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Original Paper

Urinary Level of Liver-Type Fatty Acid Binding Protein Reflects the Degree of Tubulointerstitial Damage in Polycystic Kidney Disease

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Key Words
Polycystic kidney disease • Urinary marker • Liver-type fatty acid binding protein • Tubulointerstitial damage

Abstract
Background/Aims: Polycystic kidney disease (PKD) is a common, progressive, and heritable type of kidney disease. Although certain imaging modalities are useful for the diagnosis and staging of PKD, they cannot adequately monitor the severity of interstitial inflammation and fibrosis. Therefore, the present study evaluated the urinary level of liver-type fatty acid binding protein (L-FABP) as a marker of interstitial inflammation and fibrosis in PKD. ***Methods:*** Male PCK/CrljCrl-Pkhd1pck/Crl (PCK) rats (n = 34) were used as an animal model of the PKD. Age- and sex-matched Sprague–Dawley rats (SD) (n = 34) were used as controls. Urine samples were obtained from the rats at 8, 12, 16, 20, and 24 weeks of age, and the sera and kidney tissues were obtained at 8, 16, 20, and 24 weeks of age. ***Results:*** All PCK rats developed cysts, and the degrees of tubular epithelial cell proliferation and interstitial inflammation increased linearly with age in these model rats relative to the controls. Interstitial fibrosis tended to increase in the PCK rats from 8 to 20 weeks of age, and revealed a peak level at 20 weeks. The urinary L-FABP levels increased linearly with age in the PCK rats, and the levels at 12, 16, 20, and 24 weeks were significantly higher than those in the controls. The urinary levels of L-FABP in the PCK rats correlated significantly with the severity of tubulointerstitial damage; specifically, we observed a significant correlation of the urinary levels at 16 weeks of age with the total kidney volume at 20 weeks. In contrast, both PCK and SD rats exhibited similar serum levels of

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L-FABP. ***Conclusion:*** Urinary L-FABP reflects the progression of tubulointerstitial damage, and therefore, may be a useful marker for monitoring the progression of PKD.

Introduction

Polycystic kidney disease (PKD) is a common and progressive hereditary renal disease encountered frequently in clinical nephrology practice. Two forms of PKD—autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) —have been identified, and although these forms differ in terms of gene mutation, they share a common renal pathogenesis involving the formation of renal fluid-filled cysts linked to an abnormality in the primary cilia originating in the medullar collecting ducts [1, 2]. In both forms of PKD, renal failure is a consequence of the massive enlargement of these cysts [3-5].

Imaging modalities, such as ultrasonography, computed tomography, or magnetic resonance imaging (MRI), can detect the cyst number and volume as well as the kidney volume, and are thus useful for diagnosing and staging PKD [6]; however, it is not only costly and potentially harmful by radiation but also difficult to reproduce the parameters measured using these modalities because these values depend on the type of modalities or experience of the clinical technician [7]. Therefore, these values should be measured carefully to account for changes in the modalities used at a particular facility or when comparing the values in a multicenter clinical trial. Furthermore, the roles of renal interstitial inflammation and fibrosis as exacerbating factors of renal dysfunction in PKD have recently received attention, leading to the consideration of both phenomena as crucial targets for PKD therapy [7-9]; however, the imaging modalities cannot detect these interstitial changes. Therefore, a noninvasive and high reproducible alternative is needed.

The urinary level of liver-type fatty acid binding protein (L-FABP) was identified as an excellent tubular marker of chronic kidney disease (CKD) and acute kidney injury (AKI) in a clinical setting, and was subsequently approved as a tubular biomarker by the Ministry of Health, Labor and Welfare in Japan [10-12]. Furthermore, higher urinary L-FABP levels were reported in the patients with ADPKD, compared to healthy subjects [13]; however, the relationship between the urinary L-FABP levels and the renal pathology associated with PKD, or between the change in urinary L-FABP levels and PKD progression, has not previously been investigated. The present study aimed to elucidate these points in an experimental rat model of PKD.

Materials and Methods

Animals

All animal studies were conducted in accordance with the St. Marianna University School of Medicine Institutional Guide for Animal Experiments and the Guide for the Care and Use of Laboratory Animals. In this study, we used male PCK/CrljCrl-Pkhd1pck/Crl (PCK, n = 34) rats derived from a Sprague–Dawley (SD, n = 34) colony as a model of PKD. Four-week-old male PCK rats and SD rats (controls) were purchased from Charles River Japan and were allowed free access to laboratory chow and water.

For the experiments, 8-, 12-, 16-, 20-, and 24-week-old rats were individually housed overnight in metabolic cages with free access to tap water. Urine was collected for the measurement of urinary markers, and body weight was also measured. Additionally, serum samples and kidney tissues were obtained at 8-, 16-, 20-, and 24-week-old rats. The extracted kidneys were weighted and cut into pieces for various analyzes.

Blood pressure measurement

Systolic blood pressure (SBP) was measured using a tail-cuff apparatus (Softron BP-98A; Softron, Tokyo, Japan) at every 4 weeks from 8 to 24 weeks of age. The averages of three measurements per animal per time point were recorded.

Serum and urinary biochemistry

Serum and urinary creatinine levels were measured using a QuantichromTM creatinine Assay Kit (BioAssay Systems, Hayward, CA, USA). Albuminuria was determined using a NEPHRAT III ELISA kit (Exocell, Philadelphia, PA, USA). Serum and urinary levels of rat L-FABP were measured using a Rat L-FABP ELISA kit (CMIC, Tokyo, Japan). All urinary parameters were reported as ratios relative to the urinary creatinine levels. Serum urea nitrogen (UN) levels were measured via urease-LED-ultraviolet absorption spectrophotometry, and the serum aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were measured using the Japan Society of Clinical Chemistry transferable method provided as a clinical laboratory testing service by SRL (Tokyo, Japan).

