Increased urinary excretion of angiotensinogen is associated with risk of chronic kidney disease

Katherine T. Mills1, Hiroyuki Kobori2,3,4, Lotuce Lee Hamm3,4, Arnold Brent Alper3,4, Islam Enver Khan3, Mahfuz Rahman3, Luis Gabriel Navar2,4, Yanxi Liu1, Grace M. Browne1, Vecihi Batuman3,4, Jiang He1,3,4 and Jing Chen1,3,4

1Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA, 2Department of Physiology, Tulane University School of Medicine, New Orleans, LA, USA, 3Department of Medicine, Tulane University School of Medicine, New Orleans, LA, USA, and 4Department of Physiology, Tulane University Hypertension and Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA, USA

Correspondence and offprint requests to: Jing Chen; E-mail: jchen@tulane.edu

Abstract

Background. The effect of intrarenal renin–angiotensin system (RAS) activity on risk of chronic kidney disease (CKD) has not been well studied in human subjects.

Methods. We investigated the association between urinary angiotensinogen, a reliable biomarker of intrarenal RAS activity, and risk of CKD in 201 patients and 201 controls. CKD was defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m² or presence of albuminuria (≥ 30 mg/24 h).

Results. Compared to controls, median urinary angiotensinogen excretion (45.4 versus 7.4 μg/24 h, P < 0.0001) and angiotensinogen-to-creatinine ratio (26.3 versus 4.4 μg/g, P < 0.0001) were significantly higher in patients with CKD. Log-transformed urinary angiotensinogen excretion and angiotensinogen-to-creatinine ratio were inversely correlated with eGFR (r = −0.59 and −0.57, both P < 0.0001) and positively correlated with log-transformed urinary albumin excretion (r = 0.89 and 0.87, both P < 0.0001). After adjusting for multiple covariates, including the use of angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers, diuretics and statins, the odds ratios (95% confidence interval) for CKD comparing the highest tertile to the lowest two tertiles of urinary angiotensinogen excretion and angiotensinogen-to-creatinine ratio were 6.70 (3.43, 13.1; P < 0.0001) and 6.45 (3.34, 12.4; P < 0.0001), respectively.

Conclusions. These data indicate the intrarenal RAS may play an important role in the etiology of CKD, and urinary angiotensinogen may be a useful clinical biomarker for the identification of patients at a high risk for CKD.

Keywords: albuminuria; angiotensinogen; chronic kidney disease; estimated glomerular filtration rate; renin–angiotensin system

Introduction

Chronic kidney disease (CKD) affects over 13% of the US population and is associated with an increased risk of end-stage renal disease, cardiovascular disease (CVD) and all-cause mortality [1–4]. Animal experimental studies suggest that the systemic renin-angiotensin system (RAS) plays a central role in the progression of CKD [5, 6]. Randomized clinical trials have documented that angiotensin-converting enzyme inhibitor (ACE-I) and angiotensin II receptor blocker (ARB) treatments slow the progression of CKD among patients with diabetic or non-diabetic nephropathy [7–10]. However, the RAS blocking agents do not eliminate all risk of CKD progression. In addition, the renoprotective effect of the RAS blocking agents varied among individuals and between subgroups.

In recent years, the role of the intrarenal RAS in the pathophysiology of hypertension and renal injury has become a focus of interest [11–14]. Urinary angiotensin II is unstable and, therefore, cannot be used as a reliable marker of intrarenal RAS activity in clinical settings. On the other hand, urinary angiotensinogen level was highly correlated with intrarenal angiotensinogen and angiotensin II levels and has been suggested as a reliable marker for intrarenal RAS activity [15–17]. A few clinical studies have reported an association between urinary angiotensinogen excretion and severity of CKD [18–20]. However, these small clinical studies were conducted in Asian populations [18–20]. In addition, the influence of treatment with ACE-I or ARB on this association has not been studied.

