Uromodulin and \( \alpha_1 \)-Antitrypsin Urinary Peptide Analysis to Differentiate Glomerular Kidney Diseases

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Abstract

Background/Aims: Glomerular kidney disease (GKD) is suspected in patients based on proteinuria, but its diagnosis relies primarily on renal biopsy. We used urine peptide profiling as a noninvasive means to link GKD-associated changes to each glomerular entity. Methods: Urinary peptide profiles of 60 biopsy-proven glomerular patients and 14 controls were analyzed by combining magnetic bead peptide enrichment, MALDI-TOF MS analysis, and ClinProTools v2.0 to select differential peptides. Tentative identification of the differential peptides was carried out by HPLC-MS/MS. Results: The HPLC-MS/MS results suggest that uromodulin (UMOD; m/z: 1682, 1898 and 1913) and \( \alpha_1 \)-antitrypsin (A1AT; m/z: 1945, 2392 and 2505) are differentially expressed urinary peptides that distinguish between GKD patients and healthy subjects. Low UMOD and high A1AT peptide abundance was observed in 80–92% of patients with GKD. Proliferative forms of GKD were distinguished from nonproliferative forms, based on a combination of UMOD and A1AT peptides. Nonproliferative forms correlated with higher A1AT peptide levels – focal segmental glomerulosclerosis was linked more closely to high levels of the m/z 1945 peptide than minimal change disease. Conclusion: We describe a workflow – urinary peptide profiling coupled with histological findings – that can be used to distinguish GKD accurately and noninvasively, particularly its nonproliferative forms.

Key Words
\( \alpha_1 \)-Antitrypsin • Glomerular kidney disease • MALDI-TOF MS analysis • Peptide profiling • Proteinuria • Urine proteomics • Uromodulin

Introduction

Glomerular kidney disease (GKD) is the third leading cause of end-stage renal disease in the Western world [1]. Glomerulonephritis comprises proliferative forms, such as IgA nephropathy (IgAN) and membranoproliferative glomerulonephritis (MPGN), and nonprolifera-
tive forms, such as membranous glomerulonephritis (MGN), minimal change disease (MCD), and focal segmental glomerulosclerosis (FSGS).

The suspicion of GKD is based broadly on the first clinical manifestation of glomerular injury, such as proteinuria, but proteinuria alone fails to differentiate between forms of GKD. Thus, renal biopsy is needed to diagnose the cause of glomerular injury definitively. Nevertheless, new methods must be developed to differentiate certain disease entities when forms, such as MCD and FSGS, are unable to be distinguished by histology, which has prompted the search for new noninvasive diagnostic alternatives, such as urine proteomics [2–5].

In the last decade, proteomics has been applied extensively to various fields of medicine, including nephrology, and has been used widely to study hundreds of proteins in urine simultaneously rather than individual proteins [6–11]. Urine is one of the most amenable fluids in clinical proteomics, because it can be obtained noninvasively, allowing one to identify GKD-related markers [12–22]. Profiling methods are gaining popularity in the quest for new putative biomarkers for glomerular disorders [23–31].

Magnetic bead-based fractionation methods, coupled with MALDI-TOF MS, have recently been introduced as a urinary peptide profiling strategy [32, 33] and have emerged as a suitable platform for rapid, high-throughput analysis.

Thus, we used this approach to compare urinary peptide profiles between GKD patients and healthy subjects to identify differences that are associated with GKD and correlate them with each glomerular entity.

### Materials and Methods

#### Study Population

This prospective study was performed between June 2006 and May 2009 in the Nephrology Department, Germans Trias i Pujol Hospital (Barcelona, Spain). All procedures were conducted in accordance with the Declaration of Helsinki of 1971, as revised in 2008. The local ethical committees approved the study protocol, and all patients gave written informed consent to participate.

#### Study Procedures

For inclusion, patients had to be aged older than 18 years, show clinical signs of renal disease, such as proteinuria with stable renal function, with or without hematuria, and nephrotic syndrome, and be referred for renal biopsy. Only patients with primary GKD were included. Patients with other forms of GKD, such as those that are associated with nephron mass reduction, infections, drugs, and inflammatory diseases, were excluded.

