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Renal fibrosis is the common feature of autosomal dominant tubulointerstitial kidney diseases caused by mutations in mucin 1 or uromodulin

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For decades, ill-defined autosomal dominant renal diseases have been reported, which originate from tubular cells and lead to tubular atrophy and interstitial fibrosis. These diseases are clinically indistinguishable, but caused by mutations in at least four different genes: *UMOD*, *HNF1B*, *REN*, and, as recently described, *MUC1*. Affected family members show renal fibrosis in the biopsy and gradually declining renal function, with renal failure usually occurring between the third and sixth decade of life. Here we describe 10 families and define eligibility criteria to consider this type of inherited disease, as well as propose a practicable approach for diagnosis. In contrast to what the frequently used term 'Medullary Cystic Kidney Disease' implies, development of (medullary) cysts is neither an early nor a typical feature, as determined by MRI. In addition to Sanger and gene panel sequencing of the four genes, we established SNaPshot minisequencing for the predescribed cytosine duplication within a distinct repeat region of *MUC1* causing a frameshift. A mutation was found in 7 of 9 families (3 in *UMOD* and 4 in *MUC1*), with one indeterminate (*UMOD* p.T62P). On the basis of clinical and pathological characteristics we propose the term 'Autosomal Dominant Tubulointerstitial Kidney Disease' as an improved terminology. This should enhance recognition and correct diagnosis of affected individuals, facilitate genetic counseling, and stimulate research into the underlying pathophysiology.

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Chronic kidney disease (CKD) is defined by a reduction in glomerular filtration rate and/or structural abnormalities of the kidney. It affects >10% of the adult population in Europe and North America. CKD is not only a major burden on health-care costs but also an important and independent predictor of cardiovascular morbidity and mortality.[1] Independent of the initial cause, the progression of renal disease involves increasing interstitial fibrosis and tubular atrophy (IF/TA), which is also the parameter with the highest predictive value for decline in kidney function.[2] Heterogeneous and frequent conditions (such as hypertension, diabetes mellitus, inflammatory diseases, and drug use) can lead to renal IF/TA. Multiple molecular pathways are believed to be involved in the development of IF/TA, with activation of molecules such as transforming growth factor-β1, connective tissue growth factor, and basic fibroblast growth factor-2

being some of the many leading to epithelial dedifferentiation and fibrogenesis.[3] However, the initial signals giving the impulse to fibrosis are largely unknown. In general, the clarification of monogenic hereditary diseases promises identification of priming signals. This may improve the understanding of the pathogenesis of disease far beyond the usually rare hereditary forms and lead to novel and specific ways of therapeutic intervention.

Over the past couple of decades, families with autosomal dominant tubulointerstitial kidney diseases have been repeatedly described, in which affected individuals slowly develop end-stage renal disease (ESRD) between the third and sixth decade of life because of progressive IF/TA, with or without extrarenal symptoms, such as early gout, renal cysts, or diabetes mellitus.[4] Until recently, the genes causing this disease were incompletely described; hence, the disease is not well known and it has been impossible to correctly diagnose, classify, and counsel families. Another profound difficulty, the rather unfortunate name 'medullary cystic kidney disease' (MCKD), has hampered the correct handling of the disease. Indeed, the finding of cysts in the renal medulla does not appear to be typical[4,5] and seems to have confused clinicians in correctly diagnosing the disease. Moreover, the term includes only two subtypes of the disease, when at least four can be differentiated (see below). Furthermore, numerous parallel names and abbreviations have been given to this group of diseases, such as TIN (tubulointerstitial nephritis), FJHN (familial juvenile hyperuricemic nephropathy), UMAK (uromodulin-associated kidney disease), MCD (medullary cystic disease), or ADMCKD (autosomal dominant MCKD), that has further increased the clinical bafflement. Finally, these autosomal dominant renal diseases have been clubbed together into one disease entity with the different forms of 'Familial Juvenile Nephronophthisis' (FJN) under the term 'nephronophthisis complex,'[6] despite significant clinical and genetic differences, with autosomal recessive inheritance observed in pediatric FJN patients.

Mutations in at least four genes can be implicated in the autosomal dominant disease: *MUC1* encoding mucin 1 (chromosomal location and gene at Online Mendelian Inheritance in Man (OMIM) 1q22 and 158340, respectively),[7] *UMOD* encoding uromodulin[8] (OMIM 16p12.3 and 191845), *HNF1B* encoding hepatocyte nuclear factor-1β[9,10] (OMIM 17q12 and 189907), and *REN* encoding renin[11] (OMIM 1q32.1 and 179820). Although some of these disorders may be accompanied by typical extrarenal features, these are not obligatory. The common and often the only feature of all of these variants is autosomal dominant inheritance and renal IF/TA.[4,5] Therefore, these four entities cannot be differentiated clinically but can only be reliably identified by genetic analysis.

To date, the proportion of these phenotypically similar autosomal dominant diseases due to mutations in either of the four genes is not known. However, two forms have been prominent: MCKD1 and MCKD2. MCKD2 is caused by mutations in the *UMOD* gene[8] that codes for the Tamm–Horsfall glycoprotein/uromodulin. The functions of Tamm–Horsfall glycoprotein have still not been fully resolved, but among other tasks it appears to be involved in renal clearance of uric acid, either directly or indirectly. This may explain why some families develop hyperuricemia and early gout that may precede renal insufficiency. However, numerous MCKD2 families have been described without this clinical feature (for review see Bleyer *et al.*[12]). The search for the disease-causing gene of MCKD1 has taken considerably longer. The locus on chromosome 1q21 was first identified in 1998,[13] and has subsequently been confirmed by numerous independent studies.[14–18] However, despite widespread efforts, the link to the responsible gene *MUC1* was only recently identified.[7] *MUC1* contains a coding GC-rich region of 'variable number of tandem repeat' (VNTR) sequences that was originally believed to lie between the second and third exons, consisting of up to 100 or more repeating stretches of 60 base pairs.[19] Because of this complex structure, the VNTR region of the *MUC1* gene has been impossible to analyze by straight Sanger sequencing, whole-genome, or whole-exome massive parallel sequencing. The 60 base-pair repeat includes a sequence of seven cytosine (C) residues. The only disease-causing mutation in the *MUC1* gene described to date is a duplication of one cytosine in the heptanucleotide cytosine tract that leads to a frameshift mutation and reaches an early stop after the VNTR region.[7]

In this study we analyzed 10 novel families with European ancestry with autosomal dominant CKD, and developed a systematic approach for a reliable genetic diagnosis. On the basis of clinical and genetic findings, we suggest a new term, 'Autosomal Dominant Tubulointerstitial Kidney Disease' (ADTKD). We anticipate that this improved terminology would facilitate the recognition and stimulate further research on prevalence and underlying pathophysiology.

RESULTS

Characterization of families

Aiming to improve the clinical and genetic description of the ADTKD family of renal diseases, we searched for families who fulfilled four criteria: (1) autosomal dominant inheritance; (2) development to ESRD usually between the third and sixth decade of life or at least profound CKD; (3) predominant IF/TA on renal biopsy (where available) with absence of immunohistological staining or any signs of primary glomerulopathy; and (4) bland urine sediment and absent or mild proteinuria. Our search criteria did not include the presence or absence of renal cysts.