To evaluate the renal function, creatinine clearance was measured using the following formula:

Creatinine clearance, ml/min =

[(concentration of urinary creatinine, mg/dL) × (urinary volume, ml)]

/ [(serum creatinine, mg/dL) × (time when rats were placed in the metabolic cage, min)]

Immunohistochemical Analysis

The midsection of each excised kidney was cut in the minor axial direction, fixed in 10% buffered formalin, and was embedded in paraffin. Serial sections (3-μm thickness) were then prepared for immunohistochemical analysis. An indirect immunoperoxidase method was used to detect the target antigens. Proliferating tubular epithelial cells were detected using a mouse monoclonal antibody specific for proliferating cell nuclear antigen (PCNA, 1:200; DAKO Japan, Tokyo, Japan) after the slides were microwave heated for 15 min in citric acid buffer (pH 6.0) for antigen retrieval. Additionally, myofibroblasts and tubular cells exhibiting the epithelial–mesenchymal shift were identified using a mouse monoclonal antibody specific for α-smooth muscle actin (α-SMA, 1:800; Sigma-Aldrich, St. Louis, MO, USA).

For the immunohistochemical assessment of macrophages and type I collagen, tissue specimens fixed in methyl Carnoy's solution (60% methanol, 30% chloroform and 10% glacial acetic acid) and embedded in paraffin were immunostained using a mouse monoclonal antibody specific for ED-1 (1:100; Abcam, Tokyo, Japan) to detect the rat macrophages and a goat polyclonal antibody against type I collagen (1:200; Southern Biotech, Birmingham, AL, USA). Labeled proteins were visualized using polymeric horseradish peroxidase-conjugated secondary antibodies (ready-to-use, ImmPRESS™ polymer detection kit; Vector Laboratories, Burlingame, CA, USA). Peroxidase activity was revealed via the diaminobenzidine reaction (Liquid DAB+; DAKO Japan), and sections were counterstained with hematoxylin.

Images from 10 nonoverlapping fields throughout the cortical and outer medullary region were captured at 100× magnification. The degrees of macrophage and interstitial myofibroblast infiltration in the cortical and outer medullary interstitia were automatically measured using an image analyzer (Auto/Manual Measurement Software version 6.4, WinRoof, Mitani Co., Tokyo, Japan). Briefly, the areas positively stained for PCNA, α-SMA, and ED-1 were separately measured and expressed as ratios relative to the area of the entire cortical and outer medullary region. The same method was used to measure the expression of type I collagen.

Real-time quantitative polymerase chain reaction

Total RNA was extracted from the kidney tissues using a RNeasy Midi kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, and a 0.5-μg aliquot was reverse-transcribed. TaqMan real-time polymerase chain reaction (PCR) with an Applied Biosystems StepOnePlusTM real-time PCR System (Applied Biosystems, Waltham, MA) was used to measure the mRNA levels of monocyte chemoattractant protein (MCP-1), transforming growth factor (TGF-β), α1-type I collagen, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). MCP-1 mRNA was measured to evaluate the inflammatory response, whereas TGF-β and α1-type I collagen mRNAs were measured to assess renal fibrosis. The expression levels of all mRNAs were normalized to the levels of GAPDH in all samples.

Total kidney volume (TKV)

TKV is widely considered as a surrogate marker of PKD severity. To evaluate the potential use of urinary parameters as a predictor of an increased TKV, the basic ellipsoid equation, which correlates strongly with the kidney volume measured by MRI, was used to estimate the TKV in 20- (n=12) and 24-week-old (n=12) rats as described below [14]:

TKV = (maximal longitudinal length × maximal width × maximal depth × π/6) of the left kidney + (maximal longitudinal length × maximal width × maximal depth × π/6) of the right kidney

The degrees of severe interstitial inflammation and fibrosis and increases in TKV were similar between the 20- and 24-week-old PCK rats in the present study, suggesting that interstitial damage might peak at 20-week-old rats in this model. Accordingly, the severity of kidney disease findings in the 20-week-old rats was considered almost comparable to that of the end-stage kidney disease in humans, although significant renal failure was not observed in the rats. Therefore, we evaluated correlations of the urinary parameter levels at 8, 12 and 16 weeks of age with TKV at 20 and 24 weeks of age in the PCK rats.

Statistical analysis

All values are expressed as means ± standard errors. A p value <0.05 was considered to indicate statistical significance. Multiple groups were compared using a one-way analysis of variance, followed by the Mann–Whitney U-test. Spearman's rank correlation coefficient was used to evaluate the nonparametric data and assess the correlations of the two parameters. All statistical analyzes were performed using the JMP® version 13.0.0 software (SAS Institute, Cary, NC, USA).

Results

Body weight and SBP

The body weights and SBP values were measured at every 4 weeks in rats aged between 8 and 24 weeks (Table 1). Among the PCK rats, 16-week-old rats revealed a significantly increased body weight, compared to the 8- and 12-week-old rats, whereas significant increases in the body weight were observed in the 20- and 24-week-old rats relative to earlier time points (Table 1). In contrast, the body weights of the SD rats increased gradually and significantly through the observation period (Table 1). Despite this difference, the body weights of PCK rats and SD rats did not differ significantly at any point during the observation period (Table 1). Similarly, although the SBP levels in the PCK rats were significantly higher at 16 weeks of age relative to 8 and 24 weeks, no significant differences were observed in this parameter between PCK and SD rats throughout the observation period (Table 1).

Kidney weight and renal function

In the SD rats, the kidney weights at 16, 20, and 24 weeks of age were significantly higher than those measured at 8 weeks of age. In contrast, the kidney weights of the PCK rats tended to increase throughout the observation period (Table 2), with significant increases at 16, 20, and 24 weeks relative to 8 weeks and at 20 and 24 weeks relative to 16 weeks (Table 2). Additionally, the kidney weights of the PCK rats were significantly higher than those in the age-matched SD rats at all time points (Table 2).