Healthy subjects were used as controls to establish a normal urinary peptide pattern (table 1).
Measurement of Serum Creatinine and Total Urinary Proteins

Serum creatinine levels were measured by modified Jaffe method (Roche Diagnostics, Basel, Switzerland). Total urinary proteins were measured spectrophotometrically in a 24-hour urine sample on a Cobas u 711 analyzer (Roche Diagnostics) according to the manufacturer’s instructions.

Renal Biopsy

We obtained a single renal biopsy specimen from each patient at the beginning of the study, before initiating corticosteroids or any other immunosuppressive treatment.

Percutaneous renal biopsies were performed and processed routinely for light, immunofluorescence, and electron microscopy according to standard procedures.

Light microscopy sections were stained with hematoxylin/eosin, Schiff’s periodic acid, methenamine silver, Masson trichrome, and Congo red.

Urine Samples

Fresh, first-morning urine samples were collected from the participants on the same day that the renal biopsy was performed [32]. Briefly, urine samples were centrifuged at 2,100 × g for 30 min at 4°C to remove particulate material and cellular debris. The supernatant was adjusted to 6.5 pH with NH₄HCO₃ (1 M) to minimize precipitation during storage, aliquoted to avoid freeze-thaw cycles, and stored at −80°C until use [32]. Protease inhibitors were not added. Normalization of the samples to urinary protein concentration was not necessary to analyze peptide profiles from GKD patients and healthy subjects (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000335383).

Peptide Enrichment and MALDI-TOF MS Analysis

Peptides in urine supernatant (100 μl) were extracted using magnetic beads. The beads were coated with C18 aliphatic chains, wherein poly peptides were captured by hydrophobic interactions (Dynabeads® RP-C18, Invitrogen, The Netherlands). C18 is widely used to purify and enrich peptides within the mass range that was analyzed (1–10 kDa). Preliminary experiments were conducted to optimize the protocol using different surfaces of magnetic beads (C8, IMAC, and WCX) and various MALDI matrices (online suppl. fig. 2).

Each urine sample was processed in duplicate, and each duplicate was spotted twice on the MALDI target (AnchorChip 600/384, Bruker Daltonics, Bremen, Germany). Thus, four MALDI spectra were obtained for each sample, acquired for m/z values from 1000 to 10000. Further details on the MALDI-TOF MS analysis are described in our previous report [34–36], and modified by Villanueva et al. [37].

Data Analysis (v3.4; Bruker Daltonics) was used to select the best spectrum, based on two criteria: maximum peak intensity and maximum number of peaks. The variability in peak intensities was examined in five samples within a run and between runs to assess the coefficients of variance of six peaks with different intensities and sizes (m/z). The precision of the within-run method was <15% using four spectra, and that of the between-run method was <25% over 3 days.

ClinProTools (v2.0; Bruker Daltonics) was used to analyze urinary profiles, subtract baseline values, and normalize the spectra. All spectra were normalized to their own total ion counts. The signal-to-noise ratio was ≥5. A supervised clustering algorithm with a support vector machine (leave-one-out method) was used to determine the peak alignment of the various spectra.

The peaks of all urinary patterns were sorted using significant p values from nonparametric tests to generate a peak model using a training dataset. The reliability of the peak model in predicting how a model behaves and classifies new spectra and the proper classification of a given dataset were assessed with crossvalidation and recognition capability values. The peak model was validated with an independent and equivalent dataset.

After the peak model calculation was run, the peak area data were exported in the CART (ASCII) format (*.dat; by Salford Systems, San Diego, Calif., USA). For each spectrum, the class membership and the areas of the selected peaks, given by their m/z value, were exported. The ASCII export of ClinProTools (v2.0; Bruker Daltonics) allows peak area data to be used in downstream applications, such as statistical analysis.

Peptide Identification by HPLC-MS/MS Analysis

Only those peptides that were analyzed by MALDI-TOF MS whose peak areas differed between glomerular patients and healthy subjects were analyzed by HPLC-MS/MS, as described [38]. Briefly, these peptides were identified by reverse-phase HPLC on an Agilent 1200, operated at 300 nl/min, followed by MS. The peptides that were eluted from the chromatography column were scanned and fragmented with a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, Roskilde, Denmark). Survey scans of precursor ions, ranging from 400 to 2000 m/z, were detected on an Orbitrap analyzer at high resolution (60000 full width at half-maximum of peak at 400 m/z).

