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Higher plasma renin activity is a risk factor for total mortality in older Japanese individuals: the Takahata study

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ABSTRACT

Plasma renin activity (PRA) is accepted as a marker for increased risk of cardiovascular diseases. However, the association between PRA and total mortality has not been fully explored in a general population. We here examined whether PRA is associated with increased total mortality in a general Japanese population. The participants of the Takahata study (3502 subjects; age, 62.5 ± 10.4 years), a population-based, longitudinal study of Japanese held from 2004 to 2006, were enrolled and followed up for up to 7 years. The incidence of death and causes of death were monitored annually to the end of 2010 (median follow-up, 2280 days). During the follow-up period, 143 subjects died. Kaplan-Meier analysis showed a significantly increased risk for total mortality in subjects with higher PRA (log-rank $P < .001$). Cox proportional hazard model analyses with adjustment for factors correlated with PRA (age, sex, weight, diastolic blood pressure, high-density lipoprotein cholesterol, uric acid, B-type natriuretic peptide, serum total protein, antihypertensive treatment, and diabetes) showed that higher PRA was associated with increased total mortality in linear regression models (per 1 increase in log 10 × PRA [nanograms per milliliter per hour]: hazard ratio, 2.12; 95% confidence interval, 1.47–3.06), between groups of patients stratified by quartiles of PRA (highest vs lowest quartile: 2.63, 1.57-4.41) and in subjects with high (≥ 2.0 ng/[mL h]) vs low (<2.0 ng/[mL h]) PRA (1.97, 1.37-2.83). Higher PRA was a significant and independent risk factor for increased total mortality in this Japanese population and may be a marker for subjects at an increased risk of total mortality.

Author contributions: M Daimon designed the study. M Daimon, T Konta, T Saito, I Kubota , T Kayama, and T Kato conducted the study. M Daimon, T Oizumi, S Karasawa, W Kaino, K Takase, Y Jimbu, K Wada, W Kameda, S Susa, T Konta, and T Saito were responsible for data collection. M Daimon did data analysis and wrote the report. All authors contributed to the data interpretation. This study was supervised by T Kato.

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1. Introduction

The renin-angiotensin system (RAS) is a hormonal cascade that is initiated by renin [1-4]. In the first step, renin cleaves angiotensinogen to angiotensin I, which is then converted by angiotensin-converting enzyme (ACE) to angiotensin II (AngII), the dominant effector molecule of the RAS [1-4]. The RAS is a major regulator of blood pressure through its modulation of salt and water homeostasis and is involved in the systemic regulation of cardiovascular homeostasis [1,4-8]. Local RAS activity has been identified in most organs including the liver, kidney, pancreas, brain, reproductive organs, digestive organs, vasculature, and adipose tissue [2,3,9]. The local RAS acts in an autocrine, paracrine, and/or intracrine manner and promote multiple physiological functions at local levels, including tissue angiogenesis, cellular proliferation, apoptosis, generation of reactive oxygen species, and inflammation [2,3,9]. As expected, the RAS is involved in various pathological conditions such as cancer, liver fibrosis, infection, obesity, and diabetes [2,3,10-13], as well as cardiovascular diseases (CVDs) [1,4-9]. Therefore, although the local RAS has been found to be associated with CVD and cancer [1-9], it may greatly influence other pathological conditions and hence be associated with death caused by a range of clinical conditions, not just those related to CVD or cancer.

Plasma renin activity (PRA) is accepted as a surrogate marker for the activation of the RAS and was reported to be a marker for increased risk of CVD [4,14-18]. However, few studies have investigated whether PRA is also a marker for increased mortality in a general population. Interestingly, the Framingham Heart Study of white subjects showed that plasma renin levels were associated with all-cause (total) mortality in a general population [15,16]; but no studies have examined the associations between PRA and total mortality in a general population of other ethnicities. We conducted a prospective cohort study of people living in Takahata and examined whether PRA is associated with increased total mortality in a general Japanese population.