Table 1. Body weight and systolic blood pressure (SBP). Values are the mean ± standard errors. *P<0.05 vs PCK rats at 8 weeks of age; $^{\Delta}$P<0.05 vs PCK rats at 12 weeks of age; §P<0.05 vs PCK rats at 16 weeks of age; ¶P<0.05 vs SD rats at 8 weeks of age; $^{\Diamond}$P<0.05 vs SD rats at 12 weeks of age; $^{\#}$P<0.05 vs SD rats at 16 weeks of age; $^{\&}$<0.05 vs SD rats at 20 weeks of age

Variable	Species	8 weeks of age	12 weeks of age	16 weeks of age	20 weeks of age	24 weeks of age
SBP, mmHg	PCK	121 ± 3.6	130 ± 2.2	139 ± 3.2*	130 ± 3.7	122 ± 2.6§
	SD	124 ± 2.9	125 ± 3.5	132 ± 3.2	124 ± 3.5	118 ± 5.2
Body weight,	PCK	325 ± 9.5	400 ± 6.1	487 ± 7.5$^{*\Delta}$	543 ± 5.6$^{*\Delta\S}$	563 ± 7.2$^{*\Delta\S}$
	SD	300 ± 3.5	415 ± 11.0¶	476 ± 12.1$^{\P\Diamond}$	535 ± 10.5$^{\#\P\Diamond}$	593 ± 17.6$^{\P\Diamond\&\#}$

Table 2. Kidney weight and renal function. Values are the mean ± standard errors. CCr, creatinine clearance. $^{\alpha}$P<0.05 vs SD rats at the same age; *P<0.05 vs PCK rats at 8 weeks of age; §P<0.05 vs PCK rats at 16 weeks of age; $^{\beta}$P<0.05 vs PCK rats at 20 weeks of age; ¶P<0.05 vs SD rats at 8 weeks of age; $^{\#}$P<0.05 vs SD rats at 16 weeks of age

Variable / Biomarker	Species	8 weeks of age	16 weeks of age	20 weeks of age	24 weeks of age
Kidney weight, g	PCK	1.8 ± 0.04$^{\alpha}$	2.7 ± 0.10$^{\alpha*}$	3.6 ± 0.37$^{\alpha*\S}$	4.3 ± 0.41$^{\alpha*\S}$
	SD	1.2 ± 0.06	1.5 ± 0.04¶	1.5 ± 0.04¶	1.5 ± 0.06¶
Creatinine, mg/dL	PCK	0.41 ± 0.04	0.48 ± 0.04	0.46 ± 0.03	0.55 ± 0.02$^{*\beta}$
	SD	0.40 ± 0.03	0.46 ± 0.04	0.47 ± 0.02	0.52 ± 0.02
Urea nitrogen, mg/dL	PCK	17.7 ± 0.62	19.7 ± 0.36*	27.4 ± 4.20*§	30.6 ± 2.68$^{\alpha*\S}$
	SD	18.4 ± 0.73	19.2 ± 0.85	20.6 ± 0.75¶	22.1 ± 0.93$^{\P\#}$
CCr, ml/min	PCK	1.7 ± 0.18	2.1 ± 0.15	2.5 ± 0.20	2.3 ± 0.07
	SD	1.7 ± 0.22	3.0 ± 0.61	3.0 ± 0.14	2.4 ± 0.32

Table 3. Serum biochemistry. Values are the mean ± standard errors. $^{\alpha}$P<0.05 vs SD rats at the same age; *P<0.05 vs PCK rats at 8 weeks of age; $^{\beta}$P<0.05 vs PCK rats at 20 weeks of age; $^{+}$P<0.05 vs PCK rats at 24weeks of age; ¶P<0.05 vs SD rats at 8 weeks of age; $^{\#}$P<0.05 vs SD rats at 16 weeks of age

Biomarker	Species	8 weeks of age	16 weeks of age	20 weeks of age	24 weeks of age
L-FABP, ng/mL	PCK	91.3 ± 20.3	67.3 ± 7.9	48.7 ± 12.5	67.4 ± 21.2
	SD	72.7 ± 21.9	61.0 ± 18.4	39.7 ± 17.0	101.3 ± 22.5
AST, U/L	PCK	171.6 ± 19.3	220.1 ± 30.7$^{\alpha\beta}$	103.0 ± 9.0	183.0 ± 22.1$^{\beta}$
	SD	98.6 ± 8.7	100.6 ± 7.0	123.0 ± 10.5	144.8 ± 23.1
ALT, U/L	PCK	70.7 ± 4.7	96.3 ± 5.9$^{\alpha*\beta+}$	54.4 ± 1.9$^{\alpha*}$	72.7 ± 5.0$^{\beta}$
	SD	51.3 ± 2.1	56.6 ± 4.5	38.0 ± 1.7¶	56.2 ± 14.6

Considering the renal function, the PCK rats exhibited significant increases in the serum creatinine levels at 24 weeks, compared to those at 8 and 20 weeks (Table 2). No significant differences in this parameter were observed between the PCK and SD rats at any time point (Table 2). Furthermore, the PCK rats exhibited significant increases in the serum UN levels at each 16, 20, and 24 weeks relative to 8 weeks and at 20 and 24 weeks relative to 16 weeks (Table 2). In SD rats, the serum UN levels increased significantly at 20 and 24 weeks relative to 8 weeks and at 24 weeks relative to 16 weeks (Table 2). Notably, the serum UN levels were higher in the PCK rats than in the SD rats at 24 weeks of age (Table 2); however, no changes in the levels of creatinine clearance were observed in either the PCK or SD rats throughout the observation period (Table 2).

Serum biochemistry

In both the PCK and SD rats, the serum L-FABP levels did not change significantly throughout the observation period (Table 3), with no significant differences between the groups (Table 3); however, in the PCK groups, the serum AST levels were significantly higher at 8 and 16 weeks than at 20 weeks and at 24 weeks than at 20 weeks. By contrast, the serum AST levels in the SD rats did not change significantly throughout the observation period (Table 3). The serum AST levels in the PCK rats were significantly higher than those in the SD rats at 16 weeks of age (Table 3).