Peptide sequences were identified using database searches against the International Protein Index human database (version 3.87 including 91464 entries, released on September 2011) using the Sequest search engine, integrated in Proteome Discoverer version 1.3. We specified ‘no enzyme’ as a variable, which allowed us to identify and sequence peptides that were derived from several rounds of cleavage by endoproteases, as described [39]. We considered all peptides with a false discovery rate ≤5% and Xcorr ≥1.5, 2.0, 2.25, and 3.0 to be positive identifications, corresponding to charge states of +1, +2, +3, and +4, respectively. The proteotypic peptide analysis was based on Global Proteome Machine Database [40].

Statistical Analysis

Normality of the variables was assessed by the Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation, median and range, and were compared by Mann-Whitney U test. Wilcoxon test was performed for paired samples as required to assess the variability in uromodulin (UMOD) and α₁-antitrypsin (A1AT) peptide peak areas by urinary protein concentration. Categorical variables were analyzed using χ² or Fisher’s exact probability test. Associations between variables were assessed using the Spearman correlation coefficient.

The diagnostic performance of the urinary proteomic markers was evaluated by calculating their sensitivity and specificity using the receiver operating characteristics (ROC) curves. The area under the ROC curve (AUC), 95% confidence interval (CI), and cutoff values were calculated by nonparametric method. Cutoff values were determined for the best combination of sensitivity and specificity [41].

Multiple correspondence analysis (MCA), combined with classification methods, was performed to characterize patients with similar peptide patterns [42]. To this end, we created dummy variables of UMOD and A1AT peptides using the results of the ROC curves as cutoffs.

Statistical analyses were performed using SPSS, v15.0 (SPSS Inc., Chicago, Ill., USA) and SPAD, v4.5 (Centre International de Statistiques et d’Informatique Appliquées, Saint Mandé, France). A two-tailed p value of <0.05 was considered to be statistically significant.

Results

Identification of GKD-Related Peptides in the Training Set

As shown in online supplementary figure 2, C18-coated beads were the most effective, leading to richer spectra, characterized by: (a) a higher number of peptides, (b) higher intensities, and (c) a balanced distribution of m/z values. Therefore, the combination of C18-enriched peptides and 2,6-dihydroxyacetophenone as the MALDI matrix led to significantly better results compared with other procedures in the MALDI-TOF MS analysis.

The baseline characteristics of the GKD patients and healthy subjects, randomly assigned to a training or validation set, are summarized in table 1. Significant differences were observed between groups with regard to age, renal function, and proteinuria but not gender. To establish the urinary pattern in GKD, we established a training set including 30 GKD patients and 7 healthy subjects. As shown in figure 1, the urinary peptide profile displayed by GKD patients significantly differed from that of healthy subjects, and was accompanied by a crossvalidation of 91.3% and a recognition capability of 100%.

Using Clinprotools, we identified 22 intense peaks that discriminated GKD patients from healthy subjects. As described in table 2, 11 peaks were identified – 6 achieving statistical significance.

To identify these peptides, we performed HPLC-MS/MS of the peptide preparations after magnetic bead enrichment. Although there was no direct correlation between peptide m/z ratios, as measured by MALDI-TOF (mostly singly charged species), and peptide m/z ratios, as measured by HPLC-MS/MS (mainly multiply charged species), the results strongly suggest that the three peptides that were detected by MALDI-TOF at m/z = 1682, 1898, and 1913 corresponded to uromodulin proteotypic peptides (UMOD). Similarly, the three peptides that were detected by MALDI-TOF at m/z = 1945, 2392, and 2505

Fig. 1. MALDI-TOF MS spectra of GKD patients (a) compared with healthy subjects (b). c Merged spectra of GKD patients (red) and healthy subjects (green). AU = Arbitrary units.
corresponded to α1-antitrypsin proteotypic peptides (A1AT; online suppl. fig. 3A–F).

