Low plasma renin activity is independently associated with kidney disease progression in patients with type 2 diabetes and overt nephropathy, including those with impaired kidney function: a 2-year prospective study

Kazuyoshi Kuma, Susumu Tsuda, Akiko Fukui, Ryota Yoshitomi, Naoki Haruyama and Masaru Nakayama

Division of Nephrology and Clinical Research Institute, Department of Internal Medicine, National Hospital Organization Kyushu Medical Center, Fukuoka 810-8563, Japan

Abstract. Plasma renin activity (PRA) is lower in patients with diabetic nephropathy (DN) than in healthy individuals. However, the association, if any, between PRA and renal outcomes in patients with DN remains uncertain. In a 2-year prospective observational study, we aimed to investigate the association of PRA with the decline in kidney function in patients with DN. We studied 97 patients with DN who were categorized according to tertile (T1–T3) of PRA. The annual changes in estimated glomerular filtration rate (eGFR) (mL/min/1.73 m²/year) were determined from the slope of the linear regression curve for eGFR. The secondary endpoint was defined as a composite of the doubling of serum creatinine or end-stage renal disease. Results showed that kidney function rapidly declined with lower tertiles of PRA (median value [interquartile range] of the annual eGFR changes: –8.8 [–18.5 to –4.2] for T1, –8.0 [–14.3 to –3.2] for T2, and –3.1 [–6.3 to –2.0] for T3; \( p \) for trend <0.01). Multivariable linear regression analyses showed that, compared with T3, T1 was associated with a larger annual change in eGFR (coefficient, –4.410; 95% confidence interval [CI], –7.910 to –0.909 for T1). Composite renal events occurred in 46 participants. In multivariable Cox analysis, the lower tertiles of PRA (T1 and T2) were associated with higher incidences of the composite renal outcome (T2: hazard ratio [HR], 4.78; 95% CI, 1.64–13.89; T1: HR, 4.85; 95% CI 1.61–14.65) than T3. In conclusion, low PRA is independently associated with poor renal outcomes in patients with DN.

Key words: Diabetic nephropathy, Plasma renin activity, Kidney disease progression, Renin–angiotensin–aldosterone system

DIABETIC NEPHROPATHY (DN) is the leading cause of end-stage renal disease (ESRD) worldwide [1]. The typical clinical course of classical DN is as follows: microalbuminuria develops, progresses to macroalbuminuria and sometimes leads to nephrotic syndrome; glomerular filtration rate (GFR) decreases; and eventually, patients progress to ESRD [2]. Several factors affect kidney disease progression in patients with classical DN. High systolic blood pressure, albuminuria, poor blood glucose control, hypoalbuminemia, anemia, kidney dysfunction, and smoking have been shown to be associated with poor renal outcomes [3, 4].

The renin–angiotensin–aldosterone system (RAAS) plays a pivotal role in many of the pathophysiological changes that lead to kidney disease progression [5]. Pharmacological RAAS inhibition using angiotensin (Ang)-converting enzyme (ACE) inhibitors or Ang II receptor blockers ameliorates proteinuria and the decline in kidney function in patients with DN [6-8]. Measurement of the circulating components of RAAS in animal models and humans with diabetes mellitus is an inaccurate means of predicting the state of RAAS activation or its response to intrarenal inhibition [9]. Conversely, high intrarenal concentrations of RAAS components, and particularly of Ang II, have been shown to play a role in the progression of kidney damage in an animal model [10] and in the clinical progression of IgA nephropathy [11].

DN may feature low circulating concentrations of RAAS components and high local activity of the RAAS [12]. Plasma renin activity (PRA) is lower in patients with diabetes than in normal individuals [13-17], which implies a “renin paradox” [13, 18, 19]. The intrarenal RAAS also plays an important role in the pathogenesis of DN [20]. It was previously hypothesized that low PRA reflects high intrarenal Ang II production [13].
However, the association of PRA with the progression of kidney disease in patients with DN remains to be fully characterized.

