC1* [20].

Previous reports described intracellular accumulation of uromodulin in kidney biopsies from ADTKD-*UMOD* patients [1,2]. However, such staining is not available in a large number of patients, preventing us to speculate on its value in clinical decision making. In our experience, the uromodulin 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 uromodulin levels in patients at time of diagnosis and during disease progression might offer a non-invasive diagnostic tool and biomarker in ADTKD-*UMOD*. Since urinary uromodulin levels show a positive correlation with eGFR (for eGFR <90mL/min/1.73m^2) and tubular mass [26,27], 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 uromodulin (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/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; history of gout. Of interest, renal cysts are less common in ADTKD-*UMOD* patients, in line with previous studies [15,16,21]. The delineated clinical *UMOD*-score showed an excellent negative predictive value for *UMOD* mutations (cut-off ≥5) in the Belgo-Swiss Registry (NPV: 94.3%) and in the

US ADTKD registry (NPV: 91.0%). As ADTKD-*UMOD* and ADTKD-*MUC1* present considerable clinical overlap, we were not surprised that the clinical *UMOD*-score separated modestly between these two 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 uromodulin measurements, a pathophysiological biomarker for ADTKD-*UMOD*, considerably increased the discriminating power of the score (AUC 0.89) with a positive predictive value of 84.2% for an *UMOD* mutation (cut-off ≥5 points). Since the progression of kidney disease and the prevalence and onset of gout seems dependent on the underlying genetic diagnosis, a genetic diagnosis is recommended as it might impact on the management of ADTKD patients, 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/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 neglectable risk as only 1-2 patients were in general included per family and considerable intrafamilial clinical variability exists in ADTKD [8,28]. Since kidney biopsies are rarely performed in these diseases and yield non-specific findings (e.g. interstitial fibrosis, tubular atrophy), we did not to include histopathology information in the analysis. A survey of histopathology results from the Belgo-Swiss Registry showed that interstitial fibrosis with tubular atrophy (in ca. 60% of available pathology reports) and interstitial nephritis (in ca. 40% of available pathology reports) were the preponderant histological findings in ADTKD-*UMOD* and *UMOD*-negative patients. A more detailed histological description of biopsies performed in ADTKD-*UMOD* and ADTKD-*MUC1* warrants a dedicated analysis.

It should be pointed that systematic screening for *UMOD* mutations in all 10 coding exons has only been performed in a subset of ADTKD patients. Based on previous screens and WES, we estimate that very few *UMOD* mutations outside exons 3 and 4 might have been missed in ADTKD-*UMOD* [13,16]. Furthermore, large deletions or insertions in *UMOD* are not detected by direct sequencing methods. With the availability of gene panel testing and NGS 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 NGS 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 (Cambridge, MA, USA). For these reasons, we estimate that simple clinical and biochemical tools to estimate pre-test 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 ADTKD-*UMOD* and ADTKD-*MUC1* patients. The clinical hallmarks of the two 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 uromodulin 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.

Material and Methods

International ADTKD Cohort

The International ADTKD Cohort consists of patients from the Belgo-Swiss ADTKD Registry and the US ADTKD Registry (see below). The inclusion criteria were those defined by the KDIGO consensus [2], including: a family history compatible with autosomal dominant inheritance of chronic kidney disease (CKD) with features of ADTKD including progressive loss of kidney function, bland urinary sediment, absent-to-mild albuminuria/proteinuria, normal or small-sized kidneys on ultrasound; and/or (in absence of a positive family history of CKD) a history of early-onset hyperuricemia/gout and/or the presence of interstitial fibrosis/tubular atrophy on kidney biopsy. Exclusion criteria included: a different genetic diagnosis (non-ADTKD), the presence of enlarged cystic kidneys, proteinuria (>1g/24h) and/or consistent hematuria, longstanding or uncontrolled diabetes mellitus or arterial hypertension and the consumption of drugs linked to tubulointerstitial nephritis. Only patients screened for *UMOD* and/or *MUC1* mutations were included in the Cohort. Anonymized demographics, clinical and genetic information were recorded in a database. This study was approved by the institutional review board of Wake Forest School of Medicine, Winston-Salem, NC; the UCLouvain Medical School, Brussels; and the European Community's 7th Framework Programme "European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics).

<u>*Belgo-Swiss ADTKD Registry*</u>: The Belgo-Swiss ADTKD Registry has been developed by academic partners with input from clinicians in Belgium and Switzerland. In 2019, the registry includes 275 patients enrolled since 2003. The clinical data included a family pedigree, onset and evolution of kidney function decline, onset of hyperuricemia/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<10mL/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 (Winston-Salem, NC) since 1999. Information collected included demographics, pedigree, age of ESKD (defined as above), 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.

UMOD testing: Direct sequencing of *UMOD* exons was initially performed by Sanger sequencing, as previously described [29]. More recently, *UMOD* gene is analyzed by massive parallel sequencing using a tubulopathy gene panel designed by the work package tubulopathies of the European Consortium EURenOmics [30,31] 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 to a spectrometry-based probe extension assay as previously described [7,32]. *MUC1* testing was provided by the Broad Institute of MIT and Harvard, Cambridge, MA [32] and the 1st Faculty of Medicine, Charles University, Prague, Czech Republic [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 (www.interactivebiosoftware.com) was used to assist in determining variant pathogenicity.

Identified variants were successively checked against relevant databases, such as Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/), HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), Varsome (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 (SIFT, Align GVD, mutation taster and Polyphen2) for pathogenicity. The variants were classified according to the guidelines published by the American College of Medical Genetics ACMG 2015 [33]. Variants of interest were verified by Sanger sequencing.

Measurements of urinary levels of uromodulin

A validated ELISA method was used to measure urinary uromodulin levels (second morning urine sample) from 86 patients with ADTKD [22]. Urinary creatinine was measured using a Synchron DXC800 analyzer (Beckman Coulter, Fullerton, CA) and used to normalize for urine concentration. The reference samples (n=2717) were obtained from the Cohorte Lausannoise (CoLaus), a population-based study including 6000 people aged 35–75 years from the city of Lausanne, Switzerland [23]. eGFR was calculated using the CKD-EPI equation. Informed consent was obtained from all participating individuals.

Uromodulin expression constructs

cDNA of human wild type uromodulin was cloned in pcDNA 3.1(+) (Thermofisher, Waltham, MA) and an HA tag was inserted after the leader peptide in between T26 and S27 in the protein sequence[34]. The C150S and L284P mutant isoforms were obtained by mutagenesis using the Quickchange Lightning mutagenesis kit (Agilent, Santa Clara, CA) following the manufacturer's instructions. Primers were designed using the software QuikChange® Primer Design Program.

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 (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 200 U/ml penicillin, 200 μg/ml streptomycin and 2 mM glutamine at 37°C, 5% CO_2. HEK293 cells were transfected using lipofectamine 2000 (Thermofisher) following the manufacturer’s protocol and analyzed 24 h after transfection.