Assessment of Urinary UMOD and A1AT Peptides in the Validation Set

The urinary peptide profile was tested against the validation set, which comprised 30 GKD patients and 7 healthy subjects with baseline characteristics that were equivalent to those of individuals in the training set (table 1). There were no differences in age, gender, renal function, or proteinuria between the training and validation sets, as required.

When we applied the urinary peptide profile to a blinded validation set, it correctly classified GKD patients with high sensitivity (91.7%) and specificity (85.7%), generating a positive predictive value of 95.6% and a negative predictive value of 75%.

Association between UMOD and A1AT Peptides and Clinical Parameters

As shown in figure 2, the peptides with m/z 1682, 1898, and 1913 decreased in peak area in GKD patients, and that of peptides with m/z 1945, 2392, and 2505 increased compared with healthy subjects.

To determine whether any clinical parameters influenced these results, we analyzed the urinary peptides with regard to age, gender, serum creatinine, and proteinuria but did not observe any differences in age or gender. No associations were observed between A1AT peptides and serum creatinine or proteinuria in GKD patients. But, a significant inverse correlation was noted between UMOD peptides and serum creatinine in GKD patients (fig. 3a–c). Further, we found a significant inverse correlation between a UMOD peptide (m/z 1913) and proteinuria in GKD patients (fig. 3f), and a trend toward signif-
Significance was noted between UMOD peptides and proteinuria (m/z 1682 and m/z 1898; fig. 3d, e).

**ROC Curves for UMOD and A1AT Peptides**

The sensitivity and specificity of UMOD and A1AT urinary peptides were calculated to determine their accuracy in distinguishing GKD patients from healthy subjects by ROC curve. The AUC of UMOD peptides ranged between 0.852 and 0.980, suggesting that they can establish the absence of GKD (fig. 4a). Conversely, the AUC of A1AT peptides ranged between 0.872 and 0.946, suggesting that they can detect the presence of GKD (fig. 4b).

Further, the discriminatory power of the six peptides that were selected (potentially corresponding to UMOD and A1AT) was confirmed in the validation set. As shown in table 3, a low peak area of UMOD peptides, combined with a high peak area of A1AT peptides, successfully discriminated between the disease and normal conditions, diagnosing 80–92% of patients as having GKD.

**Association between UMOD and A1AT Peptides and Histological Subtype**

As described above, based on UMOD and A1AT urinary peptides, we were able to differentiate between healthy subjects and patients with GKD (fig. 4). However, a separate analysis of UMOD and A1AT peptides did not yield enough information to distinguish between glomerular subtypes.

In our study, the MCA analysis was based on cutoff values of the ROC curves of the peptides of interest – 3 from UMOD and 3 from A1AT. These cutoffs are shown in figure 4. Those with the best equilibrium between sensitivity and specificity were chosen as the ideal cutoffs. The cutoffs refer to the values above or below which we classified a subject as healthy or a GKD patient. Moreover, these values allow us to categorize these peptides as high or low levels. To characterize patients with similar peptide patterns, we created dummy variables of UMOD and A1AT peptides using the results of the ROC curves as cutoffs.
Fig. 3. Inverse correlation of UMOD peptide peak areas with serum creatinine and proteinuria. Data for patients from the training set are shown. AU = Arbitrary units.

Fig. 4. ROC curves of UMOD and A1AT peptides in GKD and healthy subjects in the training set. Characteristics associated with AUC values. The cutoff values were the peak area values of UMOD (a), and A1AT peptides (b), that best discriminated GKD patients from healthy subjects to the best combination of sensitivity and specificity; values that exceeded the cutoff reflected a negative test result for GKD for UMOD peptides and a positive result for A1AT peptides.
Using dummy variables for UMOD and A1AT peptides, by MCA, we observed that these 6 peptides formed 2 groups in the biplot graph (fig. 5a). Healthy subjects were associated with high levels of UMOD-derived peptides (m/z 1682, 1898, and 1913), and GKD patients correlated with high levels of A1AT-derived peptides (m/z 1945, 2392, and 2505).

Nonproliferative forms of GKD were associated with high levels of the m/z 2392 and m/z 2505 peptides, whereas proliferative forms were linked to high levels of the m/z 1945 peptide and low levels of the m/z 1682, 1898, and 1913 peptides.