Overt nephropathy is defined by the presence of macroalbuminuria (urine albumin-to-creatinine ratio ≥300 mg/g creatinine [Cr]) or persistent proteinuria (urine protein-to-creatinine ratio ≥0.5 g/g Cr), according to the 2014 Japanese classification of DN [21]. In the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study, DN was diagnosed in participants if their urine albumin-to-creatinine ratio was >300 mg/g Cr or their 24-h urine protein was >500 mg. In addition, a serum Cr (SCr) concentration of 1.3–3.0 mg/dL (1.5–3.0 mg/dL in men of >60 kg) was an inclusion criterion [2]. In the study conducted by Rossing et al., patients with type 2 diabetes and DN who had macroalbuminuria (albuminuria ≥300 mg/24 h) and a median SCr of 1.20 mg/dL (interquartile range [IQR], 0.56–3.12 mg/dL) were enrolled [4]. In another study of DN, the eligibility criteria included SCr <2.59 mg/dL, 24-h urinary proteinuria >500 mg, and the presence of diabetic retinopathy [13]. In this context, several studies have been conducted in patients with type 2 diabetes and overt nephropathy who presented with various degrees of kidney dysfunction. Furthermore, a previous study divided patients with type 2 diabetes and reduced kidney function into proteinuric (urine albumin-to-creatinine ratio ≥300 mg/g Cr) and non-proteinuric (urine albumin-to-creatinine ratio <300 mg/g Cr) diabetic kidney disease (DKD). Those with proteinuric DKD (overt nephropathy) had poor renal outcomes compared with those with non-proteinuric DKD [22]. Therefore, the current study aimed to determine the association between PRA and the decline in kidney function in patients with type 2 diabetes and overt nephropathy, including those with renal impairment.

Materials and Methods

Study design and population

We performed a 2-year prospective observational study of 118 patients with DN who were admitted to our hospital for chronic kidney disease assessment between June 2009 and September 2019. The inclusion criteria were as follows: type 2 diabetes mellitus, proteinuria ≥0.5 g/day, diabetic retinopathy, and SCr ≤4.0 mg/dL. The exclusion criteria were a history of partial nephrectomy for renal cell carcinoma, staghorn calculi of the kidneys, and/or severe renal atrophy. Of the 118 enrolled patients, 14 were lost to follow-up; three required dialysis for acute exacerbation of kidney function caused by infection, congestive heart failure, or surgery for femoral neck fracture; two died; and two complied poorly with the medication, and were therefore excluded. Therefore, data from 97 participants were analyzed. All the participants were discharged from the hospital without renal replacement therapy and were subsequently followed up. The Ethics Committee of the National Hospital Organization Kyushu Medical Center approved the study (approval number: 09-09 and 11-29), and all the participants provided their written informed consent.

Definition of outcomes

The annual change in estimated GFR (eGFR) over the entire study period was determined from the slope calculated during linear regression analysis of the eGFR and is expressed as mL/min/1.73 m²/year. The alternative study endpoint was a composite of doubling of SCr or ESRD. The presence of kidney dysfunction that required maintenance hemodialysis or peritoneal dialysis was taken to indicate the development of ESRD.

Data collection

After admission, all the participants were maintained on a hospital diet that included <6 g/day of salt. In the early morning after an overnight fast, the participants were placed in a supine position for blood sample collection. On the day of admission, 24-h urine collection was started, and the 24-h urinary sodium excretion, and daily proteinuria were measured. To convert mEq of sodium to grams of salt, the quantity of sodium in mEq was multiplied by 0.0585. B-type natriuretic peptide (BNP), PRA, and plasma aldosterone concentration (PAC) were simultaneously measured in each participant. The lower limits of detection for BNP, PRA, and PAC were 5.8 pg/mL, 0.1 ng/mL/h, and 10 pg/mL, respectively. For statistical analyses, 5.8 pg/mL, 0.1 ng/mL/h, and 10 pg/mL were the assigned concentrations of four samples with BNP, seven samples with PRA, and 26 samples with PAC below the lower limits, respectively. eGFR (mL/min/1.73 m²) was calculated using the new Japanese equation as follows: eGFR = 194 × SCr⁻¹.094 × age⁻⁰.²⁸⁷ × 0.⁷³⁹ (for women) [23].

We then measured the long and short axes of the kidneys of each participant using abdominal ultrasonography and recorded the mean sizes. The cardiothoracic ratio (CTR) at the time of admission was also measured.

All the participants underwent a clinical examination and were interviewed at presentation. Their medical history and outpatient records were also evaluated in detail. Demographic information (age and sex), atherosclerotic risk factors (hypertension, history of cigarette smoking, dyslipidemia, and diabetes mellitus), and the presence or absence of diabetic retinopathy were recorded. The current status or history of cigarette smoking was recorded.
Hypertension was defined as a systolic blood pressure of >140 mmHg, diastolic blood pressure of >90 mmHg, or the use of antihypertensive drugs. Dyslipidemia was defined as a plasma triglyceride concentration of >150 mg/dL, a plasma low-density lipoprotein cholesterol concentration >140 mg/dL, a plasma high-density lipoprotein cholesterol concentration <40 mg/dL, or the use of lipid-lowering drugs. Diabetes mellitus was defined as a history of or a current fasting plasma glucose concentration >126 mg/dL or the use of hypoglycemic agents. The duration of diabetes mellitus was recorded from the time of initial diagnosis. Body mass index was calculated as the patient’s weight in kilograms divided by height in meters squared. Previous prescriptions for RAAS inhibitors, calcium blockers, α- and β-blockers, and diuretics were reviewed for each participant at presentation.

