ORIGINAL

Urinary angiotensin converting enzyme 2 and disease activity in pediatric IgA nephropathy

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Abstract : Background : Our previous studies demonstrated that the intrarenal renin-angiotensin system (RAS) status was activated in pediatric patients with chronic glomerulonephritis. In the present study, we tested the hypothesis that angiotensin-converting enzyme 2 (ACE2) expression in the kidney is associated with the development of pediatric IgA nephropathy. Methods : We analyzed urinary ACE2 levels and ACE2 expression in the kidney tissues of pediatric patients with IgA nephropathy treated with RAS blockade. Paired tests were used to analyze changes from the first to the second biopsy. Results : Urinary ACE2 levels were significantly decreased after RAS blockade treatment, accompanied by decreased ACE2 expression levels in kidney tissues, urinary protein levels and mesangial hypercellularity scores. Urinary ACE2 levels at the first biopsy were positively correlated with the ACE2 expression levels. Conclusions : These data suggest that urinary ACE2 is associated with ACE2 expression in the diseased kidney, which correlates with the pathogenesis of IgA nephropathy in pediatric patients. J. Med. Invest. 68 : 292-296, August, 2021

Keywords **:** *Angiotensin-converting enzyme 2, IgA nephropathy, renin-angiotensin system*

INTRODUCTION

The renin-angiotensin system (RAS) is a pivotal system that regulates blood pressure and controls the fluid/electrolyte balance (1). Angiotensin II is the most powerful biologically active product of the RAS, which binds to the angiotensin II type 1 (AT1) receptor (2). Local activation of the intrarenal RAS is involved in the pathogenesis of hypertension and kidney injury (1). Recent reports have shown that progression of proteinuria and kidney tissue damage is associated with intrarenal RAS activation (3-6). Indeed, treatment with angiotensin-converting enzyme (ACE) inhibitors and AT1 receptor blockers (ARBs) significantly decreases proteinuria in patients with chronic kidney disease, which is independent of blood pressure changes (1, 5).

ACE2 is a homolog of ACE that counterbalances ACE activity (7). ACE2 cleaves angiotensin I into angiotensin 1-9 and angiotensin II into the vasodilator peptide angiotensin 1-7 (7). Angiotensin 1-7 is a biologically active peptide that binds the Mas receptor to exert opposing effects on angiotensin II (8). Although many studies have reported the protective role of angiotensin 1-7 in the prevention of angiotensin II, it has been shown that angiotensin 1-7 stimulates growth factor expression and cell proliferation (9). Furthermore, the coronavirus disease 2019 (Covid-19) interfaces with the RAS through ACE2, which functions as a receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (10). The interaction between coronavirus and ACE2 has been suggested as a potential factor in infectivity (10).

Recently, we demonstrated that ACE2 expression in the kidney was increased in pediatric IgA nephropathy and was accompanied by mesangial hypercellularity (11). Furthermore, we clarified that intrarenal RAS activation was suppressed in pediatric IgA nephropathy patients treated with RAS blockade (12). In the present study, we measured urinary ACE2 levels following treatment with RAS blockade and evaluated the expression of ACE2 in the kidney tissue to test the hypothesis that ACE2 is associated with kidney damage in pediatric IgA nephropathy.

PATIENTS AND METHODS

Patients and samples

All procedures involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee. The study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The Institutional Review Board of Tokushima University approved the study protocol (IRB approval number 1085) and the use of tissue samples. Informed consent was obtained from all enrolled children.

Pediatric patients with IgA nephropathy were recruited at Tokushima University Hospital between April 1, 2011, and March 31, 2016. All study participants with other disorders, including Henoch-Schonlein purpura, systemic lupus erythematosus, chronic liver disease, diabetes mellitus, malignancies, and urinary tract infections, were excluded. At the time of study enrollment, all patients were asked to undergo a second kidney biopsy after 2 years following the end of RAS blockade (candesartan or benazepril). Clinical parameters, including sex, age, height, body weight, and blood pressure, and laboratory parameters, including serum creatinine and urinary protein and creatinine concentrations, were determined at the time of biopsy. Urine collections were combined to calculate the 24-h creatinine clearance (ml/min), which was used as an index of the glomerular filtration rate. Urinary concentrations of ACE2 were measured using a human ACE2 ELISA kit (Adipogen, Liestal, Switzerland) in accordance with the manufacturer's instructions.

