Novel MUC1 variant identified by massively parallel sequencing explains interstitial kidney disease in a large Dutch family

de Haan, Amber; van Eerde, Albertien M; Eijgelsheim, Mark; Rump, Patrick; van der Zwaag, Bert; Hennekam, Eric; Živná, Martina; Kmoch, Stanislav; Bleyer, Anthony J; Kidd, Kendrah

Published in:
Kidney International

DOI:
10.1016/j.kint.2023.02.021

Novel *MUC1* variant identified by massively parallel sequencing explains interstitial kidney disease in a large Dutch family

Check for updates

OPEN

Amber de Haan[1], Albertien M. van Eerde[2], Mark Eijgelsheim[1], Patrick Rump[3], Bert van der Zwaag[2], Eric Hennekam[2], Martina Živná[4], Stanislav Kmoch[4,5], Anthony J. Bleyer[4,5], Kendrah Kidd[4,5], Liffert Vogt[6], Nine V.A.M. Knoers[3] and Martin H. de Borst[1]

[1]Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; [2]Department of Genetics, University Medical Center Utrecht, Utrecht, the Netherlands; [3]Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; [4]Research Unit of Rare Disease, Department of Paediatrics and Inherited Metabolic Disorders, First Faculty of Medicine Charles University, Prague, Czech Republic; [5]Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; and [6]Department of Internal Medicine, Section Nephrology, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Kidney International (2023) **103,** 986–989; https://doi.org/10.1016/j.kint.2023.02.021

KEYWORDS: ADTKD-*MUC1*; autosomal dominant tubulointerstitial kidney disease; chronic kidney disease; massively parallel sequencing; *MUC1*

Chronic kidney disease (CKD) can be caused by various systemic and kidney disorders with overlapping or nonspecific clinical presentations. Despite a thorough diagnostic workup, the primary cause of CKD remains uncertain in 20% to 35% of affected individuals.[1,2] Recent studies have demonstrated that massively parallel sequencing (MPS) can be a useful additional tool in the diagnostic workup of patients with unexplained CKD, providing a molecular diagnosis in 11% to 56% of cases.[3–9] Establishing the correct diagnosis through MPS may not only have therapeutic consequences but may also improve detection of extrarenal manifestations (reverse phenotyping), improve genetic counseling of patients and their relatives, and influence donor selection for (living-related) transplantation.[S1]

Here, we describe a large Dutch family with interstitial kidney disease of unknown origin in whom we identified a novel frameshift mutation in the *MUC1* gene, encoding mucin 1, using an MPS-based multigene panel.

RESULTS

Case study

The proband (VI-96) was a 46-year-old man who presented to our clinic in 2020 for evaluation for a second kidney transplant. In 2007, he first presented with headache, fatigue, hypertension (144/90 mm Hg), serum creatinine of 400 μmol/L, renal anemia, and secondary hyperparathyroidism. Family history (Figure 1a) revealed that many family members were affected with CKD (Supplementary Methods). This family had been followed up in outpatient nephrology clinics in the northern part of the Netherlands for >60 years with unexplained kidney failure classified as "hereditary nephropathy" and was the subject of a dissertation on hereditary idiopathic kidney diseases in 1967.[S2] Urinalysis revealed no proteinuria or sediment abnormalities. Kidney ultrasonography showed increased echogenicity, with the left kidney measuring 11.2 cm and the right kidney measuring 10.3 cm. Kidney biopsy revealed sclerosed glomeruli and extensive

Correspondence: *Martin H. de Borst, Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, the Netherlands. E-mail: m.h.de.borst@umcg.nl*

Received 28 September 2022; revised 11 January 2023; accepted 13 February 2023; published online 22 March 2023