We analyzed 10 incident, unrelated families who met the inclusion criteria and were of European descent (Table 1). Two of these families were quite large (families 1 and 2); their pedigrees are shown in Figure 1 (see Supplementary Figure S1 online for the pedigrees of families 3 to 10). The inheritance pattern in these 10 families showed full penetrance in terms of each affected individual having an affected parent. Affected family members were equally dispersed in both sexes and in each generation. Two families displayed

Table 1 | Studied families

Family no.	Clinical samples/data collection	Genetical analyses	Affected gene (mutation)
1	DNA samples (46), MRI (2), RB (8)	WES, TGS, SSM, GPS, USS	*MUC1* (c.428dupC)
2	DNA samples (12), MRI (2), RB (3)	WES, TGS, SSM, GPS, USS	*MUC1* (c.428dupC)
3	DNA samples (5), MRI (2), RB (1)	SSM, TGS, GPS, USS, MLPA	NOS (*UMOD* variant c.509G>A, p.T62P)
4	DNA samples (2)	SSM, TGS, USS	*UMOD* (c.155G>A, p.C52Y)*[33]
5	DNA samples (2)	SSM, TGS, USS	*MUC1* (c.428dupC)
6	DNA samples (2)	SSM, TGS, GPS, USS, MLPA	NOS
7	DNA samples (2)	SSM, TGS, USS	*UMOD* (c.854C>A, p.A285E)[27]
8	DNA samples (2)	SSM, TGS, GPS, USS, MLPA	NOS
9	DNA samples (2), MRI (2), RB (1)	SSM, USS	*UMOD* (c.509G>A, p.C170Y)[34]
10	DNA samples (2), MRI (2), RB (1)	SSM, USS	*MUC1* (c.428dupC)

Abbreviations: GPS, gene panel sequencing; MLPA, multiplex ligation-dependent probe amplification for *HNF1B*; MRI, magnetic resonance imaging; NOS, not otherwise specified; RB, renal biopsies reviewed centrally; SSM, SNaPshot minisequencing; TGS, targeted genomic sequencing; USS, *UMOD* Sanger sequencing; WES, whole-exome sequencing.

All families studied are listed in numerical order, for whom the pedigrees with the same numbering are shown in Figure 1 and Supplementary Figure S1 online. Families 1, 2, 3, 9, and 10 are from Germany. Families 4, 5, 6, 7, and 8 are from Australia. The individual set of data, as well as the genetic result of the analyses, are summarized. Numbers in parenthesis represent the numbers of samples available or the number of patients analyzed. References for *UMOD* mutations refer to the first published report, where the asterisk marks a similar mutation with a different amino acid exchange at the same position. Following the instructions for terminology by the human genome variation, the variable number of tandem repeat (VNTR) *MUC1* mutation previously described[7] should be termed as a single base duplication. As it is not known in which repeat of the VNTR the mutation has occurred, the seventh position of the cytosine stretch of the first repeat has been chosen as the nucleotide position (c.428dupC).

slight deviations from the others. First, family 9 was the only one to show a young male with gout before the onset of renal disease. This was not reported in any other family, although hyperuricemia and occasional gout attacks did occur with progressive renal insufficiency. Second, family 3 harbored three unaffected female members who were approaching the age of 70 years and were not at ESRD but continued to remain at CKD stage 3 or 4.

Typical findings of histopathology

Next, we reviewed all available historical renal biopsies taken of the different families. As some of these were taken decades ago, we were not always successful in reordering them. In most of these cases, however, we were able to review the pathological reports. Overall, we were able to collect and centrally review 14 biopsies from 5 families (Table 1). The typical histological features that recurred in each biopsy were early and profound IF/TA, varying degree of nephrosclerosis and arteriolar thickening and hyalinosis, and negative immunofluorescence or immunostaining. Very rarely did we see widened and curled tubular segments, indicating microcystic development. Electron microscopy showed irregular width and lamelation of the basement membrane, of the glomerula and particular the tubules. Figure 2 shows a representative biopsy taken from a patient from family 1 in the third decade of life and at CKD stage 3.

Genetic studies

As the families were clinically and histologically indistinguishable, we next performed genetic analysis using different strategies (Table 1). Direct Sanger sequencing of *UMOD* detected four variants, of which three had been previously published as causative mutations (Table 1). One *UMOD* variant remains unclear (c.509G>A, p.T62P, rs143248111; Table 1, family 3), as the frequency has been reported to be ~1:2000 and the prediction of the variant is possibly damaging (Polyphen-2 score 0.66) or disease causing (MutationTaster 0.99) as per the respective mutation prediction software (see Materials and Methods). However, we performed segregation analysis in the family with two unaffected and three affected members that showed perfect segregation of this *UMOD* variant with CKD. In one single renal biopsy available from this family, immunostaining for uromodulin appeared much more cytoplasmic with much less apical enhancement. Finally, urinary uromodulin protein excretion was decreased in comparison with healthy controls (data not shown). These studies have been performed by others to characterize the effect of previously established causative *UMOD* mutations.[20] Further functional studies will have to be performed to clarify the relevance of this variant. Because of the relatively high frequency of the variant, we did not class it as causative for the purpose of this study.

The putative genetic cause could be identified for three families, but remained unclear in the other seven. Families 1 and 2 were sufficiently large and thus we were able to collect blood samples from numerous members in order to perform a genome-wide linkage analysis and subsequently a haplotype analysis narrowing down the location of a disease-linked locus. Figure 3 shows the LOD (logarithm of odds) score of the haplotype analysis for family 1 that confirms a significant 3.4 Mb locus at the predescribed site on chromosome 1q21. As the haplotype analysis of family 2 coincided (not shown) with this locus, we had a shared linkage locus with an overall LOD score clearly reaching genome-wide significance. Therefore, it is very likely that these families belonged to the formerly classed MCKD1 disease and displayed the *MUC1* mutation. We performed whole-exome sequencing (families 1 and 2) and targeted genomic sequencing for the complete linkage locus at 1q21 (families 1 to 8, see Table 1), which showed no segregating variants in any of the genes, including *MUC1*. However, it needs to be stressed that the VNTR region of the *MUC1* gene was masked in both of these analyses because of fundamental technological deficiency.[7] Therefore, we searched for the described frameshift mutation