The objective of the present study is to investigate the association between urinary angiotensinogen excretion and risk of CKD after taking into account ACE-I and/or ARB treatment in a large case-control study. In addition, the association between plasma angiotensinogen and risk of CKD will be examined.
Materials and methods

Study participants

We recruited 201 CKD patients and 201 controls without CKD in the greater New Orleans, Louisiana area from 2007 to 2010. CKD patients aged 21–74 years were identified from nephrology and internal medicine clinics in the study area. CKD was defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m² or presence of albuminuria (≥ 30 mg/24 h). Patients were excluded if they had a history of chronic dialysis, kidney transplants, immunotherapy in the past 6 months, chemotherapy within the past 2 years and current clinical trial participation that may have an impact on CKD. Additional exclusion criteria were history of HIV or AIDS and inability or unwillingness to give informed consent. Controls were recruited through mass mailing to residents aged 21–74 years living in the same area, and they had no evidence of CKD (eGFR > 60 mL/min/1.73 m² and no persistent albuminuria).

Tulane University Internal Review Board approved this study, and informed consent was obtained at the screening visit from all study participants. This study adheres to the Declaration of Helsinki.

Measurements

A standard questionnaire was administered by a trained staff at a clinical visit to obtain demographic information, lifestyle risk factors (including cigarette smoking, alcohol drinking and physical activity), self-reported history of CVD, diabetes, hypercholesterolemia and hypertension as well as medications (including use of ACE-I and ARB, diuretics and statins).

Three blood pressure (BP) measurements were obtained at a clinical visit by trained and certified staff using a standard mercury sphygmomanometer, according to a common protocol adapted from procedures recommended by the American Heart Association [21]. Body height and weight were obtained by trained staff and used to calculate body mass index (BMI = weight in kilogram/squared height in meter) and body surface area using Mosteller’s formula [(weight in kg × height in cm)/360001/2] [22].

An overnight fasting blood sample was collected to measure plasma angiotensinogen and glucose, serum creatinine and cholesterol and triglycerides. eGFR was estimated from serum creatinine (SCr), sex, age, and race using the CKD-EPI equation: eGFR = 141 × min (SCr/κ, 1)1.154 × max (SCr/κ, 1)−1.209 × 0.993SCr + 1.088 (if female) × 1.159 (if black), where κ is 0.7 for females and 0.9 for males and α = −0.329 and −0.411, respectively [23]. A 24-h urine sample was collected to measure angiotensinogen, creatinine and albumin.

Serum cholesterol and triglyceride levels were assayed using an enzymatic procedure on the Hitachi 902 automatic analyzer (Roche Diagnostics, Indianapolis, IN). Serum glucose was measured using a hexokinase enzymatic method (Roche Diagnostics, Indianapolis, IN). Serum creatinine was measured using the Roche enzymatic method (Roche Hitachi P Modular). Muscle mass was measured with Roche Creatinine Plus assay; Hoffmann-La Roche, Basel, Switzerland). Urinary concentrations of albumin and creatinine were measured with a DCA 2000 Analyzer (Bayer AG, Leverkusen, Germany). Plasma and urinary angiotensinogen were measured by a sandwich enzyme-linked immunosorbent assay method (IBL, Fujioka, Japan), which has intra-assay and inter-assay coefficients of variation of 4.4 and 4.3%, respectively [24].

Statistical analysis

Medians and interquartile ranges (IQR) for urinary angiotensinogen excretion (microgram per 24 h) and the urinary angiotensinogen-to-creatinine ratio (microgram per gram) were calculated for CKD patients and controls, and the Mann–Whitney test was used to test differences in the unadjusted medians. Quantile regression was used to obtain adjusted medians and IQR, and the Wald test was used to test differences in the adjusted medians between CKD patients and controls. Age, gender, race, high school education, current cigarette smoking, weekly alcohol consumption, physical activity (≥ twice per week), BMI, low-density lipoprotein (LDL) cholesterol, systolic BP (SBP), plasma glucose, history of CVD and use of ACE-I and/or ARB, diuretics and statins were adjusted in these analyses. Means and 95% confidence intervals (95% CI) for plasma angiotensinogen (microgram per milliliter) were calculated and a Student’s t-test was used to test for differences in unadjusted means, and analysis of variance was used for adjusted means.