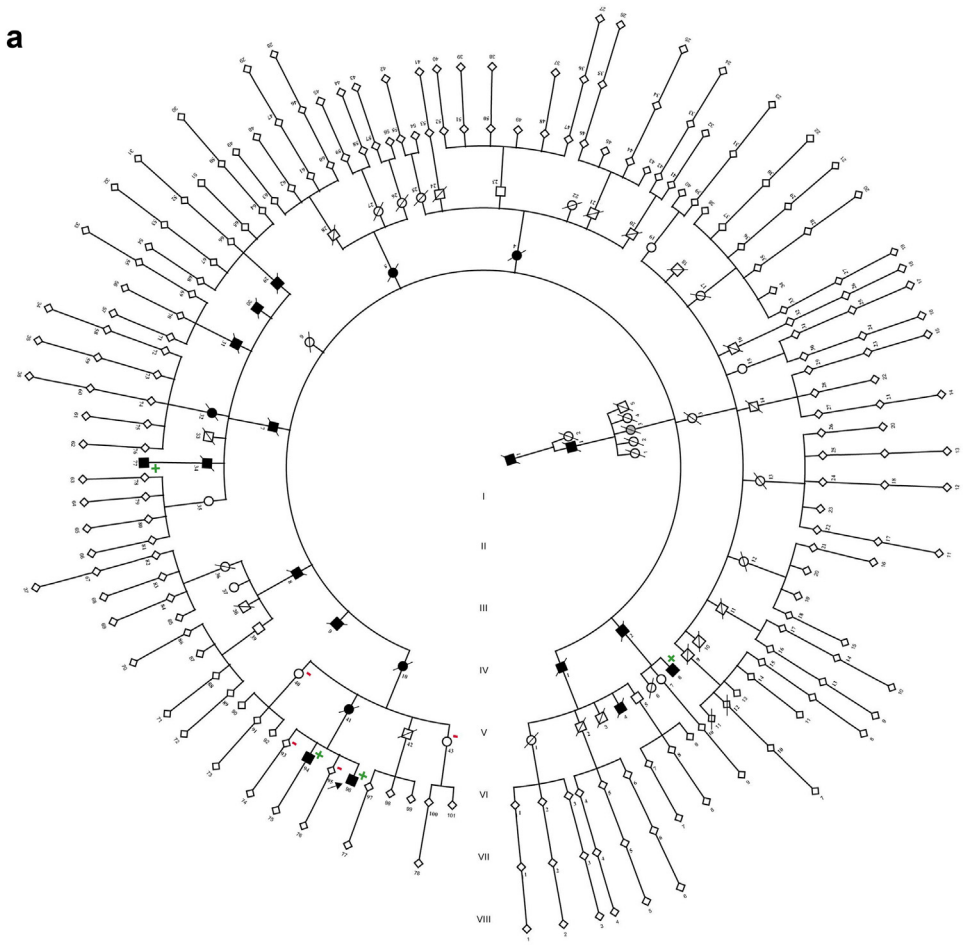

Figure 1 | (a) Extensive pedigree of the Dutch family with interstitial kidney disease of unknown origin in which we identified the novel frameshift mutation in *MUC1*. The proband is indicated by the black arrow. Solid black symbols indicate family members with chronic kidney disease. Solid gray symbols indicate family members who must have been carriers but were not formally diagnosed with kidney disease. The plus (+) symbol indicates family members with the *MUC1* variant, whereas the minus (–) symbol indicates family members without the *MUC1* variant. Individuals in generations VII (n = 134) and VIII (n = 58) are represented as singular diamonds. (**b**) Abnormal protein sequence encoded by *MUC1* exon 2 (NM_001204186.1), where the wild-type sequence starting at p.20 is indicated in black, the frameshift product by the duplication in our family starting with a proline at p.119 is indicated in red, and the start of the prototype cytosine insertion sequence 32 amino acids downstream of the frameshift is indicated in blue.

interstitial fibrosis. Electron microscopy was not performed. The patient was diagnosed with hereditary idiopathic kidney disease. Shortly after presentation, he started dialysis and underwent living-unrelated kidney transplantation at the age of 35 years. He then had progressive loss of kidney function over the next 11 years, requiring evaluation for a second kidney transplantation.

Summaries of the clinical presentation of other affected family members are provided in Supplementary Table S1 and Supplementary Data S1. Generally, family members presented between the ages of 30 and 65 years with unexplained kidney failure and histopathologic evidence of interstitial fibrosis and tubular atrophy.

Genetic testing

In VI-96, previous genetic testing restricted to *UMOD* and *HNF1-β* was negative in 2007. Therefore, additional genetic testing was performed using an MPS-based multigene panel (Supplementary Methods and Supplementary Table S2). This analysis revealed a novel frameshift variant (c.326_350dup, p.(Ser119Profs*119); NM_001204286.1) in *MUC1*. This variant is located before the variable number of tandem repeats (VNTR) domain of *MUC1* and leads to an insertion of 25 nucleotides, resulting in a 1-base frameshift in the open reading frame of the MUC1 mRNA and the introduction of a premature stop codon. The predicted C-terminal amino acid sequence of the resulting mutant protein, mucin 1 frame shift