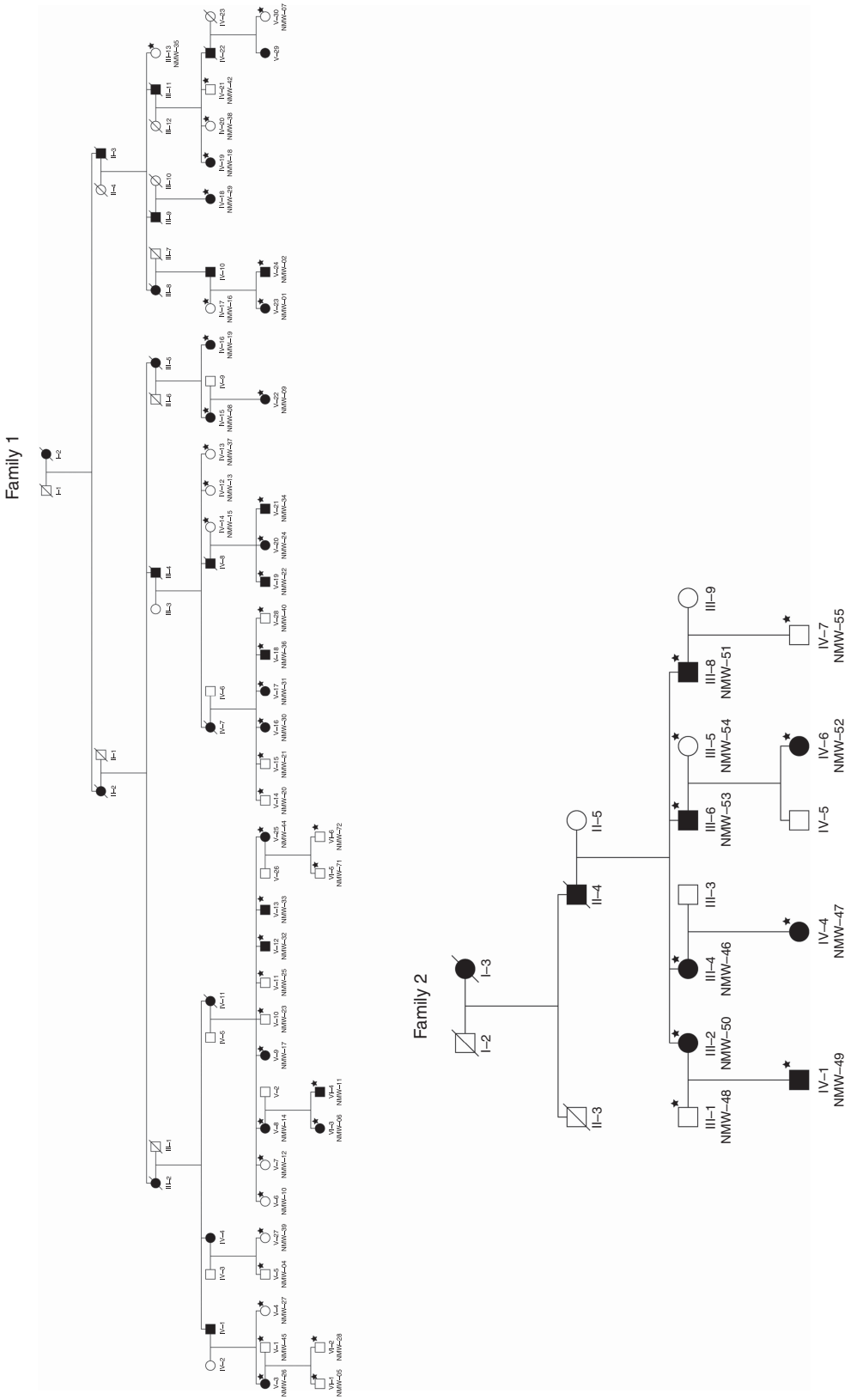


Figure 1 | Families with autosomal dominant tubulointerstitial kidney disease (ADTKD) selected for linkage analysis. Pedigrees of the two large multigeneration families investigated in the linkage analysis. Black symbols indicate affected individuals, white symbols indicate unaffected status, and slashed symbols indicate deceased individuals. DNA for *in vitro* analysis was available from all individuals numbered with NMW-xx. Generations are numbered in roman numerals. Individuals in whom SNaPshot minisequencing for the insertion mutation in *MUC1* was performed are marked by an asterisk.

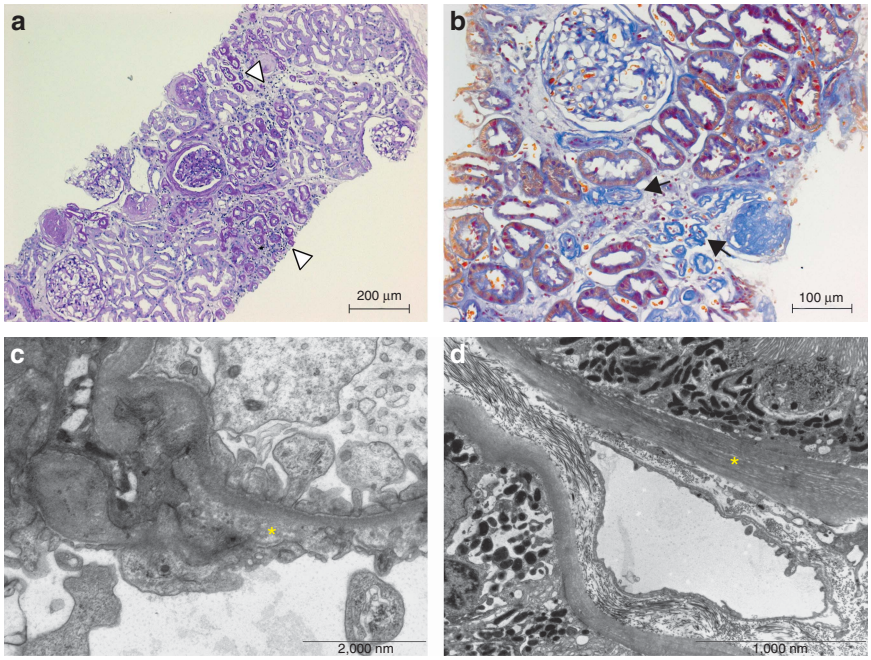


Figure 2 | Typical histological findings in autosomal dominant tubulointerstitial kidney disease (ADTKD). Representative renal biopsy from a patient of family 1 at chronic kidney disease (CKD) stage 3 (NMW-22). (**a**) Light microscopy illustrating interstitial fibrosis/tubular atrophy (IF/TA) with thickened tubular basement membranes and only mild interstitial inflammation (arrowheads; original magnification ×100; periodic acid–Schiff (PAS) stain). (**b**) Pronounced fibrosis and thickened tubular basement membranes (arrows; original magnification ×200, sour Fuchsin-orange G (SFOG) stain). (**c**) Electron microscopy illustrating multilayering of the tubular basement membranes (yellow asterisk; original magnification ×3579). (**d**) Glomerular capillary with mild irregularity of the inner layers of the basement membrane (yellow asterisk; original magnification ×12930).