2. Materials and methods

2.1. Subjects

The Takahata study is a population-based cross-sectional study of Japanese people of older than 35 years that was performed to identify possible risk factors for lifestyle-related diseases, such as diabetes and hypertension [19]. Takahata is an agricultural and suburban area about 300 km north of Tokyo. In 2005, among 26 026 people living in Takahata, 15 819 were older than 35 years. Between 2004 and 2006, 3520 residents were enrolled in the Takahata study. Of these, 3502 subjects (mean age, 62.5 ± 10.4 years; men/women, 1572/1930) who had complete clinical data at baseline were enrolled in the study. The incidence of death was monitored annually to the end of 2010. There were a total of 143 deaths in this time. The causes of the death were determined by reviewing death certificates through to the end of 2009. The causes of deaths in 2010 were unknown (n = 36). Death certificates of the deceased participants were collected with permission from the Management and Coordination Agency of the Japanese government once yearly. The death code (*International Classification of Diseases, 10th Revision*) and the date and place of death were reviewed. Subjects who moved away during the follow-up period were identified by residence transfer documents (n = 39). The median and maximum durations of follow-up were 2280 and 2385 days, respectively. To further evaluate the association between PRA at baseline and mortality, the subjects were stratified into quartiles of PRA (quartiles 1-4: ≤0.4, 0.5-0.9, 1.0-1.9 and ≥2.0 ng/[mL h], respectively) and into patients with high (≥2.0 ng/[mL h]) or low (<2.0 ng/[mL h]) PRA.

This study was approved by the Ethics Committee of the Yamagata University School of Medicine, and written informed consent was obtained from all participants. Blood samples were collected by phlebotomy, mostly between 7:00 AM and 10:00 AM, in a sitting position after at least 5 minutes of rest. Plasma renin activity was determined using a radioimmunoassay (Renin-RIA bead; Abbot Japan, Tokyo, Japan). The following clinical characteristics were also measured: height, body weight, body mass index, fasting plasma glucose (FPG), hemoglobin A_{1c} (HbA_{1c}), fasting serum insulin, insulin resistance indexes assessed by homeostasis model assessment using FPG and insulin levels (HOMA-IR), systolic blood pressure, diastolic blood pressure, total serum levels of total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, remnant-like particle (RLP) cholesterol, B-type natriuretic peptide (BNP), heart-type fatty acid–binding protein (H-FABP), total protein, uric acid, urea nitrogen, creatinine, ACE, adiponectin and homocysteine, and urine albumin levels. Hemoglobin A_{1c} (percentage) is expressed as that measured by the previous Japanese standard substance and measurement method (Japan Diabetes Society value), which is about 0.4% less than the National Glycohemoglobin Standardization Program value [20]. For precise evaluation of HOMA-IR, subjects with FPG levels of at least 140 mg/dL and on medication for diabetes were excluded from the analysis (n = 211). *Diabetes* was defined according to the 1998 World Health Organization criteria (FPG levels ≥126 mg/dL) [21]. In subjects whose FPG levels were not measured, *diabetes* was defined as postprandial glucose levels of at least 200 mg/dL. Subjects with HbA_{1c} levels of at least 6.1% were also defined as diabetic, as were those on medication for diabetes. Subjects known to have type 1 diabetes mellitus were excluded from the study. The number of subjects with diabetes was 347. *Hypertension* was defined as blood pressure of at least 140/90 mm Hg or as being on treatment of hypertension (n = 1,941). The classes of antihypertensive drugs taken were not monitored. *Hyperlipidemia* was defined as total cholesterol of at least 240 mg/dL, triglycerides of at least 150 mg/dL, or as being on treatment for hyperlipidemia. (n = 1120). Alcohol intake (current or nondrinker) and smoking habits (never, past, or current) were evaluated by questionnaire. The clinical characteristics of the study population are shown in Table 1.

In some subjects (n = 1863), daily salt consumption was estimated using the brief type of the self-administered diet

