variate analysis. For computing sex- and age-adjusted *P* values, we used analysis of covariance and logistic regression analysis as appropriate. To explore the plausibility of the Cox model, we first plotted incidence rates by tertiles of PRA, PAC, and ARR, while standardizing by the direct method for sex and age (<50, 50–65, and ≥65 years). We calculated hazard ratios of PRA, PAC, and ARR using multiple Cox regression while adjusting for sex as a categorical variable, and age, body mass index (BMI), and systolic blood pressure as continuous variables. Because subgroup analyses by salt intake were performed in previous studies of ARR,[7,17–19] we divided participants into two groups based on median salt intake. SAS version 9.1 software (SAS Institute, Cary, NC) was used for statistical analysis.

RESULTS

Participant characteristics

The 883 participants included 579 females (65.6%). Mean values were 59.0 ± 11.3 years for age, 23.5 ± 3.1 kg/m^2 for BMI, 5.02 ± 0.86 mmol/l for total cholesterol level, and 126.2 ± 12.3/79.1 ± 7.7 mm Hg for systolic/diastolic blood pressure. At enrollment, 155 (17.6%) participants were current smokers, 343 (38.8%) were current drinkers, 218 (24.7%) had hypercholesterolemia, 59 (6.7%) had diabetes mellitus, and 34 (3.9%) had a history of cardiovascular disease. The median (25th–75th percentiles) PRA level was 1.2 (0.7–2.0) ng/ml/h, the median PAC level was 6.4 (4.9–8.1) ng/dl, and the median ARR level was 5.3 (3.4 to 8.6) ng/dl per ng/ml/h. BMI, diastolic blood pressure, serum sodium, and serum potassium levels significantly increased with increases in ARR after adjustment for sex and age (**Table 1**). No other variables showed a significant and consistent association with ARR.

Association of PRA, PAC, and ARR with stroke risk

The mean duration of follow-up was 10.9 ± 2.5 years. Of the 883 subjects, a first stroke occurred in 45 subjects, including cerebral infarction in 29, intracerebral hemorrhage in 10, and subarachnoid hemorrhage in 6.

Figure 1 shows incidence rates of stroke across tertiles of PRA, PAC, and ARR. In subjects with high sodium intake (≥median of 4,058 mg/day (salt equivalent, 10.5 g/day)), the incidence rate of stroke increased with increases in ARR, whereas PRA and PAC had no consistent relation with stroke. Next, Cox regression analyses were performed using ARR as a continuous variable (**Figure 2**). Because of their positively skewed distributions, PRA, PAC, and ARR were natural-log transformed. In the Cox regression model, male gender (hazard ratio: 1.86, $P = 0.047$), older age (hazard ratio: 2.22 per 10-year increase, $P < .0001$), and systolic blood pressure

Table 1 | Clinical characteristics among groups classified by tertiles of ARR

	Tertiles of ARR (ng/dl per ng/ml/h)				
Characteristic	<4.1 *n* = 294	4.1–7.1 *n* = 294	≥7.1 *n* = 295	*P*	Sex- and age-adjusted *P*
Women, %	50.7	69.4	76.6	<.0001	—
Age, years	59.5 ± 11.3	59.2 ± 11.2	58.3 ± 11.3	0.4	—
Body mass index, kg/m^2,[a]	23.1 ± 3.2	23.5 ± 3.1	23.9 ± 3.1	0.009	0.006
Past history of heart disease, %	3.4	4.4	3.7	0.8	0.7
Diabetes, %	8.5	5.8	5.8	0.3	0.1
Hypercholesterolemia, %	23.8	23.5	26.8	0.6	0.7
Smoking, %	22.4	16.7	13.6	0.02	0.5
Drinking, %	44.2	36.7	35.6	0.07	0.6
Systolic blood pressure, mm Hg	128.6 ± 14.7	128.2 ± 12.5	129.4 ± 13.6	0.5	0.08
Diastolic blood pressure, mm Hg	71.9 ± 9.9	72.0 ± 7.7	73.0 ± 9.5	0.2	0.02
HbA_{1c}, %	5.16 ± 0.66	5.08 ± 0.55	5.12 ± 0.57	0.3	0.3
Total cholesterol, mmol/l	4.97 ± 0.90	5.03 ± 0.81	5.06 ± 0.87	0.4	0.9
eGFR, ml/min/1.73 m^2	86.3 ± 19.2	84.9 ± 18.1	85.8 ± 17.5	0.6	0.4
Serum sodium, mEq/l	141.6 ± 1.9	142.0 ± 1.7	142.2 ± 1.9	0.0007	0.001
Serum potassium, mEq/l	4.29 ± 0.38	4.29 ± 0.36	4.34 ± 0.35	0.1	0.04
Dietary intake					
Energy, kcal/day	1,912 ± 855	1,750 ± 664	1,837 ± 996	0.07	0.2
Sodium, mg/day	4,379 ± 3,320	4,408 ± 2,693	4,558 ± 2,881	0.7	0.9
Salt, g/day	11.1 ± 8.4	11.1 ± 6.8	11.5 ± 7.3	0.7	0.9
Potassium, mg/day	2,362 ± 1,018	2,441 ± 850	2,437 ± 799	0.5	0.9