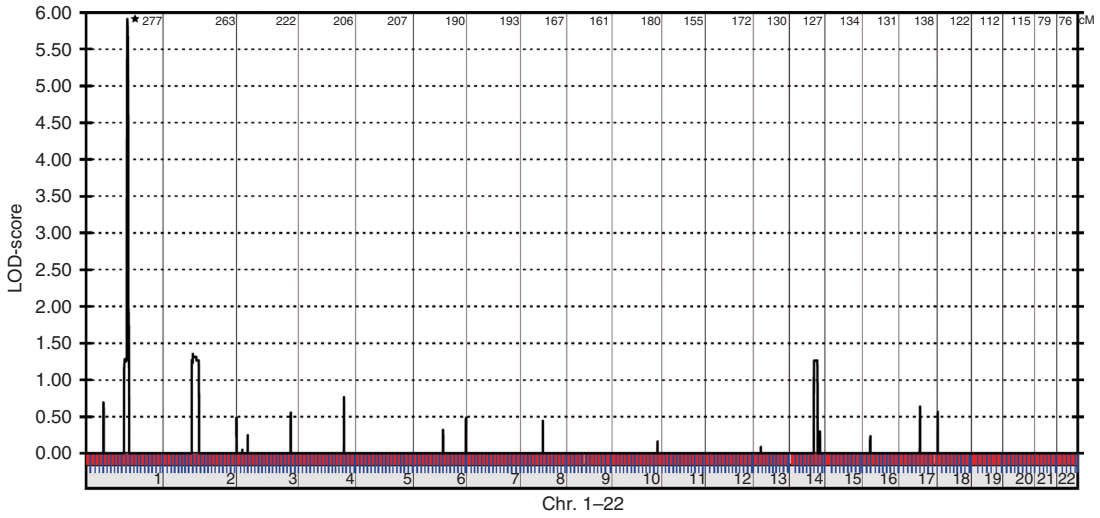


Figure 3 | Linkage analysis. Result of a whole-genome linkage analysis. Parametric multipoint LOD (logarithm of odds) scores of the pedigree of family 1. The plot indicates linkage to a single locus on chromosome (Chr.) 1 with a maximum LOD score close to 6 (indicated by an asterisk). The Y axis indicates the multipoint parametric linkage analysis LOD score and the X axis indicates the marker coverage (100 markers per vertical line) and the chromosomes (numbers under the X axis).

by duplication of a cytosine residue in the VNTR region of the *MUC1* gene in the families not bearing an *UMOD* mutation by SNaPshot minisequencing, originally established by VM and CA in Paris. We thereby found four positive families, including families 1 and 2, as expected from the linkage and haplotype analysis and locus information (Figure 4 and Table 1). We investigated their familial relationship using genome-wide comparative identity-by-state analysis and were

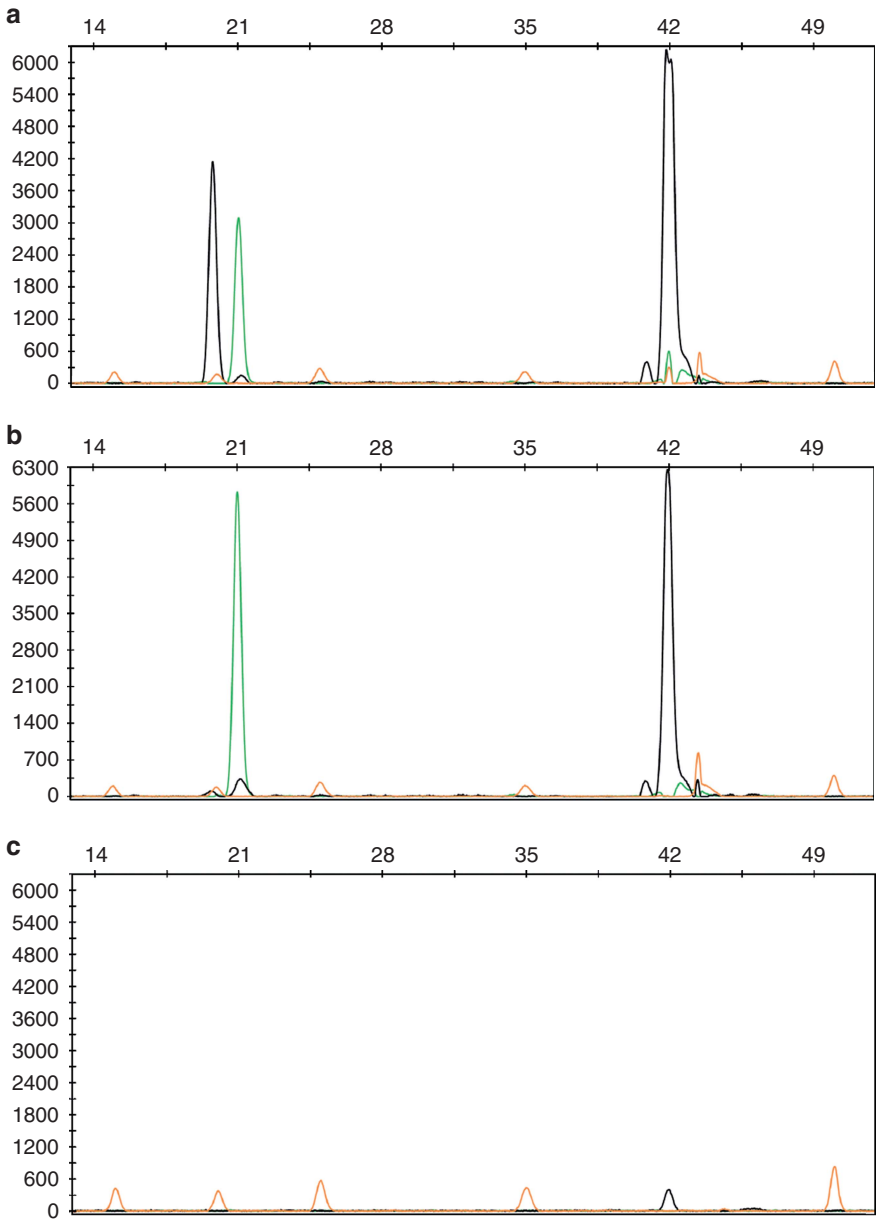


Figure 4 | Analysis of *MUC1* variable number of tandem repeat (VNTR) duplication of cytosine (C) mutations. An example of a SNaPshot electropherogram showing three individual reactions with a positive sample (**a**), a negative sample (**b**) for the cytosine duplication, and a blank control (**c**). In each reaction, 100 ng of genomic DNA was used except in the blank control (water). Numbers on the X axis above electropherograms indicate the fragment length of the extended primers in bp; the Y axis provides the RFU (relative fluorescence units). A green peak with 21 bp is the primer extended with an A (adenine) flanking immediate to the stretch of 7 Cs on the wild-type allele (**a**). A smaller black peak also appears at 21 bp after a primer extension with a C indicating the mutation due to a C duplication. The different sizes of these two peaks in the electropherogram are generated from the diverse capillary migration characteristics of the two different fluorochromes, even if the two fragments are the same base-pair length. The black peak with 39 bp is a SNaPshot control reaction on the complementary DNA strand from a larger primer extended with a C.

able to exclude a close relationship between individuals of these four families (Supplementary Figure S2 and Supplementary Table S1 online). Haplotype analyses from array genotype data showed a 9-allele haplotype shared between families 1 and 2, hinting at the possibility of an ancestral common haplotype bearing the mutation. In order to investigate this further, we extracted 24 high-confidence single-nucleotide polymorphism genotypes in and around the *MUC1* gene from genomic sequencing data. Subsequent haplotype analysis with these showed the haplotype shared by the affected individuals to be rather common (Supplementary Tables S2 and S3 online), preventing any definite statement regarding an ancestral mutated haplotype. In three families the genetic cause of their disease remains unsolved to

date (families 3, 6, and 8; Table 1); they also showed the absence of a pathogenic variant in gene panel sequencing by Ion Torrent for *MUC1*, *UMOD*, *HNF1B*, and *REN*. It is noteworthy that this analysis does not exclude copy number variations. As this is particularly relevant for *HNF1B*,[21] we performed multiplex ligation–dependent probe amplification for *HNF1B* for families 3, 6, and 8 that excluded any deletions or duplications.

