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Original Article

ORIGINAL ARTICLE

Urinary peptide signature distinguishes autosomal recessive polycystic kidney disease from other causes of chronic kidney disease

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ABSTRACT

Background. The diagnosis of autosomal recessive polycystic kidney disease (ARPKD) can be hampered by its pronounced phenotypic variability and ARPKD-mimicking phenocopies. Here, for the first time we specifically studied the urinary peptidome of patients with ARPKD with the aim of distinguishing ARPKD from other causes of chronic kidney disease (CKD).

Methods. Fifty-eight urine samples from patients with ARPKD, 662 urine samples from paediatric patients with CKD with various other CKD aetiologies and 45 samples from healthy children were included. The urinary peptidome was analysed by capillary electrophoresis/mass spectrometry.

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Results. A 77-peptide signature specific for ARPKD was identified. Application of this signature in a matched random validation set of 19 samples of patients with ARPKD, 23 samples from patients with other CKD and 21 samples from healthy individuals led to a sensitivity of 84.2% [95% confidence interval (CI) 60.4–96.6], a specificity of 100% (95% CI 92.0–100%) and an area under the receiver operating characteristics curve (AUC) of 0.994 (95% CI 0.93–1.00). The 77-peptide signature displayed a specificity of 76.1% (95% CI 72.4–79.5) and an AUC of 0.88 (95% CI 0.85–0.90) in 591 samples from non-matched children with various CKD aetiologies. The signature was primarily (83%) composed of collagen fragments indicating structural damage. Of the remaining peptides, five originated from proteins known to bind to calcium potentially linking the current work to defaults in calcium signalling in polycystic disease.
Conclusions. We determined a urinary peptide signature that identifies paediatric patients with ARPKD with high precision among a population of children with CKD. Knowledge of the identity of the underlying peptides offers a novel starting point for discussion of possible pathophysiological processes involved in ARPKD.

Keywords: ARPKD, chronic kidney disease, peptidome, polycystic kidney disease, urinalysis

KEY LEARNING POINTS

What was known:

- Molecular genetic testing including *PKHD1* is the current gold standard to confirm the diagnosis of autosomal recessive polycystic kidney disease (ARPKD), but testing is not available in all settings and circumstances.
- Urinary peptides have been used in the identification of different aetiologies of chronic kidney disease (CKD).

The study adds:

- This study represents the first specific analysis of urinary peptides of patients with ARPKD.
- It identifies with high sensitivity and specificity patients with ARPKD among patients with CKD.
- It informs non-invasively on the potential pathophysiology of ARPKD.

Potential impact:

- Such a signature could be of great assistance in distinguishing between ARPKD-mimicking phenocopies and true ARPKD patients.
- The knowledge of the identity of the underlying peptides offers a novel starting point for discussion of possible pathophysiological processes involved in ARPKD.

INTRODUCTION

Autosomal recessive polycystic kidney disease (ARPKD) is one of the leading indications for kidney replacement therapy and/or combined liver and kidney transplantation in childhood and adolescence. ARPKD is mainly caused by biallelic variants in the *PKHD1* gene encoding the cilia-associated protein fibrocystin (FC) [1, 2]. The cellular function of FC is incompletely understood [2, 3]. An ARPKD phenotype has also been described in patients with biallelic variants in *DZIP1L* [4, 5] *CYS1* [6] or biallelic hypomorphic variants in *PKD1* [7–10]. The diagnosis of ARPKD can be hampered by the pronounced phenotypic variability of ARPKD and by ARPKD-mimicking phenocopies, including cystic disorders such as (very) early onset autosomal dominant polycystic kidney disease [(V)EO-ADPKD], *HNF1B* nephropathy and nephronophthisis. The clinical diagnosis can be challenging, especially very early in life and/or when no extrarenal symptoms are present. However, correct diagnosis of ARPKD is essential as it can affect management with respect to extrarenal diagnostic workup and therapy as well as counselling of families. Extrarenal manifestations in ARPKD include obligate hepatic involvement in the form of congenital hepatic fibrosis. Molecular genetic testing, e.g. using a panel strategy covering all genes causing an ARPKD-like phenotype including *PKHD1*, is desired to confirm the diagnosis of ARPKD but testing is not available in all settings and circumstances.