Multivariable linear regression was used to examine the association of eGFR and urinary albumin with urinary angiotensinogen excretion, the angiotensinogen-to-creatinine ratio and plasma angiotensinogen levels after adjustment for the previously mentioned covariates. Log transformations were used for urinary angiotensinogen and albumin levels because they were not normally distributed [17, 18]. Multivariable logistic regression was used to obtain adjusted odds ratios comparing the highest tertile of urinary or plasma angiotensinogen levels to the lower two tertiles between CKD patients and controls. Angiotensinogen tertiles were defined based upon measurements in the control group. In addition, urinary albumin excretion was adjusted in a sensitivity analysis of the associations of urinary angiotensinogen with eGFR and risk of CKD. All analyses were performed using SAS version 9.2 statistical software (Cary, NC).

Results

The general characteristics of study participants by CKD status are presented in Table 1. Those with CKD were older, less educated, heavier and less likely to drink alcohol compared to those without CKD. In addition, they were more likely to have a history of CVD, hypertension, diabetes and hypercholesterolemia and to be taking ACE-I and/or ARB, diuretic and statin medications. Mean BMI, SBP and serum glucose were significantly higher, while LDL cholesterol, high-density lipoprotein cholesterol and eGFR were lower in CKD patients compared to controls.

The entire distributions of the log-transformed 24-h urinary angiotensinogen excretion and the angiotensinogen-to-creatinine ratio were shifted to higher levels in CKD patients compared to controls (Figure 1). Compared to controls, the median 24-h urinary angiotensinogen excretion (45.4 versus 7.4 μg/24 h, P < 0.0001) and urinary angiotensinogen-to-creatinine ratio (26.3 versus 4.4 μg/g, P < 0.0001) were significantly higher in CKD patients (Table 2). Plasma angiotensinogen levels were similar between CKD patients and their controls (24.8 versus 24.9 μg/mL, P = 0.9). After adjustment for multiple covariables, the medians of urinary angiotensinogen excretion and the angiotensinogen-to-creatinine ratio were still significantly higher in CKD patients than in controls.

Urinary angiotensinogen excretion and the angiotensinogen-to-creatinine ratio were highly correlated with each other (r = 0.87, P < 0.0001). Urinary angiotensinogen excretion was not significantly correlated with plasma angiotensinogen level (r = −0.019, P = 0.71).

The scatter plots of urinary angiotensinogen and the angiotensinogen-to-creatinine ratio versus eGFR and urinary albumin excretion show that urinary angiotensinogen was significantly associated with the severity of CKD (Figure 2). Log-transformed 24-h urinary angiotensinogen excretion and the angiotensinogen-to-creatinine ratio were significantly and inversely correlated with eGFR (r = −0.59 and −0.57, both P < 0.0001) and positively correlated with log-transformed urinary albumin excretion (r = 0.89 and 0.87, both P < 0.0001). In the linear regression analyses adjusted for multiple covariables including the use of ACE-I and/or ARB, log-transformed 24-h urinary angiotensinogen excretion and the angiotensinogen-to-creatinine ratio were significantly and inversely related to eGFR and positively related to log-transformed urinary albumin excretion (Table 3). Plasma angiotensinogen level was inversely associated with eGFR but not with
In a subgroup analysis limited to 118 CKD patients who were using ACE-I and/or ARB, the correlation coefficients were $-0.40$ (P < 0.0001) between the log-transformed 24-h urinary angiotensinogen excretion and eGFR as well as between the log-transformed angiotensinogen-to-creatinine ratio and eGFR. The correlation coefficient was $0.89$ (P < 0.0001) between log-transformed 24-h urinary angiotensinogen excretion and log-transformed urinary albumin excretion and 0.88 (P < 0.0001) between the log-transformed angiotensinogen-to-creatinine ratio and log-transformed urinary albumin excretion.

