Membrane Targeting and Secretion of Mutant Uromodulin in Familial Juvenile Hyperuricemic Nephropathy

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Familial juvenile hyperuricemic nephropathy (FJHN) is an autosomal dominant genetic disorder that is characterized by hyperuricemia, gout, and tubulointerstitial nephritis. FJHN is caused by mutations in the UMOD gene, which encodes for uromodulin, the most abundant urinary protein. Herein is demonstrated that patients with FJHN and renal insufficiency exhibit a profound reduction in urinary uromodulin together with either elevated or decreased plasma uromodulin. One young patient with FJHN, however, had normal serum creatinine and normal urinary uromodulin with elevated plasma uromodulin. These observations suggest that there are different urinary and plasma uromodulin profiles in early and late disease and that there may be an altered direction of uromodulin secretion in the course of FJHN as a result of improper intracellular sorting of the mutated protein in the thick ascending limb. With the use of immunohistochemistry and a quantitative immunoassay, targeting and secretion of wild-type and mutant (C77Y and N128S) uromodulin were investigated in the polarized renal epithelial cell line LLC-PK1. In transfected cells, uromodulin mutants were targeted properly to the apical membrane but were secreted less efficiently to the apical compartment than wild-type protein. The expression of mutant uromodulin had no effect on caspase 3 activity. These results indicate that the mutations studied do not impair glycosyl-phosphatidylinositol–mediated apical targeting of the protein but do affect apical secretion. Because the mutant proteins are secreted as efficiently as wild type to the basolateral compartment, the possibility arises that interactions with the immune system at the site of secretion are contributing factors to the development of tubulointerstitial nephritis in FJHN.


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Materials and Methods

All chemicals were obtained from Sigma (Vienna, Austria) unless otherwise stated.

Clinical Sample Collection

Urine and plasma were collected, with informed consent, from six healthy volunteers and five patients who had FJHN (four adults and one child) with the C77Y and C126R mutation of UMOD. The affected individuals are members of two FJHN kindreds published previously (2,16). Samples were aliquotted and stored at −20°C until assay. Urine and plasma were diluted and assayed for uromodulin concentration by immunoassay.

Uromodulin Immunoassay

Rabbit anti-human uromodulin antibody (3 μg/ml; Biotrend, Cologne, Germany) in bicarbonate buffer was applied to Maxisorb 96-well plates (Nunc, Neerijse, Belgium) and incubated overnight at room temperature. Plates were washed in PBS that contained 0.05% Tween 20. Nonspecific sites were blocked with 1% BSA and 5% sucrose in PBS. For clinical samples, standards and samples were diluted in 0.5% Triton X-100, 20 mM Tris, 20 mM EDTA, and 0.1% BSA (pH 7.8). This buffer helps to de-aggregate uromodulin in the samples and allows for more precise determination (17). For cell culture supernatants, culture medium was used to dilute the standards, and the supernatants were used undiluted. An 8-point standard curve was used on each plate starting at 300 ng/ml uromodulin (purified from normal urine by 0.5 M salt precipitation as described previously [18]). Diluted standards and samples were applied in duplicate to the precoated wells and incubated for 90 min with continuous orbital shaking at 450 rpm. Wells were washed and incubated for 1 h with shaking with 100 μl of polyclonal horseradish peroxidase–conjugated sheep anti-human uromodulin (Biotrend) at 1:2500 in 1% BSA, 0.05% Tween 20, 20 mM Tris Base, and 150 mM NaCl. Wells were washed and incubated with 100 μl of 20 μM Amplex Red Ultra (Molecular Probes, Leiden, Netherlands) and 5 mM H2O2 in PBS for approximately 20 min. Fluorescence was measured at 540 nm excitation and 590 nm emission on a GENios Plus reader from TECAN (Tecan, Grödig, Austria).

Construction of Mammalian Expression Vectors

Donor vectors with a pDNR-LIB backbone combining the entire coding sequence, between flanking loxP sites, for wild-type uromodulin, and the mutant C77Y and N1285S were generated as described previously (2). Cre recombinase (Invitrogen, Lofer, Austria) was used to transfer the inserts into the mammalian expression vector pLP-CMV neo (Clontech Laboratories, Palo Alto, CA). DH5α chemically competent Escherichia coli were transformed with the cre-lox reaction according to the BD Clontech creator system protocol and plated onto agar. Single clones were selected and amplified. Restriction digests of the clones were conducted using a co-digest with Apal and EcoRI (Invitrogen). Clones that demonstrated the correct pattern of restriction were amplified, and plasmid DNA was extracted using a Qiagen maxiprep kit (Qiagen Vertriebs, Vienna, Austria). Finally, the uromodulin sequence that was inserted in the plasmid DNA was sequenced (MWG, Ebersberg, Germany). Only vectors with 100% confirmed uromodulin sequence were used for transfections.