eGFR was estimated from the serum creatinine value using a Japanese equation: eGFR (ml/min/1.73 m^2) = 194 × serum creatinin$^{-1.094}$ × age $^{-0.287}$ (if female × 0.739).[13]
ARR, aldosterone-to-renin ratio; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}.
[a]Among total study subjects, 866 had data of body mass index.

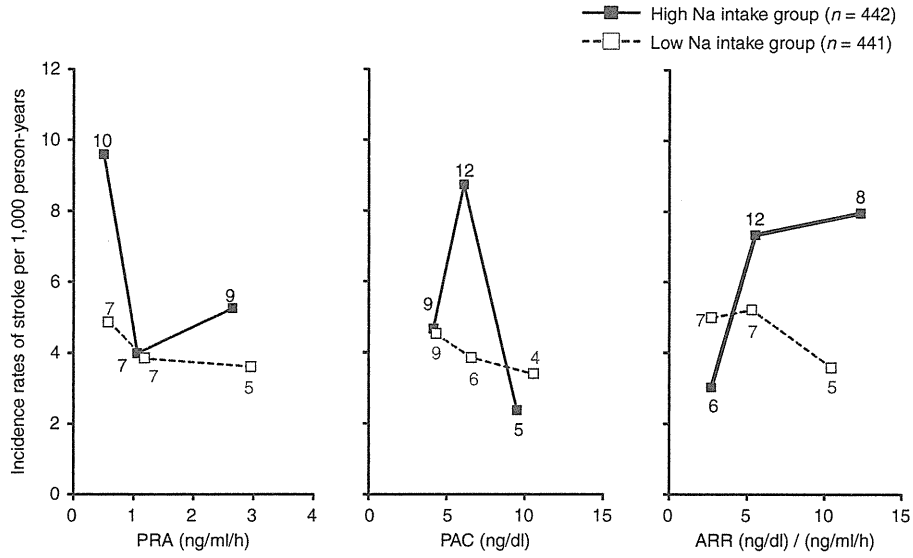

Figure 1 | Sex- and age-standardized incidence of stroke by median sodium intake. Incident rates of stroke across tertiles of plasma renin activity (PRA), plasma aldosterone concentration (PAC), and aldosterone-to-renin ratio (ARR) in all subjects (closed circles), in those with high sodium intake (≥median of 4,058 mg/day (salt equivalent, 10.5 g/day), closed squares), or in those with low sodium intake (<4,058 mg/day, open squares). Incidence rates were standardized by the direct method for sex and age (<50, 50–65, and ≥65 years). The number of events contributing to incidence rates is presented.