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Histological study

The biopsied tissues were fixed in 10% buffered formalin and embedded in paraffin according to routine clinical practice. Paraffin sections (3 μm) were stained with the periodic acid-Schiff reagent. Kidney tissue sections at least ten glomeruli were scored independently by pathologist blinded to all clinical data ; discrepancies were resolved by a third investigator. Using modifications from previous studies (12, 13), we scored histological changes as follows : mesangial hypercellularity, 0 = none ; 1 = mild (three-four mesangial per peripheral lobule) ; 2 = severe segmental (more than four mesangial cells per peripheral lobule) ; and 3 = severe global proliferation). Mesangial matrix score, 0 = none ; 1 = mild ; 2 = marked segmental (width of mesangial interspace between capillaries exceeding three mesangial cells) ; and 3 = marked global increase. Interstitial fibrosis, 0 = none ; 1 = < 25 % ; 2 = 26%-50% ; and 3 > 50% of the cortical area. Tubular atrophy, 0 = none ; 1 = < 25% ; 2 = 26%-50% ; and 3 = > 50% of the cortical tubules.

Immunohistochemistry

Formalin-fixed, paraffin-embedded kidney sections were stained with ACE2. Sections (3 μm) were incubated with anti-ACE2 antibody (ab15348 ; Abcam, Cambridge, MA, USA) overnight at 4°C, rinsed, and incubated with biotinylated secondary antibodies (Vector Labs, Burlingame, CA, USA). After rinsing, the sections were incubated with avidin-biotin-peroxidase complex (ABC Elite ; Vector Labs), followed by 3,3'-diaminobenzidine (Dojindo, Kumamoto, Japan). Each section was counterstained with Mayer's hematoxylin (Wako, Tokyo, Japan), dehydrated, and cover-slipped. The fraction of glomeruli occupied by the immunoreactive area was determined using EIS-Elements software (Nikon Corporation, Tokyo, Japan). For each glomerulus, the immunoreactive area (brown) was automatically calculated by the software, and the affected area was, in turn, divided by the total area of the glomerulus. At least ten equationally sectioned glomeruli were examined from each slide.

Statistical analysis

Pearson and Spearman correlation coefficients were used for parametric and nonparametric data, respectively. The Wilcoxon signed-rank test was used to perform paired comparisons at the first and second biopsies. All data are presented as the mean ± standard error of the mean (SEM). Statistical significance was set at $P < 0.05$. All computations, including data management and statistical analysis, were performed using JMP software version 14 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

Table 1 describes the patient's characteristics and laboratory data at the first biopsy. Kidney parameters and histological features at the first and second biopsies are shown in Table 2. The urinary protein-creatinine ratio (UP/UCre) was significantly lower at the second biopsy ; however, here was no significant change in the creatinine clearance.

Table 1. Patient characteristics

Parameters	Data
Gender, F/M	16/15
Age, Year	10.64 ± 0.74
Height, cm	139.77 ± 4.05
Body weight, kg	36.65 ± 2.90
Body mass index	17.70 ± 0.55
Serum creatinine, mg/dL	0.45 ± 0.02

Histological findings and ACE2 in the urine and kidney

Scores for mesangial hypercellularity, mesangial matrix, and glomerular expression levels of ACE2 in kidney tissues were lower in the second biopsy than in the first biopsy (Table 2 and Figure 1a). In addition, the logarithmically transformed urinary ACE2-creatinine ratio (log(UACE2/UCre)) was significantly lower in the second biopsy than in the first biopsy (Figure 1b). Figure 2 demonstrated that the single-regression analyses for log(UACE2/UCre) levels were significantly positively correlated with UP/UCre, mesangial hypercellularity, and glomerular expression levels of ACE2 in the kidney tissue. However, log(UACE2/UCre) levels were not correlated with age, height, body weight, body mass index, creatinine clearance, mesangial matrix, interstitial fibrosis, and tubular atrophy.

Table 2. Kidney parameters and histologic features at the time of kidney biopsy

Parameters	First biopsy, N = 31	Second biopsy, N = 31	Difference	P values
UP/UCre, g/g	1.45 ± 0.33	0.05 ± 0.01	-1.40 ± 0.33	< 0.0001*
CCr, mL/min	108.61 ± 1.86	109.74 ± 2.91	1.14 ± 3.88	0.9760
Mesangial hypercellularity	1.06 ± 0.07	0.41 ± 0.04	-0.65 ± 0.07	< 0.0001*
Mesangial matrix score	1.06 ± 0.10	0.74 ± 0.07	-0.32 ± 0.11	0.0081*
Interstitial fibrosis	0.05 ± 0.04	0.08 ± 0.04	0.03 ± 0.02	0.5000
Tubular atrophy	0.06 ± 0.03	0.10 ± 0.04	0.03 ± 0.05	0.7656
ACE2 expression	0.88 ± 0.08	0.22 ± 0.11	-0.66 ± 0.07	< 0.0001*

UP/UCre ; urinary protein-creatinine ratio, CCr ; creatinine clearance, * ; $P < 0.05$.