Table 1 – Baseline characteristics of the subjects

Characteristics	Men	Women	P
n	1572	1930	–
PRA			
ng/(mL h)	2.01 ± 2.74	1.22 ± 1.99	<.001 †
log 10 × ng/(mL h)	1.06 ± 0.47	0.84 ± 0.46	<.001 †
Age (y)	62.9 ± 10.4	62.2 ± 10.3	.044
Height (cm)	163.5 ± 6.9	151.2 ± 6.3	<.001 †
Body weight (kg)	63.0 ± 9.8	53.8 ± 8.6	<.001 †
Body mass index (kg/m^2)	23.5 ± 2.9	23.5 ± 3.4	.949
FPG (mg/dL) [a]	97.4 ± 19.5	92.7 ± 14.3	<.001 †
HbA_{1c} (%)	5.26 ± 0.76	5.26 ± 0.62	.836
Fasting serum insulin (μU/mL) [b]	5.1 ± 3.4	6.0 ± 3.6	<.001 *
HOMA-IR [b]	1.204 ± 0.868	1.383 ± 0.913	<.001 †
Systolic blood pressure (mm Hg)	136.1 ± 15.6	133.0 ± 15.9	<.001 †
Diastolic blood pressure (mm Hg)	82.0 ± 9.8	77.8 ± 9.8	<.001 †
Total cholesterol (mg/dL)	193.6 ± 31.4	206.8 ± 31.3	<.001 †
Triglyceride (mg/dL)	118.1 ± 79.6	98.6 ± 47.4	<.001 †
HDL cholesterol (mg/dL)	56.2 ± 14.5	61.5 ± 14.3	<.001 †
LDL cholesterol (mg/dL)	119.0 ± 29.6	128.7 ± 30.0	<.001 †
RLP cholesterol (mg/dL)	7.31 ± 5.12	6.14 ± 2.73	<.001 †
BNP (10 × log pg/mL)	12.6 ± 4.0	13.3 ± 3.4	<.001 †
H-FABP (ng/mL)	4.06 ± 2.41	3.71 ± 1.99	<.001 †
Serum total protein (g/dL)	7.48 ± 0.45	7.49 ± 0.42	.374
Serum uric acid (mg/dL)	5.79 ± 1.33	4.49 ± 1.07	<.001 †
Serum urea nitrogen (mg/dL)	16.7 ± 5.0	15.8 ± 4.1	<.001 †
Serum creatinine (mg/dL)	0.78 ± 0.27	0.59 ± 0.11	<.001 †
ACE (mg/dL)	15.1 ± 5.8	14.7 ± 5.1	.015 *
Adiponectin (10 × log μg/mL)	8.5 ± 2.3	10.2 ± 2.2	<.001 †
Homocysteine (nmol/mL)	12.6 ± 6.8	10.0 ± 3.7	<.001 †
Urinary albumin (mg/g Cr) [c]	35.9 ± 175.9	28.5 ± 102.0	.118
Hypertension, n (%)			
All	925 (58.8)	1016 (52.6)	<.001 †
On antihypertensive treatment	501 (31.9)	677 (35.1)	.046 *
Hyperlipidemia, n (%)	472 (30.0)	648 (33.6)	.025 *
Diabetes, n (%)	185 (11.8)	162 (8.4)	<.001 †
Drinking alcohol, n (%)	1144 (72.8)	315 (16.3)	<.001 †
Smoking (never/past/current), n	601/420/551	1773/53/104	<.001 †

Data are mean ± SD or number of subjects (percentage).
[a] Data were not obtained for some subjects, most of whom were known to be diabetic before the baseline examination (n [male/female]: 1476/1801).
[b] Subjects whose FPG levels were at least 140 mg/dL and who were being treated for diabetes were excluded (n [male/female]: 1358/1708).
[c] Data were not obtained for some subjects, most of whom were unable to urinate at the health examinations (n [male/female]: 1559/1898).
* $P < .05$.
† $P < .01$.

history questionnaire, which is a 58-item fixed-portion–type questionnaire developed for the assessment of Japanese diets during the previous month using semiweighed dietary records as a reference. The questionnaire requires the recall of dietary habits over a 1-month period [19,22]. Because salt consumption as a proxy for dietary sodium balance influences PRA [23], adjustment for salt consumption seems to be important to evaluate the association between PRA and total mortality. Therefore, although salt consumption was estimated in only about half of the subjects, we conducted an association study with these subjects as a subanalysis.

2.2. Statistical methods

The clinical characteristics are given as means ± SD. The statistical significance of the differences in characteristics values between 2 groups (parametric) and a case-control association between the groups stratified by PRA and the incidence of death (nonparametric) were assessed by Student *t* test and χ^2 tests, respectively. Correlations between individual factors and PRA were initially determined by univariate linear regression analyses followed by stepwise multiple linear regression analyses using those characteristics showing significant correlations in univariate analyses as covariates. For stepwise multiple linear regression analysis, the serum levels of total and HDL cholesterol were included as proxies of lipid metabolism and were correlated with PRA. For all analyses, the serum levels of BNP and adiponectin and the urine albumin levels were log-transformed (log10) to approximate a normal distribution. A value of $P < .05$ was accepted as statistically significant.