**Statistical analysis**

Continuous data are expressed as mean ± SD or median (IQR), depending on their distribution, and categorical data are expressed as number (%). Participants were categorized according to tertile (T1–T3) of PRA. Prior to statistical analysis, PRA, PAC, and BNP, which were not normally distributed, were log-transformed to achieve approximately normal distributions. For non-parametric data, the significance of differences between two groups was evaluated using the Wilcoxon rank sum test. The relationships between two continuous variables were evaluated using Spearman’s rank correlation coefficients. The relationships between log PRA and other clinical parameters and that between PRA and the annual change in eGFR were also evaluated using linear regression analyses. Survival curves were constructed using the Kaplan–Meier method and evaluated using the log-rank test. Furthermore, the association between PRA and a composite of doubling of Scr or ESRD was evaluated using a Cox proportional hazards model. We also assessed the discriminative value of the model for composite renal outcomes using Harrell’s C-statistic. The models considered were: a basic model, adjusted for age, sex, smoking, systolic blood pressure, dyslipidemia, body mass index, daily proteinuria, hemoglobin, eGFR, serum albumin concentration, and log PAC; and the basic model with the addition of log PRA. Scores of 1.0 and 0.5 indicated perfect and poor discrimination, respectively. C-indexes and 95% CIs were calculated and compared using the somersd package and lincom commands, respectively [24]. All data were analyzed using STATA version 14 (Stata Corp., College Station, TX, USA) with p < 0.05 being accepted as statistically significant.

**Results**

**Baseline characteristics**

The 97 participants comprised 77 men and 20 women and had a mean age of 65 years (range, 30–89 years). Their median (IQR) PRA was 0.6 (0.3–1.4) ng/mL/h. Three participants were diagnosed with DN using renal biopsy and the remaining 94 were diagnosed on the basis of clinical findings. Table 1 summarizes the baseline clinical characteristics of the participants, according to PRA tertile. The duration of diabetes mellitus did not differ among the PRA tertiles. Regarding the use of prescription drugs, the prevalence of the use of RAAS inhibitors did not differ between the tertiles of PRA, whereas that for diuretic use increased from T3 to T1 of PRA. The median (IQRs) PRA for participants who were or were not using an RAAS inhibitor at baseline were 0.6 (0.3–1.4) and 0.4 (0.3–1.1) ng/mL/h, respectively (p = 0.93). The median (IQR) proteinuria also did not significantly differ between participants who were or were not using an RAAS inhibitor (proteinuria: 3.49 [1.85–6.96] g/day for those using an RAAS inhibitor and 3.25 [0.93–6.71] g/day for those not using an RAAS inhibitor; p = 0.44). Conversely, the median (IQR) eGFR was lower in participants who were using an RAAS inhibitor than in those who were not (eGFR: 23.1 [15.8–32.6] mL/min/1.73 m² vs. 41.8 [28.5–53.8] mL/min/1.73 m²; p < 0.01). All 10 participants who had not previously been prescribed an RAAS inhibitor were treated in the same way during follow-up. Conversely, six participants who were taking an RAAS inhibitor at baseline discontinued their use because of their possible side effects. At the end of the study period, 91 participants were being treated using an RAAS inhibitor. Of the 97 participants, three (3%), eight (8%), 24 (25%), 46 (47%), and 16 (16%) were categorized as having eGFR of ≥60 mL/min/1.73 m², 45 to <60 mL/min/1.73 m², 30 to <45 mL/min/1.73 m², 15 to <30 mL/min/1.73 m², and <15 mL/min/1.73 m², respectively. As the PRA decreased, daily proteinuria worsened and the serum albumin concentration decreased. eGFR and urinary salt excretion showed no significant differences between the tertiles of PRA, but BNP increased from T3 to T1 of PRA.