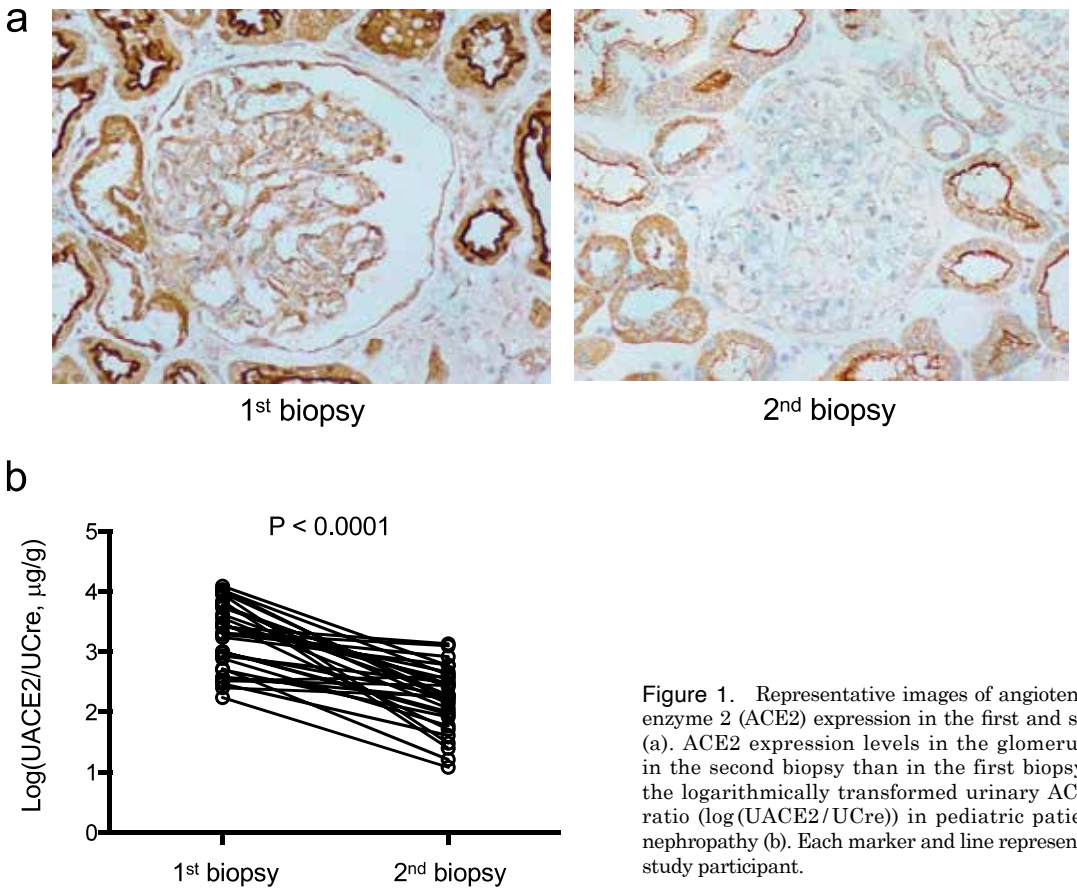

Figure 1. Representative images of angiotensin-converting enzyme 2 (ACE2) expression in the first and second biopsies (a). ACE2 expression levels in the glomeruli were lower in the second biopsy than in the first biopsy. Changes in the logarithmically transformed urinary ACE2-creatinine ratio (log(UACE2/UCre)) in pediatric patients with IgA nephropathy (b). Each marker and line represent an individual study participant.

