Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by slowly progressive bilateral renal cyst growth ultimately resulting in loss of kidney function and end-stage renal disease (ESRD). Disease progression rate and age at ESRD are highly variable. Therapeutic interventions therefore require early risk stratification of patients and monitoring of disease progression in response to treatment.

Methods: We used a urine peptidomic approach based on capillary electrophoresis–mass–spectrometry (CE-MS) to identify potential biomarkers reflecting the risk for early progression to coronary interventions: insights from the NCDR Cath-PCI registry. JACC: Cardiovascular Interv 2014; 7: 1–9.


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risk stratification of patients is of central importance to allow timely intervention in high-risk patients while avoiding unnecessary treatment of low-risk patients.

Currently, prognostic biomarkers for the clinically relevant endpoints in ADPKD are limited. Total kidney volume adjusted for height (htTKV) has been considered the best prognostic marker in ADPKD [9], however, accurate determination of htTKV is time-consuming, requires quantitative imaging algorithms and is not universally accessible. In addition, monitoring response to therapy by TKV requires relatively long follow-up times to allow for accurate determination of TKV changes over time. Common biomarkers of kidney disease, such as proteinuria/albuminuria, and tubular injury markers like KIM-1, NGAL, MCP-1, NAG and IL-18 show weak correlation with disease severity and have failed to provide prediction of outcomes [10–15]. Circulating copeptin levels, the c-terminal portion of the vasopressin precursor, moderately correlate with measures of disease severity in ADPKD [16] and have moderate predictive value for the change in htTKV and GFR decline during follow-up in two independent longitudinal studies [17, 18].

Capillary electrophoresis coupled on-line to mass spectrometry (CE-MS) allows for the simultaneous quantification of hundreds of peptides in urine samples, which can then be combined into diagnostic or prognostic scores [19]. Using this approach, we have previously identified a urinary biomarker profile of ADPKD that is highly specific for the disease and correlates with htTKV [20, 21]. To follow up on these initial observations and to determine the predictive power of proteomics in ADPKD, we identified a peptidomic pattern predictive of ESRD [10–15]. We developed a urinary test that accurately predicts relevant clinical outcomes in ADPKD patients and suggests altered proteolytic pathways involved in disease progression.

**Keywords:** ADPKD, ESRD, progression, proteases prediction, urinary peptides

**INTRODUCTION**

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease, with an incidence between 1:400 and 1:1000 live births [1, 2]. The continuous development and growth of bilateral kidney cysts leads to progressive renal enlargement and ultimately to a loss of kidney function and end-stage renal disease (ESRD) occurring at a mean age of 55 years [3–5]. Disease severity and progression are highly variable, even within families. Despite massive cystic enlargement, polycystic kidneys retain a remarkable compensatory capacity through hyperfiltration of remnant nephrons, where kidney function is maintained, making the disease course hard to predict. A significant increase in serum creatinine ensues late in the course of the disease and is typically followed by a uniform rapid decline of glomerular filtration rate (GFR) and progression to ESRD, often too late to consider therapeutic interventions [6].

Until recently, treatment for ADPKD has been largely symptomatic and supportive, including management of complications and ultimately renal replacement therapy. Rapidly accumulating molecular insights into disease pathogenesis and progression have resulted in the development of several targeted treatment approaches [7]. Rigorous blood pressure control using renin–angiotensin–aldosterone system (RAAS) blockade has been shown to reduce cyst growth in early ADPKD in the HALT PKD study, with improvement in the chronic slope of estimated GFR (eGFR) [8]. Recently, tolvaptan has been approved for ADPKD treatment in Japan, Canada and Europe. Since loss of GFR occurs only once massive structural damage to the kidneys is present, late treatment initiation for ADPKD is considered unlikely to significantly affect outcomes. Thus, early risk stratification of patients is of central importance to allow