In the logistic regression analyses adjusted for age, gender and race, participants in the highest tertiles of urinary angiotensinogen excretion and the angiotensinogen-to-creatinine ratio had a significantly higher odds of CKD compared to those in the lower tertiles (Table 4). Plasma angiotensinogen level was also borderline, significantly associated with increased odds of CKD, though the magnitude of this association was less than that for urinary angiotensinogen. After further adjustment for education, current cigarette smoking, weekly alcohol drinking, physical activity, BMI, LDL cholesterol, SBP, plasma glucose and history of CVD, urinary angiotensinogen excretion, the angiotensinogen-to-creatinine ratio and plasma angiotensinogen levels were significantly associated with increased odds of CKD in the highest tertiles compared to the lower two tertiles. In a final model with additional adjustment for the use of ACE-I and/or ARB, diuretics and statin medications, the increased odds of CKD in the highest tertiles of urinary angiotensinogen excretion, angiotensinogen-to-creatinine ratio and plasma angiotensinogen level compared to the lower two tertiles remained statistically significant.

### Sensitivity analysis

We conducted a sensitivity analysis to investigate the association of urinary angiotensinogen excretion with decreased eGFR and risk of CKD beyond proteinuria. After additionally adjusting for urinary albumin excretion in the multivariable linear regression analyses, a one SD increase in the log-transformed urinary angiotensinogen excretion (1.86 μg/24 h) and the angiotensinogen-to-creatinine ratio (1.78 μg/g) was significantly associated with a reduction in eGFR of $-17.5$ (−21.7 to −13.3, P < 0.0001) and $-15.2$ (−19.2 to −11.2, P < 0.0001) mL/min/1.73 m², respectively. In addition, after further adjustment for urinary albumin excretion in the logistic regression analyses, those...
Table 2. Urinary and plasma levels of angiotensinogen according to CKD status

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<th>Unadjusted median (IQR)</th>
<th>Multivariable-adjusted median (IQR)b</th>
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<tr>
<td></td>
<td>CKD patients (n = 201)</td>
<td>Non-CKD controls (n = 201)</td>
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<td></td>
<td>P-value for difference</td>
<td></td>
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<tr>
<td>Urinary AGT (µg/24 h)</td>
<td>45.4 (10.2, 227.2)</td>
<td>7.4 (4.4, 11.5)</td>
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<tr>
<td>Urinary AGT-to-Cr ratio (µg/g)</td>
<td>26.3 (5.6, 167.2)</td>
<td>4.4 (3.0, 7.0)</td>
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<tr>
<td>Plasma AGT (µg/mL)c</td>
<td>24.8 (23.6, 26.0)</td>
<td>24.9 (23.4, 26.4)</td>
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a AGT, angiotensinogen; Cr, creatinine.
b Adjusted for age, gender, race, high school education, current cigarette smoking, weekly alcohol drinking, physical activity, BMI, LDL cholesterol, SBP, plasma glucose, history of CVD and use of ACE-I and/or ARB, diuretic and statin medications.
c Mean (95% CI).

Fig. 2. Scatter plots of log transformation of 24-h urinary excretion of angiotensinogen (left upper panel) and urinary angiotensinogen-to-creatinine ratio (right upper panel) against eGFR and scatter plots of log transformation of 24-h urinary excretion of angiotensinogen (left lower panel) and urinary angiotensinogen-to-creatinine ratio (right lower panel) against log transformation of 24-h urine albumin with fitted regression lines and 95% CIs.