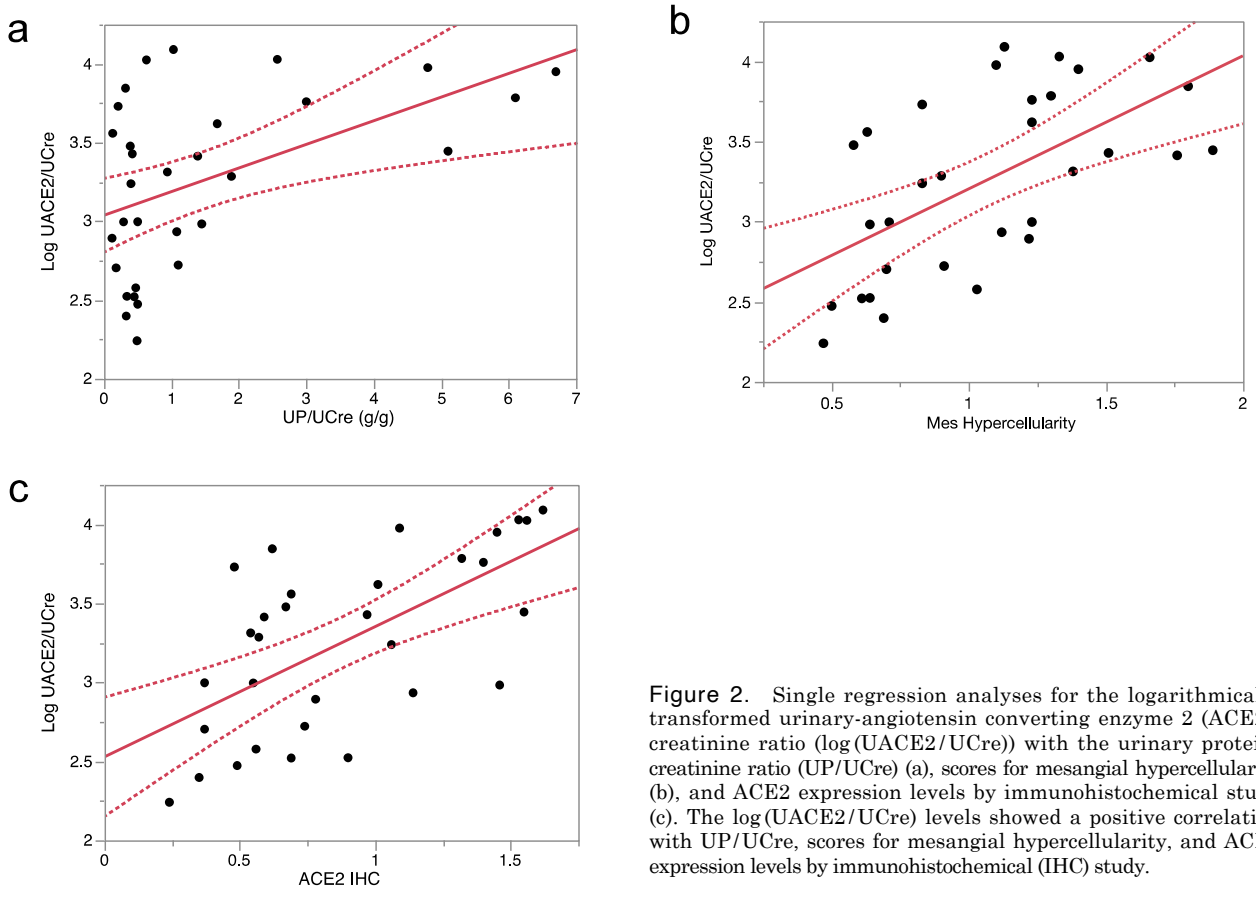

Figure 2. Single regression analyses for the logarithmically transformed urinary-angiotensin converting enzyme 2 (ACE2)-creatinine ratio (log(UACE2/UCre)) with the urinary protein-creatinine ratio (UP/UCre) (a), scores for mesangial hypercellularity (b), and ACE2 expression levels by immunohistochemical study (c). The log(UACE2/UCre) levels showed a positive correlation with UP/UCre, scores for mesangial hypercellularity, and ACE2 expression levels by immunohistochemical (IHC) study.

DISCUSSION

Activated intrarenal RAS is involved in the progression of kidney injury (3, 4). Urinary angiotensinogen is a specific biomarker of intrarenal RAS activation in various kidney diseases (1, 5). Previously, we reported that ACE2 expression in the kidney was increased in pediatric IgA nephropathy and was accompanied by mesangial hypercellularity (11). However, urinary ACE2 excretion and kidney ACE2 expression associated with the pathophysiology of kidney injury have not been studied extensively in humans. Our study is the first to demonstrate that urinary ACE2 and glomerular ACE2 expression levels at the second biopsy were lower than those at the first biopsy in pediatric IgA nephropathy. We also found that urinary ACE2 levels positively correlated with urinary protein levels, scores for mesangial hypercellularity, and expression levels of ACE2 in the kidney tissue.