Urinary markers identified by omics-based strategies can capture the heterogeneity and complexity of diseases. In particular, analysis of the urinary low molecular weight proteome (the peptidome) has emerged as an attractive area in the identification of biomarkers for characterizing chronic kidney disease (CKD) and its progression [11, 12]. Recent studies also suggest that urinary peptides could inform the underlying aetiology of CKD. For example a urinary peptide signature was developed that differentiated between primary and secondary focal segmental glomerular sclerosis [13]. Additional urinary peptide signatures allowed differential diagnosis of patients with diabetic kidney disease, immunoglobulin A nephropathy, vasculitis and healthy controls with a 70% overall accuracy [14]. Finally, urinary peptidome analysis was used to distinguish renal cysts and diabetes (RCAD) syndrome from autosomal dominant polycystic kidney disease, congenital nephrotic syndrome and CKD [15]. From a practical clinical point of view peptide signatures have been shown to be very stable even after repetitive freeze–thaw cycles [16]. Thus urine samples from a peripheral setting could potentially be sent to centralized institutions for urinary peptidome analysis.

These studies suggest that urinary peptide signatures might also help to identify the underlying aetiology of CKD in patients with an ARPKD phenotype. Therefore, the aim of the current study was to evaluate the possibility of distinguishing ARPKD from other causes of CKD using urinary peptidome analysis.

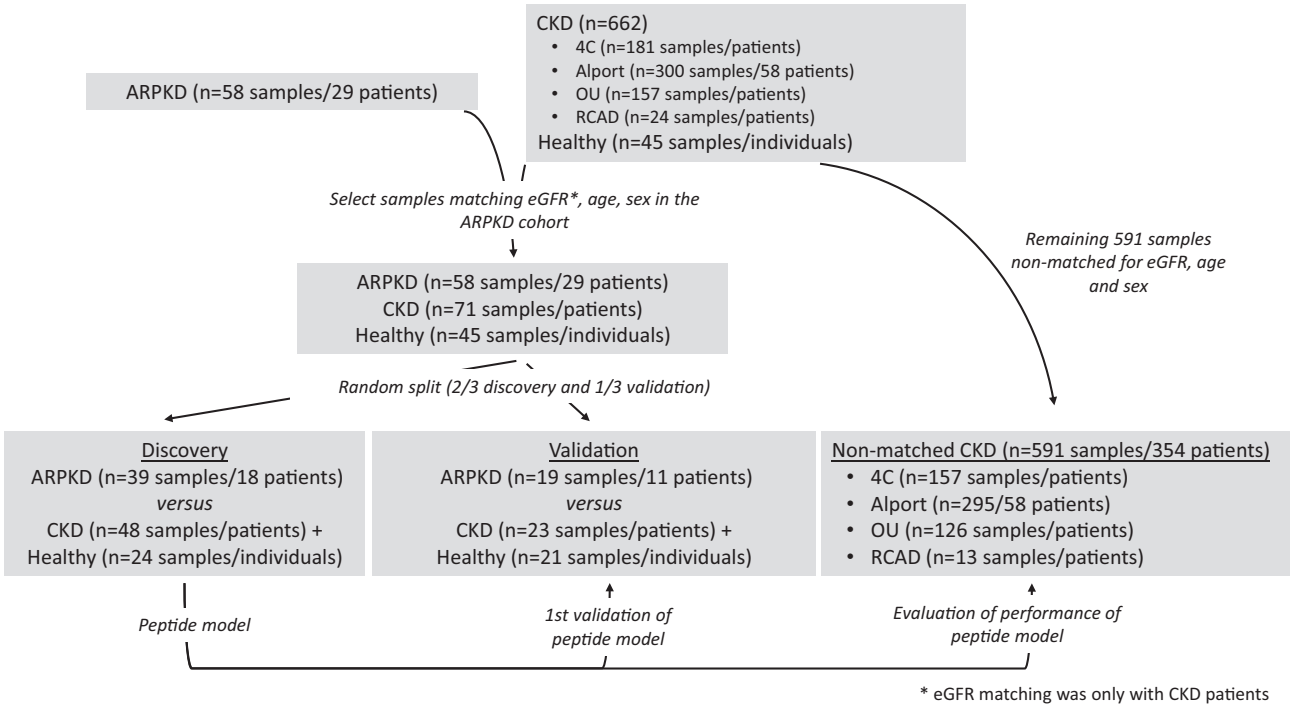

Figure 1: Flow chart of the study design and inclusion of samples. Samples from individuals with ARPKD were matched to CKD samples deriving from different cohorts according to eGFR, age and sex. After random split, 2/3 of the matched samples were used to establish a peptide signature that was afterwards tested in the validation cohort (1/3 of matched samples). In a second approach, the performance of the peptide signature was evaluated in the remaining non-matched CKD sample cohort. For ARPKD and Alport syndrome patients, a number of patients contributed with multiple urine samples with ≥6 months between sampling as indicated by '$n = x$ samples/y patients'. For all other individuals one sample corresponds to one patient as indicated by '$n = x$ samples/patients' or '$n = x$ samples/individuals'.