Cell Culture and Transfection

LLC-PK1 cells (porcine proximal tubular cell line, ATCC no. CL-101) were cultured in DMEM, 5 mM glucose, and 7% FCS with 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere that contained 5% CO2/95% air. Serial passages from 185 to 240 were used. Approximately 95% confluent cells were transfected with UMOD constructs and empty vectors using a 2:1.5 DNA/lipofectamine 2000 mixture diluted in DMEM/F12. Cells were transfected in 10-cm dishes, glass coverslips, or 96-well plates, depending on the experiment. After 4 to 6 h, the transfection reagent was removed and growth medium was reapplied. For selection of stably transfected cells, G418 (neomycin) at a concentration of 1 mg/ml was added to growth medium 24 h after transfection. Stable transfects were cultured for 12 passages in G418 medium and immunopurified using a polyclonal anti-uromodulin antibody bound to Dynal magnetic beads (Dyna1, Hamburg, Germany). Cells were cultured for an additional seven passages in medium that contained G418. After this time, G418 was omitted from the medium. Stably transfected LLC-PK1 cells at passages 20 to 45 were used in these experiments (where passage was reset to 0 at transfection).

Immunohistochemistry

Cells that were cultured and transfected on glass coverslips were fixed either in 4% paraformaldehyde (nonpermeabilized) for 30 min at 4°C or in 99% methanol (permeabilized) for 10 min at −20°C. Fixed cells were washed in PBS and blocked in 5% BSA in PBS for 30 min. Rabbit anti-human primary antibody (Biotrend) at 1:500, diluted in 1% BSA in PBS, was applied for 1 h. Coverslips were washed and incubated for 30 min in Texas Red–conjugated secondary antibody (Molecular Probes), diluted at 1:400 in 1% BSA in PBS. Coverslips were washed and mounted on microscope slides in 3 mg/ml p-phenylene-diamine glycerol. For the permeabilized protocol, Triton X-100 was included in the blocking (1% vol/vol) and antibody steps (0.2% vol/vol). Fluorescence images were obtained using a Zeiss Axiohot fluorescence microscope and captured with a cooled CCD camera (Spot Diagnostics, Sterling Heights, MI) using METAVUE image processing software (Universal Imaging, Downingtown, PA). Z-scan images were obtained from stable transfects using a 63× oil immersion objective (1.4 numerical aperture; Zeiss, Oberkochen, Germany) equipped with a piezo stepper (Visitron Systems, Puchheim, Germany) and stacked.

Time Course

LLC-PK1 cells were cultured to near confluence on 96-wells plates. pLP-CMV neo UMOD constructs were transfected for various time intervals. Supernatant was removed and the cell monolayers were lysed in 1% Triton X-100, 10 mM Tris, and 2 mM EDTA. Caspase 3–like activity was measured in lysates by following the cleavage of Z-DEVD-R110 (Molecular Probes). Fluorescence was measured at 485 nm excitation and 535 nm emission using a TECAN GENios plus multiplate reader. Uromodulin was measured by immunoassay in supernatants and lysates.

Uromodulin Secretion and Reabsorption Assay

Secretion. Stable transfects were seeded at approximately 2 × 105 cells/ml on 2.5-cm-diameter, 0.2-μm-pore size, aluminum oxide filter inserts (Nunc, Wiesbaden, Germany). Cells were cultured to full confluence and trans epithelial electrical resistance (TEER) of monolayers was measured using the Endohm and EVOM systems from World Precision Instruments (Berlin, Germany). TEER of blank filters was subtracted from all samples. TEER values were expressed as ohm cm2. Monolayers were washed in PBS and incubated with fresh medium. Apical medium contained 25 μg/ml FITC-conjugated inulin. After 3 d, supernatant medium was collected from apical and basolateral chambers. Uromodulin was determined in the medium by immunoassay, and inulin permeability was quantified by measuring fluorescence at 485 nm excitation and 535 nm emission.
Reabsorption. Conditioned medium from stable transfects was collected, and uromodulin was quantified by immunoassay. Conditioned medium was diluted in fresh medium to approximately 7 ng/ml uromodulin for all cell types. This medium then was applied to the apical compartment of confluent filter–grown nontransfected LLC-PK1 cells. Uromodulin was measured in the apical and basolateral compartments after a 24-h incubation.