Intrarenal RAS is independently regulated, and its inappropriate activation contributes to the pathogenesis of hypertension and kidney disease (1, 4). The RAS in the kidney is unique because all of the components necessary to generate intrarenal angiotensin II are present along the nephron in both the interstitial and intratubular compartments (2). It is well known that the major fraction of angiotensin II present in kidney tissues is generated from angiotensinogen and then subsequently delivered to the kidney, as well as from angiotensinogen produced by proximal tubule cells (2). Renin is secreted by juxtaglomerular and proximal tubular cells (2). The brush border membrane of the proximal tubules also expresses abundant levels of ACE. ACE was detected in proximal and distal tubular fluids, with higher concentrations observed in the proximal tubule fluid, and catalyzes the transformation of angiotensin I to angiotensin II (14). Therefore, all of the major components required to generate angiotensin II are expressed in the kidney (1, 5). In our previous study, cultured glomerular mesangial cells produced ACE2 mRNA and protein (11). Furthermore, glomerular ACE2 expression and urinary ACE2 levels were found to be involved in the pathogenesis of pediatric IgA nephropathy in the current study. These findings suggest that ACE2 plays an important role in the intrarenal RAS activation during the course of pediatric IgA nephropathy.

Previous studies have shown that urinary angiotensinogen levels were increased not only in hypertensive subjects but also in patients with chronic kidney disease (5). Recently, urinary angiotensinogen have been used as an index of intrarenal RAS activation (1, 5). We previously reported that urinary angiotensinogen levels were increased in patients with chronic glomerulonephritis compared with control subjects and that treatments with RAS blockers suppressed these augmentations (15). In addition, we found that urinary angiotensinogen levels and kidney angiotensinogen expression were decreased in patients with pediatric IgA nephropathy treated with RAS blockade (12). These findings suggest that urinary angiotensinogen could be a novel biomarker of intrarenal RAS status in pediatric patients. In the present study, we demonstrated that elevated urinary ACE2 levels were suppressed by RAS blockade in pediatric IgA nephropathy. Furthermore, urinary ACE2 levels correlated with glomerular damage and ACE2 expression levels. Taken together, urinary ACE2 could be associated with glomerular ACE2 expression involved in intrarenal RAS activation in the development of kidney injury.

ACE is also a functional receptor for SARS-CoV-2, the virus responsible for the Covid-19 pandemic (10). SARS-CoV-2 within the host cell is a putative link between Covid-19 and hypertension, cardiovascular disease, and kidney disease (16). The SARS-CoV-2 spike protein binds to membrane-bound ACE2 on the surface of respiratory epithelial cells to gain cell entry (17, 18). SARS-CoV-2 infection can cause renal involvement, and severe renal dysfunction is more common among patients with chronic kidney disease (19). SARS-CoV-2 infection fundamentally initiates a mechanism of renal injury through highly expressed ACE2 in renal tissue, suggesting that ACE2 signaling pathway plays a key role in the development of the disease (19). Furthermore, systemic effects such as host immune clearance and immune tolerance disorders, endothelial cell injury, thrombus formation, glucose and lipid metabolism disorder, and hypoxia aggravate this renal injury (19). Endocytosis of SARS-CoV-2 combined with ACE2 contributes to decreased ACE2 expression and activity in infected cells (20, 21). It has been suggested that the loss of ACE2 functionality is critically important to Covid-19 pathophysiology (16). Several studies have suggested that RAS inhibitors may increase ACE2 expression, raising concerns regarding their safety in patients with Covid-19 (10). However, sufficient data on human studies are not available, and it is recommended to continue RAS inhibitors because the abrupt withdrawal of RAS inhibitors in high-risk patients may result in clinical instability and adverse health outcomes (10). In the future, further investigations to define risk factors and determine the pathophysiology of SARS-CoV-2 and Covid-19 are important. In fact, our current findings demonstrated that ACE2 in the kidney is crucial in glomerular damage in pediatric IgA nephropathy ; however it is difficult to determine whether ACE2 drives the activity of IgA nephropathy.

The relatively small number of subjects in this study is a potential limitation. However, our observations showed that glomerular ACE2 expression and urinary ACE2 levels were significantly decreased by the treatment with RAS blockade. In addition, urinary ACE2 levels were correlated with urinary protein excretion, glomerular mesangial hypercellularity, and glomerular ACE2 expression levels.

These data strongly support the hypothesis that RAS plays an important role in the pathogenesis of IgA nephropathy and is associated with increased ACE2 expression in the kidney. We emphasize that measuring urinary ACE2 levels is a useful tool to assess glomerular damage in pediatric IgA nephropathy. However, we recognize that the future evaluation of urinary ACE2 in prospective studies with a large number of patients and an extended, long observation period is required. Subsequently, a prospective study was conducted to clarify the novel mechanism of ACE2, which may provide useful information for optimizing the approach for treating pediatric IgA nephropathy.

CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists.

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