MATERIALS AND METHODS

Patients

Fifty-eight urine samples from 29 patients from the international ARPKD registry study ARegPKD were included (Fig. 1) [17]. The study protocol including patient information and consent forms was reviewed and approved by the Ethics Commission of the Faculty of Medicine of Cologne University. Patients had the clinical diagnosis of ARPKD and carried at least one relevant [according to the American College of Medical Genetics and Genomics (ACMG) ≥3] [18] *PKHD1* variant. A total of 662 urine samples from patients with CKD other than ARPKD were also included (Fig. 1): 181 samples obtained from 181 paediatric patients were part of the Cardiovascular Comorbidity in Children with Chronic Kidney Disease (4C) Study [19] (NCT0104644832), 300 samples obtained from 58 children with Alport syndrome were from participants of the Early Protect trial [20] (NCT01485978), 157 samples from 157 paediatric patients with obstructive uropathy (OU) from the Chronic Kidney Disease in Children (CKiD) Study [21] (NCT01485978), anonymized urinary peptidome data from 24 children with RCAD syndrome and 45 healthy children from the Human Urinary Proteome Database [22] (Fig. 1). For individuals with ARPKD estimated glomerular filtration rate (eGFR) was based on the full age spectrum formula [23]. For participants of the 4C Study, the Early Protect trial and children with RCAD, eGFR was determined using the Schwartz formula [24]. For individuals in the CKiD Study eGFR was based on the U25 equation [25]. Basic characteristics of the study population were described by mean and standard deviation (SD) for continuous variables and by absolute and relative frequencies for categorical variables (Table 1).

Sample preparation and capillary electrophoresis–mass spectrometry (CE-MS) analysis

Urine aliquots of 750 μl were thawed and diluted with the same volume of 2 M urea and 10 mM ammonium hydroxide containing 0.2% sodium dodecyl sulphate. To eliminate high molecular weight compounds, the sample was ultrafiltered with a Centristat 20-kD cutoff centrifugal filter device (Sartorius Göttingen, Germany) at 2000 *g* for 60 min at 4°C. Subsequently, the filtrate was applied onto a PD-10 desalting column (GE Healthcare Chicago, IL, USA) to remove urea, electrolytes and salts and thereby to decrease matrix effects. Finally, all samples were lyophilized in a Savant SpeedVac SVC100H connected to a VirTis 3 L Sentry freeze dryer (Thermo Fisher Scientific Waltham, MA, USA) and stored at 4°C until use. For CE-MS analysis, samples were resuspended in high-performance liquid chromatography–grade water to a final protein concentration of 1 µg/µl. CE-MS analysis was performed on a PrinCE Next 840 capillary electrophoresis system (Prince Technologies Emmen, The Netherlands) or P/ACE MDQ CE electrophoresis system (Beckman Coulter Brea, CA, USA) coupled on-line to a micrOTOFII MS (Bruker Daltonics, Billerica, MA, USA) as previously described [26]. The CE was coupled to the MS using a grounded electro-ionization sprayer (Agilent Technologies Santa Clara, CA, USA) with an ion spray interface potential between −4 and −4.5 kV. Data acquisition and MS acquisition methods were

Table 1: Basic characteristics of individuals with ARPKD or CKD and healthy individuals providing urinary samples in the discovery and validation cohorts. Samples from individuals with ARPKD were matched by eGFR, age and sex with samples from patients with various CKD aetiologies and by age and sex with samples from healthy children.

	Discovery			Validation		
Characteristics	ARPKD ($n = 39$)[a]	CKD[c] ($n = 48$)	Healthy ($n = 24$)	ARPKD ($n = 19$)[b]	CKD[d] ($n = 23$)	Healthy ($n = 21$)
Age, years, mean (SD)	10.2 (2.4)	10.4 (5.0)	11.0 (4.9)	7.5 (4.1)	9.4 (4.4)	9.5 (4.5)
Female, %	48.7	48.0	44.0	40.0	43.0	33.3
eGFR (ml/min/1.73 m^2), mean (SD)	73.0 (34.7)	62.9 (32.0)	n.a.	58.2 (31.6)	59.6 (26.1)	n.a.