**MATERIALS AND METHODS**

**Patients and sample analysis**

ADPKD urine samples were obtained as fasting, first-morning void at the baseline visit of the CRISP study [22]. Detailed inclusion criteria for the CRISP study have been reported elsewhere [23], the most relevant of which were age 15–46 years and creatinine clearance >70 mL/min. After the first 3 years of follow-up, participants entered an extension study (CRISP II) with an additional follow-up of 5 years. Study visits were scheduled at baseline and years 1, 2, 3, 6, and 8, including routine urine and blood laboratory evaluation, renal MRI, iothalamate-based GFR measurement (mGFR) and bio-banking. Details of the study protocol have been described elsewhere [22, 23]. After CRISP II, participants gave informed consent to be further followed in CRISP III, which included phone interviews and evaluations for major outcomes, including ESRD. The CRISP cohort includes a total of 241 ADPKD patients, 221 of which had a baseline urine sample available. In this study, we used the previously acquired peptidomic datasets on these patients [21] and correlated them with extended longitudinal clinical follow-up information. Urine sample storage, processing and capillary electrophoresis–mass spectrometry
(CE-MS) analysis have been described in detail elsewhere [20, 21] and are summarized in the Supplementary methods.

Clinical data of non-ADPKD chronic kidney disease (CKD) patients (n = 522) with different CKD stages have been previously described [24]. Participants in this cohort had an average of five follow-up measurements of eGFR during a period of 54 ± 28 months. The mean eGFR at baseline and mean eGFR change per year during follow-up were 76 ± 24 mL/min/1.73 m² and -1.24 ± 5.03%, respectively. The mean urinary albumin concentration of these patients was 127 ± 415 mg/L at baseline. Rapid progressors were defined by a decrease of eGFR >5% per year [24].

Statistical analysis

The selection of patients from the entire CRISP cohort for biomarker identification and all internal validation analyses are depicted in Figure 1. For the identification of biomarkers associated with ESRD risk, we compared patients reaching ESRD (defined as initiation of renal replacement therapy or kidney transplantation) during follow-up with control patients characterized by a slow progression rate as defined by no ESRD during follow-up AND serial mGFR and eGFR determinations available over >7 years AND at least six study visits including eGFR determination available AND both mGFR and eGFR slope (as calculated by linear regression of mGFR and eGFR over time in the individual patients) of no more than -3 mL/min/1.73 m² per year AND age at baseline >24 years. Two-thirds of patients from both cases and controls were randomly chosen to create a development cohort for biomarker identification, leaving the remaining patients for validation. A sensitivity analysis was performed excluding patients reaching ESRD that were >40 years of age at baseline. The biomarker model was subsequently tested in several internal validation cohorts (the numbers given here correspond to the labelling in Figure 1): (i) the above-mentioned randomly chosen one-third of patients excluded from the development cohort, consisting of patients reaching ESRD and of patients with stable disease; (ii) the entire CRISP cohort, i.e. including also patients with intermediate progression that exhibited relevant GFR decline but did not reach ESRD during follow-up. To avoid bias due to differences in follow-up duration and baseline age, only patients with >10 years of follow-up and >24 years of age at baseline were included as controls for this validation cohort; (iii) this same cohort, but excluding all patients used in the development cohort, as well as a proportional 75% of patients with intermediate progression (aiming to create a completely independent

FIGURE 1: Schematic study flow chart depicting the subgroups of the CRISP cohort used for biomarker model generation and validation. 1) For the definition of stable disease, see methods section.
validation cohort not including any patients from the development cohort); (iv) patients < 24 years of age at baseline that had been excluded from the development cohort and the formerly mentioned validation cohorts. Because ESRD would be very unlikely in these young patients, we used a > 30 mL/min/1.73 m² decline in mGFR from baseline to year 8 as a surrogate outcome measure.

**In silico protease prediction**

*In silico* protease mapping was performed using Proteasix [25], a bioinformatic tool that predicts the proteases involved in naturally occurring peptide generation. N- and C-terminal cleavage sites for each of the peptides were used to predict the associated proteases, as described in the Supplementary methods. Based on the mean of log2 transformed intensities of all peptides associated with ADPKD progression that were likely generated by a particular protease, the activity of this protease was calculated for each patient and estimated protease activities were compared between cases and controls.

