Characterization of a Recurrent In-frame UMOD Indel Mutation Causing Late-onset Autosomal Dominant End-Stage Renal Failure

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Summary
Background and objectives In a single-center renal clinic, we have established routine mutation testing to diagnose UMOD-associated kidney disease (UAKD), an autosomal dominant disorder typically characterized by gout, hyperuricemia, and renal failure in the third to sixth decades.

Design, setting, participants, & measurements Four probands and their multigeneration kindreds were assessed by clinical, historical, and biochemical means. Diagnostic UMOD sequencing was performed, and mutant uromodulin was characterized in vitro.

Results All available affected members of the four kindreds harbored the same complex indel change in UMOD, which was associated with almost complete absence of gout and a later onset of CKD; the youngest age at ESRD or death was 38 years (range, 38 to 68 years) compared with 3 to 70 years in other reports. Three mutation carriers (all <35 years) are currently asymptomatic. The indel sequence (c.278_289delTCTGCCCCGAAGinsCCGCCTCCT; p.V93_G97del/ins AASC) results in the replacement of five amino acids, including one cysteine, by four novel residues, also including a cysteine. Uromodulin staining of the only available patient biopsy suggested disorganized intracellular trafficking with cellular accumulation. Functional characterization of the mutant isoform revealed retarded intracellular trafficking associated with endoplasmic reticulum (ER) retention and reduced secretion into cell culture media, but to a lesser extent than we observed with the previously reported C150S mutation.

Conclusions The indel mutation is associated with a relatively mild clinical UAKD phenotype, consistent with our in vitro analysis. UAKD should be routinely considered as a causative gene for ESRD of unknown cause, especially where there is an associated family history or where biopsy reveals interstitial fibrosis.


Introduction
Familial juvenile hyperuricemic nephropathy (FJHN; MIM 162000) and medullary cystic kidney disease type 2 (MIM 603860) are autosomal dominant disorders characterized by progressive renal tubulointerstitial fibrosis, hyperuricemia, and gout. About 45% of cases of FJHN and medullary cystic kidney disease type 2 are caused by mutations in three genes, UMOD (1,2), REN (3), and HNF1β (4), with mutations in UMOD accounting for the majority of these. A further locus for FJHN at 2p22.1-p21 has also recently been described (5), but some FJHN families show no linkage to any of the known loci, suggesting further genetic heterogeneity.

UMOD encodes uromodulin (or Tamm-Horsfall protein). Uromodulin is a heavily glycosylated 640-amino acid protein containing an N-terminal signal peptide, four potential EGF-like domains, a central region of unknown function containing eight conserved cysteine residues, a zona pellucida domain, and a C-terminal glycosylphosphatidylinositol anchor site (Figure 1). It is secreted into the nascent urine in the loop of Henle, the only segment in which it is expressed, and is the most abundant urinary protein. However, there is no consensus as to its physiologic function. Postulated roles for uromodulin have included providing a water-tight boundary to the thick ascending limb of the loop of Henle (6); prevention of urinary tract infections (7) or renal calculi formation (8); and cilial signaling (9). One certainty is that UMOD does not encode a urate transporter (10), so the etiology of the hyperuricemia in FJHN remains unexplained.

Disease-causing UMOD mutations studied to date have mostly been shown to result in impaired trafficking and secretion of uromodulin, with intracellular accumulation in the ER (11–13). Because many are mis-sense changes and disrupt cysteine residues that
are predicted to be important for correct domain folding, this is probably because of misfolding. Mutant uromodulin is likely acting in a dominant-negative manner, resulting in toxic effects independent of normal function. In contrast, other UMOD variants are reportedly associated with increased risk of developing CKD in the general population (14), suggesting that subtle alterations in expression of wild-type protein might also lead to impaired renal function as part of a complex trait.

Here, we report the identification of a recurrent UMOD mutation in a series of families ascertained from a single center renal out-patient service. This mutation (c.278-289delTCTGCCCCGAAGinsCCGCCTCCT; p.V93_G97del/ins AASC) is associated with a relatively mild clinical phenotype, and our in vitro analysis has revealed defective but milder abnormalities of intracellular trafficking and cellular localization compared with a previously reported severe mutation, C150S (15).