n.a.: not applicable.
No significant differences were seen between age, eGFR and sex between the discovery and validation cohorts (one-way ANOVA for age and eGFR, Fischer's exact for sex).
[a]39 samples/29 patients.
[b]19 samples/11 patients.
[c]OU ($n = 19$), CAKUT ($n = 16$), RCAD ($n = 8$), Alport ($n = 2$), glomerulopathy ($n = 1$), tubulointerstitial disease ($n = 1$), nephrosclerosis ($n = 1$).
[d]Posterior urethral valves ($n = 12$), CAKUT ($n = 3$), Alport ($n = 3$), RCAD ($n = 3$), tubulointerstitial disease ($n = 2$).

automatically controlled by the CE via contact closure relays. Spectra were accumulated every 3 s, over a mass:charge (m:z) ratio range of 350–3000.

Data processing

CE-MS data assessment was performed using the MosaicFinder software [22]. The mass spectral ion peaks representing identical molecules at different charge states were deconvoluted into single masses. Only signals with $z > 1$ observed in a minimum of three consecutive spectra with a signal:noise ratio ≥ 4 were considered. The resulting peak list characterizes each polypeptide by its mass and migration time. Data were calibrated utilizing 3151 internal standards as reference data points for mass and migration time by applying global and local linear regression, respectively. To correct for variability, a linear regression algorithm was applied for normalization, using internal standard peptides as a reference. Urine contains a number of highly abundant endogenous collagen fragments that function as 'housekeeping' peptides [27]. These peptides are present in at least 75% of all urine samples and exhibit relatively low variability. Normalization to those multiple collagen fragments achieves a performance comparable to 24-h urinary excretion and provides a better compensation for marker level variations in single-voided urine samples compared with creatinine-based normalization [27]. Technical aspects have been described in more detail previously [28, 29]. The obtained peak list characterizes each polypeptide by its calibrated molecular mass (Da), calibrated CE migration time (min) and normalized signal intensity.

Statistics

Statistical analysis was performed using GraphPad Prism version 10.3.1 (GraphPad Software, Boston, MA, USA). Differences in patient characteristics between the discovery and validation cohorts were evaluated by one-way analysis of variance (ANOVA) for age and eGFR and with the Fischer's exact test for sex. To identify specific peptide biomarkers associated with ARPKD, only peptides present in $\geq 70\%$ of the urine samples in one of the groups examined were considered. Peptide data were arcsine transformed before analysis. Peptides were considered significantly different between groups with a P-value $< .05$ (Wilcoxon test followed by Benjamini–Hochberg-based false discovery rate correction). Differences between peptide signature scores were analysed by one-way ANOVA with a Tukey post hoc test with a P-value $< .05$ considered significant. Diagnostic performances were assessed by calculating the area under the receiver operating characteristics curve (AUC) and 95% confidence interval (CI; DeLong method). Sensitivities and specificities were calculated taking the threshold identified by the Youden index in the discovery cohort.

RESULTS

Characteristics of the study population and study setup

A total of 765 urine samples were included in the study (Fig. 1). In the first step, 58 samples from 29 individuals with ARPKD were matched by eGFR, age and sex with samples from patients with various CKD aetiologies other than ARPKD and by age and sex with samples from healthy children. The CKD cohort of the discovery and validation cohorts encompassed 71 patients with obstructive uropathy (OU; $n = 31$), congenital anomalies of the kidney and urinary tract systems (CAKUT; $n = 19$), RCAD syndrome ($n = 11$), Alport syndrome ($n = 5$), tubulointerstitial disease ($n = 3$), nephrosclerosis ($n = 1$) and glomerulopathy ($n = 1$). This resulting set of samples from individuals with ARPKD or CKD and from healthy children was split randomly in discovery (2/3; $n = 111$) and validation (1/3; $n = 63$) cohorts (Table 1). The discovery cohort was used for the definition of ARPKD-specific peptides and to generate a peptide signature that was, in the next step, tested in the validation cohort. Subsequently the predictive value of the peptide signature was evaluated in the non-matched remaining samples from patients with CKD ($n = 591$).