Western blot

Cells were lysed in octylglucoside lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 60 mM octyl β-D-glucopyranoside, 10 mM NaF, 0.5 mM Sodium orthovanadate, 1 mM glycerophosphate and protease inhibitor cocktail (Sigma)) for 1 h at 4 °C under rotation followed by 10 min centrifugation at 17,000 g. Soluble fractions were quantified by the Bio-Rad Protein Assay (Bio-Rad). Western blot experiments were performed as described in Schaeffer et al [34]. Antibodies: Mouse purified anti-HA.11 Epitope Tag antibody (Cat# 901502, Biolegend, San Diego, CA, dilution 1:1,000), mouse monoclonal anti-β-actin (A2228, Sigma, dilution 1:20,000).

Immunofluorescence

<u>Kidney biopsies:</u> Immunodetection of uromodulin and GRP78 was performed on 5 μm-thick kidney sections obtained from nephrectomy samples of ADTKD-*UMOD* (Female, 41-year-old, ESKD; Male, 42-year-old, ESKD) and ADTKD-*MUC1* patients (Female, 60-year-old, ESKD;

Male, 47-year-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; Abcam ab21685), followed by incubation with AlexaFluor555-conjugated goat anti-rabbit antibody for 45 minutes (1/200; Invitrogen). The slides were probed with sheep anti-uromodulin primary antibody (1/800; Meridien Life Science Inc. K90071C), followed by AlexaFluor488-conjugated donkey anti-sheep (1/200; Invitrogen). Coverslips were mounted with Prolong gold antifade reagent with 4′,6-diamidino-2-phenylindole (DAPI, Invitrogen) and analyzed under a Zeiss LSM 510 Meta Confocal microscope (Carl Zeiss, Jena, Germany) with high numerical aperture lenses (Plan-Neofluar 20x/0.5). The use of these samples has been approved by the UCLouvain Ethical Review Board [35].

<u>HEK293 cells:</u> Cells grown on coverslip were fixed in 4% paraformaldehyde (PFA) for 15 min, permeabilized 10 min with 0.5 % triton and blocked 30 min with 10 % donkey serum. Cells were labelled for 1 h 30 min at room temperature with a mouse purified anti-HA.11 Epitope Tag antibody (Cat# 901502, Biolegend, dilution 1:500) and a rabbit polyclonal anti-calreticulin (C4606, Sigma, dilution 1:500) followed by 1h incubation with the appropriate Alexa-Fluor conjugated secondary antibodies (Thermofisher, dilution 1:500). Cells were stained with 4,6-diamidino-2-phenylindole (DAPI) 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 (ie. providing alternative explanation for CKD: proteinuria/hematuria, diabetes/uncontrolled hypertension, renal cysts/enlarged kidneys) [2,16,21]. 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. In order to discriminate ADTKD-*UMOD* from ADTKD-*MUC1*, we defined a normal range of urinary uromodulin (mg/g creatinine/eGFR) using 2717 urine samples from the general population. Based on the pathophysiology of ADTKD-*UMOD*, on previous reports [36] as well as on our findings (Figure 5A), we assigned respectively +1 and +3 points for urinary uromodulin values between the median and 25th percentile and below the 25th percentile of normal urinary uromodulin levels. Similarly, we assigned respectively -1 and -3 points for urinary uromodulin values between the median and 75th percentile and above the 75th percentile of normal urinary uromodulin levels. Conceptualization of the score was based on the previously published HNF1β score [37].

Statistical analysis

Quantitative parameters are presented as median and interquartile range (25th to 75th percentiles) (for scale variables) or means ± standard deviation (for continuous variables), and qualitative parameters are presented as fractions with percentages. Categorical variables were compared using the chi-squared test. Continuous variables were compared using the Mann–

Whitney U test or unpaired *t*-test. ANOVA testing with Tukey's multiple comparison test was used to compare urinary uromodulin levels. Kaplan-Meier curves were generated to display ESKD- 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 as 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 area under the curve of the receiver operating characteristic (ROC) curve. The Youden's index was used to define the optimal discriminatory cut-off point for the *UMOD*-score. Statistical analysis was performed using SPSS Statistics (Armonk, NY: IBM Corp). $p<0.05$ was considered statistically significant, two sided tests were used.

Disclosure

The authors declare no potential conflict of interest relevant to this article.

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Parts of these data have been presented as a poster during the 2017 ASN Kidney Week (October 31-November 5, 2017, New Orleans, Louisiana).

Supplementary Material

Referring Physicians
Supplementary Figures S1-S6
Supplementary Tables S1-S5
Supplementary information is available at *Kidney International*'s website.

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Figure Legends

Figure 1. Design and flowchart of mutation detection in the International ADTKD Cohort

[a]Clinical characteristics of ADTKD are based on the KDIGO Consensus Report [2], see Material & Methods for more details.

n=number of patients; N=number of families. ADTKD, autosomal dominant tubulointerstitial kidney disease; CKD, chronic kidney disease; *UMOD*, gene encoding uromodulin; *MUC1*, gene encoding mucin-1.

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**: Prevalence of different *UMOD* mutations: missense mutations (101/106; 95.3%), affecting cysteine (57/106; 53.8%) or non-cysteine (44/106; 41.5%) amino acids and insertion/deletions (5/106; 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). TM, transmembrane domain; SEA domain, self-cleavage module. **D**: Prevalence of identified *MUC1* mutations in reported ADTKD-*MUC1* families.

Figure 3. Clinical characteristics of ADTKD-*UMOD* and ADTKD-*MUC1*

A: Quantitative parameters are presented as median and quartiles or means±SD. Qualitative parameters are presented as fractions with percentages. Chi-square test for categorial variables, Mann-Whitney U and unpaired *t*-test for quantitative parameters were used. # and $ represent gender comparison within ADTKD-*UMOD* and ADTKD-*MUC1*, respectively. Column n (*UMOD*/*MUC1*) denotes the number of ADTKD-*UMOD* and ADTKD-*MUC1* patients analyzed for the respective parameter. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESKD, end stage kidney disease. **B**: Scatter plots for age at ESKD and onset of gout for ADTKD-*UMOD* and ADTKD *MUC1* patients. Bars indicate means±SD.

Figure 4. Freedom from ESKD and gout in ADTKD-*UMOD* and ADTKD-*MUC1*

A: Kaplan-Meier curve of renal survival in ADTKD-*UMOD* and ADTKD-*MUC1* patients. Median renal survival was 54 years (95% 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 ADTKD-*UMOD* and ADTKD-*MUC1* patients. 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.