To discriminate between nonproliferative and proliferative forms, we performed an analysis, excluding healthy subjects. Figure 5b shows that among the nonproliferative forms of GKD, MCD and MGN correlated with high levels of the m/z 2392 and m/z 2505 peptides, whereas proliferative forms were linked to high levels of the m/z 1945 peptide and low levels of the m/z 1682, 1898, and 1913 peptides. FSGS patients had high levels of the m/z 1945 peptide, like other proliferative forms of GKD (MPGN and IgAN).

**Discussion**

Several proteomic approaches have been taken to demonstrate the efficacy of urinary proteomics in determining whether histological alterations that are associated with GKD are reflected in urine [12–29, 33]. Thus, clinical urinary proteomics is an invaluable approach in nephrology and other pathologies.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Peak area, AU</th>
<th>Healthy subjects, % observed</th>
<th>Glomerular patients, % observed</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMOD</td>
<td></td>
<td>n.a.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>1682 m/z</td>
<td>&lt;21.9</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.a.</td>
</tr>
<tr>
<td>1898 m/z</td>
<td>&lt;61.1</td>
<td>14.3</td>
<td>80.8</td>
<td>0.001</td>
</tr>
<tr>
<td>1913 m/z</td>
<td>&lt;342.4</td>
<td>14.3</td>
<td>92.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A1AT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1945 m/z</td>
<td>&gt;14.4</td>
<td>0</td>
<td>84.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2392 m/z</td>
<td>&gt;27.7</td>
<td>0</td>
<td>84.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2505 m/z</td>
<td>&gt;20.8</td>
<td>0</td>
<td>80.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

p values were calculated using the Fisher’s exact probability test. AU = Arbitrary units; n.d. = not detected; n.a. = not applicable.

Peptidome-based methods using MS are valid and useful approaches to identify small proteins and peptides [32] in biological fluids. Nevertheless, urine is a complex bio-fluid that contains a wide range of proteins and peptides [for review, see 11]. Thus, peptides need to be enriched prior to MALDI-TOF MS analysis. Magnetic bead-based enrichment of urinary peptides is ideal in generating proteomic patterns for MALDI-TOF MS [37]. Magnetic beads have many advantages, such as reduced sample requirements and high-throughput capacity, versus classical 2-dimensional electrophoresis, which is too time-consuming for routine use [11, 43, 44].

Magnetic bead-based profiling and MALDI-TOF MS constitute a new strategy that assesses differences between GKD patients and a control group. Few studies have examined the urinary peptidomes of renal disease patients by peptide enrichment and MALDI-TOF MS to identify urinary biomarkers [30, 31, 33]; only one has focused on differentiating IgAN from other GKD forms using our proteomics approach [33].

In this report, we have demonstrated that magnetic bead technology, combined with MALDI-TOF MS, assesses differences in urinary peptide patterns in GKD patients with high sensitivity (91.7%) and specificity (85.7%). We suggest that the differential peptides correspond to UMOD and A1AT cognate proteins, as derived after separation and fragmentation of the peptides by HPLC-MS/MS analysis. Construction of a differential peptide pattern is the initial step toward the classification of GKD patients, including the proliferative and nonproliferative forms.

We consistently observed significant decrease in three peptides (m/z 1682, 1898, and 1913) in GKD patients. We suggest that these peptides identified by HPLC-MS/MS correspond to UMOD. Currently, it has been reported that the so-called ‘proteotypic’ peptides are gaining interest in the validation of protein identification [40]. These peptides are repeatedly and consistently identified for any given protein in a complex mixture, such as urine. Indeed, it has been successfully argued that as few as one or two identified proteotypic peptides were sufficient to positively declare the presence of a particular protein in a sample. Therefore, in our study the use of proteotypic peptides seems to be a useful approach for identifying differentially expressed urinary peptides that distinguish between GKD patients and healthy subjects.

UMOD (Tamm-Horsfall protein) is the most abundant protein excreted in the urine under physiological conditions. UMOD is a kidney-specific protein that is exclusively expressed by epithelial cells lining the thick ascending limb of Henle’s loop [45–51]. Indeed, UMOD is syn-
thesized as a pro-form anchored by its C-terminus to the apical plasma membrane of epithelial cells and released into the urine via a conserved proteolytic cleavage [52]. In our study, we found UMOD peptides that correspond to the C-terminal region of the protein, which mainly remains attached to the apical plasma membrane of tubular epithelial cells after ectodomain cleavage, but is released into the urine through an unknown mechanism.