Identification of a specific ARPKD signature

With the aim of identifying a specific ARPKD signature, we compared the urinary peptidome of 39 samples from 18 patients with ARPKD with 48 eGFR-, age- and sex-matched samples from other CKD patients and with 24 samples from age- and sex-matched healthy children (discovery cohort; Table 1). The statistical analysis of the urinary peptidome led to the identification of 904, 819 and 1178 peptides with significantly different abundance ($P < .05$, after Benjamini–Hochberg-based false discovery rate correction) in the comparisons ARPKD/healthy, ARPKD/CKD and healthy/CKD, respectively. In order to obtain an ARPKD-specific signature, we then selected the 77 peptides overlapping (and

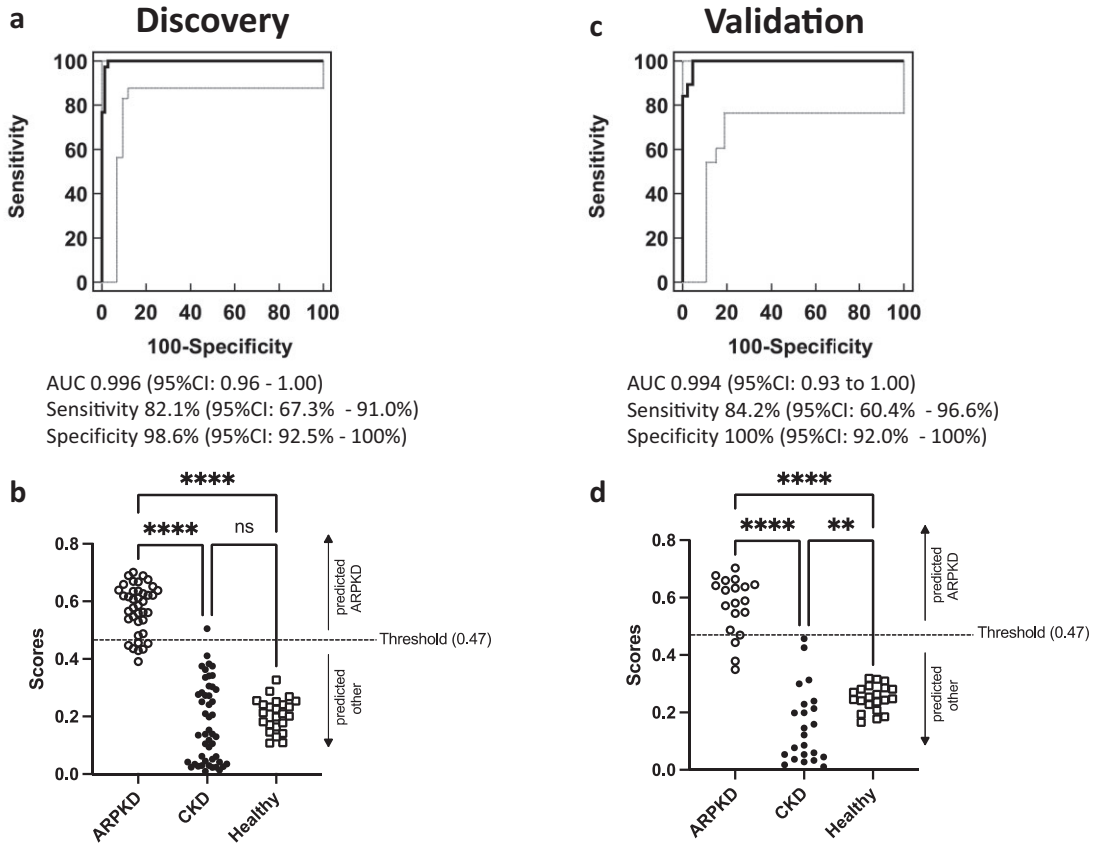

Figure 2: Discovery and blinded validation of the urinary 77-peptide signature in separate ARPKD sample populations together with healthy controls and individuals suffering from other CKDs. Receiver operating characteristics curves for the 77-peptide signature based on all samples in the **(a)** discovery and **(c)** validation cohorts. Dot plots for the classification of all patient cohorts (ARPKD samples, other CKD samples, healthy samples) of the **(b)** discovery and **(d)** validation cohort according to the 77-peptide signature. **P < .01; ****P < .0001; ns: non-significant.

with an identical direction of alteration) in the ARPKD/healthy and ARPKD/CKD comparisons but that did not overlap with the healthy/CKD comparison (Supplementary Fig. S1). These peptides were included in a random forest–based diagnostic signature. This signature distinguished samples of patients with ARPKD from samples of other children (CKD or healthy) with an AUC of 0.996 (95% CI 0.96–1.00) (Fig. 2a). The achieved scores were significantly higher in the ARPKD cohort compared with both the CKD and healthy cohorts (Fig. 2b). On the basis of a peptide signature threshold score of 0.47 (defined by the Youden index) this signature identified with a sensitivity of 82.1% (95% CI 67.3–91.0) and a specificity of 98.6% (95% CI 92.5–100) samples of patients with ARPKD in this discovery cohort (Fig. 2a and b). The 77-peptide signature was for the major part [64/77 (83%)] composed of different collagen fragments (26 with reduced and 38 with increased abundance in ARPKD) indicating structural damage. Of the other 13 peptides, 5 originate from proteins known to bind to calcium, including fibrinogen alpha chain, hornerin, peflin, protocadherin-7 and 36-kDa vesicular integral membrane protein (VIP36) (Table 2).