The serum ALT levels in the PCK rats were significantly higher at 16 weeks than at 8, 20 and 24 weeks and at 24 weeks than at 20 weeks (Table 3). Furthermore, the serum ALT levels in the PCK rats were significantly higher than those in the SD rats at both 16 and 20 weeks of age (Table 3).

Immunohistological analysis of the kidneys

To evaluate the tubular epithelial cell proliferation, we conducted an immunohistochemical analysis of the PCNA expression (Fig. 1). In the PCK rats, PCNA expression was detected in the tubular epithelial cell nuclei (Fig. 1A, B, C and D), and the degrees of PCNA positivity tended to increase with age. Specifically, the PCNA-positive areas at 16, 20, and 24 weeks were significantly greater than those at 8 weeks, and the areas at 24 weeks were significantly

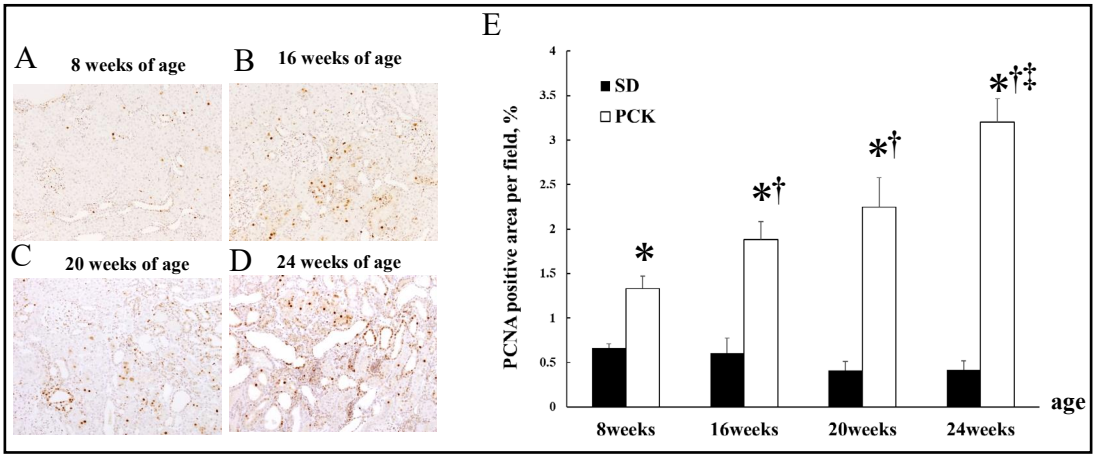


Fig. 1. Immunohistological staining using an antibody against PCNA in the PCK rats (A, B, C, D) and the PCNA-positive areas per field were assessed quantitatively (E). Original magnification, x100. *P<0.05 vs SD rats at the same age; †P<0.05 vs PCK rats at 8 weeks of age; ‡P<0.05 vs PCK rats at 16weeks of age.

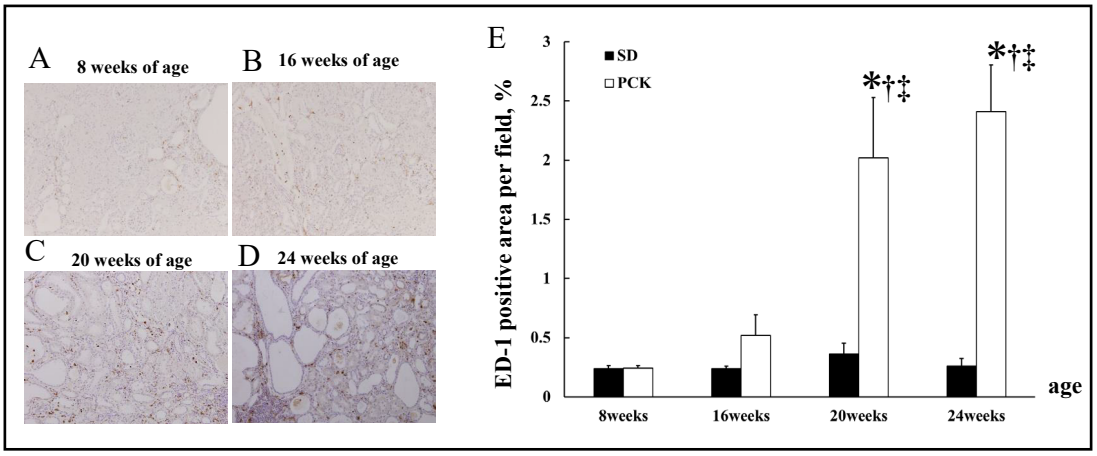


Fig. 2. Immunohistological staining using an antibody against ED-1 in the PCK rats (A, B, C, D) and the ED-1 positive areas per field (E). Original magnification, x100. *P<0.05 vs SD rats at the same age; †P<0.05 vs PCK rats at 8 weeks of age; ‡P<0.05 vs PCK rats at 16 weeks of age.

greater than those at 16 weeks (Fig. 1E). Furthermore, significantly greater PCNA expression was observed in the PCK rats relative to the age-matched SD rats throughout the observation period (Fig. 1E).

To evaluate the degree of renal interstitial inflammation, we immunohistochemically detected the macrophage marker ED1 (Fig. 2). In the PCK rats, macrophages were detected in the interstitium (Fig. 2A, B, C, and D), and the degree of infiltration tended to increase with age. The degrees of infiltration were significantly greater in the PCK rats than in the SD rats at both 20 and 24 weeks of age and, than in the PCK rats at both 8 and 16 weeks (Fig. 2E).

Additionally, an immunohistochemical analysis of α-SMA and type I collagen expression was performed to evaluate the degree of tubulointerstitial fibrosis. In the PCK rats, α-SMA-positive areas were observed in the tubules and interstitium. The extent of these areas tended to increase with age (Fig. 3A, B, C, and D), and was significantly greater in the PCK rats at all time points relative to the age-matched SD rats (Fig. 3E). Furthermore, the extent of α-SMA-positive areas in the 20- and 24-week-old PCK rats was significantly greater than that in the 8-week-old rats and in the 24-week-old rats relative to the 16-week-old rats (Fig. 3E).