The pathophysiological mechanisms of UMOD are unknown [45–47, 53], but it has been suggested that UMOD peptides are associated with impaired renal function [28, 30, 31, 54]. We observed that levels of UMOD-related peptides decreased as renal disease progressed in patients with GKD, consistent with other studies that have proposed UMOD as a renal disease biomarker [30, 31, 33]. The UMOD peptide at m/z 1913 was recently reported as a biomarker for IgAN [33]. We observed decreased levels of m/z 1913 in urine from GKD patients, consistent with Wu et al. [33]. Nevertheless, we noted that m/z 1913 alone could not distinguish between forms of GKD, even with three individual UMOD peptides.

We speculate that three of the six differential peptides that we identified by HPLC-MS/MS belong to A1AT, consistent with the reported increase in A1AT levels in urine from patients with nephrotic syndrome [15, 17]. A1AT is
a protease inhibitor in plasma and urine [11]. This protein has been proposed to be a biomarker for various pathologies, such as liver cirrhosis [55] and urologic carcinomas, as reviewed in Casado-Vela et al. [11]. A1AT has also been suggested to regulate inflammation and the accumulation of mesangial matrix [56, 57].

Similar to reports that observed A1AT protein or its fragments [4, 15, 17–19, 21, 22], we identified three peptides – potentially derived from A1AT cleavage from its C-terminus during protease inhibition and elevated in GKD patients. Although examining urinary peptides by immunoassay is difficult, e.g. when peptides share an antigenic epitope or are too small [58], the fact that A1AT urinary protein was detected in GKD patients but was virtually undetectable in healthy subjects (data not shown) confirms our conjecture.

Remarkably, we noted an increase in three peptides in GKD – potentially belonging to cognate A1AT protein – independent of the degree of proteinuria. Therefore, the identification of these peptides, which are not modified by proteinuria, offers additional information in the diagnosis of GKD, because proteinuria alone fails to discern between glomerular disease forms.

The analytical workflow that we have described allows the unambiguous differentiation of GKD patients versus healthy subjects, based on the differential protein profile of six peptides. We believe that this workflow is easy to implement in clinical practice to distinguish GKD subtypes. Moreover, interpreting the results is easy for a clinician because the intensity of peptides with diagnostic value changes drastically in healthy versus GKD patients. Similarly, the composite of differential levels of six peptides (three from UMOD and three from A1AT) allowed us to differentiate between proliferative (i.e. IgAN, MPGN) and nonproliferative (i.e. MCD, FSGS, and MGN) forms of GKD; in particular, it seems to be useful in distinguishing MCD using A1AT peptides.

We observed a decrease in UMOD urinary peptides and an increase in A1AT urinary peptides in GKD patients, demonstrating that MB technology, coupled with MALDI-TOF MS, can be used to assess urine from GKD patients, as has been reported in other proteomic approaches [12–29, 33].

Proteinuria and renal biopsy are used in clinical practice as useful diagnostic markers to predict nephropathy, but they are insufficient to predict the evolution of GKD and the response to treatment. Thus, nephrologists should develop new proteomics approaches regarding the development of noninvasive diagnostic tools and novel therapeutic targets.

To this end, our findings constitute a novel method of diagnosing and making prognoses for certain GKD forms, when histology is unable to distinguish them, as in MCD and FSGS, allowing clinicians to provide more accurate treatment. Perhaps, well-established proteomic patterns will complement or surpass histological findings in diagnosing GKD.

In conclusion, urinary peptide profiling can be used to identify GKD-related peptides. We propose that the analysis of urinary UMOD and A1AT peptides, simultaneous renal biopsy, proteinuria, and serum creatinine measurements can aid in establishing the diagnosis of GKD. Further studies are necessary to determine the functions of markers in GKD and recapitulate our findings in a larger sample of patients.

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Disclosure Statement

The authors declare no potential conflicts of interest with respect to the authorship or publication of this article.

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