**Pathway enrichment analysis**

In order to investigate the molecular pathways altered during ADPKD progression, we performed pathway enrichment analysis using Cytoscape [26], an open source software for visualization of molecular interaction networks with high-throughput expression data. From the prognostic model based on 20 peptides, all 16 peptides with available sequence information were examined using Cytoscape’s plug-ins GlueGO and Clue Pedia [26, 27]. Identification of significantly affected molecular pathways were performed using the Reactome pathway database [28].

**RESULTS**

**Biomarker identification**

During a follow-up of up to 13 years, 28 of the 221 CRISP patients with available baseline urine samples have reached ESRD. Baseline characteristics of relevant patient groups are shown in Table 1. A total of 2247 urinary peptides were detectable in > 40% of either patients reaching ESRD or patients with a relatively stable renal function during follow-up. A total of 1020 of these peptides showed a significantly different urinary excretion rate between cases and controls. Using liquid chromatography–tandem mass spectrometry, the amino acid sequence of 16 of the 20 peptides could be identified (Table 2).

The sensitivity analysis excluding ESRD patients > 40 years of age at baseline (n = 9) yielded 16 peptides, 10 of which were among the 20 peptides from the original analysis (Supplementary data, Table S1).

**Prognostic biomarker models**

We next used a support vector machine (SVM)-based approach to generate a prognostic biomarker model based on the identified biomarkers. This model achieved an area under the receiver operating characteristics curve (AUC) of 0.95 to classify patients into low or high risk for ESRD when applied to the development cohort after total cross-validation (21 ESRD and 39 control patients) and an AUC of 0.83 [95% confidence interval (CI) 0.60–0.96; P = 0.0011] when applied to the validation cohort (7 ESRD and 14 control patients) (Figure 2).

Sensitivity, specificity and positive and negative predictive values are given in Table 3. A second model was generated based on the 16 peptides from the sensitivity analysis that resulted in a very similar performance (Supplementary data, Figure S1).

Since the prognostic model was derived by comparing the extremes of stable disease and progression to ESRD over 13 years, we next evaluated its more general utility by applying it to

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
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<tbody>
<tr>
<td><strong>Variable</strong></td>
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<tr>
<td><strong>n</strong></td>
</tr>
<tr>
<td>Baseline age (years)</td>
</tr>
<tr>
<td>Gender (% female)</td>
</tr>
<tr>
<td>Baseline TKV (mL)</td>
</tr>
<tr>
<td>Baseline hTKV (mL/m)</td>
</tr>
<tr>
<td>Baseline mGFR (mL/min/1.73 m²)</td>
</tr>
<tr>
<td>Baseline eGFR (mL/min/1.73 m²)</td>
</tr>
<tr>
<td>GFR slope (mL/min/1.73 m²/year)</td>
</tr>
<tr>
<td>Genotype (PKD1/PKD2/no detectable mutation; %)</td>
</tr>
<tr>
<td>Duration of follow-up (years)</td>
</tr>
</tbody>
</table>

Values are mean ± SD if not otherwise stated.

TKV, total kidney volume; hTKV, height-adjusted TKV; mGFR, measured GFR; eGFR, estimated GFR.
the entire CRISP cohort, including all patients with follow-up >10 years and age >24 years at baseline, i.e. also patients with intermediate progression (validation cohort 2; n = 142). Here, the biomarker model achieved an AUC of 0.86 (95% CI 0.79–0.91; P < 0.0001) corresponding to an overall sensitivity of 86% and specificity of 71% at a predetermined cut-off level of -0.033 for the dimensionless biomarker score to detect patients reaching ESRD during follow-up (Figure 3A). When excluding all patients used in the development cohort from this analysis, as well as a proportional 75% of patients with intermediate progression (validation cohort 3), the AUC remained high at 0.80 as well as a proportional 75% of patients with intermediate progression (validation cohort 2; n = 99). From this analysis, PKD2 mutations or no identifiable mutation were excluded from the validation cohorts (Supplementary data, Table S2).