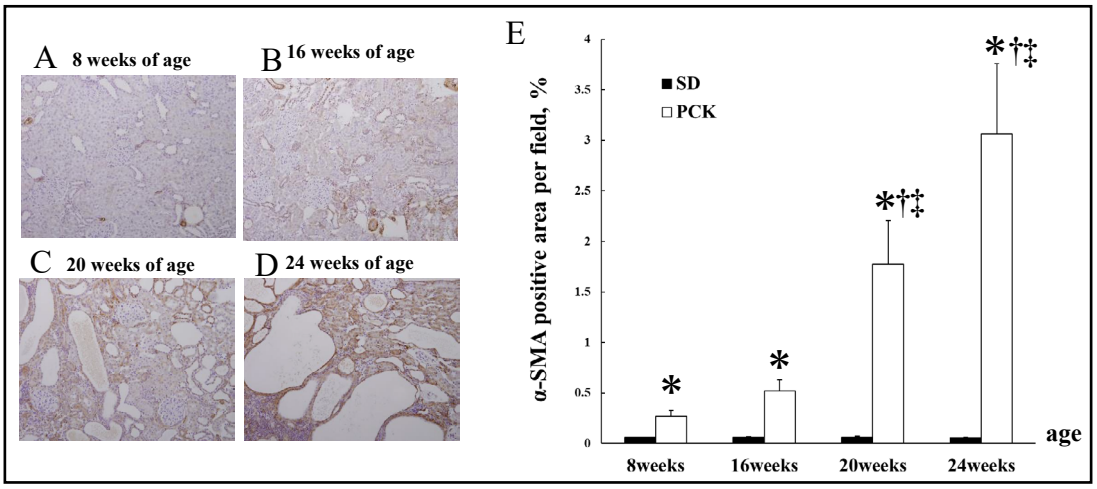


Fig. 3. Immunohistological staining using an antibody against α-SMA in the PCK rats (A, B, C, D) and the α-SMA positive areas per field (E). Original magnification, x100. *P<0.05 vs SD rats at the same age; †P<0.05 PCK rats at 8 weeks of age; ‡P<0.05 vs PCK rats at 16weeks of age.

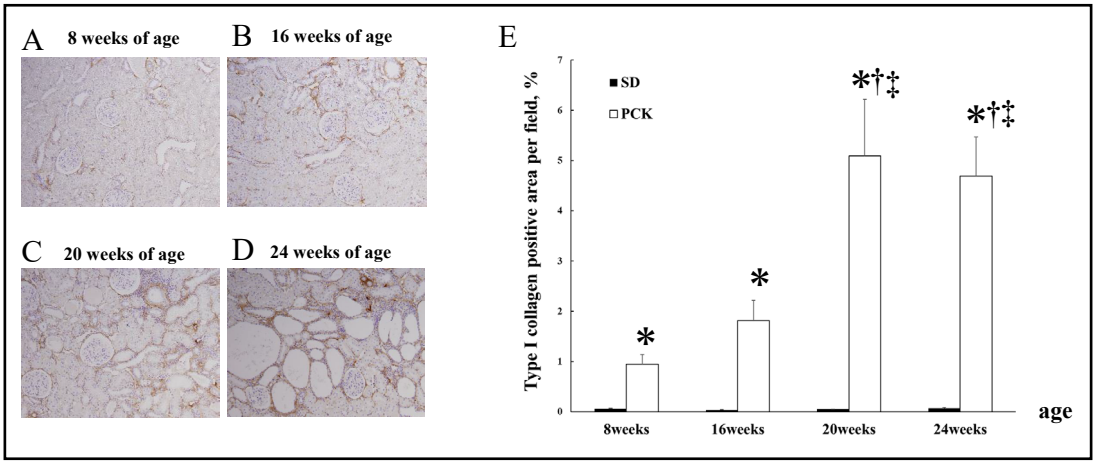


Fig. 4. Immunohistological staining using an antibody against type I collagen in the PCK rats (A, B, C, D) and the type I collagen positive areas per field (E). Original magnification, x100. *P<0.05 vs SD rats at the same age; †P<0.05 vs PCK rats at 8 weeks of age; ‡P<0.05 vs PCK rats at 16 weeks of age.

Further analysis revealed type I collagen expression in the interstitium (Fig. 4A, B, C, and D). In the PCK rats, the degree of type I collagen expression tended to increase with age up to 20 weeks and remained significantly higher than the degree of expression in the SD rats throughout the observation period (Fig. 4E). Furthermore, the degrees of type I collagen expression in the 20- and 24-week-old PCK rats were significantly greater than those in the 8- and 16-week-old rats (Fig. 4E).

Gene expression analysis in the kidneys

In the PCK rats, MCP-1 mRNA levels tended to increase with age, and the expression of this gene was significantly higher than in the age-matched SD rats throughout the observation period (Fig. 5A). Furthermore, the MCP-1 mRNA levels were significantly higher at 20 and 24 weeks relative to 8 weeks and at 24 weeks relative to 16 weeks. Similarly, the TGF-β mRNA levels increased with age in the PCK rats, and the levels at 16, 20, and 24 weeks were significantly higher than those in the age-matched SD rats (Fig. 5B). In the PCK rats, the