Figure 5. Uromodulin processing in ADTKD-*UMOD* and ADTKD-*MUC1*

A: Urinary uromodulin excretion normalized to urinary creatinine (mg/g creatinine) (*left panel*) and normalized to urinary creatinine and eGFR (mg/g creatinine/eGFR) (*right panel*) in ADTKD-*MUC1* patients, ADTKD-*UMOD* patients and a reference population. Median, 25th percentile and 75th percentile values in the reference population are indicted in Figure 5A right panel. Numerical values (median and quartiles) for urinary uromodulin, eGFR and sample size are below the graph. Outlier removed with GraphPad (ROUT Q=1%), One-way ANOVA p<0.0001 for both graphs, Tukey's multiple comparison test was applied. **B**: Immunofluorescence staining for uromodulin (green) and GRP78 (red) in ADTKD-*MUC1*, ADTKD-*UMOD* and normal human kidney (NHK) biopsy. Scale bar: 50µm

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, kidney imaging; [b] Interstitial fibrosis, tubular atrophy, thickening and lamellation of tubular basement membranes, tubular dilatation (microcysts), negative immunofluorescence for complement and immunoglobulins; [c] Proteinuria >300mg/dL, persistent hematuria (both eumorphic and dysmorphic) in repeated urinalysis; [d] HbA1c >10% or repeated blood pressure measurements > 160/100mmHg and/or corresponding clinical findings of hypertensive cardiopathy/nephropathy; [e] ≥1 cyst at any location diagnosed by ultrasonography, CT-scan or MRI. Example: 35-year-old patient, gout onset 32y (+1), serum uric acid 550µmol/L (+3), eGFR 55mL/min/1.73m^2, bland urine analysis and sediment, kidneys without cysts and normal size on MRI, no diabetes or hypertension (+2 for CKD of unknown origin), family history of CKD documented on three generations (+2), total clinical *UMOD*-score of 8 points. Abbreviations: CKD, chronic kidney disease; ADTKD, autosomal dominant tubulointerstitial kidney disease. **B**: Receiver operating characteristics (ROC) curve of the clinical *UMOD*-score in the Belgo-Swiss Registry (n=211 ADTKD patients with available data), AUC 0.72 , 95% 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, NPV 94.3%, PPV 59.1%. **C**: Histogram of clinical *UMOD*-score results in *UMOD*-positive (n=106) and *UMOD*-negative (n=105) patients. The red horizontal line indicates the cut-off value of 5.

Figure 7. *UMOD*-score comparing ADTKD-*UMOD* vs. ADTKD-*MUC1* in the US ADTKD Registry

A: *Left panel*: Receiver operating characteristics (ROC) curve of the clinical *UMOD*-score in the US Registry (n=205 ADTKD-*UMOD* and *MUC1* patients with available data), AUC 0.69 , 95% 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. *Right panel:* Histogram of clinical *UMOD*-score results in ADTKD-*UMOD* (n=125) and ADTKD-*MUC1* (n=80) patients. B: *Left panel:* Receiver operating characteristics (ROC) curve of the clinical *UMOD*-score including urine uromodulin levels in the US Registry (n= 86 ADTKD-*UMOD* and *MUC1* patients with available urinary uromodulin data), 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, NPV 89.7%, PPV 84.2%. *Right panel:* Histogram of clinical + urinary uromodulin *UMOD*-score results in ADTKD-*UMOD* (n=51) and ADTKD-*MUC1* (n=35) patients. The red horizontal line indicates the cut-off value of 5.

Figure 8. Diagnostic algorithm for suspected ADTKD based on clinical *UMOD*-score and urinary uromodulin levels

[a]Progressive loss of renal function, bland urinary sediment, normal-to-mild albuminuria/proteinuria, normal sized kidneys on ultrasound, no consumption of drugs linked to tubulointerstitial nephritis.

[b]Assessed by validated ELISA and normalized to urinary creatinine and eGFR. Obtained values should be interpreted against *UMOD*-negative family members or reference populations [26,27]. See results and discussion section for more details.

[c]For diagnostic algorithm including other ADTKD genes, refer to Devuyst et al.[1]. Alternative diagnosis include nephronophthisis (autosomal recessive), ADPKD (large cystic kidneys), autosomal dominant glomerulopathies (proteinuria/hematuria), other causes of tubulointerstitial kidney disease (autoimmune, TINU) including drugs and toxins (NSAID, aristolochic acid, calcineurin inhibitors, lithium).

Abbreviations: ADTKD, autosomal dominant tubulointerstitial kidney disease; CKD, chronic kidney disease; *UMOD*, gene encoding uromodulin.

Table 1. Clinical and genetic characteristics of ADTKD patients

	International ADTKD Cohort (n=726)	Belgo-Swiss ADTKD Registry (n=275)	US ADTKD Registry (n=451)	n (BE-CH/US)
Number of families	N=585	N=156	N=429	
Sex (%)				
- Female	332/726 (46)	115/275 (42)	217/451 (48)	
Age at presentation (y)	45 (31; 58)	34 (22; 49)	49 (37; 62)	174/377
Positive family history (Gout/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 (y)	44 (32; 55)	44 (33; 56)	44 (32; 55)	245/146
Serum uric acid (µmol/L)	472.0 ± 140.7	479.4 ± 145.3	454.6 ± 128.4	173/74
- Female	452.2 ± 148.8	456.7 ± 158.4	443.1 ± 128.7	67/33
- Male	485.4 ± 133.7	493.8 ± 135.2	463.9 ± 129.0	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 (y)	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
Mutations				
- *UMOD*	N=216/562 (38.4%)	N=54/156 (34.6%)	N=162/406 (40.0%)	

Quantitative parameters are presented as median and quartiles or means±SD. Qualitative parameters are presented as fractions with percentages. N=families, n=patients; Column n(BE-CH/US) denotes the number of patients from the Belgo-Swiss and US Registry analyzed for the respective parameter; BE-CH, Belgo-Swiss; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease.