Comparison of the biomarker model’s prognostic performance to total kidney volume

We next compared the prognostic performance of the biomarker model to that of hTKV. In validation cohort 2, hTKV achieved an AUC of 0.89 (95% CI 0.83–0.94; P < 0.0001), corresponding to a sensitivity and specificity of 82% and 84%, respectively, with a cut-off hTKV >825 mL/m² (Figure 3B). When directly comparing the biomarker score with hTKV in individual patients, the vast majority of patients who were classified as high risk for progression based on hTKV were also classified as high risk by the biomarker score at the threshold of >-0.033; in contrast, several patients classified as low risk based on baseline hTKV scored positive in the biomarker score (Table 4 and Figure 3C), some of which progressed to ESRD. Thus, the biomarker score exhibited a higher sensitivity for progression compared with hTKV at the expense of a loss of specificity. However, many of the patients scoring as high risk in the biomarker model exhibited a pronounced loss of kidney function, albeit not progressing to ESRD: from baseline to Year 8 (the last follow-up for mGFR), patients scoring as high risk in the biomarker score exhibited a GFR loss of 27 ± 24 mL/min/1.73 m² as compared with 16 ± 28 mL/min/1.73 m² for patients deemed low risk by the biomarker model (P < 0.05). By logistic regression analysis, we next evaluated whether the combination of hTKV with the 20-biomarker pattern would further improve prediction. The combined model achieved an AUC of 0.92 (95% CI 0.86–0.96; P < 0.0001) when applied to validation cohort 2.

Performance of the prognostic biomarker model in young patients

Prediction of progression is particularly important in young patients because specific treatments may be more promising if initiated early, before the burden of irreversible damage is high. Although case and control patients were age matched, we first initiated early, before the burden of irreversible damage is high. Therefore, we applied the biomarker panel to patients <24 years of age. Due to their young age, none of these patients have reached ESRD. We therefore used a >30 mL/min/1.73 m² to calculate the GFR. Given are internal peptide ID and mean amplitude of the peptides in the development cohort, the false discovery rate (FDR)-adjusted P-value according to the method of Benjamini and Hochberg [29] for the comparison of cases (reaching ESRD) and controls (stable disease). Amino acid position, ESRD - end-stage renal disease; ADPKD - autosomal polycystic kidney disease; SVM - support vector machine. Prediction of progression is particularly important in young patients because specific treatments may be more promising if initiated early, before the burden of irreversible damage is high. Although case and control patients were age matched, we first initiated early, before the burden of irreversible damage is high. Therefore, we applied the biomarker panel to patients <24 years of age. Due to their young age, none of these patients have reached ESRD. We therefore used a >30 mL/min/1.73 m² to calculate the GFR. Given are internal peptide ID and mean amplitude of the peptides in the development cohort, the false discovery rate (FDR)-adjusted P-value according to the method of Benjamini and Hochberg [29] for the comparison of cases (reaching ESRD) and controls (stable disease). Amino acid position, ESRD - end-stage renal disease; ADPKD - autosomal polycystic kidney disease; SVM - support vector machine.
decline in mGFR from baseline to Year 8 as an outcome measure. Thirty patients had mGFR available at baseline and Year 8 (validation cohort 4 in Figure 1). Here, the prognostic biomarker model achieved an AUC of 0.92 (95% CI 0.76–0.99; P < 0.0001) (Figure 4). In comparison, htTKV, applied to the same patient groups, achieved an AUC of 0.96 (95% CI 0.82–1.00; P < 0.0001).