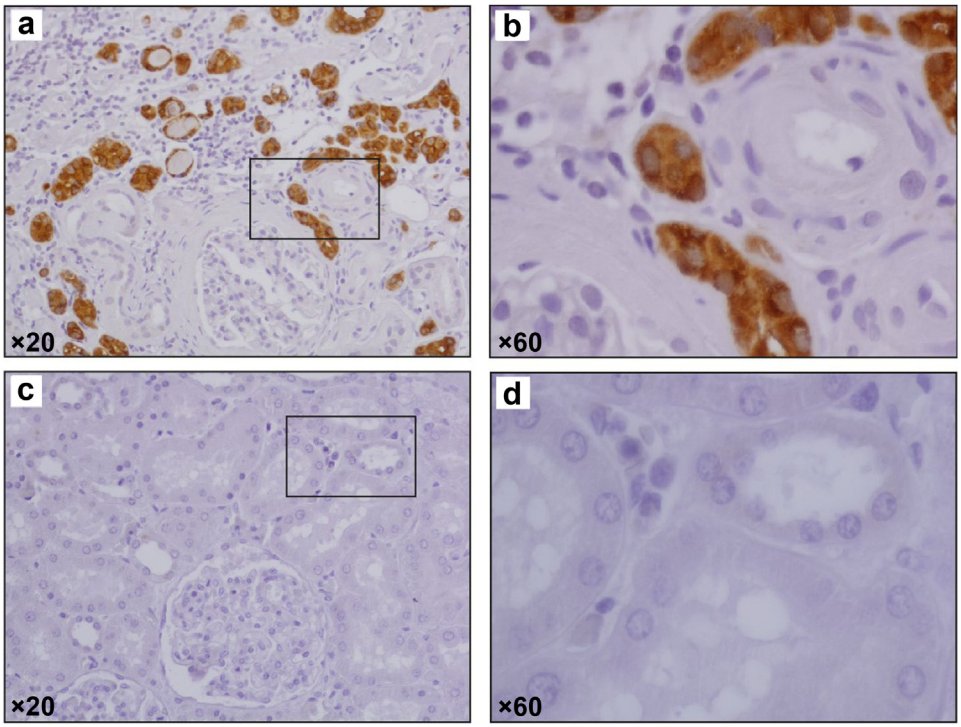

Figure 2 | Immunohistochemical detection of the mucin 1 frame shift (MUC1fs) protein in kidney tissue from the proband and a control. (**a,b**) Kidney tissue from the proband (VI-96) showing interstitial inflammation and granular staining of MUC1fs in distal tubules and collecting ducts. MUC1fs staining in the proximal tubules not expressing MUC1 was negative. (**c,d**) Kidney tissue from an age-matched control showing negative staining for MUC1fs in the distal tubules, collecting ducts, and proximal tubules. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

(MUC1fs), is the same as the previously reported pathogenic cytosine duplication within the VNTR (Figure 1b).[S3] An additional restriction-specific enrichment assay of the *MUC1* VNTR (Supplementary Methods) did not identify classic pathogenic variants in the VNTR. The variant cosegregated with the kidney phenotype in this family. No other (potentially) pathogenic variants were found.

Kidney biopsy

To determine if the variant resulted in the intracellular deposition of MUC1fs in a similar manner to autosomal dominant tubulointerstitial kidney disease–*MUC1* (ADTKD-*MUC1*) due to the classic cytosine duplication, specific MUC1fs immunohistochemistry was performed in kidney tissue from VI-96 (Supplementary Methods). Granular MUC1fs positivity was detected in distal tubules and collecting ducts (Figure 2a and b). In kidney tissue from an age-matched healthy control, MUC1fs immunostaining was negative (Figure 2c and d).

DISCUSSION

We describe a large family with a novel mutation in *MUC1* causing ADTKD. ADTKD-*MUC1* is characterized by tubulointerstitial fibrosis, an autosomal dominant inheritance pattern, and no extrarenal manifestations.[S4] Urinary sediment is usually bland, and disease severity and age of onset of kidney failure vary within and between families, with most patients presenting between the ages of 20 and 70 years.[S5–S7] The phenotype of this family matches the ADTKD-*MUC1* phenotype, and the variation in age at the start of renal replacement therapy in affected family members is consistent with this diagnosis.

The most common cause of ADTKD-*MUC1*, a cytosine duplication within the VNTR, is responsible for >90% of ADTKD-*MUC1* cases, whereas other frameshift mutations within the VNTR may also cause ADTKD-*MUC1*.[S8] In contrast, affected members of our family carry a novel mutation located before the first VNTR repeat unit. The novel mutation in this family resulted in the same MUC1fs protein as reported in all previous ADTKD-*MUC1* cases.[S8] Although the pathophysiology of ADTKD-*MUC1* is not fully understood, it is considered a toxic proteinopathy with accumulation of MUC1fs protein being central to the disease pathogenesis, as *Muc1* knockout mice do not have kidney disease[S9] and all known pathogenic variants in *MUC1* lead to expression of the same MUC1fs.[S3,S8,S10] Our findings support this hypothesis by showing that a new variant outside the VNTR results in the same MUC1fs protein.