Western Blot
Cells were cultured to confluence on filters as described previously, washed, and incubated in serum-free medium for 3 d. Medium was collected and concentrated using Amicon Ultra centrifuge filters (Millipore, Vienna, Austria) with a molecular weigh cutoff of 50 kD. Typically, a 100× concentrate was achieved. Samples were mixed with Laemelli buffer and run on 8% acrylamide resolving gels under denaturing conditions. Proteins were transferred to Immobilon-P membranes (Millipore, Vienna, Austria), blocked with 5% nonfat milk and probed with sheep anti-human uromodulin (Biotrend) in 1% nonfat milk. Rabbit anti-sheep IgG horseradish peroxidase (Invitrogen) was used as the secondary antibody. An enhanced chemiluminescence assay (Cell Signaling, Danvers, MA) was used for blot development.

Cell Treatment
Stable transfects were cultured to confluence on 96-well plates. Cells were treated with 0.6% DMSO (vehicle), MG-132 (Calbiochem, Bad Soden, Germany) in DMSO (1, 10, and 50 µM), or cycloheximide in DMSO (3.6 µM) for 24 h. Uromodulin was measured in supernatants. Both uromodulin and caspase 3 activity was measured in cell lysates.

Protein Assay
Protein was measured by the Bradford method using BSA to generate a standard curve.

Statistical Analyses
Statistical analysis was performed using GraphPad Prism version 3.03 for Windows (GraphPad Software, San Diego, CA). An unpaired t test was used for assessing statistical significance between experimental groups. All experiments were carried out at least three times with at least three replicates per experiment.

Results

Daily Variation in Control Individuals
To investigate individual daily variation in plasma and urinary uromodulin concentration, samples were collected during a consecutive 5-d period from six healthy volunteers. Uromodulin was determined in samples by immunoassay. Urinary uromodulin was corrected to creatinine measured by the picric acid method. The results are shown in Figure 1 and summarized in Table 1. The percentage coefficients of variance in urine samples for volunteers 1 through 6 were 27.37, 29.94, 53.04, 57.93, 59.61, and 54.65% with a mean of 47.09% and in plasma samples were 26.83, 27.83, 6.08, 18.96, 12.12, and 10.71% with a mean of 17.09%.

Clinical Samples
Urinary and plasma uromodulin were measured in five patients with FJHN, three with the C77Y mutation and two with the C126R mutation. These data were compared with urinary and plasma uromodulin that was taken from either single, one-time samples that were collected from six healthy, nonaffected individuals (control group II) or from five consecutive daily measurements from six healthy, nonaffected individuals (control group I). Patients with FJHN, presenting with renal insufficiency and elevated serum creatinine, had abnormally low urinary uromodulin (Table 1, C77Y-1/2 and C126R-1/2). In these patients, one had abnormally low plasma uromodulin (Table 1, C77Y-1) and two had elevated plasma uromodulin (Table 1, C126R1/2). A 12-yr-old child with no symptoms of renal insufficiency (Table 1, C77Y-3) exhibited low but within normal range urinary uromodulin and highly elevated plasma uromodulin.