Morphological evaluation by MRI

Having performed broad genetic testing for the four genes in question, we finally wished to characterize the kidney morphology, particularly the search for the existence and location of renal cysts. As renal ultrasound has too little sensitivity for small changes, we screened our patients using magnetic resonance imaging (MRI). From five families (3× *MUC1*, 1× *UMOD* mutations, and 1× not otherwise specified) we analyzed one affected member with mild and severe renal disease each. Figure 5 shows representative images from eight affected probands. The images clearly show that renal cysts occur only occasionally and there were no medullary cysts in any case. Thus, the development of renal and/or medullary cysts does not appear to be a typical or early sign of ADTKD. Interestingly, some but not all of the patients with *MUC1* mutations develop a moderate number of cysts. However, these cysts can be seen in the parenchyma as well as at the corticomedullary boundary. Figure 6 shows several sections through the kidneys of a single female patient with the *MUC1* mutation who had the most pronounced number of cysts of all patients. In this patient, every single cyst that could be found is depicted.

DISCUSSION

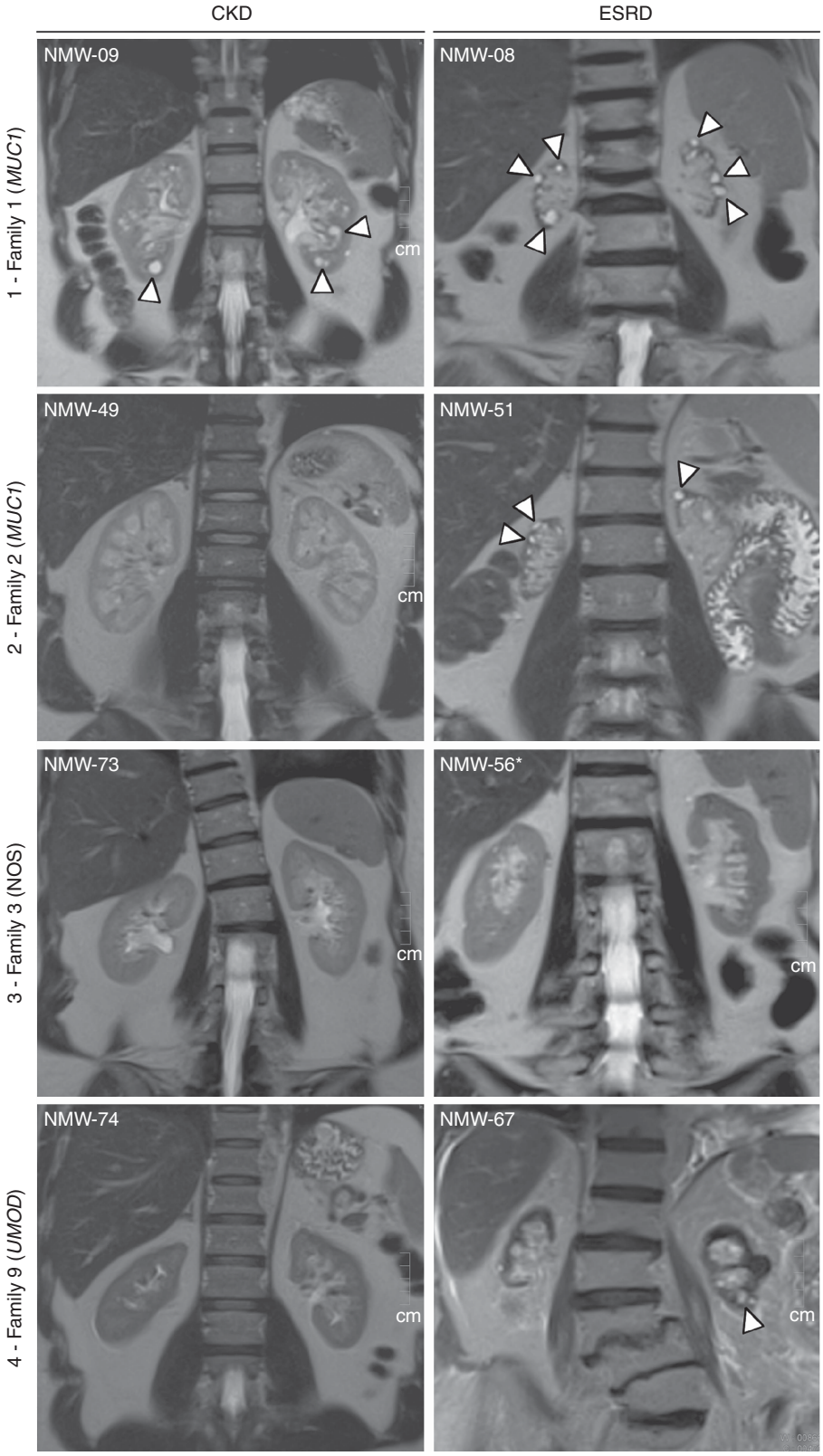
In daily nephrological practice, many patients enter ESRD with unrecognized origin of renal disease. In a large population-based sample of patients with ESRD, Freedman *et al.*[22] identified >20% of dialysis patients having a positive family history for ESRD in first- or second-degree relatives, excluding known Mendelian diseases and urological causes. A similar rate of 18.2% of all patients with ESRD with first- or second-degree relatives also having ESRD was recently reported from Cyprus.[23] Interestingly, this figure rose to 27% in these patients with ESRD due to uncertain etiology that will mostly have excluded autosomal dominant polycystic kidney disease. Together, these studies document a strong genetic component among the reasons leading to ESRD. It is noteworthy that even if a positive family history is present and thereby a hereditary cause likely, the exact diagnosis is frequently not established. This precludes genetic counseling and handicaps the risk assessment for disease recurrence after transplantation. Importantly, an exact genetic diagnosis of a hereditary disease will put the need of renal biopsies in the affected families into perspective. This point is especially problematic in diseases focused on in this study, as renal biopsy does not usually lead to a solid diagnosis, as there are no specific findings. Moreover, the uncertainty about the underlying diagnosis hampers research into the epidemiology, clinical course, and pathogenesis. Thus, efforts to clarify the genetic causes of renal disease should be promoted, wherever possible.