Figure 1

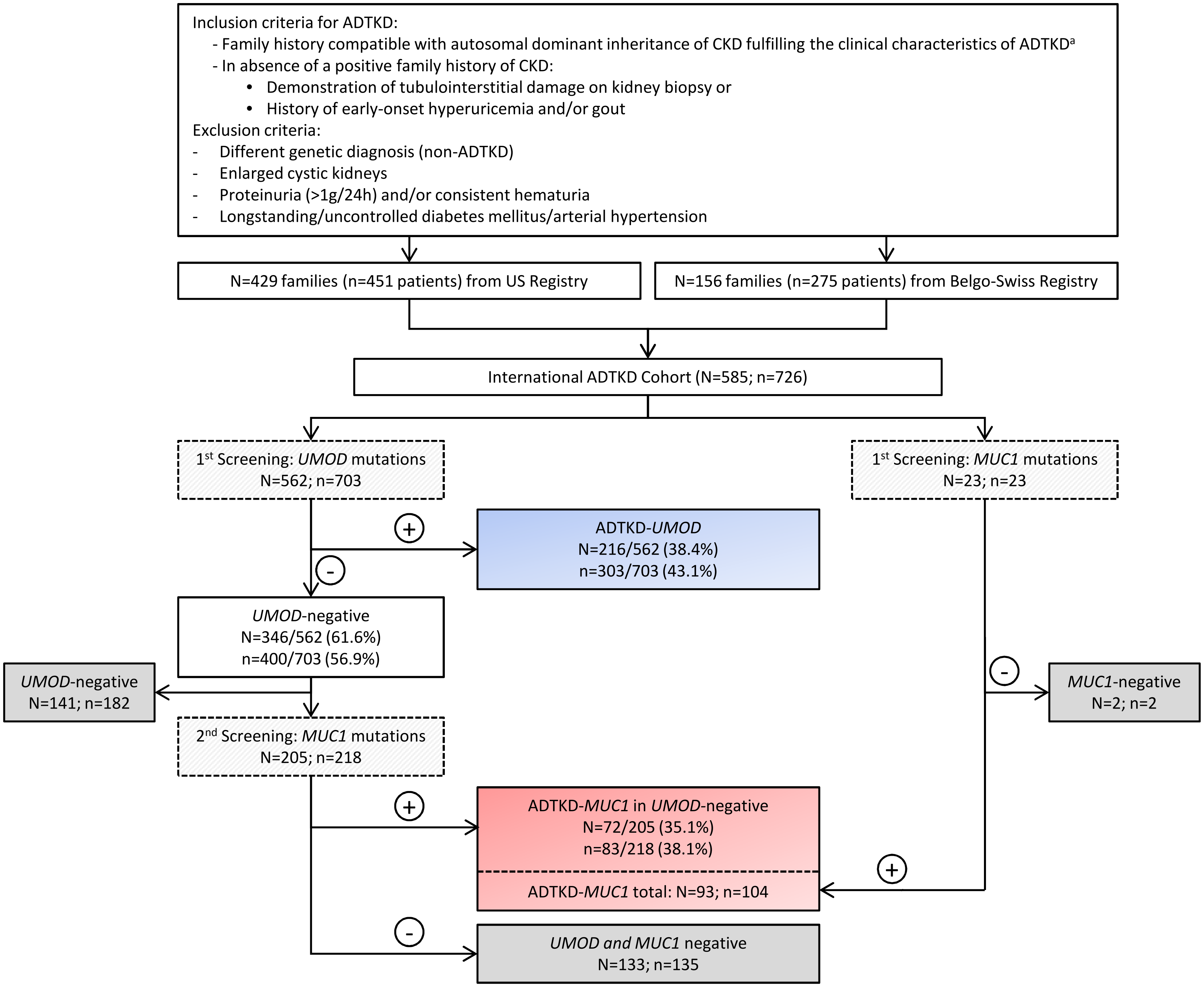
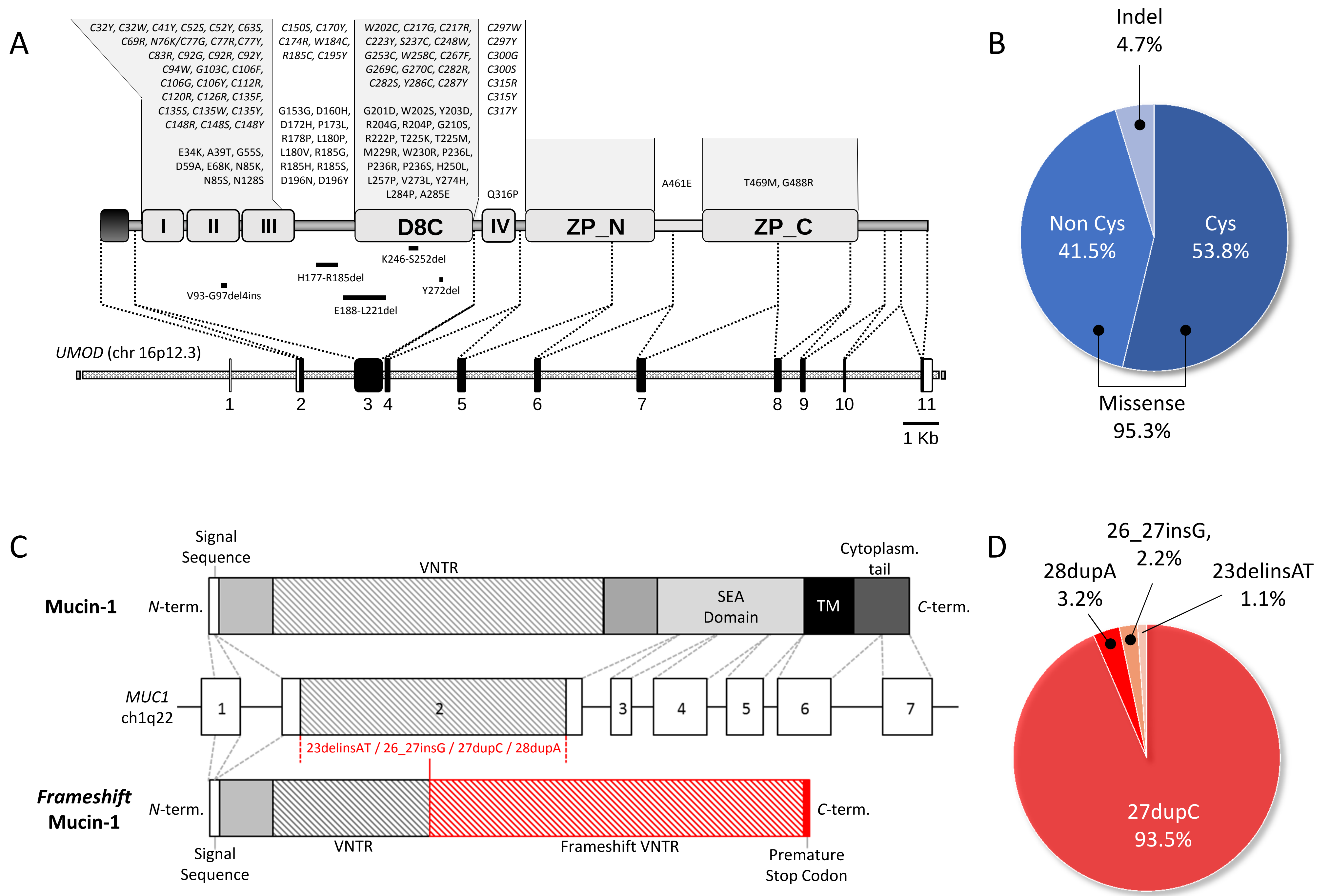

Inclusion criteria for ADTKD:
- Family history compatible with autosomal dominant inheritance of CKD fulfilling the clinical characteristics of ADTKD[a]
- In absence of a positive family history of CKD:
• Demonstration of tubulointerstitial damage on kidney biopsy or
• History of early-onset hyperuricemia and/or gout
Exclusion criteria:
- Different genetic diagnosis (non-ADTKD)
- Enlarged cystic kidneys
- Proteinuria (>1g/24h) and/or consistent hematuria
- Longstanding/uncontrolled diabetes mellitus/arterial hypertension
N=429 families (n=451 patients) from US Registry
N=156 families (n=275 patients) from Belgo-Swiss Registry
International ADTKD Cohort (N=585; n=726)
1st Screening: UMOD mutations
N=562; n=703
1st Screening: MUC1 mutations
N=23; n=23
+
ADTKD-UMOD
N=216/562 (38.4%)
n=303/703 (43.1%)
-
UMOD-negative
N=346/562 (61.6%)
n=400/703 (56.9%)
UMOD-negative
N=141; n=182
-
MUC1-negative
N=2; n=2
2nd Screening: MUC1 mutations
N=205; n=218
+
ADTKD-MUC1 in UMOD-negative
N=72/205 (35.1%)
n=83/218 (38.1%)
ADTKD-MUC1 total: N=93; n=104
+
-
UMOD and MUC1 negative
N=133; n=135