**Materials and Methods**

**UMOD Patients and Kindreds**

All probands (Figure 2 and Table 1) were referred to a regional renal genetics clinic (http://www.addenbrookes.org.uk/serv/clin/renal/services/renal_genetics/renal_genetics.html) because of a strong family history of renal disease for which no specific diagnosis had been made. In addition to usual workup for CKD, investigations included interrogation of genomic DNA for sequence variation in UMOD, Western blotting of urine from available pretransplant patients, and calculation of fractional excretion of uric acid. All participants gave informed consent (NRES 08/H0306/62).

**Mutation Screening and Genotyping**

The East Anglian Regional Genetics Service performed direct sequencing of UMOD according to a UKGTN-approved protocol (http://www.cuh.org.uk/addenbrookes/services/clinical/genetics/genetics_index.html). Intragenic single nucleotide polymorphisms were typed by direct sequencing. Polymorphic markers closely flanking UMOD were PCR-amplified using a fluorescent reporter primer, and products were electrophoresed according to standard methods.

**Expression Constructs and Site-directed Mutagenesis**

Full-length UMOD cDNA was amplified from normal human kidney using gene-specific primers and cloned into the mammalian expression vector pcDNA3.1. Site-directed mutagenesis was performed using mutagenic oligonucleotides and the QuikChange kit (Stratagene), and all constructs were sequence-verified before use.

**Cell Culture, Transient Transfection, and Secretion Assays**

HEK293 cells were cultured in DMEM containing 5 mM glucose and 10% FCS at 37°C in 5% CO₂/95% air. 2 × 10⁵ cells were plated into each well of a six-well plate and transfected using Lipofectamine Plus (Invitrogen) according to the manufacturer’s recommendations. At specified time intervals between 4 and 72 hours post-transfection, 150 μl aliquots of media were removed and analyzed by Western blot. At 72 hours, the cells were lysed with radiolabels, and the results were considered statistically significant at P < 0.05.

**Urine Samples**

Urine was collected into sterile receptacles containing 0.02% sodium azide and stored at −80°C until required. For Western blotting, thawed samples were diluted in 5 mM Tris-Cl (pH 8.0) to achieve equivalence of urinary creatinine, denatured by the addition of an equal volume of protein loading buffer containing β-mercaptoethanol, and boiled before separation on a 10% SDS-polyacrylamide gel.

**Immunofluorescence and Immunohistochemistry**

Transfected HEK293 cells grown on coverslips coated with poly-L-lysine (100 μg/ml) were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.2% Triton X-100 for 8 minutes, and washed with Hanks’ balanced salt solution containing 5% FCS. Fixed cells were incubated with sheep anti-uromodulin antibody at 1:1000 dilution and mouse anti-PD1 (Abcam) at 1:300 overnight at 4°C. The cells were washed three times in Hanks’ balanced salt solution containing 5% FCS and incubated with AlexaFluor 488 donkey anti-sheep and AlexaFluor 546 donkey anti-mouse antisera (Invitrogen) at 1:1000 dilution for 2 hours at room temperature.
Formalin-fixed, paraffin-embedded sections were deparaffinized in xylene, rehydrated, and permeabilized with proteinase K (50 μg/ml). Sections were blocked with 3% BSA/TBST for 15 minutes and incubated with sheep anti-uromodulin antibody (1:2000) in a humidified atmosphere for 24 hours at 4°C. Sections were washed in TBST and incubated for 2 hours with a fluorescently labeled anti-sheep antibody (1:1000) (Invitrogen). All of the sections were washed and mounted using Vectashield mounting medium, and all of the images were captured using a Zeiss LSM510 META confocal microscope.

Results
UMOD Kindreds
The four kindreds reported here all had an extensive family history of renal disease, with hypertension as a prominent feature. We were able to obtain variable amounts of data on 30 individuals, of whom only one (CIII.1; Figure 2) has had clinical gout. Macroscopic hematuria was absent from the cohort. Two women (BII.6 and DIII.3) reported recurrent urinary tract infections.