Validation of the 77-peptide signature

Next this 77-peptide signature was applied in an eGFR-, sex- and age-matched random holdout set of 19 samples from 11 patients with ARPKD, 23 samples of patients with CKD other than ARPKD and 21 samples from healthy individuals. Again, the peptide signature scores were significantly higher in the ARPKD cohort compared with both the other CKD aetiology and healthy cohorts using the 0.47 threshold previously defined in the discovery cohort (Fig. 2d). The signature identified samples from ARPKD patients with a sensitivity of 84.2% (95% CI 60.4–96.6), a specificity of 100% (95% CI 92.0–100) and an AUC of 0.994 (95% CI 0.93–1.00) (Fig. 2c and d).

For patients with ARPKD who had multiple urine samples available (at least three), the coefficient of variation of the 77-peptide signature scores was 3.3–15.7% over a maximum time span of 6 years. The 77-peptide signature scores in patients with ARPKD correlated with eGFR ($r = -0.5205$, $P < .0001$; Supplementary Fig. S2) and height-adjusted total kidney volume (haTKV) at the time of sampling ($r = 0.7090$, $P = .0002$; Supplementary Fig. S3).

Evaluation of the 77-peptide signature in non-matched children with CKD

We next evaluated the capacity of the 77-peptide signature to score the remaining 591 samples from children with various CKD aetiologies. These patients were non-eGFR, -age and -sex matched with the discovery and validation cohorts (Fig. 1, Table 3). The 77-peptide signature displayed a specificity of 76.1% (95% CI 72.4–79.5) and was associated with an AUC of 0.88 (95% CI

Table 2: Thirteen peptides identified in the 77-peptide signature that do not assign to collagen fragments derived from the comparison between ARPKD and CKD and healthy samples.

Protein name	Protein fragments *n*	Uniprot accession number	Molecular function[a]	Biological process[a]	Ligand[a]	Regulation in ARPKD
Beta-2 microglobulin	1	P61769		Immunity		Up
Complement factor B	1	P00751	Hydrolase protease serine protease	Complement alternate pathway Immunity Innate immunity		Up
Fibrinogen alpha chain	1	P02671		Adaptive immunity blood coagulation haemostasis immunity innate immunity	Calcium metal binding	Up
Haemoglobin subunit alpha	1	P69905		Oxygen transport transport	Haem iron metal binding	Down
Hornerin	1	Q86YZ3	Developmental protein	Keratinization	Calcium metal binding	Up
Myristoylated alanine-rich C-kinase substrate	1	P29966	Actin binding calmodulin binding			Up
Neurosecretory protein VGF	1	O15240	Antibiotic antimicrobial growth factor			Up
Peflin	1	Q9UBV8			Calcium metal binding	Down
Polymeric immunoglobulin receptor	2	P01833				Up
Protocadherin-7	1	O60245		Cell adhesion	Calcium	Down
Retinol-binding protein 4	1	P02753		Sensory transduction transport vision	Retinol-binding vitamin A	Up
VIP36	1	Q12907		Protein transport transport	Calcium lectin metal binding	Down

[a]Annotation based on Uniprot keywords (https://www.uniprot.org/). An empty box means the absence of a keyword for that category.

Table 3: Basic characteristics of samples from the non-matched CKD population.

Characteristics	All non-matched CKD population ($n = 591$)[a]	4C ($n = 157$)	Alport ($n = 295$)[b]	OU ($n = 126$)	RCAD ($n = 13$)
Age (years), mean (SD)	10.2 (4.5)	11.9 (3.2)	10.7 (4.9)	8.4 (4.6)	5.1 (3.5)
Female, %	15.6	28.2	13.5	4.8	0
eGFR (ml/min/1.73 m^2), mean (SD)	50.1 (34.3)	28.2 (11.1)	109 (25.3)	48.2 (18.7)	111 (24.4)

[a]$n = 591$ samples/354 patients.
[b]$n = 295$ samples/58 patients.

0.85–0.90) for this mix of CKD aetiologies (Fig. 3a and b). Patients with Alport syndrome displayed the lowest specificity [68.1% (95% CI 62.5–73.4) ($n = 295$) when studying the CKD aetiologies separately.