Protein Expression
Wild-type and mutated uromodulin was expressed in cultured polarized renal epithelial cells (LLC-PK1) and analyzed for uromodulin expression and secretion patterns. Transient transfection of the UMOD constructs resulted in the expression of uromodulin in LLC-PK1 cells (Figure 2). Uromodulin expression was apparent at 5 h and more intense at 24 h, for both wild-type and mutant constructs (Figure 2). Using nonpermeablized cells, uromodulin was seen to be expressed abundantly at the apical membrane. Wild-type and mutant UMOD constructs exhibited similar expression patterns. To investigate the long-term expression patterns of uromodulin, we generated stable transfects. Similar expression patterns were observed in LLC-PK1 cells that stably expressed wild-type and mutant uromodulin (Figure 3). Stacked Z-scan
Table 1. Uromodulin concentrations in urine and plasma from control and FJHN affected individuals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum Creatinine (mg/dl)</th>
<th>Urinary Uromodulin (mg/g Creatinine)</th>
<th>Plasma Uromodulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group I</td>
<td>ND</td>
<td>46.7 ± 25.02b</td>
<td>14.81 ± 5.28c</td>
</tr>
<tr>
<td>(five consecutive daily measurements from six individuals)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group II</td>
<td>0.80 ± 0.16</td>
<td>26.62 ± 17.27d</td>
<td>19.88 ± 5.97e</td>
</tr>
<tr>
<td>(one-time measurement; n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C77Y-1 (III:6)</td>
<td>2.91</td>
<td>0.17</td>
<td>0.70</td>
</tr>
<tr>
<td>C77Y-2 (III:1)</td>
<td>1.41</td>
<td>0.04</td>
<td>NA</td>
</tr>
<tr>
<td>C77Y-3 (IV:1)</td>
<td>0.81</td>
<td>7.44</td>
<td>137.97</td>
</tr>
<tr>
<td>C126R-1</td>
<td>4.37</td>
<td>0.07</td>
<td>200.42</td>
</tr>
<tr>
<td>C126R-2</td>
<td>2.44</td>
<td>1.09</td>
<td>39.38</td>
</tr>
<tr>
<td>C77Y-1 (III:6)</td>
<td>2.91</td>
<td>0.17</td>
<td>0.70</td>
</tr>
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<td>4.37</td>
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</tr>
<tr>
<td>C126R-2</td>
<td>2.44</td>
<td>1.09</td>
<td>39.38</td>
</tr>
</tbody>
</table>

aUrine and plasma were collected from healthy, unaffected individuals (control groups I and II) and from familial juvenile hyperuricemic nephropathy (FJHN) affected individuals with the C77Y and C126R UMOD mutation. Urinary uromodulin was corrected to urinary creatinine concentration. Values represent the mean ± SD. Numbers in parentheses correspond to pedigree position previously published with clinical data (16). NA, not available; ND, not determined.
bUrine uromodulin range was 10.64 to 126.45 mg/g creatinine.
cPlasma uromodulin range was 8.59 to 27.33 ng/ml.
dUrine uromodulin range was 7.36 to 48.97 mg/g creatinine.
ePlasma uromodulin range was 13.39 to 28.74 ng/ml.

Figure 2. Uromodulin expression in transiently transfected LLC-PK1 cells. LLC-PK1 cells that were cultured on glass coverslips were transfected with UMOD variants for 5 (A) and 24 h (B). Cells were processed with either permeabilized or nonpermeabilized protocols. Immunohistochemistry was conducted using a polyclonal primary antibody, followed by a Texas Red–conjugated secondary antibody. Cells that were transfected with the empty vector were used as a transfection control. Vector alone images were overexposed to show background staining. Magnification, ×630.
images showed uromodulin to be present in high abundance at the luminal membrane of nonpermeablized cells (Figure 3), thereby demonstrating a normal sorting of mutated uromodulin to the luminal membrane.

**Time Course in Uromodulin Expression**

The time course of uromodulin expression and secretion was investigated using LLC-PK1 cells that had been transiently transfected with the wild-type or mutant UMOD constructs. Uromodulin concentrations in the supernatant and lysates were determined by immunoassay. The results showed that during the time frame studied, the UMOD mutants expressed less cytosolic uromodulin (Figure 4A) and consequently secreted less uromodulin into the supernatant medium (Figure 4B). Uromodulin was not detected from cells that were transfected with the empty vector or from untransfected cells.

Uromodulin expression and secretion also were investigated in LLC-PK1 cells that were stably transfected with wild-type or UMOD mutants. LLC-PK1 cells that stably expressed wild-type, C77Y, and N128S uromodulin did not exhibit an altered morphology (Figure 5). All cell types exhibited cobblestone morphology and were capable of vectorial transport of water and solutes as evidenced by the formation of domes (Figure 5). Wild-type and C77Y stable transfects exhibited the same amount of cellular uromodulin (Figure 6A). N128S stable transfects had elevated cellular uromodulin concentrations (Figure 6A). However, both UMOD mutations exhibited a reduced secretion of uromodulin when compared with wild type (Figure 6, B and C).