Mortality rates were compared among the subjects stratified by PRA using the Kaplan-Meier method. Multivariate Cox regression models were used to calculate the hazard ratios (HRs) of PRA for mortality with adjustment for factors correlated with PRA (ie, age, sex, weight, hypertension, diastolic blood pressure, HDL cholesterol, uric acid, BNP, urea nitrogen, serum protein, and diabetes). Odds ratios (ORs) for mortality among subjects with high PRA vs those with low PRA were calculated using multiple logistic regression analysis with adjustment for the factors listed above. All analyses were done using StatView software version 5.0 (SAS Institute, Cary, NC).

3. Results

3.1. Clinical characteristics of the study subjects

The clinical characteristics of the subjects at baseline are shown in Table 1. Most of the clinical characteristics, including PRA ($P < .001$), were significantly different between men and women. Therefore, we examined the factors associated with PRA in each sex separately. Plasma renin activity was not normally distributed and was highly skewed toward larger values, as previously reported [24]. Therefore, PRA values were log-transformed to approximate a normal distribution for statistical analyses. The minimum, mean, median, and maximum values of PRA (in nanograms per milliliter per hour) were 0.1, 2.01, 1.2, and 38.5 for men and 0.1, 1.22, 0.7, and 43.8 for women, respectively.

3.2. Factors correlated with PRA

As shown in Table 2, univariate linear regression analyses showed that antihypertensive treatment; diabetes and its related characteristics such as FPG and HOMA-IR; hyperlipidemia; and serum levels of total cholesterol, H-FABP, total protein, uric acid, creatinine, and homocysteine were positively correlated with PRA, whereas blood pressures and serum BNP levels were negatively correlated with PRA in both sexes. In men, serum levels of HDL cholesterol and urea

Table 2 – Correlations between clinical characteristics and PRA

Characteristics	Men			Women		
	Univariate	Stepwise		Univariate	Stepwise	
		Model 1	Model 2		Model 1	Model 2
r^2	–	0.176	0.187	–	0.132	0.145
Age (y)	0.016	–	–	–0.019	–	–
Height (cm)	–0.050 *	–	–	0.039	–	–
Body weight (kg)	–0.062 *	–0.120	–0.168	0.012	NA	NA
Body mass index (kg/m^2)	–0.044	–	–	–0.013	–	–
FPG (mg/dL)[a]	0.113 †	–	0.074	0.136 †	–	0.134
HbA_{1c} (%)	0.034	–	–	0.057 *	–	–
Fasting serum insulin (μU/mL)[b]	0.077 †	–	–	0.146 †	–	–
HOMA-IR[b]	0.093 †	–	0.077	0.157 †	–	NA
Systolic blood pressure (mm Hg)	–0.052 *	NA	–0.129	–0.093 †	–0.096	–0.125
Diastolic blood pressure (mm Hg)	–0.067 †	–0.111	NA	–0.117 †	–0.115	–0.100
Total cholesterol (mg/dL)	0.069 †	NA	NA	0.071 †	NA	NA
Triglyceride (mg/dL)	0.048	–	–	0.006	–	–
HDL cholesterol (mg/dL)	0.063 *	0.058	0.073	0.026	NA	0.056
LDL cholesterol (mg/dL)	0.031	–	–	0.078 †	–	–
RLP cholesterol (mg/dL)	0.012	–	–	–0.055 *	–	–
BNP (10 × log pg/mL)	–0.220 †	–0.265	–0.264	–0.213 †	–0.220	–0.226
H-FABP (ng/mL)	0.056 *	–	–	0.054 *	–	–
Serum total protein (g/dL)	0.203 †	0.153	0.124	0.179 †	0.147	0.138
Serum uric acid (mg/dL)	0.140 †	0.116	0.114	0.129 †	0.088	0.074
Serum urea nitrogen (mg/dL)	0.106 †	0.072	0.061	0.044	NA	NA
Serum creatinine (mg/dL)	0.053 *	NA	NA	0.089 †	NA	NA
ACE (mg/dL)	0.012	–	–	–0.056 *	–	–
Adiponectin (10 × log μg/mL)	–0.055 *	NA	NA	–0.040	NA	NA
Homocysteine (nmol/mL)	0.063 *	NA	NA	0.065 †	0.071	0.072
Urinary albumin (10 × log mg/g Cr)	–0.001	–	–	–0.056 *	–	–
Hypertension, n (%)						
All	0.009	–	–	0.012	–	–
On antihypertensive treatment	0.181 †	0.221	0.219	0.090 †	0.153	0.145
Hyperlipidemia, n (%)	0.071 †	–	–	0.063 †	–	–
Diabetes, n (%)	0.068 †	0.058	–	0.051 *	0.054	–
Drinking alcohol, n (%)	0.029	–	–	0.014	–	–
Smoking (never/past/current), n	–0.027	–	–	–0.001	–	–