Figure 2



A
C32Y, C32W, C41Y, C52S, C52Y, C63S, C69R, N76K/C77G, C77R,C77Y, C83R, C92G, C92R, C92Y, C94W, G103C, C106F, C106G, C106Y, C112R, C120R, C126R, C135F, C135S, C135W, C135Y, C148R, C148S, C148Y
E34K, A39T, G55S, D59A, E68K, N85K, N85S, N128S
C150S, C170Y, C174R, W184C, R185C, C195Y
G153G, D160H, D172H, P173L, R178P, L180P, L180V, R185G, R185H, R185S, D196N, D196Y
W202C, C217G, C217R, C223Y, S237C, C248W, G253C, W258C, C267F, G269C, G270C, C282R, C282S, Y286C, C287Y
G201D, W202S, Y203D, R204G, R204P, G210S, R222P, T225K, T225M, M229R, W230R, P236L, P236R, P236S, H250L, L257P, V273L, Y274H, L284P, A285E
C297W
C297Y
C300G
C300S
C315R
C315Y
C317Y
Q316P
A461E
T469M, G488R
I
II
III
D8C
IV
ZP_N
ZP_C
K246-S252del
H177-R185del
Y272del
V93-G97del4ins
E188-L221del
UMOD (chr 16p12.3)
1
2
3
4
5
6
7
8
9
10
11
1 Kb
B
Indel
4.7%
Non Cys
41.5%
Cys
53.8%
Missense
95.3%
C
Signal
Sequence
VNTR
Cytoplasm.
tail
Mucin-1
N-term.
SEA
Domain
TM
C-term.
MUC1
ch1q22
1
2
3
4
5
6
7
23delinsAT / 26_27insG / 27dupC / 28dupA
Frameshift
Mucin-1
N-term.
C-term.
Signal
Sequence
VNTR
Frameshift VNTR
Premature
Stop Codon
D
26_27insG,
2.2%
28dupA
3.2%
23delinsAT
1.1%
27dupC
93.5%

Figure 3

A

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

B

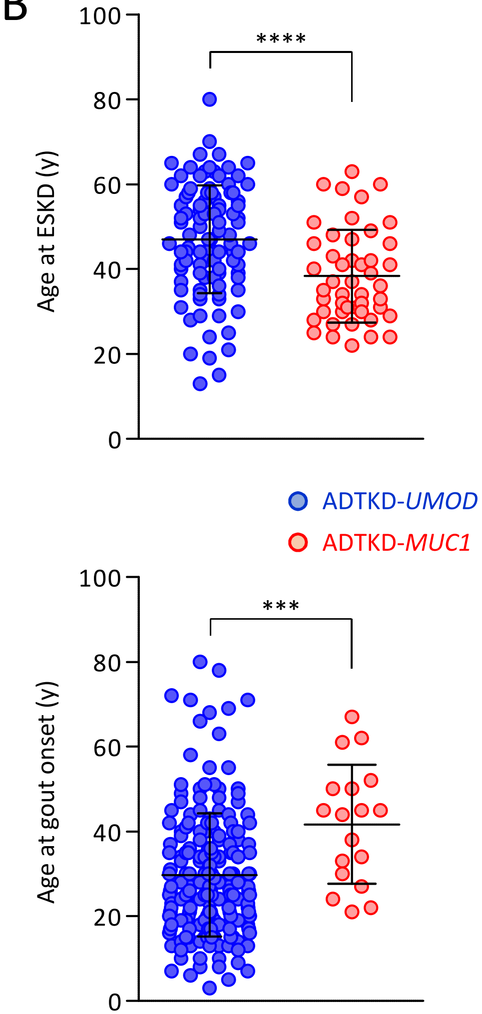


Figure 4

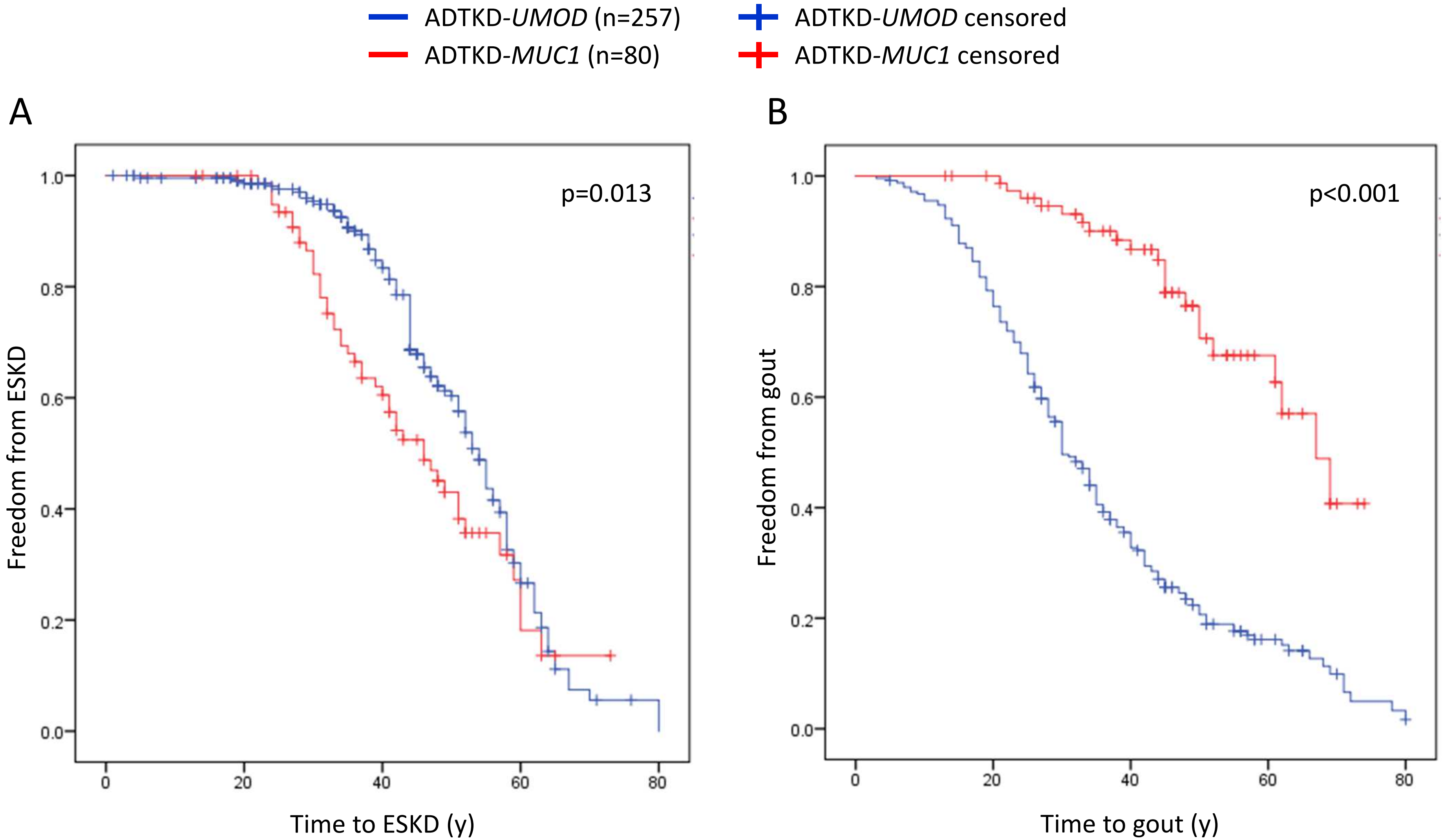
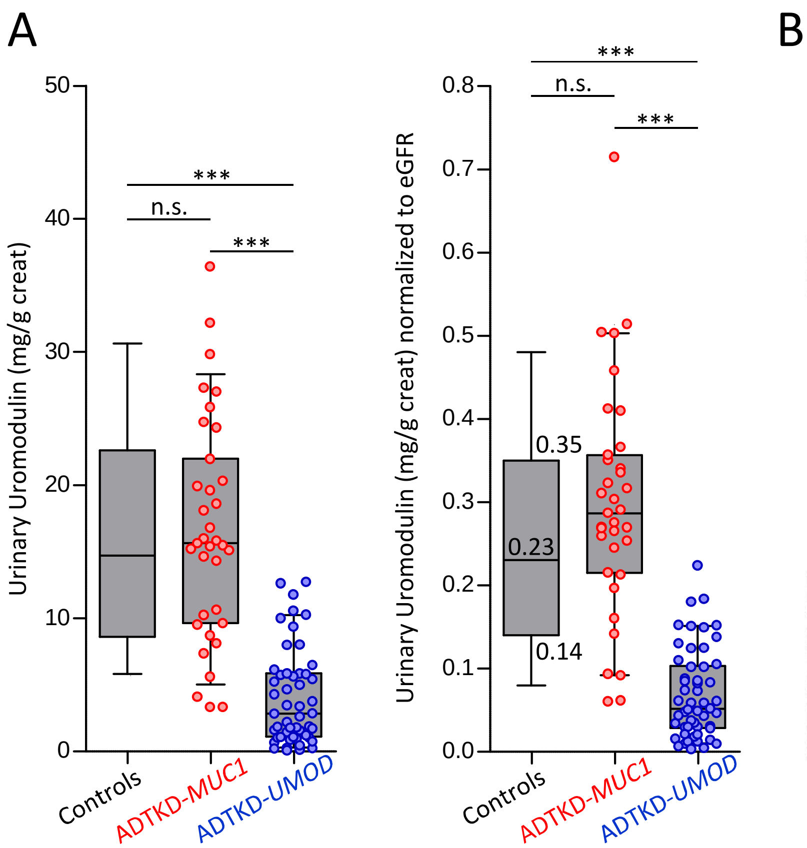