Specificity of the biomarker model for ADPKD

To evaluate whether the identified prognostic peptidome signature is reflecting ADPKD-specific processes or unspecific mechanisms of progressive renal injury, we applied our model to a cohort of 522 patients with CKD due to a variety of other identified renal disorders with an average follow-up of five eGFR measurements over a period of 3 years [24]. In this cohort, divided into rapid (n = 89) and slow (n = 433) progressors based on the mean annual eGFR change, the prognostic ADPKD model achieved an AUC of 0.70 (95% CI 0.66–0.74; P < 0.0001) to predict progression, whereas the previously published biomarker model CKD_273, developed in a non-ADPKD CKD population [24, 30], yielded an AUC of 0.82 (95% CI 0.79–0.85; P < 0.0001) (Figure 5A). In contrast, the CKD_273 model achieved an AUC of 0.77 (95% CI 0.69–0.83; P < 0.0001) to predict ESRD in the ADPKD cohort (validation cohort 2 from Figure 1) and an AUC of 0.76 (95% CI 0.57–0.90; P = 0.023) to predict a 30 mL/min/1.73 m 2 GFR reduction over 8 years in the ADPKD patients <24 years of age (validation cohort 4 from Figure 1) (Figure 5B and C, respectively). When individual peptides were compared from the ADPKD 20 marker panel and the general CKD prognostic marker panel, there was a relatively small overlap of six peptides common to both models.

Prediction of proteolytic pathways involved in disease progression

The urinary peptides detected by CE-MS (molecular weight <20 kDa) represent naturally occurring peptides generated during proteolytic processing and breakdown of parental proteins. We therefore aimed to identify the specific proteolytic pathways involved in the generation of prognostic peptides. Based on the mean intensities (urinary concentrations) of the 16 prognostic peptide fragments with available sequence information in ESRD versus control patients in the development cohort, in silico analysis revealed nine proteases that showed a significant correlation of their predicted activity with ADPKD progression (Table 5), including prominent alternations in cathepsin activities. We mapped the identified peptides and the predicted proteases using the Reactome pathways database [28] and Cytoscape’s plug-ins ClueGo and CluePedia [26, 27]. This resulted in the identification of several molecular pathways that may be involved in progression of renal disease in ADPKD (Figure 6).

**DISCUSSION**

We have identified a urinary peptidomic biomarker pattern that reliably predicts progression to ESRD in ADPKD patients based on a single baseline urine sample.

For prognostic biomarkers for ADPKD, age at ESRD would be clinically the most relevant endpoint, but given the prolonged asymptomatic disease progressive phase, this would require extremely long follow-up times. Alternatively, using GFR slope or a predefined GFR change as an outcome requires accurate determination of GFR. GFR can be acutely influenced by common medications such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. Specifically in ADPKD, GFR is maintained for decades with episodes of hyperfiltration,
followed by an accelerated decline to ESRD, indicating that the GFR slope is age dependent and not constant. We therefore chose to divide our cohort into two age subgroups. ESRD was used as the outcome in patients >24 years of age at baseline, whereas a predefined GFR loss of 30 mL/min/1.73 m² over 8 years, based on accurate iothalamate-based measurements, was used as the outcome in patients <24 years of age at baseline. To avoid bias of the duration of follow-up time and age at baseline, we minimized these two sources by (i) restricting this analysis to CRISP participants with a follow-up time >10 years and (ii) age matching the control group to the ESRD cases. An additional sensitivity analysis, excluding all patients reaching ESRD that were >40 years of age at baseline, yielded very similar results.

We intentionally included both patients with PKD1 and PKD2 mutations as well as patients without detectable mutation, because genetic analysis is not routinely performed in clinical practice. Hence, a clinically useful biomarker should be applicable to all ADPKD patients without requiring knowledge of their genotype. Nevertheless, we tested the model in validation cohorts including only patients with PKD1 mutations, where its predictive power was only slightly reduced. Thus, the identity of the mutated PKD gene does not account for the identified peptidomic pattern to a relevant degree.

The prognostic performance of the biomarker model was similar to that of baseline htTKV. Importantly, CE-MS analysis of urine is not operator dependent, as in the case of ultrasound imaging, nor does it require timely and expensive image analysis algorithms that may not be widely available, as in the case of magnetic resonance (MR)-based determination of htTKV. In several clinical settings, CE-MS analysis performed in Clinical Laboratory Improvement Amendments–certified laboratories would be easier and less expensive to implement than standardized image-based TKV measurements. In this study, we used urine collected in a standardized format without special preparation in an automated set-up for urine peptidome analysis that yields reproducible results and is available for clinical applications.