Table 3. Multivariable-adjusted regression coefficients (95% CIs) of GFR and urinary albumin associated with a one SD difference in urinary and plasma angiotensinogen

<table>
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<th>eGFR, mL/min/1.73 m²</th>
<th>Log (urinary albumin, mg/24 h)</th>
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<td></td>
<td>β (95% CI)b</td>
<td>P-value</td>
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<tr>
<td>Log (urinary AGT, 1.86 µg/24 h)</td>
<td>−16.3 (−19.0, −13.6)</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Log (urinary AGT-to-Cr ratio, 1.78 µg/g)</td>
<td>−14.7 (−17.4, −12.1)</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Plasma angiotensinogen (9.55 µg/mL)</td>
<td>−3.68 (−4.68, −0.89)</td>
<td>0.01</td>
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a AGT, angiotensinogen; Cr, creatinine.
b Adjusted for age, gender, race, high school education, current cigarette smoking, weekly alcohol consumption, physical activity, BMI, LDL cholesterol, SBP, plasma glucose, history of CVD and use of ACE-I and/or ARB, diuretic and statin medications.
with a urinary angiotensinogen excretion in the highest tertile (≥ 9.85 μg/24 h) had a 3.39-fold (95% CI 1.66–6.91; P = 0.0008) increased odds of CKD, and those with a urinary angiotensinogen-to-creatinine ratio in the highest tertile (≥ 5.92 μg/g) had a 3.02-fold (95% CI 1.47–6.21; P = 0.003) increased odds of CKD compared to their counterparts in the lower two tertiles.

Discussion

The results of the present study indicate that urinary angiotensinogen excretion is significantly higher in patients with CKD compared to those without. In addition, urinary angiotensinogen is significantly associated with declined kidney function and increased albuminuria. These associations are independent of multiple established risk factors for CKD, including BP, plasma glucose, history of CVD and treatment with ACE-I and/or ARB. Furthermore, the association of urinary angiotensinogen with reduced eGFR and increased risk of CKD is independent from urinary albumin excretion, an index of kidney structure damage and urinary protein leakage. Previous studies have indicated that urinary angiotensinogen is a reliable maker for intrarenal RAS activity [15–17]. Our study findings suggest that intrarenal RAS may play an important role in the risk of CKD, and urinary angiotensinogen may be useful for risk classification and prediction of CKD.

Kobori et al. [15, 16] first reported that urinary angiotensinogen level was highly correlated with intrarenal angiotensinogen and angiotensin II levels in angiotensin II-induced and spontaneous hypertensive rats. The production of intrarenal angiotensinogen in the glomerulus and proximal tubule cells was increased under pathological conditions and through positive feedback from angiotensin II [25, 26]. Urinary angiotensinogen excretion has been suggested as a reliable marker for intrarenal RAS activity in animal experiments and clinical studies [11, 17].

A few small clinical studies have reported increased urinary angiotensinogen excretion in patients with CKD [18–20]. For instance, Yamamoto et al. [19] found that urinary angiotensinogen excretion was inversely correlated with eGFR among 80 Japanese patients with CKD. Kobori et al. [18] reported that the log-transformed urinary angiotensinogen-to-creatinine ratio was significantly higher in 80 Japanese CKD patients compared to seven healthy controls. They also reported a significantly positive correlation of the log-transformed angiotensinogen-to-creatinine ratio with the urinary albumin-to-creatinine ratio and an inverse association with eGFR [18]. In addition, Kim et al. [20] found that urinary angiotensinogen levels correlated positively with proteinuria but negatively with eGFR in 58 Korean patients with severe glomerulosclerosis, tubular atrophy and interstitial fibrosis. Patients who were taking ACE-I and/or ARB were excluded from these studies. Our study conducted in white and black patients in the USA supports the previous reports from clinical studies in Asian populations. In addition, our study indicates that the association between urinary angiotensinogen excretion and declined glomerular filtration rate and albuminuria is independent from established CKD risk factors, as well as the use of ACE-I and/or ARB. Therefore, urinary angiotensinogen excretion might provide additional information for risk classification and prediction in CKD patients. Furthermore, targeting intrarenal RAS might provide a novel approach for reducing the risk of CKD. Future longitudinal studies and clinical trials should be conducted to establish the causal effect of intrarenal RAS on the risk of CKD.