**Relationships of log PRA with other clinical parameters**

Table 2 shows the relationships between log PRA and other clinical parameters, assessed using univariable linear regression analyses. Log PRA was positively associated with serum albumin but inversely associated with systolic blood pressure, daily proteinuria, log BNP, and CTR. In contrast, there were no relationships between log PRA and RAAS inhibitor use, diuretic use, eGFR,
Table 1 Baseline clinical characteristics of patients according to tertiles of PRA

| Variables                  | All (n = 97) | PRA-T1 (n = 34) (≤-0.3 ng/mL/h) | PRA-T2 (n = 31) (0.4–0.9 ng/mL/h) | PRA-T3 (n = 32) (1.0–10.0 ng/mL/h) | p for trend |
|----------------------------|--------------|---------------------------------|----------------------------------|----------------------------------|-------------|
| Age (years)               | 65 ± 11      | 66 ± 11                         | 64 ± 10                          | 64 ± 12                          | 0.59        |
| Male, n (%)               | 77 (79)      | 26 (76)                         | 24 (77)                          | 27 (84)                          | 0.43        |
| Duration of diabetes (years) | 15 (9–22)  | 16 (10–24)                      | 13 (9–20)                        | 17 (10–23)                       | 0.85        |
| Smoking, n (%)            | 76 (78)      | 28 (82)                         | 22 (71)                          | 26 (81)                          | 0.90        |
| Dyslipidemia, n (%)       | 84 (87)      | 28 (82)                         | 27 (87)                          | 29 (91)                          | 0.33        |
| Hypertension, n (%)       | 96 (99)      | 34 (100)                        | 31 (100)                         | 31 (97)                          | 0.22        |
| RAAS inhibitors, n (%)    | 87 (90)      | 31 (91)                         | 27 (87)                          | 29 (91)                          | 0.93        |
| Calcium blockers, n (%)   | 79 (81)      | 30 (88)                         | 24 (77)                          | 25 (78)                          | 0.29        |
| α blockers, n (%)         | 13 (13)      | 7 (21)                          | 5 (16)                           | 1 (3)                            | 0.04        |
| β blockers, n (%)         | 28 (29)      | 9 (26)                          | 10 (32)                          | 9 (28)                           | 0.88        |
| Diuretics, n (%)          | 48 (49)      | 24 (71)                         | 11 (35)                          | 13 (41)                          | 0.01        |
| SBP (mmHg)                | 144 ± 18     | 149 ± 14                        | 145 ± 18                         | 137 ± 19                         | <0.01       |
| Body mass index (kg/m²)   | 22.6 (20.9–25.5) | 22.9 (20.9–25.5) | 22.1 (21.1–25.9) | 22.6 (20.8–25.4) | 0.97      |
| Daily proteinuria (g)     | 3.36 (1.77–6.76) | 5.00 (2.67–8.59) | 4.59 (2.56–7.14) | 2.70 (0.95–3.67) | <0.01     |
| Hemoglobin (g/dL)         | 10.5 ± 2.0   | 10.0 ± 2.0                      | 10.9 ± 2.0                       | 10.6 ± 2.0                       | 0.13       |
| Serum albumin (g/dL)      | 2.9 (2.3–3.5) | 2.7 (2.0–3.2)                   | 2.9 (2.3–3.4)                    | 3.4 (3.0–3.7)                    | <0.01      |
| Hemoglobin A1c (%)        | 6.6 (6.2–7.2) | 6.3 (6.0–7.2)                   | 6.7 (6.2–6.9)                    | 6.9 (6.3–7.4)                    | 0.03       |
| eGFR (mL/min/1.73 m²)     | 23.6 (16.6–36.2) | 19.7 (15.5–38.4) | 25.1 (17.4–42.9) | 23.7 (16.3–33.1) | 0.74      |
| eGFR ≥60 (mL/min/1.73 m²), n (%) | 3 (3)    | 1 (3)                          | 2 (6)                            | 0 (0)                            | 0.51        |
| eGFR 30 to <60 (mL/min/1.73 m²), n (%) | 8 (8)    | 3 (9)                          | 3 (10)                           | 2 (6)                            | 0.71        |
| eGFR 15 to <30 (mL/min/1.73 m²), n (%) | 24 (25)  | 8 (24)                         | 7 (23)                           | 9 (28)                           | 0.67        |
| eGFR <15 (mL/min/1.73 m²), n (%) | 46 (47)  | 15 (44)                        | 15 (48)                          | 16 (50)                          | 0.63        |
| eGFR (mmHg)               | 74 ± 11      | 76 ± 10                         | 74 ± 10                          | 71 ± 12                          | 0.09        |
| Daily proteinuria (g)     | 51.5 (26.9–111.9) | 70.3 (44.5–188.5) | 63.9 (26.4–138.3) | 31.6 (13.2–78.2) | <0.01      |
| PAC (pg/mL)               | 29.7 (10.0–65.9) | 22.8 (10.0–93.3) | 25.7 (14.6–43.0) | 44.5 (13.9–64.2) | 0.52       |
| Urinary salt excretion (g/day) | 7.12 (4.74–10.22)  | 7.32 (5.32–9.93)  | 8.80 (5.19–10.40) | 6.04 (4.38–10.48) | 0.64       |
| CTR (%)                   | 48.3 (43.9–51.0) | 49.5 (43.5–55.6) | 49.1 (44.7–52.0) | 46.8 (43.5–49.5) | 0.053      |
| Long axis of kidney (cm)  | 10.1 (9.3–11.0) | 10.3 (9.8–11.0) | 10.4 (9.5–11.1) | 10.0 (9.2–10.6) | 0.15       |
| Short axis of kidney (cm) | 5.3 ± 0.7    | 5.4 ± 0.6                       | 5.2 ± 0.6                        | 5.3 ± 0.8                        | 0.24       |