The table below compares the prognostic scoring of patients by the biomarker model and by htTKV and their outcome:

<table>
<thead>
<tr>
<th>Biomarker model</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>htTKV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>No ESRD</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>23</td>
<td>72</td>
<td>No ESRD</td>
</tr>
</tbody>
</table>

Table 4. Comparison of the prognostic scoring of patients by the biomarker model and by htTKV and their outcome

FIGURE 3: Performance of (A) the prognostic biomarker model and (B) height-adjusted TKV (htTKV) to predict ESRD during follow-up when applied to the whole cohort of 142 patients >24 years of age at baseline with either a follow-up duration >10 years or reaching ESRD (validation cohort 2). (C) Direct comparison of the biomarker model score with htTKV in individual patients. Patients reaching ESRD during follow-up are shown as black dots, patients not reaching ESRD are shown as open circles. Optimal cut-off values for both, the biomarker score and htTKV are depicted as dotted line.
FIGURE 4: Performance of (A) the biomarker model and (B) hTKV to predict a 30 mL/min/1.73 m² GFR decline from baseline to Year 8 in young patients <24 years of age at baseline (validation cohort 4).

FIGURE 5: (A) Prognostic performance of the ADPKD biomarker model as compared with the CKD_273 biomarker model to predict CKD progression in a non-ADPKD CKD patient cohort (n = 522). (B) Prognostic performance of the CKD_273 biomarker model as compared with the ADPKD biomarker model to predict ESRD when applied to validation cohort 2. (C) Prognostic performance of the CKD_273 biomarker model as compared with the ADPKD biomarker model to predict an absolute 30 mL/min/1.73 m² GFR decline from baseline to Year 8 in ADPKD patients <24 years of age at baseline (validation cohort 4).
that use less automated, more laborious and less reproducible analytical methods and usually require the development of alternative quantitative methods (such as enzyme-linked immu-

nosorbent assays) for identified candidate markers before clinical application, whereas peptidomic profiling by CE-MS yields reproducible quantitative results and can be directly used to analyse clinical samples.

Urine peptidome analysis may add prognostic information that is not reflected by TKV measurements. A subset of ADPKD patients that progressed to ESRD with relatively small kidney volumes were correctly classified by the urine peptidome score. Finally, the combination of the proteomic score with hTKV led to an improvement of prediction beyond that of hTKV or the urine peptidome score alone, although our study was not sufficiently powered to evaluate whether this improvement was significant. Further larger studies are needed to evaluate whether urine peptidome patterns provide additional prognostic information for disease progression for ADPKD and whether imaging and urinary peptidomic measures reflect the same causal pathways in disease progression in ADPKD. In this regard, an important question is whether the peptidomic pattern merely reflects the extent of cyst burden or whether it actually reflects disease ‘activity’, i.e. the rate of disease progression. This question is of particular relevance, since in the latter case, urine peptidome analysis might reflect response to treatment. Future studies analysing the effect of pharmaceutical interventions, such as tolvaptan, on the biomarker pattern will be required to address this question.

In addition to serving prognostic purposes, the identified peptide panel provides mechanistic insights into molecular pathways of disease progression. We identified the sequences of 80% of the prognostic peptides. These peptides are endogenous proteolytic cleavage products of larger proteins. Their excretion in urine is therefore not only dependent on expression of the parental proteins but also—or even primarily—on the activity of the proteases generating them. The parental proteins antithrombin III, fibrinogen alpha chain, alpha-1 antitrypsin and apolipoprotein A1 of five peptides among the biomarker panel that we