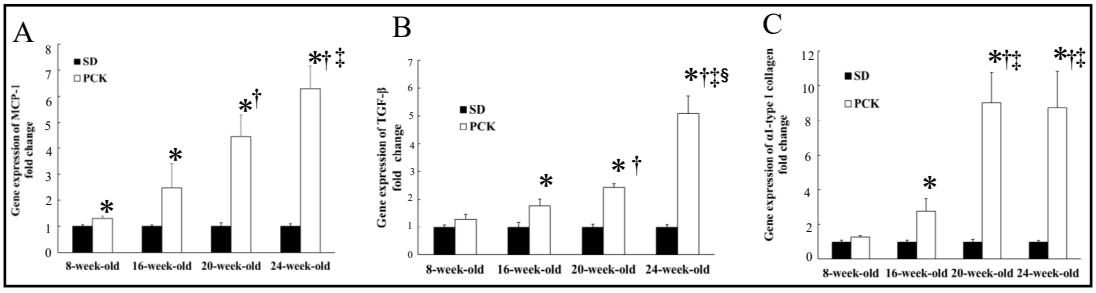


Fig. 5. mRNA transcript expressions of MCP-1(A), TGF-β (B), and α1-type I collagen (C). *P<0.05 vs SD rats at the same age; †P<0.05 vs PCK rats at 8 weeks of age; ‡P<0.05 vs PCK rats at 16 weeks of age; §P<0.05 vs PCK rats at 20 weeks of age.

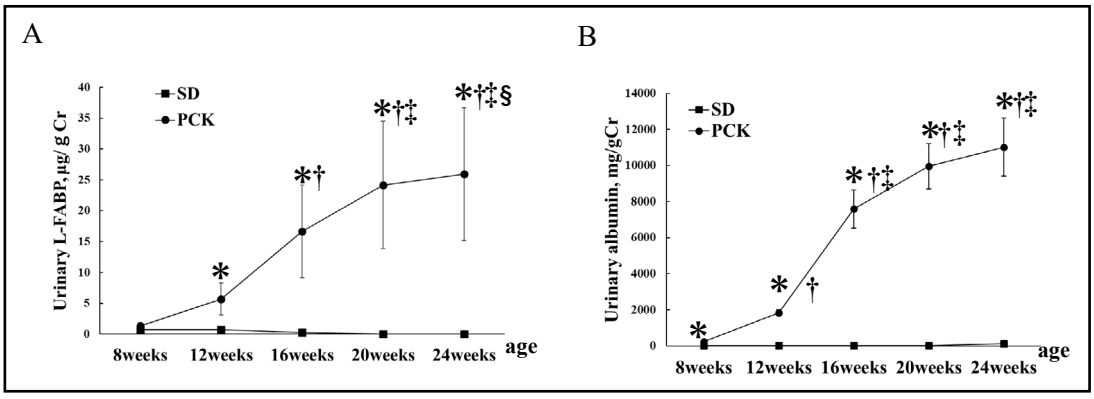


Fig. 6. Change in urinary L-FABP (A), and urinary albumin (B). *P<0.05 vs the SD rats at the same age; †P<0.05 vs PCK rats at 8 weeks of age; ‡P<0.05 vs PCK rats at 12 weeks of age.;§P<0.05 vs PCK rats at 16 weeks of age.

TGF-β mRNA levels were significantly higher at 20 and 24 weeks relative to 8 weeks and at 24 weeks relative to 16 and 20 weeks.

The expression of α1-typeI collagen mRNA in the PCK rats tended to increase with age up to 20 weeks and remained high at 24 weeks and were significantly higher at 16, 20, and 24 weeks relative to those in the age-matched SD rats (Fig. 5C). Furthermore, the mRNA levels in the PCK rats were significantly higher at 20 and 24 weeks, compared to 8 and 16 weeks (Fig. 5C).

Change in the urinary biomarkers

Both the urinary L-FABP and urinary albumin levels were measured at every 4weeks from 8 to 24 weeks of age (Fig. 6). Notably, the urinary L-FABP levels tended to increase with age in the PCK rats and were significantly higher than those in the age-matched SD rats between 12 and 24 weeks of age (Fig. 6A). In the PCK rats, these levels were significantly higher at 16, 20, and 24 weeks relative to 8 weeks, at 20 and 24 weeks relative to 12 weeks, and at 24 weeks relative to 16 weeks (Fig. 6A).

In the PCK rats, urinary albumin levels similarly tended to increase with age and were significantly higher than those in the age-matched SD rats between 8 and 24 weeks of age (Fig. 6B). Furthermore, the levels in the PCK rats were significantly higher at 12, 16, 20, and 24 weeks relative to 8 weeks and at 16, 20, and 24 weeks relative to 12 weeks (Fig. 6B).