With respect to CKD aetiologies with phenotypes similar to ARPKD, interestingly, the highest specificity [100% (95% CI 75.3–100.0) was observed for patients with RCAD syndrome ($n = 13$; Fig. 3a and b). Additionally, within the 4C cohort ($n = 157$), all four patients flagged with 'renal dysplasia with cysts' tested negative for ARPKD. Similarly, 13 of 16 patients classified under 'nephronophthisis' also tested negative for ARPKD.

DISCUSSION

In a cohort of 29 individuals with the clinical diagnosis of ARPKD and at least one relevant (ACMG ≥3) *PKHD1* variant deriving

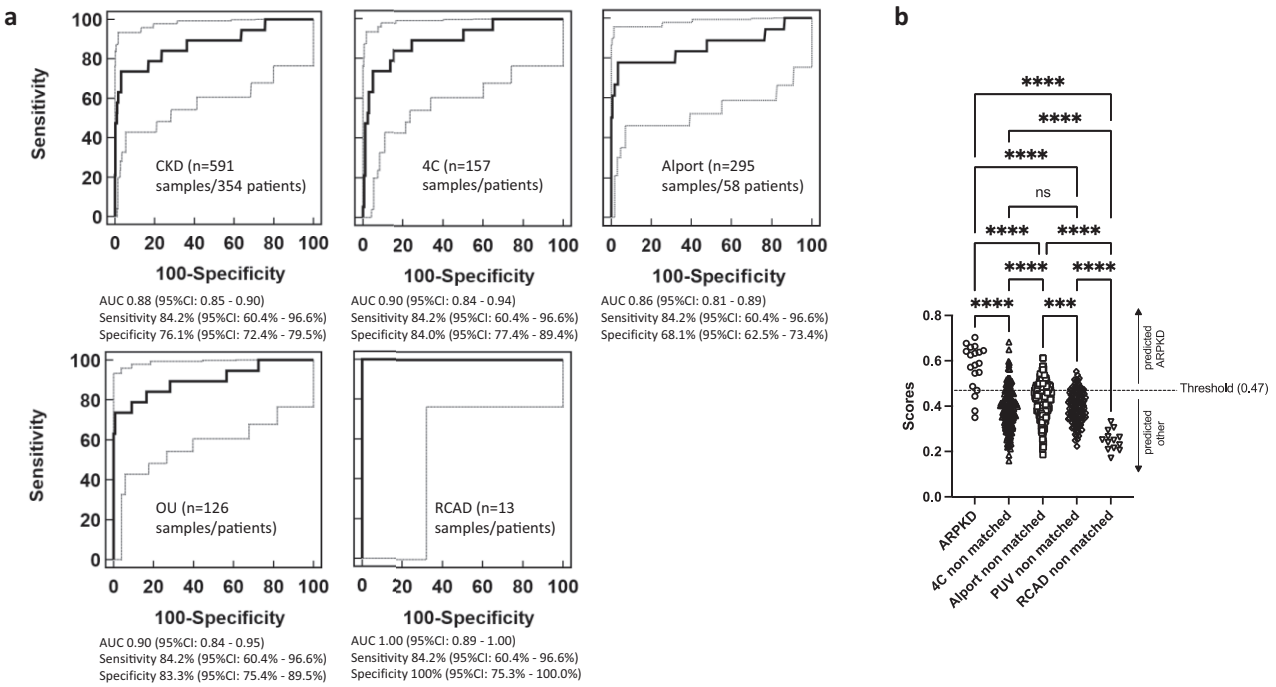

Figure 3: Application of the urinary 77-peptide signature in various CKD subcohorts that were not matched with the ARPKD samples. **(a)** Receiver operating characteristics curves for the 77-peptide signature based on samples in the non-matched cohorts of all remaining CKD samples as well as 4C, Alport, OU and RCAD subcohorts. **(b)** Dot plots of scores for the classification of selected non-matched cohorts (4C, Alport, OU, RCAD non-matched) compared with the ARPKD samples. ***$P < .001$; ****$P < .0001$; ns: non-significant. For the CKD and Alport syndrome patients, a number of patients contributed with multiple urine samples with ≥6 months between sampling as indicated by '$n = x$ samples/y patients'. For all other individuals one sample corresponds to one patient as indicated by '$n = x$ samples/patients'.