<table>
<thead>
<tr>
<th>Protease</th>
<th>Number of cleavage sites</th>
<th>Activity in ESRD/controls (mean ± SEM)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin D</td>
<td>2</td>
<td>4.5 ± 1.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Cathepsin E</td>
<td>2</td>
<td>4.9 ± 1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Cathepsin L</td>
<td>2</td>
<td>−2.7 ± 0.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Meprin A</td>
<td>2</td>
<td>4.3 ± 1.1</td>
<td>0.0004</td>
</tr>
<tr>
<td>Mmp2</td>
<td>2</td>
<td>3.9 ± 1.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mmp3</td>
<td>2</td>
<td>3.1 ± 0.8</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mmp8</td>
<td>2</td>
<td>3.5 ± 1.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Mmp9</td>
<td>3</td>
<td>−1.8 ± 0.8</td>
<td>0.036</td>
</tr>
<tr>
<td>Pepsin A</td>
<td>2</td>
<td>4.9 ± 1.3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 5. The proteases involved in generation of the 20 peptide fragments, number of cleavage sites associated with each protease (i.e. number of N- or C-termini of the 20 peptides that likely arise from cleavage by the respective protease) and the estimated fold difference of activity of each protease in patients progressing to ESRD versus controls (with mean ± SEM and adjusted P-value for the comparison)

**FIGURE 6:** Network of the molecular pathways associated with ADPKD progression. Each pathway is represented as an individual large octagonal node. Small octagonal nodes represent the predicted proteases that generated the identified risk-associated biomarkers; triangles denote the biomarkers. The edges (links) between pathways indicate an approximation of biological interaction between pathways based on the cross-pathway feature overlap. Given are identified peptides and proteases. AGRN, agrin; ALB, serum albumin; APOA1, apolipoprotein A-I; COL1A1, collagen alpha-1(I) chain; COL3A1, collagen alpha-1(III) chain; FGA, fibrinogen alpha chain; OGN, mimecan; SERPINA1, alpha-1-antitrypsin; SERPING1, antithrombin-III; SERPING1, plasma protease C1 inhibitor; TTR, transthyretin; TSD, cathepsin D; CTSE, cathepsin E; CTSL1, cathepsin L; MEP1A, meprin A subunit alpha; MMP, matrix metalloproteinase; PGA, pepsin A.
describe here have been previously identified in cystic fluid from ADPKD patients [31], suggesting that these peptide fragments directly derive from cystic kidney tissue. Using a bioinformatic approach, we identified several proteases likely responsible for generation of the urinary peptides in the peptidomic panel that associate with ADPKD disease progression. Elevated activity of matrix metalloproteases has been previously demonstrated in ADPKD and may play a role in the extracellular matrix turnover during cyst expansion [32–34]. In addition, we found a shift in cathepsin activity from cathepsin L (the activity of which was reduced) to cathepsin D and E (which showed increased activity). Decreased activity of cathepsin L has been demonstrated in human ADPKD cells and murine Pkd null kidneys and might promote cyst growth through reduced proteolytic processing of Cux1, a homeobox gene that represses the cyclin kinase inhibitors p21 and p27 [35]. The other two proteases that were upregulated, pepsin A and meprin A, have not yet been implicated in ADPKD and warrant further investigations.

Our study has several limitations. First, although it was based on the largest observational cohort of ADPKD patients with phenotypic MR imaging, GFR measurements, and a long follow-up duration, the number of patients is still small for a proteomic analysis with a need for rigorous adjustment for multiple testing. Second, even longer follow-up duration would be desirable. Reanalysis of the data set several years from now with a larger number of patients reaching ESRD, allowing for an analysis of time to ESRD, will thus be valuable. Third, although we used an independent development subcohort and performed several internal validations of the model, the study lacks an external validation.

In summary, we have identified a urinary peptidome signature that allows stratification of ADPKD patients according to their risk to develop ESRD within the next 10–13 years. Future studies will address further validation of these biomarkers and evaluate their response to treatment.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://ndt.oxfordjournals.org.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST STATEMENT**

H.M. is founder and co-owner of Mosaiques Diagnostics, who developed CE-MS technology. M.P., J.S., J.M. and M.D. are employees of Mosaiques Diagnostics.

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Estimating glomerular filtration rate for the full age spectrum from serum creatinine and cystatin C

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Background. We recently published and validated the new serum creatinine (Scr)-based full-age-spectrum equation (FAScrea) for estimating the glomerular filtration rate (GFR) for healthy and kidney–diseased subjects of all ages. The equation was based on the concept of normalized Scr and shows equivalent to superior prediction performance to the currently recommended equations for children, adolescents, adults and older adults.