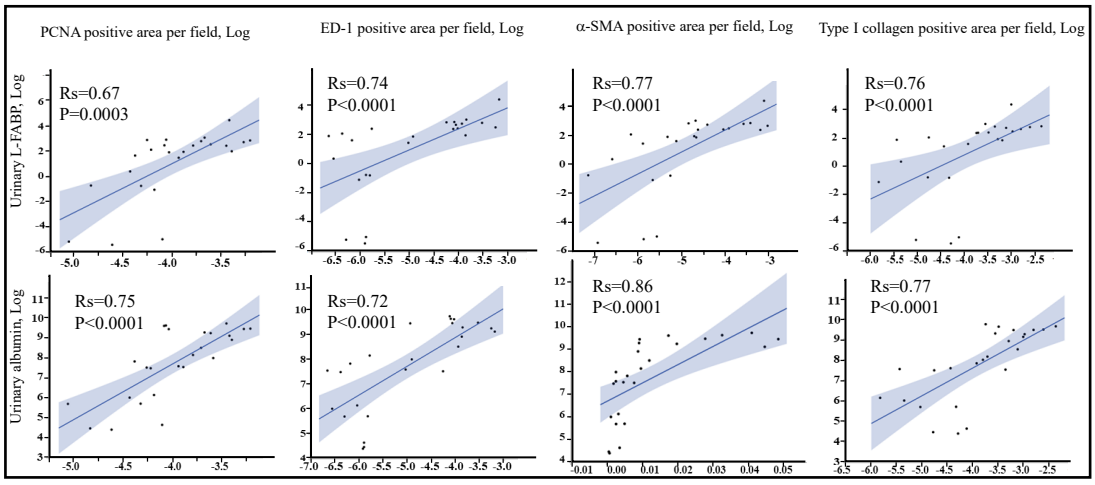
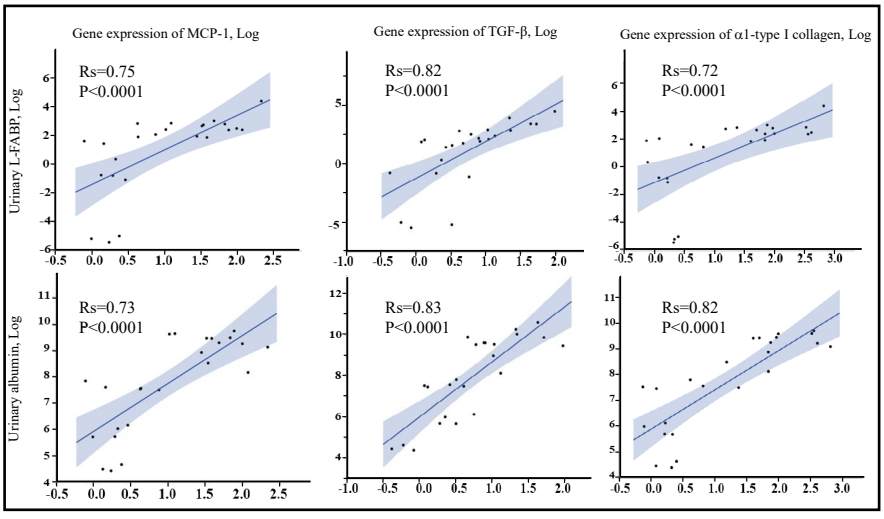


Fig. 7. Correlations between each urinary marker and each histological evaluation.

Fig. 8. Correlations between each urinary marker and each gene expression.



Correlation between the urinary marker levels and histological changes

The urinary levels of both L-FABP and albumin were found to correlate significantly with the degree of tubular epithelial cell proliferation (i.e., PCNA-positive area), macrophage infiltration (i.e., ED-1-positive area), and tubulointerstitial fibrosis (i.e., α-SMA-positive area and type I collagen deposition; $p < 0.0005$, Fig. 7).

Correlation between the urinary marker levels and gene expression

The urinary levels of both L-FABP and albumin were found to correlate significantly with the mRNA expression of MCP-1 (inflammatory cytokine), TGF-β (profibrotic cytokine), and α1-type I collagen (universal fibrotic marker; $p < 0.0001$, Fig. 8).

Correlation between the urinary parameters in the 8-, 12-, and 16-week-old PCK rats and TKV in the 20- and 24-week-old rats

Two rats each in the 20- and 24-week-old groups of PCK rats died during the observation period. Therefore, this evaluation included the 11 remaining PCK rats in each group. Notably, the 20- and 24-week-old PCK rats revealed a significantly greater TKV, compared to the age-matched SD rats; however, the TKVs of the 20- and 24-week-old PCK rats were similar (Fig. 9A).

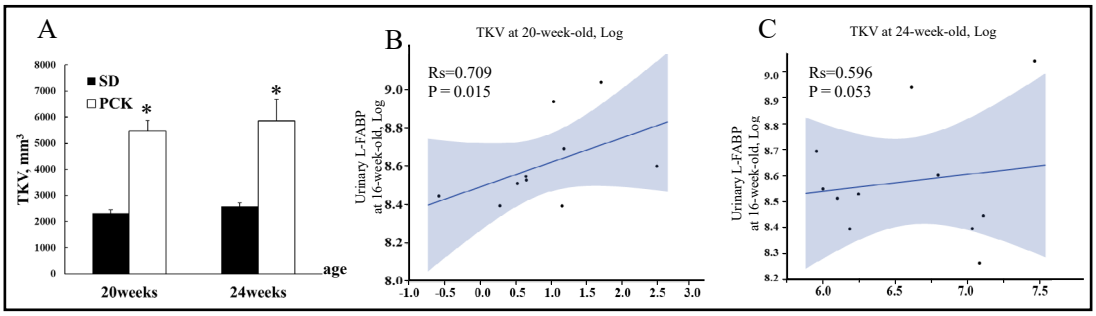


Fig. 9. TKV at both 20 and 24 weeks of age (A). Correlations between urinary L-FABP at 16 weeks of age and TKV at each 20 (B) and 24 weeks of age (C).

Furthermore, the urinary L-FABP levels of the 16-week-old PCK rats were found to correlate significantly with the TKV at 20 weeks of age (Rs = 0.709, $p < 0.0001$, Fig. 9B) and tended to correlate with the TKV at 24 weeks of age (Rs = 0.596, $p = 0.053$, Fig. 9C); however, the urinary L-FABP at 8 and 12 weeks and urinary albumin levels at 8, 12, and 16 weeks did not correlate significantly with the TKV at 20 and 24 weeks in the PCK rats (data not shown).

Discussion

In the present PCK rat model study, we observed increasing urinary levels of L-FABP and albumin with age and found that these markers correlated significantly with the degrees of tubular epithelial cell proliferation, interstitial inflammation, and fibrosis. Furthermore, the urinary L-FABP level may be a marker of PKD progression, as this parameter at 16 weeks was found to correlate significantly with the TKV at 20 weeks and tended to correlate with the TKV at 24 weeks. These results suggest that both the urinary L-FABP and urinary albumin levels might be clinically useful for monitoring the PKD progression. Despite the growing evidence of a relationship between the urinary L-FABP levels and tubulointerstitial damage or renal prognosis in both CKD and AKI [15-17], the correlations of this parameter with PKD have not yet been investigated. Therefore, the present study is the first to report the potential utility of urinary L-FABP as a monitoring marker of PKD progression.