Correlation coefficients (r) are shown. Model 1: Body weight, systolic and diastolic blood pressures, total and HDL cholesterol, BNP, total protein, uric acid, urea nitrogen, creatinine, adiponectin, homocysteine, antihypertensive treatment, and diabetes (vs nondiabetes) were included as covariates. Model 2: Covariates were the same as those included in model 1 except that diabetes was replaced with continuous variables (FPG and HOMA-IR). "–" indicates not included in multiple regression analysis; NA, not accepted as significant in stepwise multiple regression analysis.

* $P < .05$ (univariate regression analysis).

† $P < .01$ (univariate regression analysis).

nitrogen were positively correlated with PRA, whereas body weight and serum adiponectin levels were negatively correlated with PRA. In women, serum LDL cholesterol levels were positively correlated with PRA, whereas serum levels of RLP cholesterol and ACE and urine albumin levels were negatively correlated with PRA. Stepwise multivariate linear regression analysis showed that antihypertensive treatment; diabetes and its related characteristics; serum levels of HDL cholesterol, BNP, total protein, and uric acid; and blood pressures were independently associated with PRA in both sexes.

3.3. *Higher PRA is associated with increased risk of total mortality*

Mortality rates among groups of subjects stratified by PRA were compared using the Kaplan-Meier method, as shown in Fig. 1. During the follow-up, 143 (4.08%) subjects died. The rates of total mortality were significantly higher among subjects belonging to quartile 4 of PRA (the number of death of quartiles 1-4 were 24, 32, 32, and 55, respectively) or the high-PRA group compared with the other groups of subjects ($P < .001$). Of the high-PRA group, 52 subjects had very high PRA (>10 ng/[mL h]), of which 5 subjects died during the follow-up. The rate of total mortality of the very high-PRA group was, although not significant, even higher than that of the high-PRA group (9.1% vs 6.9%, $P = .454$).

Next, Cox proportional hazard model analysis was used to adjust for the differences in clinical characteristics among the groups, which could account for the observed differences in the mortality. Because several characteristics were independently associated with PRA in this study, we included all of these characteristics as covariates in the model, although for the characteristics belonging to a certain condition, such as hypertension, dyslipidemia, and diabetes, the characteristic

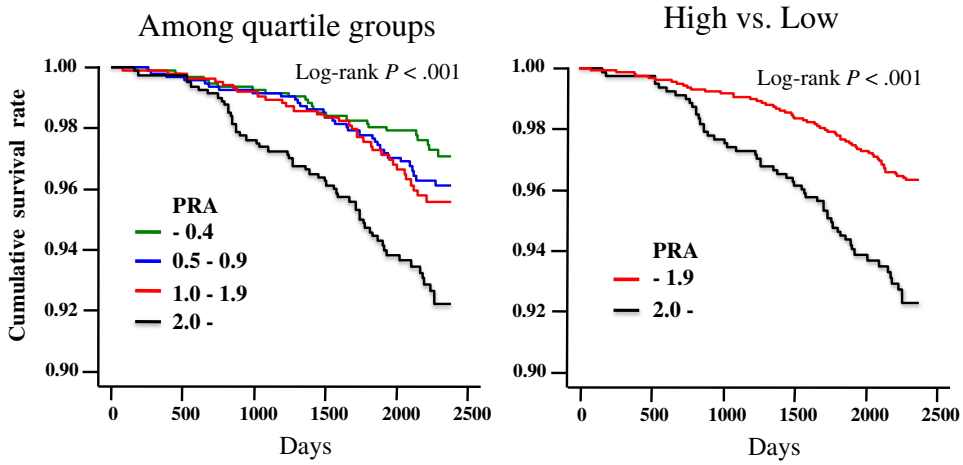

Fig. 1 – Kaplan-Meier survival curves for the quartiles of PRA (left panel) and in 2 groups of subjects with high or low PRA (right panel). The significances of the differences among the groups were assessed using log-rank tests, and the P values are indicated on each panel. Values of P < .05 were considered statistically significant.