Kindred A. The 61-year-old proband (AIII.4; Figure 2) presented elsewhere at age 47 with polydipsia and polyuria. Investigations revealed bilateral small kidneys and impaired excretory function. She developed end-stage renal failure at age 54 and received a living unrelated kidney transplant the following year. Her deceased mother and three deceased maternal relatives were all described as having either CKD or “chronic nephritis” (Figure 2 and Table 1). Her mother had presented immediately predialysis at age 61 and underwent renal transplantation 2 years...
Table 1. Clinical and biochemical features of index cases and affected family members

<table>
<thead>
<tr>
<th>Age (years)a</th>
<th>Gender</th>
<th>Presentation</th>
<th>Initial Ultrasound Findings</th>
<th>FEUr (%)</th>
<th>Age at RRT (years)</th>
<th>Age at Tx (years)</th>
<th>Age at Death (years)</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>Index cases</td>
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<tr>
<td>AII.4</td>
<td>47</td>
<td>F</td>
<td>HT, CKD, polyuria, polydipsia</td>
<td>“Small kidneys”</td>
<td>54</td>
<td>55</td>
<td></td>
<td>Indel</td>
</tr>
<tr>
<td>BII.4</td>
<td>43</td>
<td>M</td>
<td>HT, CKD3, proteinuria</td>
<td>“Small kidneys”</td>
<td>48</td>
<td>50</td>
<td></td>
<td>Indel</td>
</tr>
<tr>
<td>CIV.3</td>
<td>35</td>
<td>M</td>
<td>Asymptomatic, normal biochemistryb</td>
<td>“Normal” (at 30 years)</td>
<td>9.2</td>
<td></td>
<td></td>
<td>Indel</td>
</tr>
<tr>
<td>DII.3</td>
<td>46</td>
<td>F</td>
<td>HT, CKD4, recurrent UTI</td>
<td>“Normal IVU” (at 46 years)</td>
<td>52</td>
<td></td>
<td></td>
<td>Indel</td>
</tr>
<tr>
<td>Family members</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AII.1</td>
<td>50</td>
<td>F</td>
<td>HT, “chronic nephritis,” CVAc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NK</td>
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<td>AII.2</td>
<td>NK</td>
<td>M</td>
<td>HT, CKD, LVF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NK</td>
</tr>
<tr>
<td>AII.3</td>
<td>45</td>
<td>F</td>
<td>“Died of renal failure”</td>
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<td></td>
<td>NK</td>
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<tr>
<td>AII.5</td>
<td>61</td>
<td>F</td>
<td>HT, CKD5</td>
<td>Small kidneys (“like walnuts”)</td>
<td>61</td>
<td>63</td>
<td>63</td>
<td>NK</td>
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<td>AIV.8</td>
<td>28</td>
<td>F</td>
<td>Asymptomatic, urate 0.37 mmol/L,d</td>
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<td>8.0</td>
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<td></td>
<td>Indel</td>
</tr>
<tr>
<td>BL1</td>
<td>49</td>
<td>M</td>
<td>“Died of renal failure”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NK</td>
</tr>
<tr>
<td>BII.1</td>
<td>51</td>
<td>M</td>
<td>HT, CKD4, proteinuria (NB has diabetes)</td>
<td>Left 10.5 cm, right 10 cm</td>
<td>38</td>
<td>48</td>
<td>51</td>
<td>NK</td>
</tr>
<tr>
<td>BII.3</td>
<td>38</td>
<td>F</td>
<td>HT, CKD5</td>
<td></td>
<td></td>
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<td></td>
<td>NK</td>
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<tr>
<td>BII.5</td>
<td>41</td>
<td>F</td>
<td>HT, CKD</td>
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<td>BII.6</td>
<td>39</td>
<td>F</td>
<td>CKD, polydipsia, proteinuria, recurrent UTI</td>
<td>Left 8.3 cm, right 8.5 cm</td>
<td>41</td>
<td>44</td>
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<td>Indel</td>
</tr>
<tr>
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<td>NK</td>
<td>M</td>
<td>Malignant HT, CKD, anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NK</td>
</tr>
<tr>
<td>CII.2</td>
<td>NK</td>
<td>F</td>
<td>HT, “chronic pyelonephritis” MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NK</td>
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<tr>
<td>CII.3</td>
<td>57</td>
<td>M</td>
<td>HT, CKD3, polyuria, gout</td>
<td>Left 9.5 cm, right 10.5 cm</td>
<td>67</td>
<td>68</td>
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<td>Indel</td>
</tr>
<tr>
<td>CII.4</td>
<td>60</td>
<td>M</td>
<td>HT, CKD 4, proteinuria</td>
<td>Horseshoe kidney</td>
<td>65</td>
<td></td>
<td></td>
<td>Indel</td>
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<tr>
<td>CIV.4</td>
<td>34</td>
<td>M</td>
<td>HT, CKD</td>
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<td></td>
<td></td>
<td>NK</td>
</tr>
<tr>
<td>CIV.5</td>
<td>30</td>
<td>F</td>
<td>Asymptomatic, normal biochemistryb</td>
<td>Both 10.7 cm</td>
<td>13.5</td>
<td></td>
<td></td>
<td>Indel</td>
</tr>
<tr>
<td>DI.8</td>
<td>49</td>
<td>F</td>
<td>Malignant HT, “uremia”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NK</td>
</tr>
<tr>
<td>DII.1</td>
<td>50</td>
<td>M</td>
<td>HT, CKD4</td>
<td></td>
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<td></td>
<td>NK</td>
</tr>
<tr>
<td>DII.5</td>
<td>NK</td>
<td>F</td>
<td>HT, CKD</td>
<td></td>
<td></td>
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<td></td>
<td>NK</td>
</tr>
<tr>
<td>DIV.2</td>
<td>NK</td>
<td>F</td>
<td>HT, CKD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NK</td>
</tr>
</tbody>
</table>