Values are expressed as the means ± SD, number (percent), or median (interquartile range).

Abbreviations: PRA, plasma renin activity; RAAS, renin–angiotensin–aldosterone system; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide; PAC, plasma aldosterone concentration; CTR, cardiothoracic ratio.

* Missing for 2 patients in T1 of PRA.

† Missing for 1 patient in T1 of PRA, 3 patients in T2 of PRA, and 2 patients in T3 of PRA.

* Missing for 6 patient in T1 of PRA, 5 patients in T2 of PRA, and 6 patients in T3 of PRA.

Changes in eGFR in each tertile of PRA were −8.8 (−18.5, −4.2) for T1, −8.0 (−14.3, −3.2) for T2, and −3.1 (−6.3, −2.0) for T3 (p for trend < 0.01). As shown in Fig. 1, log PRA had a significant positive relationship with the annual change in eGFR.
Table 3 shows the relationships between PRA and the annual change in eGFR in multivariable linear regression analyses. In the fully adjusted model (Model 3), log PRA (1-log unit increment) was significantly associated with the annual change in eGFR. Compared with T3 of PRA, T1 showed a significant inverse association with the annual change in eGFR.

Associations of PRA with composite renal outcomes
During the study, 46 participants manifested renal events (a composite of doubling of SCr or ESRD). In Kaplan–Meier analysis, the prevalence of renal events was significantly higher in participants in the lower tertiles (T1–T2) of PRA (Fig. 2). Table 4 shows the HRs for the composite renal outcomes associated with each PRA. In the fully adjusted model (Model 3), log PRA (1-log unit increment) was significantly associated with the composite renal outcomes, and T1 and T2 were significantly associated with poorer renal outcomes than T3.

Discriminative values for predicting composite renal outcomes
Regarding the discriminative values for composite renal outcomes, the C-indexes for the basic model and for the basic model + log PRA were 0.8148 (95% CI, 0.7583–0.8713) and 0.8474 (95% CI, 0.8001–0.8946), respectively. Thus, the addition of log PRA to the basic model significantly improved the discrimination for composite renal outcomes, showing an increase in Harrell’s C-statistic, with a difference of +0.0326 (p = 0.02).

Table 2  Association of log PRA with clinical parameters in univariable linear regression analyses

| Variables                                      | Coefficient (95% CI)     | p    |
|------------------------------------------------|--------------------------|------|
| Age (per 10-years increase)                    | –0.095 (–0.304, 0.114)   | 0.37 |
| Male                                           | 0.252 (0.328, 0.832)     | 0.39 |
| Smoking                                        | 0.035 (0.537, 0.607)     | 0.90 |
| Dyslipidemia                                   | 0.286 (0.403, 0.975)     | 0.41 |
| Use of RAAS inhibitors                         | 0.049 (0.725, 0.824)     | 0.90 |
| Use of diuretics                               | –0.436 (0.899, 0.026)    | 0.06 |
| Systolic blood pressure (per 10-mmHg increase) | –0.212 (–0.3338, –0.087) | <0.01|
| Body mass index (per 1-kg/m² increase)         | 0.022 (0.043, 0.087)     | 0.50 |
| Daily proteinuria (per 1-g/day increase)       | –0.114 (–0.175, –0.053)  | <0.01|
| Hemoglobin (per 1-g/dL increase)               | 0.073 (0.043, 0.188)     | 0.21 |
| Serum albumin (per 1-g/dL increase)            | 0.860 (0.562, 1.158)     | <0.01|
| eGFR (per 1-mL/min/1.73 m² increase)           | –0.007 (–0.021, 0.008)   | 0.34 |
|^Log BNP (per 1-log unit increase)              | –0.399 (–0.580, –0.219)  | <0.01|
|^Log PAC                                        | 0.238 (0.006, 0.483)     | 0.06 |
|^Urinary salt excretion (per 1-g/day increase)  | –0.033 (–0.110, 0.044)   | 0.39 |
|^CTR (per 1-% increase)                         | –0.042 (–0.082, –0.003)  | 0.04 |