found to be most correlated was included as a characteristic representing such condition. The covariates included age; weight; antihypertensive treatment; diabetes; diastolic blood pressure; serum levels of HDL cholesterol, uric acid, BNP, and total protein; and, when appropriate, sex. As shown in Table 3, higher PRA was associated with increased mortality in linear analyses (per 1 log10 × ng/[mL h] PRA increase: HR, 2.12; 95% confidence interval [CI], 1.47-3.06), among quartiles (Q4 vs Q1: 2.63, 1.57-4.41), and in the high- vs low-PRA groups (1.97, 1.37-2.83). Although no correlation between PRA and hypertension was observed, hypertension rather than antihypertensive treatment might be an important confounder for the analysis because the association between PRA and coronary heart disease has been postulated to be specific in hypertensive subjects [25]. However, substituting hypertension for antihypertensive treatment as a covariate for the analysis did not change the association between PRA and total mortality substantially (eg, 1.91, 1.34-2.74 for the high- vs low-PRA groups).

3.4. *Association between PRA and CVD-related mortality*

We next examined the association between PRA and cause of death-specific mortality. The causes of death were varied, and the total number of death was relatively low in this period. Therefore, we grouped the causes of death into the following categories: cancer (n = 49), CVD (n = 22), others (infections [n = 11], accidents [n = 8], heart diseases other than coronary heart disease [which belongs to CVD] [n = 6], lung diseases [n = 4], kidney diseases [n = 2], intoxication [n = 2], digestive organ diseases [n = 1], collagen diseases [n = 1]),

Table 3 – Mortality according to PRA

	Men			Women			Total		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Per 1 log 10 × ng/(mL h) PRA increase									
Crude	1.74	1.15-2.65	.009†	2.78	1.44-5.37	.002†	2.51	1.77-3.54	<.001†
Age adjusted	1.64	1.09-2.45	.017*	2.38	1.28-4.43	.006†	2.22	1.59-3.08	<.001†
Age and sex adjusted	NA	NA	NA	NA	NA	NA	1.83	1.31-2.57	<.001†
Adjusted for multiple factors[a]	2.10	1.35-3.29	.001†	2.11	1.07-4.16	.032*	2.12	1.47-3.06	<.001†
Among quartiles of PRA (PRA, n)[b]									
Q1 (≤0.4, 927)	Ref	–	–	Ref	–	–	Ref	–	–
Q2 (0.5-0.9, 925)	1.54	0.77-3.07	.223	1.46	0.63-3.42	.380	1.51	0.88-2.58	.132
Q3 (1.0-1.9, 850)	1.65	0.86-3.19	.134	0.79	0.26-2.36	.673	1.46	0.85-2.50	.176
Q4 (≥2.0, 800)	2.68	1.40-5.13	.003†	2.44	1.02-5.84	.045*	2.63	1.57-4.41	<.001†
High vs low PRA (PRA, n)[b]									
Low (<2.0, 2702)	Ref	–	–	Ref	–	–	Ref	–	–
High (≥2.0, 800)	1.86	1.22-2.85	.004†	2.25	1.13-4.49	.021*	1.97	1.37-2.83	<.001†

Cox proportional regression analysis was used.
[a] Adjusted for age; body weight; diastolic blood pressure; HDL cholesterol; BNP; total protein; uric acid; antihypertensive treatment; diabetes (vs nondiabetes); and, when appropriate, sex.
[b] Adjusted for multiple factors.
* P < .05.
† P < .01.

Table 4 – Associations between PRA and cause of death-specific mortality

	PRA (n)			High vs low PRA		
	High ≥2 (800)	Low <2 (2702)	P (χ^2)	OR	95% CI	P
Total	55	88	<.001 †	1.96	1.32-2.90	<.001 †
Cancer	17	32	.047 *	1.53	0.80-2.92	.195
CVD	11	11	.002 †	4.11	1.60-10.56	.003 †
Others [a]	18	18	<.001 †	4.01	1.69-9.56	.002 †
Unknown	9	27	.757	0.79	0.35-1.80	.789

Multiple logistic regression analysis was used with adjustment for age, sex, weight, diastolic blood pressure, HDL cholesterol, BNP, total protein, uric acid, antihypertensive treatment, and diabetes (vs nondiabetes).

[a] Other causes included the following (n): infections (11), accidents (8), heart diseases (other than CVD) (6), lung diseases (4), kidney diseases (2), intoxication (2), digestive organ diseases (1), collagen diseases (1), and neurodegenerative diseases (1).

* $P < .05$.

† $P < .01$.