CKD, chronic kidney disease; CVA, cerebrovascular accident; FEUr, fractional excretion of urate, which is equal to [(urine urate × serum creatinine)/(serum urate × urine creatinine)] × 100%; HT, hypertension; IVU, intravenous urogram; LVF, left ventricular failure; MI, myocardial infarction; NK, not known; RRT, renal replacement therapy; Tx, transplant; UTI, urinary tract infection.

aAge at first presentation.
bSerum sodium, potassium, estimated GFR, venous bicarbonate, corrected calcium, phosphate, urate.
cClinical information in quotation marks is from historical documents or patient recollection.
dNormal range, 0.15 to 0.35 mmol/L.
later. All of the proband’s children were asymptomatic and, after appropriate counseling, elected to undergo diagnostic testing.

Kindred B. The proband, a 48-year-old man (BII.4; Figure 2), was already receiving renal replacement therapy. Initial investigations carried out elsewhere 5 years earlier had revealed hypertension, CKD, and bilateral small kidneys. A renal biopsy had not been performed. In light of his family history, he was concerned whether his children should be offered any screening. His father and three of his five siblings had renal disease that had progressed to end-stage renal failure.

Kindred C. The 35-year-old index case (CIV.3) presented because of a family history of renal disease affecting his brother, father, paternal uncle, grandfather, and great aunt (Figure 2). He was normotensive with no urinary dipstick abnormality and had normal renal imaging and function. Because his affection status was uncertain, initial UMOD sequencing was performed on his clinically affected brother (CIV.4).

Kindred D. The proband (DIII.3) was a 78-year-old woman who had received a renal transplant in 1984 at age 52, having presented at age 46 with hypertension and progressive CKD. Her mother died at age 59 from uremia, and there were six other maternal relatives with a diagnosis of renal disease or uremia (Figure 2 and Table 1). She was concerned about the implications for her own offspring.

In the 26 individuals where age of ESRD/death was recorded, the median was 50.5 years (range, 38 to 68 years).