Abbreviations: PRA, plasma renin activity; CI, confidence interval; RAAS, renin-angiotensin-aldosterone system eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide; PAC, plasma aldosterone concentration; CTR, cardiothoracic ratio.

a Missing for 6 patients.
b Missing for 17 patients.
Sensitivity analyses

Log BNP showed a strong inverse association with log PRA (Table 2). Therefore, we also performed sensitivity analyses for the 91 participants with available BNP data (Supplementary Table 1). After adjustment for the addition of log BNP to the covariates included in Model 3 (Tables 3 and 4), log PRA (1-log unit increment) was associated with the annual change in eGFR (coefficient,
analyses showed that log PRA (1-log unit increment) (the lowest tertile of PRA) was associated with the annual change in eGFR (T1: coefficient, –4.536; 95% CI, –8.263 to –0.809). Additionally, multivariable Cox analyses showed that log PRA (1-log unit increment) was associated with composite renal outcomes (HR, 0.62; 95% CI, 0.42–0.90) and that T1 and T2 were associated with significantly higher risks of poor renal outcomes than T3 (T2: HR, 4.44; 95% CI, 1.52–12.93; T1: HR, 4.26; 95% CI, 1.39–13.02; p for trend = 0.02).

After the exclusion of 10 participants who were not taking an RAAS inhibitor at baseline, 87 were evaluated using alternative sensitivity analyses (Supplementary Table 2). After adjustment for the covariates included in Model 3 (Tables 3 and 4), log PRA (1-log unit increment) was associated with the annual change in eGFR (coefficient, 1.698; 95% CI, 0.221–3.175); and compared with T3, T1 was associated with the annual change in eGFR (coefficient, –4.732; 95% CI, –8.541 to –0.922 for T1). In multivariable Cox analyses, log PRA (1-log unit increment) was associated with the composite renal outcome (HR, 0.58; 95% CI, 0.39–0.87), and T1 and T2 were associated with poorer renal outcomes than T3 (T2: HR 4.16, 95% CI; 1.41–12.22; T1: HR, 4.62; 95% CI, 1.50–14.27; p for trend <0.01).

Discussion

In this 2-year prospective observational study, we investigated the associations of PRA with the progression of kidney disease in patients with DN. Low PRA was found to be associated with a rapid decline in kidney function and participants with low PRA were found to be at a higher risk of a composite of doubling of Scr or ESRD. To the best of our knowledge, this is the first study to demonstrate a significant association between PRA and renal outcomes in patients with DN.

Local activation of the RAAS is important in various tissues, including the brain, heart, adrenal glands, vasculature, and kidneys [25]. In particular, the intrarenal RAAS is unique because all the components necessary to generate intrarenal Ang II are present along the nephron, in the glomerulus and interstitial and intratubular compartments [25-27]. In a diabetic milieu, angiotensinogen expression is high in proximal tubular cells and is induced in glomerular mesangial cells [28-30]. Urinary angiotensinogen may also represent a biomarker of early dysregulation of the intrarenal RAAS in DN [25]. In diabetes, high glomerular ACE activity and low glomerular ACE2 activity result in the excess accumulation of glomerular Ang II, causing albuminuria and/or glomerular injury [26]. Compared with healthy individuals, low ACE2 and high ACE expression have been shown in both the tubulointerstitium and glomeruli of patients with type 2 diabetes and overt nephropathy [27]. Hyperglycemia may activate the intrarenal RAAS within glomeruli and proximal tubules, thereby triggering the production of local Ang II, which may cause feedback inhibition of systemic renin release [9]. Accordingly, high intrarenal Ang II production might explain the low PRA in patients with DN [13]. Furthermore, greater Ang II generation is attributable to the progression of DN, involving several hemodynamic, tubular, and growth-promoting effects [19]. Given these findings, the declining kidney function in patients with low PRA that was identified in the present study might explain the link between low PRA and the activation of the intrarenal RAAS, generating Ang II.