ADTKD-UMOD (n=257)
ADTKD-MUC1 (n=80)
ADTKD-UMOD censored
ADTKD-MUC1 censored
A
p=0.013
Freedom from ESKD
Time to ESKD (y)
B
p<0.001
Freedom from gout
Time to gout (y)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80

Figure 5



Urinary uromodulin /creatinine
Controls (n=180) eGFR: 55.5 (51.7; 58.0) uUMOD: 14.7 (8.6; 22.6)
ADTKD-*MUC1* (n=35) eGFR: 55.1 (45.0; 68.3) uUMOD: 15.7 (9.7; 22.0)
ADTKD-*UMOD* (n=51) eGFR: 51.6 (37.0; 69.2) uUMOD: 2.8 (1.1; 5.9)

Urinary uromodulin /creatinine and eGFR
Controls (n=2717) eGFR: 78.0 (70.3; 84.1) uUMOD: 0.23 (0.14; 0.35)
ADTKD-*MUC1* (n=35) eGFR: 55.1 (45.0; 68.3) uUMOD: 0.29 (0.22; 0.36)
ADTKD-*UMOD* (n=53) eGFR: 53.5 (37.0; 73.6) uUMOD: 0.052 (0.029; 0.10)

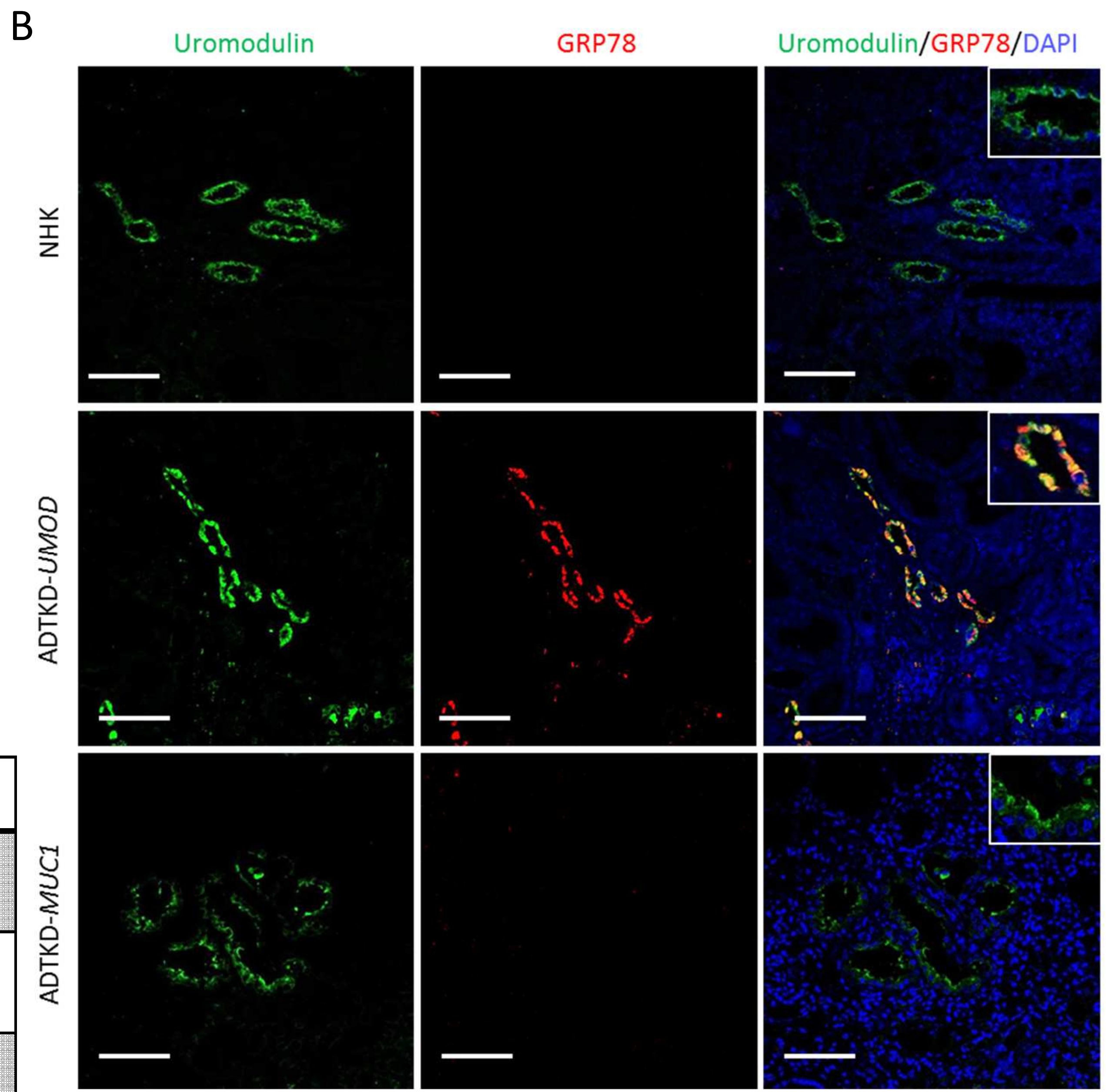
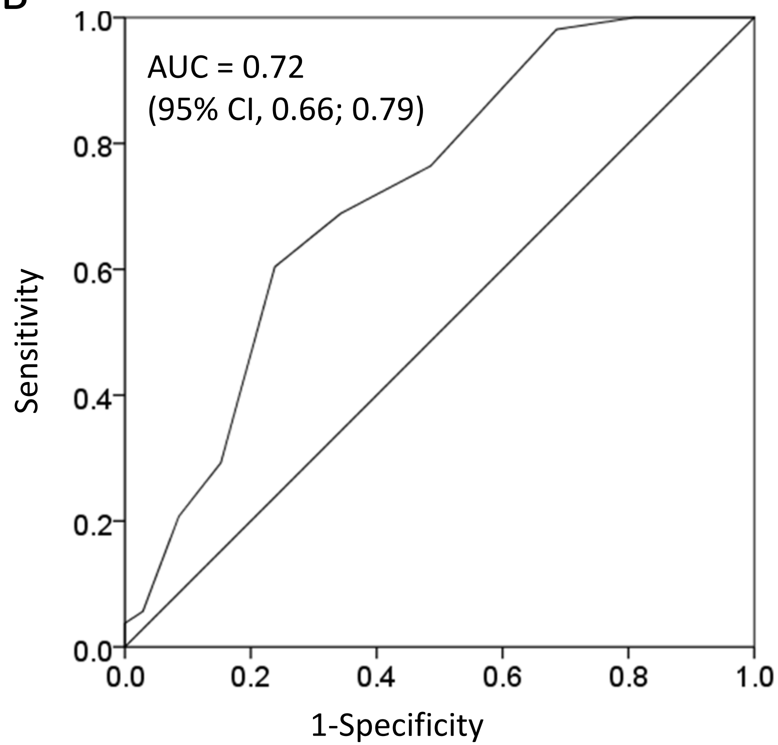