Identification of UMOD Mutation

In all of these kindreds, the family history was compatible with an adult-onset autosomal dominant nephropathy. In the absence of other diagnostic markers, UMOD testing was offered as a first-line diagnostic test, because the presentations with CKD and small kidneys were suspicious for UMOD-associated kidney disease (UAKD). The same indel mutation (c.278_289delTCTGCCCCGAAGinsCCG CCTCTC; p.V93_G97del/ins AASC) was identified in all four probands and in all clinically affected individuals who were available for testing. Predictive and diagnostic testing was subsequently offered to other family members who requested it, after formal nephrology and genetics review. Evidence for a founder effect was sought by haplotype analysis of available polymorphic microsatellite markers and intragenic single nucleotide polymorphisms. No shared common haplotype was found in all four families (data not shown).

The Indel Sequence Change Predicts a Structural Change in Uromodulin

The replacement of c.278_289 TCTGCCCCGAAG with CCGCCTCCT results in the replacement of five amino acids including a cysteine residue in the second uromodulin calcium-binding EGF-like domain, with four novel residues also including a cysteine residue (Figure 2, lower panel). Structural alignments using Phyre (16) and nFOLD3 (17) confirmed the presence of an EGF-like domain from residues 65 to 107 (UniProt reference P07911). This region contains six highly conserved cysteine residues that are predicted to disulfide-bond in a 1–3, 2–4, 5–6 pattern. Figure 3 (left panel) demonstrates a model of this domain on the basis of the corresponding structure of a fibrillin-1 EGF-like domain (Protein Data Bank code 1emo), with the three disulfide bridges maintaining a compact fold of two major β sheets and N- and C-terminal loops. A similar model for the indel mutant reveals a loss of disulfide bridging at the fifth cysteine, even although it is replaced with a new cysteine, with resultant unfolding of the N-terminal region (Figure 3, right panel).

The Indel Mutation Results in Trafficking Defects

To assess the effect of the indel mutation on uromodulin processing and trafficking, equal amounts of expression constructs containing wild-type (WT), indel, C150S UMOD, or empty vector were transfected into HEK293 cells. The C150S substitution has previously been shown to result in marked ER retention of mutant uromodulin (11). Transfection efficiency among the different expression constructs was verified by cotransfection with an enhanced green fluorescent protein-expressing plasmid.

The media and cell lysates were initially collected at 72 hours post-transfection and were analyzed by Western blotting. Each experiment was carried out in triplicate and independently repeated three times. Transfection efficiencies for wild type, indel, and C150S were not different at 33, 34, and 31%, respectively. Comparison of total cell lysates (Figure 4A) shows that both the 82-kD precursor (ER resident) and the 100-kD mature, glycosylated uromodulin protein could be detected in all transfected cells. Densitometric analysis confirmed twice as much mature protein as precursor in wild-type transfected cells, compared with 1.5 and 0.4 times for indel and C150S transfected cells, respectively (P = 0.041 and 0.003 versus WT). The mature form of uromodulin was also readily detected in the media of wild type–expressing cells but was approximately halved in the indel-expressing cells (Figure 4A). As expected from previous observations, very little of the C150S mature form was detectable in media (11).

Figure 3. | Structural models of wild-type (WT) and indel uromodulin isoforms. Shown are models of part of the WT and indel uromodulin isoform, on the basis of the solved EGF-like domain of fibrillin-1 (Protein Data Bank code 1emo). The indel isoform loses disulfide bridging at the fifth cysteine, with consequent unfolding of the N-terminal region.
To assess glycosylation of the uromodulin isoforms, total cell extracts from wild-type and mutant isoforms of uromodulin-expressing cells were treated with Endo H or PNGase F and changes visualized by Western blot. Endo H cleaves high mannose oligosaccharides of ER-resident glycoproteins and PNGase F cleaves all types of \(N\)-linked glycosylation. The 82-kD (or ER resident) WT uromodulin is cleaved by Endo H to a 60-kD deglycosylated form, whereas PNGase F cleaves both the 100- and 82-kD forms to the 60-kD form. Figure 4B shows that the indel behaved as did WT in this respect, whereas C150S displayed a previously reported intracellular processing defect (11).