As outlined above, the spectrum of autosomal dominant tubulointerstitial diseases has not yet reached clinical recognition, and this we intend to improve. A very confusing issue of this disease has been the terminology, in particular the focus on medullary cysts. It is noteworthy that one of the very first cases reported in 1945, following which the terminus 'medullary cysts' was introduced, did show a profound number of cysts in the renal medulla on autopsy.[24] However, considering the age of 8 years at the time of presentation and the empty family history, we would suspect autosomal recessive FJN in this single case. Most of the recently published work has stressed that medullary cysts are not usually present in the adult forms of the disease,[25] and this is also confirmed by our MRI studies.[4,5] Therefore, we would support a new terminology and would consider the term ADTKD appropriate, because the elements of this name summarize the conformity between the different disease types and genes in question and will remain correct irrespective of future gains in pathomechanistic knowledge. However, a panel of experts should agree on the future terminology. Other autosomal dominant diseases also stem from tubular cells, such as autosomal dominant polycystic kidney disease or distal tubular acidosis. These diseases should not be included in this terminology as they have different clinical characteristics (that is, enlarged cystic kidneys and acid base disorders). As the uniform histological feature of ADTKD is IF/TA, we feel that it is justified and correct to name the disease 'tubulointerstitial.' Clearly, for *MUC1* and *UMOD* mutations, the primary cellular origin of the disease is indeed the renal tubular apparatus. Hence, we would discourage naming the disease 'cystic,' as the appearance of renal cysts is neither an early nor a specific feature of the disease. It is to be noted that any renal disease has the potential to lead to tubular dedifferentiation and development of degenerative cysts.[26] Furthermore, the small to moderate number of cysts that can be found in ADTKD patients (if any) is certainly not the reason for the decline in renal function. Further studies with more patients and preferably MRI will have to verify our findings that occasional cysts at the corticomedullary boundary can be found in *MUC1*-associated disease. Whether this is linked to the frameshift protein and its localization is pure speculation to date.

ADTKD is considered a rare disease, but as clinical presentation and biopsy findings are unspecific and genetic analyses not regularly offered, it is likely that a substantial proportion of families remain unidentified so far. Furthermore, a significant number of patients could be affected following a *de novo* mutation. It can be speculated that the occurrence of the duplication of another C in a stretch of preexisting 7 Cs in the VNTR region of *MUC1* might not be a rare event. This could mean that there may be a significant number of

sporadic patients with *MUC1* mutations that are even more difficult to diagnose without a positive family history and may easily be mistaken as hypertensive nephropathy, with IF/TA and glomerulosclerosis in renal biopsy.

We do not yet know which of the respective genes are more or less frequent within the spectrum of ADTKD. In our study, a relevant *MUC1* or *UMOD* mutation was found in 7/10 families. A recent study from France analyzed a large



number of individuals with diseases compatible with our definition of ADTKD.[27] Among 136 unrelated probands, 24 (17.6%) carried an *UMOD* mutation and 5 probands (3.7%) showed an *HNF1B* mutation. The remaining 107 probands remained unclear (79.3%). With the identification of *MUC1* as the disease-causing gene for MCKD1,[7] it can now be presumed that some or many of these patients will harbor the *MUC1* mutation. Therefore, we would assume that a majority of ADTKD indeed stem from *MUC1* or *UMOD* mutations. It is noteworthy that similar families have been identified with gene loci outside of the known genes to date.[28,29] Thus, it is quite likely that there is at least one additional gene that could cause the clinical condition of ADTKD, marked by 'NOS' for the purpose of this study.

As the four known genes described above will possibly include the great majority of cases with ADTKD, sequencing of these four genes appears appropriate as the primary screening effort, possibly by gene panel sequencing as performed on families 1, 2, 6, and 8 in our series (Table 1). To date, no other mutation in the *MUC1* gene outside the VNTR region has been described, neither in the seminal identifying paper by massive parallel sequencing[7] nor in other studies by Sanger sequencing,[30] or in our analyses by whole-exome, targeted genomic, or gene panel sequencing. Therefore, the existence of any such mutation in ADTKD patients would be of great interest. If sequencing of the four genes does not reveal a result, in our view testing for the C duplication in the VNTR region of the *MUC1* gene should follow. If this technique is more broadly used and reliable and most cases of ADTKD turn out to be *MUC1* frameshift mutations, this test can be elevated to the position of the first diagnostic procedure in the future. Interestingly, the specific

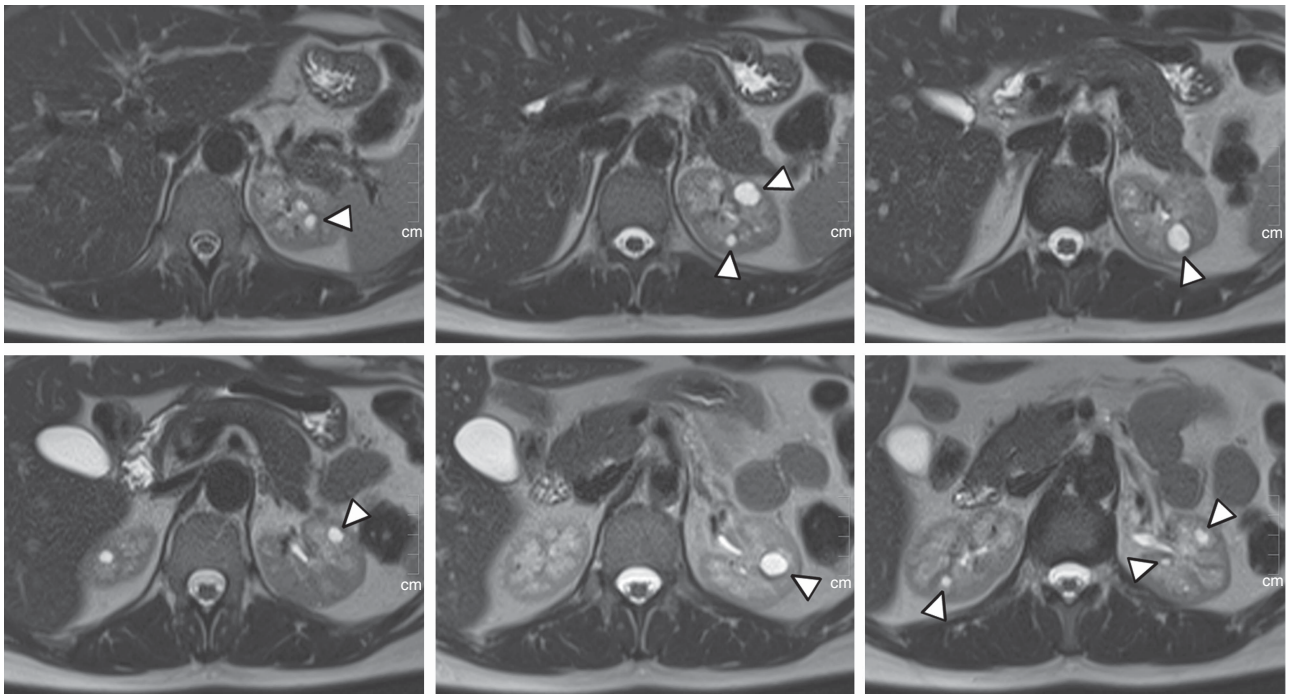


Figure 6 | Magnetic resonance imaging (MRI) images of a single affected female with the *MUC1* mutation. T2-weighted axial sections through native kidneys are shown. This female patient (NMW-70, family 10) at 37 years of age has a glomerular filtration rate (GFR) of 51 ml/min and bears the *MUC1* mutation. The number of cysts in this patient is the most pronounced of all patients studied, located either in the parenchyma or at the corticomedullary boundary (indicated by arrowheads).