Table 5 – Mortality and PRA (high vs low) with adjustment for salt consumption

	PRA (n)		P
	High ≥2 (414)	Low <2 (1449)	
Salt consumption (g/d)	12.7 ± 4.1	12.9 ± 4.1	.330
Total mortality [a]			
No. of death	26	41	
HR (95% CI)	2.06 (1.20-3.54)	Ref	.009 *

[a] Cox proportional regression analysis was used with adjustment for age, sex, weight, diastolic blood pressure, HDL cholesterol, BNP, total protein, uric acid, antihypertensive treatment, diabetes (vs nondiabetes), and salt consumption.

* $P < .01$.

and unknown (n = 36). As shown in Table 4, the rates of death caused by cancer (2.13% vs 1.18%, $P = .047$), CVD (1.38% vs 0.41, $P = .002$), and other causes (2.25 vs 0.67, $P < .001$) were significantly higher in the high-PRA group than in the low-PRA group. Multiple logistic regression analysis with the adjustment for the factors described above confirmed the independent association between PRA and CVD-specific mortality (multivariate-adjusted OR, 4.11; 95% CI, 1.60-10.56), but did not confirm the association with cancer-specific mortality. Multivariate logistic analysis also showed a significant association between PRA and deaths caused by other causes (4.01, 1.69-9.56), although we did not determine the associations with the specific causes of death in this category.

3.5. *Independent association between PRA and total mortality from salt consumption*

Influence of salt consumption on the association between PRA and total mortality was examined using the subjects whose daily salt consumption was estimated. Although a linear regression analysis showed a significant but modest correlation between salt consumption and PRA ($r = –0.073$, $P = .002$), no significant difference in salt consumption was observed between the high- and the low-PRA groups (Table 5). Furthermore, as shown in Table 5, Cox proportional hazard model analysis with multiple factors, including salt consumption, as covariates showed significant and substantial association between PRA and total mortality independently from these factors or salt consumption.

4. Discussion

Because the RAS is involved in various pathological conditions, PRA, which is accepted as a surrogate marker for the activation of the RAS, may also be associated with a range of clinical characteristics. Therefore, we first examined the correlations between PRA and clinical characteristics and found that many factors were significantly correlated with PRA. Therefore, these factors were included as covariates in analyses to determine the independent association between PRA and mortality. These analyses were conducted in 2 ways by entering PRA into the model as either categorical or continuous values. These analyses clearly showed that higher PRA was an independent risk factor for total mortality. Because the analyses were statistically adjusted for multiple factors and included a relatively large population-based/general sample of individuals, these results indicate that higher PRA is a marker for increased total mortality in a general population.

Antihypertensive drugs have a substantial influence on PRA. Especially, ACE inhibitors, angiotensin II type I receptor blockers (ARBs), and mineralocorticoid receptor antagonists induce an increase in PRA by a feedback mechanism. Some diuretics and β-blockers also induce an increase in PRA. However, calcium channel blockers have no influence. Furthermore, direct renin inhibitors such as aliskiren inhibit PRA, although direct renin inhibitors were very recently approved for clinical use in Japan and, thus, no subjects were on the drugs at baseline. Therefore, the classes of antihypertensive drugs used appeared to be a confounding factor for the analysis. However, we evaluated the influences of antihypertensive treatment on the association between PRA and total mortality without regard to the classes of drugs used. This fact is a limitation of the study. As expected, antihypertensive treatment was significantly positively correlated with PRA in both sexes (Table 2); the subjects on antihypertensive treatment had higher PRA than those who were not in both sexes (2.92 ± 4.06 vs 1.58 ± 1.66 ng/[mL h], $P < .001$ and 1.60 ± 2.92 vs 1.01 ± 1.17, $P < .001$ for men and women, respectively). Therefore, antihypertensive treatment seemed to have some influence in the results obtained here. However, Cox proportional hazard model analysis with adjustment for age and sex showed no significant association between antihypertensive treatment and total mortality ($P = .861$). Furthermore, the association between PRA and total mortality was always examined with adjustment for antihypertensive treatment. Therefore, although the classes of drugs used were not considered, antihypertensive treatment did not seem to have a substantial influence on the results.