The high GC-rich content and variable length of the VNTR in *MUC1* prevents identification of typical variants by MPS[S11]; therefore, ADTKD-*MUC1* is not commonly diagnosed via MPS. The common cytosine duplication and other variants within the VNTR can be identified by multiple other techniques.[S12–S15] In our family, common pathogenic variants

in the VNTR of VI-96 were excluded. Another family with a frameshift *MUC1* mutation immediately before the VNTR has previously been identified using MPS.[S10] Genetic testing in patients with unexplained (interstitial) kidney disease or with suspected ADTKD should thus include both MPS-based genetic testing, which can identify pathogenic variants outside the VNTR, and analysis of mutations within the VNTR.[S16] This latter analysis is not provided in routine panels or with whole exome or whole genome sequencing but can be obtained at several institutes.

This study also demonstrates the broader significance of genetic testing in patients with kidney failure of unknown etiology. Identification of a monogenic kidney disease provided a diagnosis for a family who had experienced unexplained CKD for many generations. In addition, a genetic diagnosis allows counseling for family planning and can guide donor selection for living-related kidney donation. Predonation genetic testing in ADTKD-*MUC1* families is pivotal, as urinary sediment and kidney ultrasound are often normal in this condition, and some affected individuals may have normal serum creatinine values up until the age of 30 years. In addition, promising leads to a treatment for ADKTD-*MUC1* are emerging,[S17] and affected individuals may be able to participate in clinical trials or receive treatments in the future. This study also underlines the importance of reevaluation and updated genetic testing several years after initial negative test results in familial kidney disease.

Currently, there are 4 confirmed carriers of the *MUC1* variant in this family, and clinical evaluation is ongoing to characterize and potentially identify more affected individuals in generations VI to VIII. If more carriers are confirmed, this large family could be useful for future genetic studies. For example, it would allow us to study the influence of genetic modifiers on the age of onset of kidney failure.[S18]

In conclusion, we identified a novel frameshift mutation in *MUC1* using MPS, explaining severe interstitial kidney disease in a large family. This study highlights that *MUC1* variants positioned before the VNTR region can lead to ADTKD and that genetic testing in patients with suspected ADTKD-*MUC1* should also include sequencing of the region before the VNTR. In addition, it demonstrates the value of genetic retesting in patients with persistent suspicion of hereditary kidney disease.

DISCLOSURE

All the authors declared no competing interests.

DATA STATEMENT

The data underlying this article cannot be shared publicly because of concerns regarding the privacy of individuals participating in this study. The data will be shared on reasonable request to the corresponding author.

ACKNOWLEDGMENTS

This work is cofunded by Sanofi Genzyme and the PPP Allowance made available by Health ~ Holland, Top Sector Life Sciences and Health, to stimulate public-private partnerships (grants RVO/6320 and IMAGEN/LSHM20009). AMvE was supported by the Dutch Kidney Foundation (18OKG19). SK, MŽ, KK, and AJB were supported by the Ministry of Health of the Czech Republic (grant NU21-07-00033), the Ministry of Education of the Czech Republic (grant LTAUSA19068), and institutional programs of Charles University in Prague (UNCE/MED/007). Several authors of this article are members of the European Reference Network for Rare Kidney Diseases project identifier 739532.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary Table S1. Overview of affected family members.

Supplementary Table S2. List of the 141 genes included on the Chronic Kidney Disease in Young Patients (CKD-Y) panel v18 at University Medical Center Utrecht.

Supplementary Data S1. Clinical summaries of affected family members.

Supplementary References.

REFERENCES

1. Kramer A, Pippias M, Noordzij M, et al. The European Renal Association – European Dialysis and Transplant Association (ERA-EDTA) Registry Annual Report 2015: a summary. *Clin Kidney J*. 2018;11:108–122.
2. Boenink R, Astley ME, Huijben JA, et al. The ERA Registry Annual Report 2019: summary and age comparisons. *Clin Kidney J*. 2022;15:452–472.
3. Lata S, Marasa M, Li Y, et al. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. *Ann Intern Med*. 2018;168:100–109.
4. Groopman E, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med*. 2018;380:142–151.
5. Ottlewski I, Münch J, Wagner T, et al. Value of renal gene panel diagnostics in adults waiting for kidney transplantation due to undetermined end-stage renal disease. *Kidney Int*. 2019;96:222–230.
6. Connaughton DM, Kennedy C, Shril S, et al. Monogenic causes of chronic kidney disease in adults. *Kidney Int*. 2019;95:914–928.
7. Mann N, Braun D, Amann K, et al. Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. *J Am Soc Nephrol*. 2019;30:201–215.
8. Rao J, Liu X, Mao J, et al. Genetic spectrum of renal disease for 1001 Chinese children based on a multicenter registration system. *Clin Genet*. 2019;96:402–410.
9. Schrezenmeier E, Kremerskothen E, Halleck F, et al. The underestimated burden of monogenic kidney disease in adults waitlisted for kidney transplantation. *Genet Med*. 2021;23:1219–1224.