In the PCK rats, cyst formation is induced via a splicing mutation of the human orthologous polycystic kidney and hepatic disease 1 (PKHD1) gene, which encodes the polycystin proteins [18]. Consequently, the PCK rats suffer from congenital hepatic fibrosis [19]. Therefore, these rats are presumed to mimic human ARPKD. In contrast, Lager et al. reported that the slow progression of renal cyst growth in a PCK rat model of a hepatic disorder resembled the human ADPKD, a disease first diagnosed in children or adolescents that is characterized by the prolonged (i.e., several decades) and persistent expansion of renal cysts [20]. We further note that the changes in renal pathology, such as interstitial inflammation and fibrosis, as well as tubular epithelial cell proliferation leading to cyst enlargement, are related to renal dysfunction during PKD progression and occur similarly in ARPKD and ADPKD, despite their different genetic etiologies [21]. Therefore, we assume that our PCK rat model is instrumental to evaluations of the relationship between the renal pathological changes occurring during PKD progression and the urinary marker levels.

TKV can be measured via imaging and used as a surrogate marker of PKD. In particular, the TKV determined using high-resolution MRI was confirmed to correlate strongly with a reduced estimated glomerular filtration rate and PKD progression [5]. In general, however, MRI is costly and only available at the medical university or advanced treatment hospitals. Furthermore, the requirement for sedation makes it difficult to perform MRI in children [22]. Therefore, a simple, urine-based clinical marker of PKD is needed. Considering our observation of a significant association between the urinary L-FABP level at 16 weeks and

the TKV at 20 weeks (estimated using the basic ellipsoid equation rather than MRI), urinary L-FABP may be useful as a marker for the routine clinical monitoring of PKD.

Tubulointerstitial damage, which is induced by inflammatory cytokines and growth factors and characterized by interstitial inflammation and subsequent fibrosis, is more associated with CKD progression than the degree of glomerular damage [23, 24]. Similarly, tubulointerstitial pathology is useful for monitoring the slow progression of ADPKD and some cases of ARPKD because the inflammatory cytokines and growth factors produced by the cyst epithelial cells induce macrophage infiltration and provoke interstitial fibrosis. These processes, collectively, with the exacerbation of cyst enlargement, eventually lead to renal function impairment [8, 9,21]. In the present study, MCP-1 expression was upregulated in the 8-week-old PCK rats consequent to tubular epithelial cell proliferation, and subsequently induced macrophage infiltration began at16 weeks, although this change was not significant. Although early (8-week) increases were observed in both the α-SMA positive area and type I collagen deposition, significant increases in both TGF-β and type I collagen mRNA were not observed until 16 weeks. Collectively, the changes in the interstitial pathophysiology and PKD progression observed were similar to the features of the noncystic CKD. A previous study found a significant correlation of urinary L-FABP with the degree of tubulointerstitial damage in noncystic CKD [25]. Therefore, urinary L-FABP might reflect the degree of PKD-associated interstitial damage.

The present study further revealed a significant and early increase in the urinary albumin levels in the PCK rats, compared to the SD rats. A previous morphometric study of glomerular damage found that segmental glomerular basement membrane thickening and foot process effacement was observed via transmission electron microscopy in the 10-week-old PCK rats [20]. Furthermore, the urinary albumin level, which increases as a consequent to dysfunctional reabsorption in the proximal tubules, may be useful as a tubular marker [15, 16, 26]. This marker might be particularly sensitive because it reflects both glomerular and tubulointerstitial pathological changes that occur during the early disease phase in the PCK rats.

In our previous report, we confirmed an increase in the serum level of L-FABP in human patients with hepatic disease [27]; however, we did not find a correlation between the serum and urinary levels of L-FABP in CKD. Another research group reported a significant correlation of the urinary level of L-FABP with changes in the serum levels of various liver enzymes in critically ill patients admitted to an intensive care unit [28]. Furthermore, a previous report identified a progressive hepatic disorder in the PCK rats [19]. In our study, significantly higher changes in the serum liver enzyme levels, especially ALT, were observed in the PCK rats relative to the SD rats at two points; however, we did not observe a significant increase in the serum levels of L-FABP in the PCK rats relative to the SD rats. Therefore, the observed increase in the urinary L-FABP levels was not attributed to an increase in the serum L-FABP level.

Certain strategies for preventing PKD progression are presently at the research stage or in clinical practice; however, the present study did not examine the effects of these interventions on the urinary marker levels in the PCK rats. Although the vasopressin V2-receptor antagonist, Tolvaptan, was reported to slow the increase in TKV and the decline in renal function in patients with ADPKD [3, 4], it did not trigger a decrease in the urinary albumin level [4]. In contrast, an angiotensin II receptor blocker known to inhibit renal dysfunction [29] was reported to reduce the urinary L-FABP levels in patients with ADPKD [13]. These findings suggest that the urinary level of the tubular specific marker L-FABP may determine the effect of Tolvaptan treatment on the effects of PKD.

This study had some limitations, notably, pertaining the inability of animal models to completely mimic human pathophysiology. First, the degree of cyst growth differs between PCK rats and humans with PKDs. Although the cysts in human patients spread from the surface of the kidney and compress the surrounding organs, most cysts formed in the PCK rats remained within the enlarged kidneys [20, 30, 31]. Second, the degree of renal

dysfunction in the PCK rats was relatively slight, compared to the degree of tubulointerstitial damage. Third, the SBP did not increase significantly in the PCK rats relative to the SD rats; in contrast, hypertension is a frequent characteristic of slow progressive human PKD.

Conclusion

The urinary levels of L-FABP and albumin appear to reflect the degree of tubulointerstitial damage and may be useful for monitoring PKD in clinical practice. Furthermore, clinical studies are necessary to validate the clinical superiority of urinary markers relative to imaging modalities for monitoring the progression of PKD or therapeutic responses to the interventions.

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Disclosure Statement

T. Sugaya is the Director and Senior Scientist and K. Ohata is a Scientist of CMIC HOLDINGS Co., Ltd., which produced the kits used for L-FABP analysis. None of the other authors had conflicts of interest or financial disclosures relevant to the present study.

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