To investigate whether there is an alteration in the direction in which uromodulin is secreted, stably transfected cells were cultured on microporous supports. Supernatants that were collected over 3 d from the apical and basolateral compartments were assayed for uromodulin by immunoassay. Transepithelial electrical resistance and paracellular permeability to FITC-Inulin was the same in the cells that expressed the wild-type or UMOD mutants (Figure 7, B and C). Wild-type and mutant uromodulin proteins were secreted into both the apical and basolateral compartments (Figure 7A). The UMOD mutants exhibited a decreased apical uromodulin secretion when compared with wild type but exhibited similar basolateral secretion. To exclude a potential preferential reuptake of secreted mutant uromodulin, we incubated nontransfected LLC-PK1 cells with conditioned medium from the transfected cells to assess reabsorption rates of the wild-type and mutant uromodulin proteins. All variants exhibited approximately 6% apical-to-basolateral reabsorption rate per 24 h (Figure 7D), thereby demonstrating that under these conditions, the UMOD mutants do not exhibit an altered reabsorption rate. This result demonstrates that the decreased appearance of mutated uromodulin in the apical medium is not due to an enhanced apical-to-basolateral transepithelial transfer.

We conducted Western blot analysis on concentrated supernatants that were collected from apical and basolateral compartments. (These experiments were conducted in serum-free medium to prevent the co-concentration of FCS proteins.) There was no difference in the apparent molecular weight of either apically or basolaterally secreted uromodulin (Figure 8). In addition, the secreted mutations were of the same apparent molecular weight as secreted wild-type and urinary uromodulin (approximately 85 kD). This suggests that apically and
basolaterally secreted wild-type and mutated uromodulins are mature proteins with a similar glycosylation profile.

To investigate whether uromodulin mutants were preferentially degraded, we treated LLC-PK1 cells that stably expressed wild-type and UMOD mutants with the proteasome inhibitor MG-132 and with the protein synthesis inhibitor cycloheximide. Cycloheximide, as expected, reduced the amount of cytosolic and secreted uromodulin in all cell types (Figure 9, A and B). MG-132 at 1 and 10 μM had no significant effect on cytosolic or secreted uromodulin from any of the cell types (Figure 9, A and B). MG-132 at 50 μM, however, decreased the amount of secreted uromodulin but not cytosolic uromodulin (Figure 9, A and B). This effect may be due to a partial inhibition of the enzyme that is responsible for proteolytic cleavage of membrane-anchored uromodulin, at this high MG-132 concentration. Caspase 3 activity was measured in cell lysates as an indicator of apoptosis. Cycloheximide and MG-132 resulted in elevated apoptosis (Figure 9C). The levels for basal and induced apoptosis were similar for the wild-type and UMOD mutants, C77Y- and N128S-expressing cells. Basal apoptosis also was measured in transient and stable transfects, and this also revealed that caspase 3 activity was similar for the wild-type and UMOD mutants (Figure 9D).
Discussion

This study aimed to characterize the effects of uromodulin mutations on membrane targeting and direction of secretion. Often mutations that result in an altered protein folding lead to intracellular accumulation and cell death (19). The possibility that mutated uromodulin results in cytosolic accumulation and hampered urinary secretion is supported by the observations that patients with FJHN and with MCKD2 exhibit patchy intracellular uromodulin deposits in the TAL and distal tubule (3) and have severely decreased urinary uromodulin when compared with normal individuals (13). However, it is possible that such observations are a consequence of disease progression and
Figure 8. Western blot analysis of concentrated apical and basolateral supernatants. Supernatants were collected and concentrated from apical and basolateral compartments, subjected to SDS-PAGE (8%) and Western blot. Mwm, molecular weight markers. (A and B) Apical (A) and basolateral (B) concentrates. uTHP, is urinary uromodulin. Apical and basolateral uromodulin concentrates exhibited the same molecular weight as each other and as urinary uromodulin (approximately 85 kD), irrespective of the presence of mutation.

not of disease initiation. We analyzed urinary and plasma uromodulin from C77Y and C126R variant individuals with FJHN. A 12-yr-old boy with normal renal function, representing an early stage of disease progression, had low but within normal range urinary uromodulin and elevated plasma uromodulin. Patients with renal insufficiency had, as expected, very low urinary uromodulin. Two patients with the C126R mutation had elevated plasma uromodulin, whereas one with the C77Y mutation had attenuated plasma uromodulin. These observations suggest that there are different urinary and plasma uromodulin profiles in early and late disease stages and that there may be an altered direction of uromodulin secretion in the course of FJHN.