Figure 5 | Representative magnetic resonance imaging (MRI) of native kidneys from patients. T2-weighted coronal slices showing two affected individuals from each of four families (numbers 1, 2, 3, and 9) with autosomal dominant tubulointerstitial kidney disease (ADTKD). The first four probands (panels 1 and 2) belong to *MUC1*-associated disease and the lower two probands (panel 4) to *UMOD*-associated disease. Panel 3 depicts probands from a family with ADTKD not otherwise specified (NOS). The individual families can be found for further reference in Table 1; the respective individuals can be identified by their study number in the pedigrees (NMW-xx). The left-hand column shows individuals with milder CKD (Modification of Diet in Renal Disease (MDRD) glomerular filtration rate (GFR): 1 NMW-09 19 ml/min, 2 NMW-49 33 ml/min, 3 NMW-73 49 ml/min and 4 NMW-74 32 ml/min). The right-hand column shows individuals with end-stage renal disease (ESRD; NMW-08, NMW-51, and NMW-67 are transplanted), with the asterisk pointing at the one single exception (panel 3, NMW-56 with GFR at 21 ml/min) where no family member with ESRD is alive. Occasional cysts can be seen either in the renal parenchyma or at the corticomedullary boundary, in particular in *MUC1*-associated probands. In the applied T2-weighted sequence, the cysts can be seen as circular shapes of different sizes with a high signal (larger cysts marked with an arrowhead).

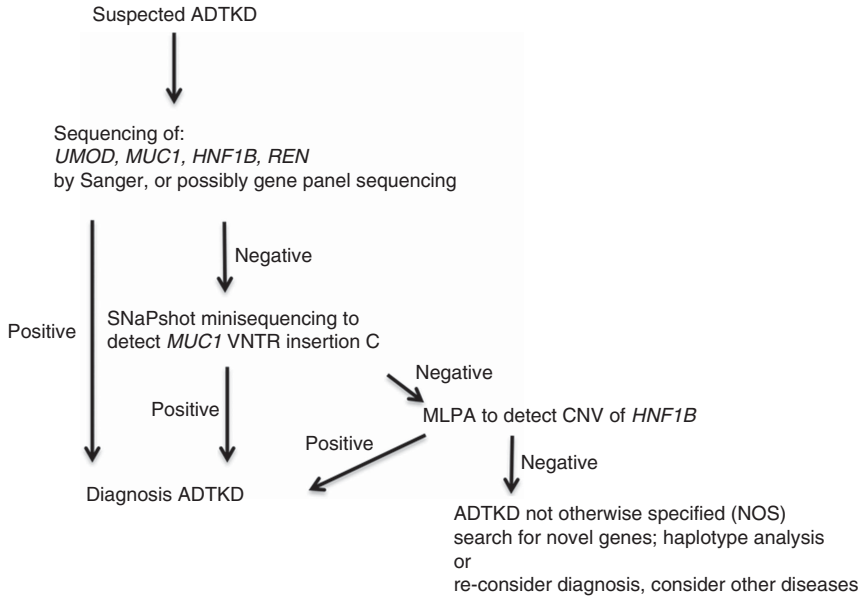


Figure 7 | Suggested diagnostic workup of clinically suspected autosomal dominant tubulointerstitial kidney disease (ADTKD). If a patient and/or family presents with typical clinical features of ADTKD, which is the absence of any specific renal signs and symptoms, bland urine, and predominant interstitial fibrosis/tubular atrophy (IF/TA) on renal biopsy, we would suggest sequencing the four candidate genes first. This can be done by direct Sanger sequencing or by gene panel sequencing. If none of these show a pathogenic mutation, a test for the described frameshift mutation[7] of the variable number of tandem repeat (VNTR) of the *MUC1* gene should be performed that is, to date, available only in specialized laboratories. If also negative, copy number variations (CNVs) of the *HNF1B* gene should be considered that could be analyzed by multiplex ligation-dependent probe amplification (MLPA). Should none of these tests show a causative mutation, the patient and/or family may be deemed to have an unknown cause of the disease, or the diagnosis should be reconsidered.

technique that we used for detecting the duplication of the C mutation would miss the insertion of an adenosine (A), as in most repeats an A follows the C stretches. In these cases, possibly nongenomic analysis such as immunohistochemical analysis for the frameshift mutation in renal biopsies (as shown previously[7]) may help identify the disease, as the frameshift protein would be identical. If the VNTR region of *MUC1* is also not affected, we would suggest searching for copy number variations in the *HNF1B* gene. Approximately 50% of *HNF1B*-associated renal diseases are indeed caused by copy number variations[21] that are usually overseen with the different technologies of next-generation sequencing or direct Sanger sequencing. Should this also not be positive, then other kidney diseases should be considered, or an unknown gene may be considered as being responsible and a haplotype analysis of the kindred could be debated. Figure 7 summarizes these diagnostic steps toward a genetic diagnosis.

We anticipate that using the novel, more systematic nomenclature together with the proposed diagnostic algorithm will simplify and facilitate recognition of affected families and individuals and help to establish the prevalence of the disease and its subtypes. This should also promote pathomechanistic studies that will hopefully unravel the priming signals of fibrosis in the kidney. It will be of utmost interest to identify the molecular pathways shared by the four genes in question. Besides the immense gratification felt by physicians and families when the correct diagnosis is made, the recent scientific advances should be able to aid in the development of specific and novel strategies for therapeutic intervention of the disease.

MATERIALS AND METHODS

The study was approved by the local ethics committee (protocol no. 4103). All patients included in this study signed a written informed consent form. Patient information included genetic analysis and a review of historical renal biopsies.

Patient samples

Genomic DNA from whole EDTA blood was extracted using the automated magnetic bead–based chemagic MSM I technology (PerkinElmer chemagen, Baesweiler, Germany) according to the instructions of the provider.

Genetical analyses

Direct Sanger sequencing of the *UMOD* gene was performed as a routine clinical analysis in the molecular genetics laboratories of the University of Cologne. The detected variants were analyzed with the mutation prediction software MutationTaster[31] and the Polyphen-2 score.[32] For analysis of copy number variants we used the SALSA reagents and a predesigned kit (P241-D1 MODY, Kit-ID MRCH-41211) for multiplex ligation-dependent probe amplification (MRC-Holland, Amsterdam, The Netherlands) for *HNF1B*, according to the manufacturer's instructions. For a detailed description of linkage and haplotype analysis, identity-by-state analysis for family relationship, whole-exome and genomic sequencing of the linkage

locus, SNaPshot minisequencing of *MUC1*-VNTR, and the parallel sequence variation detection with semiconductor sequencing, please refer to the methods in the Supplementary Material online.

MRI

MRI was performed on a 1.5 T scanner (Magnetom Aera Siemens, Erlangen, Germany) without gadolinium on selected patients after written informed consent was obtained. Applied sequences were as follows: T2 HASTE (slice thickness: 5 mm; TR: 1200 ms; TE: 92 ms; coronal orientation), T2 turbo spin-echo with fat saturation (slice thickness: 4 mm; TR: 4600 ms; TE: 137 ms; coronal orientation), and T2 HASTE (slice thickness: 4 mm; TR: 1000 ms; TE: 112 ms; axial orientation).