To study the time course of the observed behavior of mutant uromodulin, glycosylation and secretion were analyzed at a series of intermediate time-points from 4 to 72 hours post-transfection. The 82-kD immature protein was detectable from 4 hours in cell lysates of all constructs (Figure 5A). Mature (100 kD) protein was first detectable at 5 hours in wild-type and indel but not C150S transfected cells. By 10 hours, there was a greater amount of the mature 100-kD WT protein compared with the same time point in both the indel and C150S, irrespective of comparable quantities of the immature form at this and later time points. However, by 24 hours, the ratio of immature to mature protein was comparable in both WT and indel transfected cells. These temporal differences indicate delayed processing of the misfolded indel uromodulin.

Analysis of media from these transfected cells (Figure 5B) showed that secreted WT uromodulin could be detected at 24 hours, whereas no mutant protein could be detected from either the indel or C150S transfected cells at this time. The levels of indel uromodulin were lower compared with WT at 48 and 72 hours, whereas levels of C150S were lowest at all time points. Detection of the ER-resident immature 82-kD protein in the media of C150S transfected cells at the 72-hour time point suggested decreased cell viability.

### Localization and Trafficking Defects of the Indel Isoform

We next assessed whether the delay in protein secretion displayed by the indel protein \textit{in vitro} was mirrored by ER retention, as reported for C150S. As shown in Figure 6, immunofluorescence microscopy of transiently transfected cells 48 hours after transfection revealed little colocalization of WT uromodulin with the ER marker, PD1. In contrast, both the indel and C150S mutant isoforms clearly colocalized with PD1, C150S to a greater extent. Taken together with the Western blot experiments, these data are suggestive of an intermediate phenotype for the indel protein.

### Histology and Urine Biochemistry

Of the three individuals from this cohort who underwent renal biopsy, only one had tissue available for review (Figure 2, patient CIV.4), which revealed streaky tubulo-interstitial atrophy and fibrosis, with secondary focal segmental glomerulosclerosis (Figure 7B). Mild nonspecific lymphocytic inflammation was observed in atrophic foci.
but not elsewhere. Small arteries showed prominent myointimal thickening. An earlier review elsewhere reported the absence of immune deposits on fluorescence or electron microscopy, the latter revealing very focal foot process effacement only and no glomerular basement membrane abnormalities. Uromodulin localization was determined in residual tissue from this biopsy and compared with normal kidney (Figure 7, C through F). Similar to other reports (9,18), apical staining was seen in the latter (Figure 7, C and E), whereas in the indel biopsy, uromodulin was located throughout the cell (Figure 7, D and F), suggesting cellular retention and/or disorganized trafficking.

Western blotting of urine from the four available pre-transplant affected individuals not on allopurinol (AIV.8, CIV.3, CIV.4, and CIV.5; amounts corrected for urine creatinine) revealed variable levels trending toward a reduction in uromodulin compared with a group of unaffected relatives and healthy volunteers (Figure 7G; \( P = 0.25 \)). Notably, the fractional excretion of uric acid was within normal limits in the available affected subjects (Table 1).

**Discussion**

In this report we have functionally studied a recurrent but previously uncharacterized uromodulin mutation identified in four autosomal dominant families seen in a single center renal clinic, all of UK origin. This cohort contains 12% of the index cases we have genetically screened (three other non-indel UMOD mutations have also been found). A founder effect appears unlikely, because closely linked markers were not haploidentical in all four families. Mechanisms for such recurrent sequence alterations are poorly understood.