In transiently transfected LLC-PK1 cells, we could demonstrate that both C77Y and N128S mutants are targeted to the apical membrane and are secreted from the cell. However, mutants exhibited a delay in both cytosolic accumulation and secretion. This suggests that the mutants are synthesized at a slower rate than wild-type protein, perhaps as a result of slower processing in the ER, as previously demonstrated with seven different uromodulin mutations (3,14,15). Because uromodulin-associated kidney disease follows a chronic course, we also established stable cell lines, which better represent a persistent synthesis and processing of the wild-type and mutated proteins. LLC-PK1 cells that were stably transfected with C77Y and N128S exhibited no differences in morphology, barrier function, or proliferation as compared with wild-type uromodulin expressing cells over 45 passages. Concentrated luminal expression was observed in all transfects, demonstrating that these mutations do not impair the GPI-mediated apical targeting of the protein. Wild type and C77Y exhibited similar cytosolic uromodulin concentration, whereas N128S had a higher cytosolic content. Although there may be a slower synthesis of the mutated uromodulin, the proteins accumulate to a similar extent with time. We also cultured these cells in filter cultures to create two compartments, mimicking the lumen and interstitium. Both mutants exhibited impaired apical secretion, whereas all cell lines had similar quantities of basolateral uromodulin. Mutants also exhibited no alteration in apical-to-basolateral reabsorption rate, ruling out the possibility that C77Y and N128S are reabsorbed more efficiently than wild-type uromodulin.

C77Y and C126R result in the loss of an evolutionary conserved cysteine, which is likely to be involved in disulphide bridge formation and thus potentially affects protein folding (2). N128S occurs in exon III and is hypothesized to result in the loss of calcium coordination, which may destabilize the EGF III–like domain (2). Often alterations in protein folding are detected by ER quality control mechanisms, and the mutations are eliminated by the proteasome (20). We excluded this possibility for mutated uromodulin by demonstrating that proteasome blockade using MG-132 neither increases cytosolic uromodulin nor restores apical secretion. Neither basal nor MG-132–induced apoptosis was regulated differently in cells that expressed wild-type or mutated uromodulin. Thus, cells that stably expressed these mutations do not have a higher threshold to enter apoptosis. Because total cytosolic uromodulin concentration was not less than wild type and apical-to-basolateral transfer was unchanged, the decreased apical secretion for mutations could be due to a decreased synthesis rate and thus a decreased apical flux out of the cell. This would be in line with previous findings that demonstrated ER retention of other uromodulin mutations (5). However, an effect of the mutation on proteolytic cleavage from the cell membrane anchor cannot be ruled out at this time.

The function of uromodulin in the blood is unclear. It has been demonstrated in several animal studies that elevated serum uromodulin causes a cellular immune response that is directed against uromodulin, resulting in tubulointerstitial nephritis (21–23). We determined the plasma uromodulin concentration in healthy volunteers to be 19.88 ± 5.97 ng/ml. Because uromodulin is targeted specifically to the apical membrane, its presence in the blood may seem puzzling. However, an alternative minor pathway for GPI-anchored proteins has been proposed, whereby the carboxy-terminal is proteolyzed intracellularly without addition of the GPI anchor (24). The intracellularly truncated form of the protein subsequently is secreted from the cell via exocytosis. Western blots of renal tissue homogenates exhibit a band with the same mobility as urinary uromodulin (6). This provides evidence that a fraction of uromodulin is present intracellularly with a cleaved C-terminus without the addition of GPI anchor. This non–GPI-anchored fraction represents a secondary pathway for uromodulin secretion and may represent the major pathway for basolateral secretion into the interstitial space. Although we observed no increase in basolateral secretion with the two mutants, we have shown an increased plasma uromodulin content in three patients...
with FJHN. However, it is not possible to say, at this time, whether such an elevation is due to enhanced secretion of mutated uromodulin or due to TAL injury. Our in vitro model does not support an enhanced basolateral secretion of mutated uromodulin. Because we have shown that both N128S and C77Y mutations are secreted efficiently to the basolateral side of the cell, this raises the possibility that mutational alteration of the uromodulin protein could induce an immune activation at lower concentrations than required for the wild-type protein. Such an event would result in TAL injury, causing a further elevation of interstitial uromodulin, leading to a perpetuation of inflammatory processes and TAL damage.

Conclusion
This study demonstrates that C77Y and N128S mutations of uromodulin decrease apical uromodulin secretion without affecting apical targeting or causing damage to the synthesizing cells. Because the mutant proteins are secreted as efficiently as wild type to the basolateral compartment, the possibility arises that interactions with the immune system at the site of secretion contributes to the development of tubulointerstitial nephritis in uromodulin-associated kidney diseases.

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Disclosures
None.

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See the related editorial, “Mechanism of Injury in Uromodulin-Associated Kidney Disease,” on pages 10–12.

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