from the international ARPKD registry study ARegPKD, we were able to determine a signature containing 77 peptides that allows us to identify with paediatric patients with ARPKD high precision among a population of children with CKD. Such a signature could be of great help to distinguish between ARPKD-mimicking phenocopies and true ARPKD patients. Indeed, patients with *HNF1B* variants are known to be possible ARPKD phenocopies [8]. In the RCAD cohort—which are patients with *HNF1B* variants—the majority of the patients displayed cystic or hyperechogenic kidneys. For the latter we have recently shown that hyperechogenic kidneys correspond to microcysts [30]. Interestingly, all patients with RCAD syndrome were clearly distinguished from patients with ARPKD using the peptide signature. Similarly, within the 4C cohort [19] all patients with 'renal dysplasia with cysts' scored negative for ARPKD, while 13 of the 16 patients labelled 'nephronophthisis' also scored negative for ARPKD. Overall, these data suggest that the 77-peptide signature could identify ARPKD-mimicking phenocopies.

As shown in previous studies in ADPKD and RCAD syndrome [15, 31], the majority of identified peptides derived from collagen reflecting structural damage. Interestingly, of the non-collagen-derived peptides, five of the parental proteins are known to bind to calcium (Table 2). Little is known about the cellular function of FC. Two studies have linked FC to regulation of calcium in ARPKD [32, 33] and a link to the ADPKD protein polycystin-2 (PC2) was suggested [33]. Imbalance between functional PC1 and PC2 proteins in the polycystin complex in ARPKD might disrupt calcium channel activities at various subcellular locations including the cilium, the plasma membrane and the endoplasmic reticulum (ER), thereby altering intracellular calcium signalling and leading to the aberrant cell proliferation and apoptosis associated with the development and growth of renal cysts [34]. Our findings might reflect altered calcium signalling specific for ARPKD versus other CKD aetiologies. Two of the parental proteins, peflin [35] and VIP36 [36]—known to bind to calcium—are involved in cargo transport from the ER. Peflin is a protein that binds in a calcium-dependent manner to other proteins involved in ER export of endogenous collagen I and peflin depletion increases collagen I transport [35] and thus might be involved in the observed remodelling of the extracellular matrix in ARPKD. Recent work in the PKD field has also highlighted the role of signalling at the dynamic physical interaction of mitochondria and the ER, mitochondria-associated membranes [37] that are integral structures of intracellular calcium transport and intracellular calcium homeostasis [38]. More functional work will be required to understand the link between FC and calcium signalling.

From a clinical point of view the proposed peptide signature could reliably identify individuals with ARPKD related to at least one relevant *PKHD1* variant. Peptidomic analysis of urine is robust [16] and pre-analytic workup is relatively easy, thus potentially making the testing available for patients in regions with limited access to or availability of genetic analysis.

The current study has several strengths. This is the first time that a non-genetic test makes it possible to identify patients with ARPKD. It is also the first specific urinary peptidome in individuals with the clinical diagnosis of ARPKD and at least one relevant *PKHD1* variant. The knowledge of the peptides specifically associated with ARPKD offers unique starting points for discussion of possible pathophysiologic processes in the light of previously characterized urinary proteomes in other cystic and

non-cystic kidney diseases [13–15, 39]. Furthermore, the potential of urinary peptidome analysis as a tool in prediction and understanding the mechanisms of progression to kidney failure has been elucidated recently [40].

Limitations encompass variable numbers and time points of urine sampling in different patients. As multiple samples from the same patients (16 of the 29 individuals with ARPKD had multiple samples) were included in the analyses, validation cannot be considered as fully independent. The study setup was conducted in a retrospective manner, with post hoc inclusion and comparison of various control cohorts from previous studies. Furthermore, information on albuminuria/proteinuria was sparse in some of the examined individuals and samples of individuals with *DZIP1L*-dependent ARPKD were not available for analysis. Finally, the available follow-up data were insufficient to assess the potential prognostic value of the 77-peptide signature in relation to kidney survival. However, we observed a strong correlation between the signature and haTKV. This points to a potential prognostic relevance of the peptide signature, but clearly further investigations in additional cohorts and with longer follow-up are required to confirm this potential association.

In conclusion, a urinary peptide signature was identified that can be used to select paediatric patients with ARPKD with high precision among a population of children with CKD. Such a signature could be of great assistance in distinguishing between ARPKD-mimicking phenocopies and true ARPKD patients. In addition, knowledge of the identity of the underlying peptides offers a unique starting point for discussion of possible novel pathophysiological processes involved in ARPKD.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online

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DATA AVAILABILITY STATEMENT

Data of this study is available upon reasonable request.

CONFLICT OF INTEREST STATEMENT

JS is an employee of Mosaiques Diagnostics.

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