Clinical severity increased with age but notably, gout was not a prominent feature in any of these families, which was also noted in the only other clinical report featuring this sequence change (19). Relatively late presentation was also described there, similar to our median age to ESRD or death. We have shown that the trafficking defects, ER retention, and reduction in the amount of protein secreted both in vitro and in vivo by indel uromodulin is immediately severe in comparison with the C150S mutant or indeed the majority of other reported mutations involving
cysteine residues (19–22). This predicts the observed milder clinical course and perhaps the predominant absence of gout in our cohort. Some of our patients are currently devoid of clinical features despite carrying the mutation, which is entirely consistent with the experience of the majority of the older cases, where late but rapid deterioration of renal function occurred.

Pathogenicity of the indel is predicted because it disrupts the structure of the second EGF-like domain. Pathogenic UMOD mutations are mostly clustered in the N-terminal region containing three EGF-like domains and eight conserved cysteine residues. EGF domains are protein folds widely distributed in nature, which are often involved in cell adhesion and numerous protein-protein interactions. Mutations in other EGF domains are responsible for disorders such as Marfan syndrome (MIM 154700) and familial hypercholesterolemia (MIM 143890). The replacement of one cysteine with another in the indel mutation, even in a closely related position, is not able to maintain structural or functional integrity of uromodulin (Figure 3). The indel amino acid change also lies adjacent to the proposed Ca\(^2\)\(^+\)-binding site within this region (23), suggesting that calcium binding within EGF-like 2 could be disrupted.

Uromodulin has recently been shown to interact with and regulate the renal outer medullary potassium channel ROMK2, which is also present in the loop of Henle (24). In that study, a variety of point mutant uromodulin proteins still possessed the ability to interact with ROMK2 \textit{in vitro} but failed \textit{in vivo} to increase ROMK2’s surface expression and current amplitude. Also, given that ROMK2 functionally interacts with the apical sodium potassium chloride cotransporter (NKCC2), one might speculate that uromodulin might therefore have been predicted in our cohort, but none was observed.

Of note, UMOD C147W transgenic (18) and Nkcc2\(^-/-\) mice (25) share a number of similarities including a urine concentrating defect, which has been avidly reported in children with UAKD (20). Interestingly, polydipsia and/or polyuria have been reported within our cohort (Table 1). Another similarity between our cohort and UMOD transgenic strains is the absence of hyperuricemia, which of itself cannot therefore be a major contributor to CKD (18,26).

Because fractional excretion of urate has been previously proposed as a biochemical screening tool for UAKD (27,28), it is also notable that in the available genetically confirmed, but as yet clinically unaffected subgroup of our cohort, this parameter was not reduced. In these cases, it cannot be accounted for by CKD. Occasional normal levels were in fact reported in one study (10), and further evaluation in larger groups would be useful. This subgroup also had normal serum potassium, bicarbonate, calcium, and phosphate levels.

Although UAKD is judged to be rare, its true prevalence is not known, particularly among CKD patients presenting with already-reduced renal size. Our approach is proactively to screen UMOD in such individuals, particularly if they present with a positive family history of renal disease, no underlying diagnosis, and/or notable interstitial fibrosis on renal biopsy (the latter a feature here and in other UMOD reports) (9,18,23). This strategy has yielded important results both for these patients and their families and for those considering living related kidney donation, where establishing a negative genetic diagnosis in a potential donor will be important given the relatively late emergence of the phenotype. We therefore propose that genetic screening should be more widely applied. Allied to this, identification of mutations in additional genes (to explain causation in the approximately 50% of patients unaccounted for by UMOD) is an important goal.

With anecdotal evidence that it is useful, it has been proposed that allopurinol, a urate-lowering agent, should be offered to all those affected by UAKD, to slow progression (29,30). There is a need for proper, controlled trials of this and/or other potential therapies, to which end accurate genotypic as well as phenotypic characterization are important. The cohort reported here provides a unique opportunity to contribute to this debate, because it includes currently clinically unaffected gene carriers.

The absence of validated preventive therapies makes decisions regarding screening of clinically unaffected but at-risk relatives more difficult. It is clearly an issue for patients including some within this cohort. Whether or not genetic screening is pursued, we suggest that at-risk relatives should remain under some form of continuing surveillance for hypertension, CKD, and other cardiovascular risk factors.

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Disclosures
None.

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