Several studies have addressed the association between the intrarenal RAAS and proteinuria/albuminuria in patients with diabetes. Proteinuria positively correlates with the mRNA expression of ACE and ACE2 in the urinary tract of patients with DN [31]. In patients with type 1 diabetes, a high urine albumin/creatinine ratio is associated with high urinary tract angiotensinogen and ACE activities [18]. Urinary tract angiotensinogen is higher in patients with type 2 diabetes than in healthy individuals, and it progressively increases as they transition from normo- to micro- to macroalbuminuria [32]. Furthermore, Sawaguchi et al. demonstrated that urinary tract angiotensinogen positively correlates with the urine albumin/creatinine ratio and that high urinary tract angiotensinogen expression is associated with a decline in kidney function in patients with diabetes. In addition, high urinary tract angiotensinogen expression may be associated with greater intrarenal RAAS activation in such patients [33]. Therefore, substantial proteinuria or albuminuria may be associated with high intrarenal RAAS activity. Furthermore, a previous study demonstrated that PRA is significantly lower in patients with diabetes and macroalbuminuria than in those with normo- or microalbuminuria [17]. In the present study, lower PRA was also found to be associated with greater proteinuria (Tables 1 and 2), which might reflect activation of the intrarenal RAAS.

It has been demonstrated that high plasma prorenin activity is common in patients with diabetes and that high plasma prorenin concentration is associated with microalbuminuria [34] and DN [35]. (Pro)renin receptor (P)RR is a single transmembrane protein that binds renin and prorenin with equal affinity and is widely expressed, including in the brain, heart, liver, and kidney [36]. The binding of prorenin to the extracellular domain of (P)RR causes non-proteolytic activation of renin [37], which accelerates the conversion of angiotensinogen to Ang I. Therefore, (P)RR plays an important role in tissue Ang II
generation [36]. Ichihara et al. showed that diabetic rats have significantly lower PRA and higher prorenin concentrations than control rats, and also that the development and progression of DN is associated with more marked increases in kidney Ang I and II concentrations. Moreover, treatment with the “handle region” peptide of prorenin, which acts as a decoy peptide to inhibit the non-proteolytic activation of prorenin, reduces the renal concentrations of Ang I and II and inhibits the development of DN. These findings suggest that the non-proteolytic activation of prorenin contributes to the activation of the intrarenal RAAS [38]. In contrast, soluble (P)RR is generated intracellularly by the cleavage of furin, is found in rat and human plasma, and can bind prorenin [39]. In patients with essential hypertension, the soluble (P)RR concentration positively correlates with urinary tract angiotensinogen expression [40], which is a biomarker of intrarenal RAAS in these individuals [41]. It has also been suggested that soluble (P)RR might represent a marker of intrarenal RAAS activation in patients with diabetes [42].

Mineralocorticoid receptors (MRs) are expressed in various tissues in humans, including the kidney [43]. The pathophysiological implications of an increase in MR expression and activation (either aldosterone-dependent or direct ligand-independent activation) and its blockade have been documented in in vitro and in vivo experimental studies [44]. The small GTPase Rac1 has been identified as a ligand-independent modulator of MR activity [45]. In hypertensive mice, high salt intake led to hypertension and kidney injury, and simultaneously reduced PAC [46]. In contrast, renal MR expression increases in diabetic rats [47]. It has been reported that high glucose stimulates MR transcriptional activity via Rac1 in a ligand-independent manner [48]. In patients with DN, the albinuria-lowering effect of the MR blocker spironolactone is independent of both the baseline levels and changes in PAC [49]. In addition, a previous study demonstrated that hyperglycemia in diabetes, independent of PAC, induces podocyte injury through MR-mediated reactive oxygen species (ROS) production and leads to proteinuria in diabetic rats, and that spironolactone prevents kidney injury by reducing ROS production [50]. In addition, it has been suggested that the activation of the Rac1-MR pathway by a high glucose concentration might explain the relationship between diabetic milieu, kidney injury, and MR-mediated ROS production, independent of PAC [48].