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Families with ADTKD included in the study.
Figure S2. Relationship analysis of families with MUC1 duplication of C mutation.
Table S1. Comparative identity-by-state (IBS) analysis of distinct families.
Table S2. Haplotypes derived from genomic sequencing of the linkage locus on chromosome 1.
Table S3. SNPs used for haplotype analysis from genomic sequencing data.
Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

REFERENCES

1. Hallan SI, Matsushita K, Sang Y *et al.* Age and association of kidney measures with mortality and end-stage renal disease. *JAMA* 2012; **308**: 2349–2360.
2. Bohle A, Mackensen-Haen S, von Gise H. Significance of tubulointerstitial changes in the renal cortex for the excretory function and concentration ability of the kidney: a morphometric contribution. *Am J Nephrol* 1987; **7**: 421–433.
3. Liu Y. Cellular and molecular mechanisms of renal fibrosis. *Nat Rev Nephrol* 2011; **7**: 684–696.
4. Bleyer AJ. Improving the recognition of hereditary interstitial kidney disease. *J Am Soc Nephrol* 2009; **20**: 11–13.
5. Srivastava S, Sayer JA. Hereditary interstitial kidney disease: known genes and opportunities for diagnosis. *OA Nephrology* 2013; **1**: 5.
6. Hildebrandt F, Waldherr R, Kutt R *et al.* The nephronophthisis complex: clinical and genetic aspects. *Clin Investig* 1992; **70**: 802–808.
7. Kirby A, Gnirke A, Jaffe DB *et al.* Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in MUC1 missed by massively parallel sequencing. *Nat Genet* 2013; **45**: 299–303.
8. Hart TC, Gorry MC, Hart PS *et al.* Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. *J Med Genet* 2002; **39**: 882–892.
9. Lindner TH, Njolstad PR, Horikawa Y *et al.* A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1beta. *Hum Mol Genet* 1999; **8**: 2001–2008.
10. Bingham C, Ellard S, Allen L *et al.* Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1 beta. *Kidney Int* 2000; **57**: 898–907.
11. Zivna M, Hulkova H, Matignon M *et al.* Dominant renin gene mutations associated with early-onset hyperuricemia, anemia, and chronic kidney failure. *Am J Hum Genet* 2009; **85**: 204–213.
12. Bleyer AJ, Hart PS, Kmoch S. Hereditary interstitial kidney disease. *Semin Nephrol.* 2010; **30**: 366–373.
13. Christodoulou K, Tsingis M, Stavrou C *et al.* Chromosome 1 localization of a gene for autosomal dominant medullary cystic kidney disease. *Hum Mol Genet* 1998; **7**: 905–911.
14. Cohn DH, Shohat T, Yahav M *et al.* A locus for an autosomal dominant form of progressive renal failure and hypertension at chromosome 1q21. *Am J Hum Genet* 2000; **67**: 647–651.
15. Auranen M, Ala-Mello S, Turunen JA *et al.* Further evidence for linkage of autosomal-dominant medullary cystic kidney disease on chromosome 1q21. *Kidney Int* 2001; **60**: 1225–1232.
16. Fuchshuber A, Kroiss S, Karle S *et al.* Refinement of the gene locus for autosomal dominant medullary cystic kidney disease type 1 (MCKD1) and construction of a physical and partial transcriptional map of the region. *Genomics* 2001; **72**: 278–284.
17. Parvari R, Shnaider A, Basok A *et al.* Clinical and genetic characterization of an autosomal dominant nephropathy. *Am J Med Genet* 2001; **99**: 204–209.
18. Wolf MT, Mucha BE, Hennies HC *et al.* Medullary cystic kidney disease type 1: mutational analysis in 37 genes based on haplotype sharing. *Hum Genet* 2006; **119**: 649–658.
19. Hattrup CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. *Annu Rev Physiol* 2008; **70**: 431–457.
20. Vylet'al P, Kublova M, Kalbacova M *et al.* Alterations of uromodulin biology: a common denominator of the genetically heterogeneous FJHN/MCKD syndrome. *Kidney Int* 2006; **70**: 1155–1169.
21. Faguer S, Decramer S, Chassaing N *et al.* Diagnosis, management, and prognosis of HNF1B nephropathy in adulthood. *Kidney Int* 2011; **80**: 768–776.
22. Freedman BI, Volkova NV, Satko SG *et al.* Population-based screening for family history of end-stage renal disease among incident dialysis patients. *Am J Nephrol* 2005; **25**: 529–535.
23. Connor TM, Oygar DD, Gale DP *et al.* Incidence of end-stage renal disease in the Turkish-Cypriot population of Northern Cyprus: a population based study. *PLoS One* 2013; **8**: e54394.
24. Smith CH, Graham JB. Congenital medullary cysts of the kidneys with severe refractory anemia. *Am J Dis Child* 1945; **69**: 369–377.
25. Stavrou C, Koptides M, Tombazos C *et al.* Autosomal-dominant medullary cystic kidney disease type 1: clinical and molecular findings in six large Cypriot families. *Kidney Int* 2002; **62**: 1385–1394.
26. Ishikawa I. Cancer in dialysis patients. In Eric P Cohen (ed). *Cancer and the Kidney*. Oxford Medical Publications, Oxford University Press: Oxford, UK, 2005; pp 227–247.
27. Bollee G, Dahan K, Flamant M *et al.* Phenotype and outcome in hereditary tubulointerstitial nephritis secondary to UMOD mutations. *Clin J Am Soc Nephrol* 2011; **6**: 2429–2438.
28. Stiburkova B, Majewski J, Hodanova K *et al.* Familial juvenile hyperuricaemic nephropathy (FJHN): linkage analysis in 15 families, physical and transcriptional characterisation of the FJHN critical region on chromosome 16p11.2 and the analysis of seven candidate genes. *Eur J Hum Genet* 2003; **11**: 145–154.
29. Hodanova K, Majewski J, Kublova M *et al.* Mapping of a new candidate locus for uromodulin-associated kidney disease (UAKD) to chromosome 1q41. *Kidney Int* 2005; **68**: 1472–1482.
30. Wolf MT, Karle SM, Schwarz S *et al.* Refinement of the critical region for MCKD1 by detection of transcontinental haplotype sharing. *Kidney Int* 2003; **64**: 788–792.
31. Schwarz JM, Rodelsperger C, Schuelke M *et al.* MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* 2010; **7**: 575–576.
32. Adzhubei IA, Schmidt S, Peshkin L *et al.* A method and server for predicting damaging missense mutations. *Nat Methods* 2010; **7**: 248–249.
33. Kudo E, Kamatani N, Tezuka O *et al.* Familial juvenile hyperuricemic nephropathy: detection of mutations in the uromodulin gene in five Japanese families. *Kidney Int* 2004; **65**: 1589–1597.
34. Dahan K, Devuyst O, Smaers M *et al.* A cluster of mutations in the UMOD gene causes familial juvenile hyperuricemic nephropathy with abnormal expression of uromodulin. *J Am Soc Nephrol* 2003; **14**: 2883–2893.