Because the RAS is activated physiologically when blood pressure or extracellular fluid volume (ECFV) decreases [1,26],

blood pressure and ECFV seemed to be important confounders for the analysis. In the study, blood pressures were included as covariates; but ECFV per se was not. However, BNP that may represent, at least in part, ECFV was included. Dietary sodium (salt) consumption is a potent factor affecting ECFV and thus PRA. Therefore, we included dietary salt consumption as a covariate for the analysis in the subanalysis of about half of the subjects and found that, although PRA was negatively associated with salt consumption, salt consumption did not differ between the high- and low-PRA groups (about 13 g/d for each group) and higher PRA was still significantly associated with increased risk for total mortality after the adjustment for salt consumption. Therefore, we believe that higher PRA found as a risk for total mortality in the study reflects not just a physiologically increased but, at least in part, a pathologically high RAS.

Although the associations between the RAS with CVD and cancer have been examined in much detail, there are some questions that need to be answered before we can reach a definitive conclusion. Activation of the RAS is associated with increased CVD risk in many intervention studies in which hypertensive patients were treated with RAS inhibitors (ie, ACE inhibitors and/or ARBs) [1,27-29]. However, the subjects included in those studies had specific diseases, such as hypertension, diabetes, and other CVD risk factors [1,27-29]. Associations between the RAS and cancer have also been reported in many epidemiological and intervention studies, most of which showed that RAS activation is associated with the incidence and/or progression of cancer [2,3,30-34]. However, the effects of RAS inhibitors on cancer risk are inconsistent [2,3,30-38]. For example, several studies showed significantly decreased cancer risk with the use of ACE inhibitors [2,3,30-33], although other studies did not confirm these effects [2,3,35-37]. Surprisingly, the use of ARBs was associated with increased cancer risk [35]. Therefore, the effects of RAS activation on the risk of CVD or cancer in the general population have not been fully clarified. Therefore, we also determined whether PRA is associated with CVD- and cancer-related mortality in the general population and found that there were significantly more CVD- ($P = .002$) and cancer-related ($P = .047$) deaths among subjects belonging to the high-PRA group compared with the low PRA group. This indicates that PRA may also be a marker for CVD and cancer risks in the general population.

In multivariate logistic regression analysis with adjustment for factors correlated with PRA, the association between PRA and cancer became insignificant, whereas that with CVD remained significant. The ORs for these associations between PRA with CVD and cancer were very different (4.11 vs 1.53), which substantially affects the statistical power of these analyses. We used SampSize software (http://sampsize.sourceforge.net/iface/index.html) to determine the statistical power of the differences in the frequencies of CVD- and cancer-related deaths between the high- and low-PRA groups. At a significance level of .05, there was 89.7% power to detect an OR of 4.11 for CVD and 28.9% to detect an OR of 1.53 for cancer. Furthermore, adjustment for multiple factors can substantially reduce statistical power. Therefore, because the statistical power for the association between PRA and cancer is very low, the statistically nonsignificant results may not exclude the possibility that PRA is weakly, if at all, associated with cancer in a general population.

In this study, although the serum levels of several clinical characteristics, including BNP, were considered as confounding factors, several others that link to PRA and have substantial influence on mortality, such as AngII, aldosterone, and vasopressin, were not. Therefore, such characteristics not examined here might influence the association between PRA and mortality examined in this study. Despite this limitation, because PRA is responsible for the initial activation of the RAS, it can be considered as an upstream marker of the above-mentioned characteristics and can, most importantly, be easily measured in everyday clinical settings. Thus, we suggest that PRA is an appropriate marker for total mortality.

This study has several strengths and limitations. As for the strengths, statistical adjustments were made for multiple factors that could confound the results; and a relatively large population-based/general sample of individuals was used. As for the limitations, blood samples were collected in a sitting position after at least 5 minutes of rest, which does not seem to be sufficient to overcome the large variability in the influence of posture on PRA. Furthermore, information about the classes of antihypertensive drugs used was not monitored; and daily salt consumption was estimated in only about half of the subjects. In addition, dietary sodium balance by 24-hour urine collections or spot urine-to-creatinine ratios were not used. Instead, salt consumption was used as a proxy for dietary sodium balance; and several characteristics that link to PRA, such as AngII, aldosterone, and vasopressin, were not measured.

In conclusion, we found that higher PRA was significantly and independently associated with increased risk for total mortality in a general Japanese population. This finding indicates that PRA is an appropriate marker for total mortality, at least in Japanese, and seems to warrant further examination to determine whether or not higher PRA is associated with an increased risk for total and/or cause of death-specific mortalities in other ethnicities.

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Conflict of Interest

The authors have nothing to disclose.

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