Figure 6

A

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

B



C

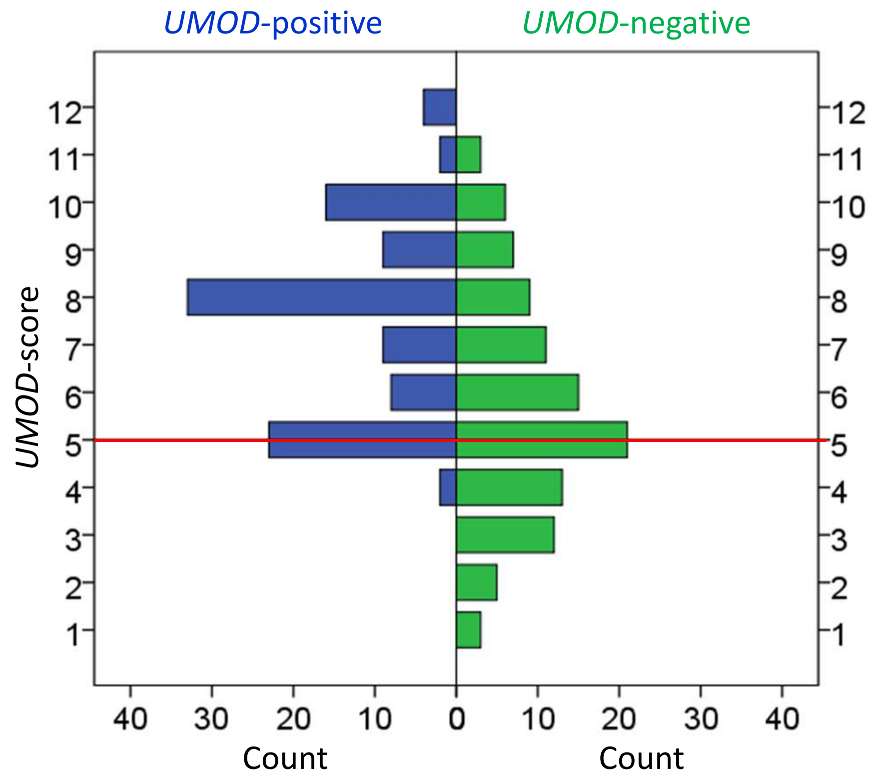


Figure 7

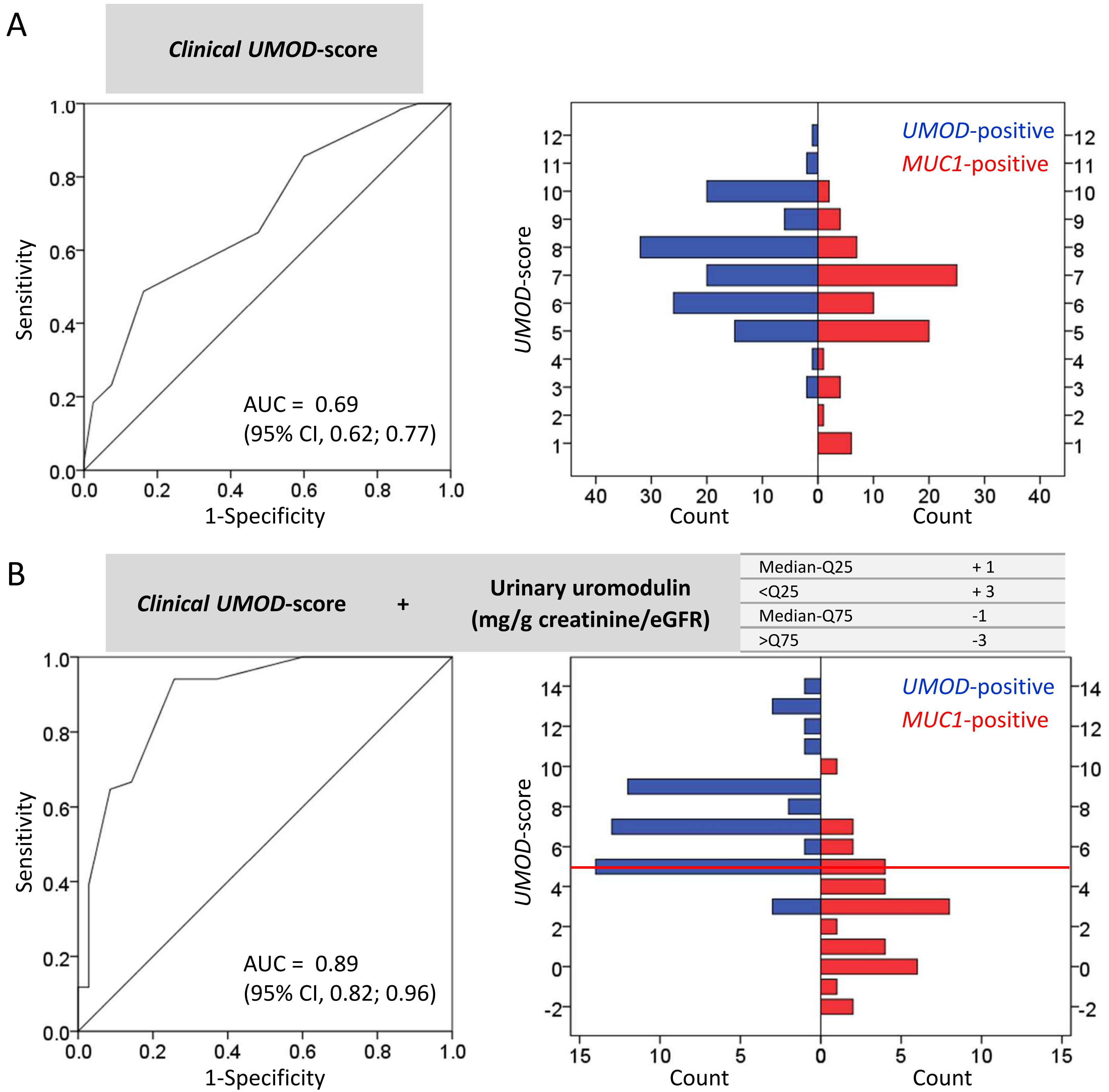
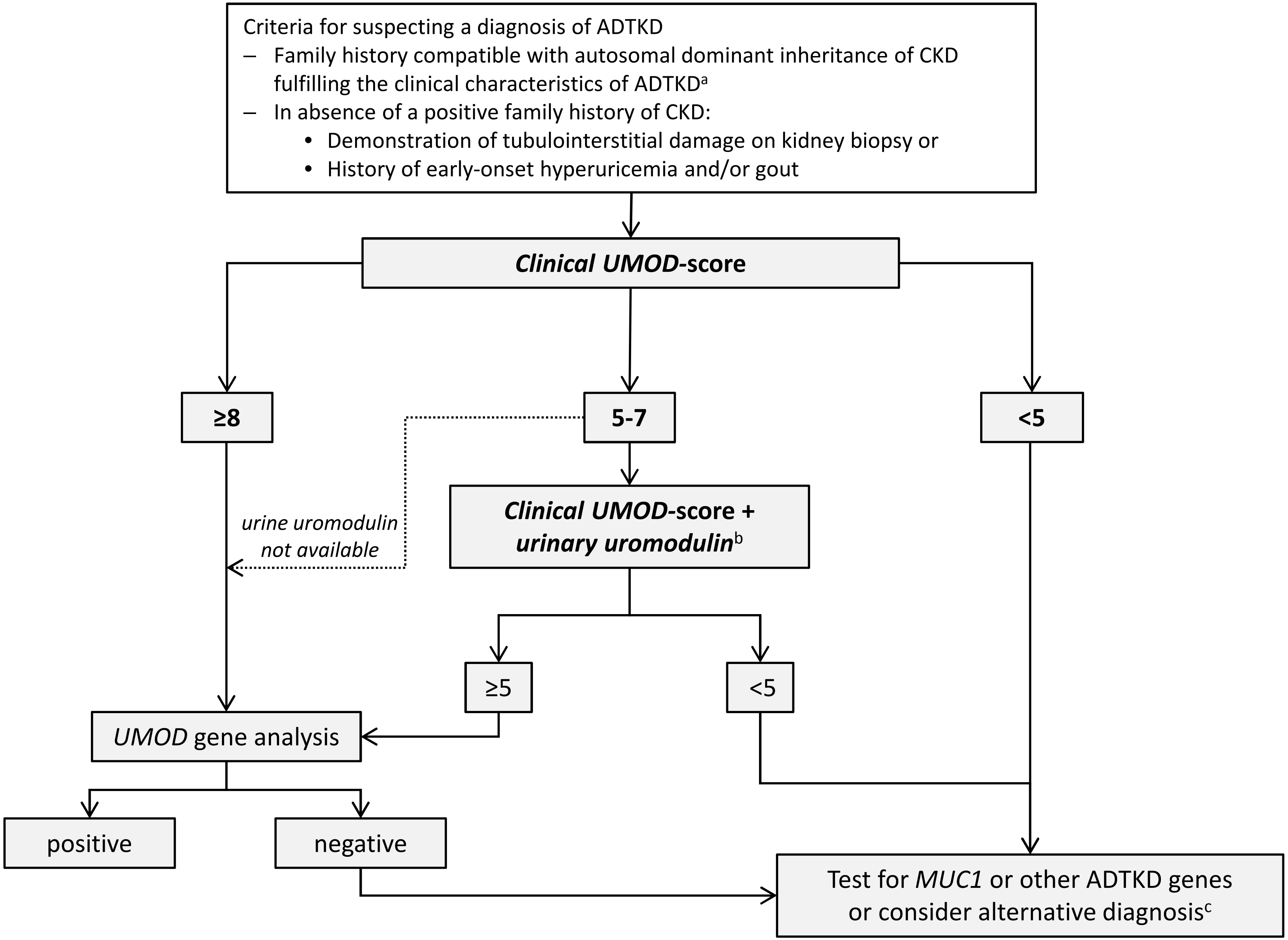

A
Clinical UMOD-score
AUC = 0.69
(95% CI, 0.62; 0.77)
Sensitivity
1-Specificity
UMOD-positive
MUC1-positive
UMOD-score
Count
Count
B
Clinical UMOD-score + Urinary uromodulin (mg/g creatinine/eGFR)
Median-Q25 + 1
<Q25 + 3
Median-Q75 -1
>Q75 -3
AUC = 0.89
(95% CI, 0.82; 0.96)
Sensitivity
1-Specificity
UMOD-positive
MUC1-positive
UMOD-score
Count
Count

Figure 8


Criteria for suspecting a diagnosis of ADTKD
– Family history compatible with autosomal dominant inheritance of CKD fulfilling the clinical characteristics of ADTKD[a]
– In absence of a positive family history of CKD:
• Demonstration of tubulointerstitial damage on kidney biopsy or
• History of early-onset hyperuricemia and/or gout
Clinical UMOD-score
≥8
5-7
<5
urine uromodulin not available
Clinical UMOD-score + urinary uromodulin[b]
≥5
<5
UMOD gene analysis
positive
negative
Test for MUC1 or other ADTKD genes or consider alternative diagnosis[c]