In response to myocardial stretching, atrial natriuretic peptide (ANP) and BNP are secreted from cardiomyocytes into the circulation [51], and high ANP and BNP concentrations are indicative of volume overload [52, 53]. In a previous study, patients with diabetes who had poor glycemic control also had a higher ANP concentration and lower PRA than those with moderate glycemic control or healthy individuals, and ANP concentration was inversely related to PRA in patients with diabetes [54]. Similarly, another study showed that patients with diabetes have higher ANP concentrations and lower PRAs than healthy individuals [14]. In addition, high tubular Ang II activity, which may be followed by hyperglycemia-induced angiotensinogen synthesis in proximal tubular cells, can directly stimulate sodium and water reabsorption, thereby promoting extracellular fluid volume expansion and subsequently inhibiting the release of juxtaglomerular cell-derived renin into the circulation [9]. Thus, low PRA may reflect fluid retention status.

In the present study, CTR, which is commonly used to assess volume status [55], was measured alongside the BNP concentration. Univariable linear regression analyses showed that both log BNP and CTR were inversely associated with log PRA, suggesting that patients with low PRA tend to be in a state of volume overload. We also conducted sensitivity analyses of data from 91 participants for whom BNP data were available. After adjustment by the addition of log BNP to the other covariates included in Model 3 (Tables 3 and 4), low PRA was found to be associated with poor renal outcomes (Supplementary Table 1). Hence, low PRA may indicate a significant risk of kidney disease progression in patients with DN, independent of fluid retention. In general, the administration of diuretics increases PRA by causing water and electrolyte deprivation, but in the present study, the prevalence of diuretic administration increased from T3 to T1 of PRA. Given the possibility that patients with lower PRA had volume overload, the higher prevalence of diuretic use in participants in the lower tertiles of PRA might be attributable to the higher prevalence of edema in patients with low PRA, although we could not assess the edema of each participant at enrollment.

In the present study, the prevalence of RAAS inhibitor use at baseline did not differ among the tertiles of PRA. The median PRA also did not differ between participants who were or were not using an RAAS inhibitor at baseline. However, the differences between the two groups were difficult to evaluate because the number of participants who were not taking an RAAS inhibitor was much lower than the number who were taking such medication. Nevertheless, these results suggest that RAAS inhibitor use did not affect PRA in participants with DN at baseline. To reduce the influence of the use
Low PRA was found to be independently associated with kidney disease progression in the 87 participants who were using an RAAS inhibitor (Supplementary Table 2). Low PRA was found to be independently associated with kidney disease progression, similar to the results shown in Tables 3 and 4. Therefore, patients with low PRA were at a higher risk of a decline in kidney function, despite their use of RAAS inhibitors.

The present study had some limitations that should be acknowledged. First, all the participants were recruited from a single regional hospital. Therefore, the sample was fairly homogeneous and the sample size was relatively small, which might imply selection bias. We calculated the required sample size using Fisher’s z test, comparing one correlation to a reference value. For a significance level of 0.05, a power of 0.8, and a correlation coefficient of 0.300, the estimated required sample size was 85. In the present study, the correlation coefficient for relationship between log PRA and the annual change in eGFR was 0.407, as shown in Fig. 1. Therefore, a sample size of 97 should have been appropriate for the evaluation of this relationship. Second, although DN was clinically diagnosed, a histological diagnosis was not documented in 94 participants. Further studies are also needed to investigate whether PRA is related to the degree of glomerulosclerosis or tubulointerstitial damage. Third, we could not evaluate the duration of RAAS inhibitor use or the dose, which could affect PRA, before enrollment. Fourth, we could not assess whether a sufficient quantity of the hospital diet was consumed by each participant. Therefore, it is possible that some of the participants did not consume enough of the diet, such that their salt intake may have been underestimated. In addition, 24-h urine collections were performed between the first and second days of admission, and this timing does not fully exclude the possibility that the 24-h urinary salt excretion measured in the present cohort may have been affected by dietary sodium intake prior to admission. Fifth, the findings of the present study should be interpreted with some caution. Given the role of PRA in the regulation of fluid volume, it remains unknown whether low PRA is the cause or consequence of greater proteinuria, low serum albumin, high BNP, high CTR, and high systolic blood pressure. In addition, in the present cohort, the prevalence of advanced kidney dysfunction (eGFR <30 mL/min/1.73 m²) at baseline was high, as shown in Table 1. Accordingly, when exploring the risk factors for poor renal outcomes in the present cohort, which included a large number of participants with advanced kidney dysfunction, it should be borne in mind that those with advanced kidney dysfunction would have been at a fundamentally higher risk of progression to ESRD during the 2 years of the study. Finally, a single PRA measurement may not have been a sufficiently accurate means of predicting renal outcomes.

In conclusion, low PRA is associated with poor renal outcomes in patients with DN, which suggests that PRA may represent a useful predictor of the renal prognosis of such patients.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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