It has been suggested that urinary excretion of angiotensinogen reflects intrarenal angiotensin II levels [15, 16]. Angiotensin II is a central mediator of renal injury because of its ability to produce glomerular capillary hypertension that results in damage to glomerular epithelial, endothelial and mesangial cells [27]. Furthermore, angiotensin II and aldosterone have several non-hemodynamic effects that are also important in the pathogenesis of CKD, including activation of pathways associated with inflammation, fibrosis, extracellular matrix accumulation, reactive oxygen species and endothelial dysfunction [6, 27].

This cross-sectional analysis cannot establish whether intrarenal RAS activation, measured by urinary angiotensinogen excretion, causes renal damage or the damaged kidney increases urinary protein, including angiotensinogen and

<table>
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<th>Tertiles of angiotensinogen</th>
<th>OR (95% CI)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
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<th>OR (95% CI)</th>
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<tr>
<td><strong>Urinary AGT (μg/24 h)</strong></td>
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<td>&lt; 9.85</td>
<td>1.0 (ref)</td>
<td>&lt; 0.0001</td>
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<td>≥ 9.85</td>
<td>7.66 (6.49, 12.51)</td>
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<td>6.93 (3.81, 12.61)</td>
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<td>6.70 (3.43, 13.06)</td>
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<td><strong>Urinary AGT/Cr ratio (μg/g)</strong></td>
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<td>&lt; 5.92</td>
<td>1.0 (ref)</td>
<td>&lt; 0.0001</td>
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<td>≥ 5.92</td>
<td>5.99 (3.80, 9.46)</td>
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<td>6.47 (3.62, 11.55)</td>
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<td>6.45 (3.34, 12.43)</td>
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<td><strong>Plasma AGT (μg/mL)</strong></td>
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<td>&lt; 25.24</td>
<td>1.0 (ref)</td>
<td>0.05</td>
<td>1.0 (ref)</td>
<td>0.0004</td>
<td>1.0 (ref)</td>
<td>0.03</td>
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<td>≥ 25.24</td>
<td>1.56 (1.00, 2.44)</td>
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<td>2.86 (1.60, 5.11)</td>
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<td>2.18 (1.14, 4.16)</td>
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*AGT, angiotensinogen; Cr, creatinine; OR, odds ratio.

*Adjusted for age, gender, race, high school education, current cigarette smoking, weekly alcohol drinking, physical activity, BMI, LDL cholesterol, SBP, plasma glucose and history of CVD.

*In addition, adjusted for use of ACE-I and/or ARB, diuretic and statin medications.
excretion in CKD patients. However, our study indicates that there is an association between urinary angiotensinogen excretion and CKD independent from albuminuria, a sensitive marker for kidney structure damage and urinary protein leakage. These results suggest that the increased urinary angiotensinogen in CKD patients is not entirely due to leakage from the glomerular barrier. Previous studies suggested that increased urinary angiotensinogen occurred prior to albuminuria in diabetic nephropathy [11, 12]. Another limitation of this study is that data on dietary sodium intake were not collected. It is well known that dietary sodium intake affects RAS and BP. The association between dietary sodium intake and risk of CKD, however, has not been established. In addition, BP was adjusted in the multivariable analyses as a confounding factor in our study.

In conclusion, our study indicates that urinary angiotensinogen excretion is associated with risk and severity of CKD independent from the use of ACE-I and ARB and albuminuria. These results imply that intrarenal RAS may play an important role in the development of CKD. Furthermore, blockade of intrarenal RAS may be an important strategy for CKD prevention and treatment. Our study also suggests that urinary angiotensinogen excretion may be useful for risk classification and prediction among CKD patients. These findings need to be further explored in prospective cohort studies and clinical trials.

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Conflict of interest statement. None declared.


References


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