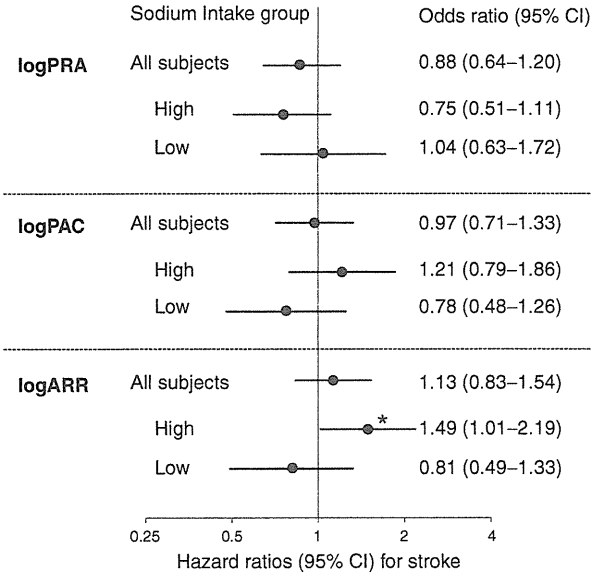

Figure 2 | Adjusted hazard ratios (95% CIs) for stroke by median sodium intake. Hazard ratios (95% CIs) indicated the stroke risk associated with each 1 s.d. increase in logPRA, logPAC, or logARR by median sodium intake (4,058 mg/day (salt equivalent, 10.5 g/day)) after adjusting for sex, age, body mass index, and systolic blood pressure. Stroke incidence occurred in 26 and 19 subjects with high and low sodium intake, respectively. *$P < 0.05$. ARR, aldosterone-to-renin ratio; CI, confidence interval; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

(hazard ratio: 1.25 per 10 mm Hg increase, $P<0.038$) were significantly associated with stroke risk. However, logARR ($P = 0.4$) and BMI ($P = 0.2$) were not significant predictors for stroke after adjustments in all subjects. Then, we stratified subjects by median sodium intake (4,058 mg/day (salt equivalent, 10.5 g/day)). In subjects with high sodium intake, each 1 s.d. increase in logARR (hazard ratio: 1.49, $P = 0.04$), male gender (hazard ratio: 3.26, $P = 0.005$), older age (hazard ratio: 2.58 per 10-year increase, $P \leq .0001$), and systolic blood pressure (hazard ratio: 1.41 per 10 mm Hg increase, $P = 0.034$), but not BMI ($P = 0.07$), were significantly associated with an increased hazard ratio for stroke. The interaction between logARR and sodium intake on stroke was not statistically significant ($P = 0.2$).

For the sensitivity analyses, we adjusted diastolic blood pressure or pulse pressure instead of systolic blood pressure, and we additionally adjusted for estimated glomerular filtration rate, serum sodium and serum potassium, because these variables were significantly associated with ARR as shown in **Table 1**. Even after applying these adjustments, the hazard ratio of logARR was almost consistent (hazard ratio: ≥1.46, $P \leq 0.057$). When logPRA and logPAC were simultaneously included in the same Cox regression model, similar results were observed; the hazard ratios were 0.67 ($P = 0.051$) for logPRA and 1.47 ($P = 0.1$) for logPAC. The association between ARR and stroke was not observed in subjects with low sodium intake ($P = 0.7$). When we repeated all of the analyses using logPRA or logPAC instead of logARR, we did not find any significant associations.

Among 442 subjects with high sodium intake, 356 (80.5%) had intermediate follow-up data regarding hypertensive status based on blood pressure and antihypertensive medication use after 8.0 ± 1.7 years of the baseline examination. Among these 356 patients, 134 patients became hypertensive, defined as a blood pressure of 140 mm Hg systolic or 90 mm Hg diastolic or the use of antihypertensive drugs. Then hypertensive status was further adjusted in the Cox regression analysis using two design variables: (1) hypertensive status (present = 1, absent = 0) and (2) missing value for hypertensive status (missing = 1, present = 0). With this analysis, the association of 1 s.d.

increase in logARR with stroke in subjects with high sodium intake was slightly weakened to the nonsignificant level (hazard ratio: 1.37, $P = 0.1$).

Although we also stratified subjects according to sex (men/women), age (<65/≥65 years), BMI (<25/≥25 kg/m^2), total energy intake (below or above median of 1,656 kcal/day), or potassium intake (below or above median of 2,366 mg/day) instead of sodium intake, we did not find any statistically significant association of logPRA, logPAC, and logARR with stroke (all $P \geq 0.2$). When we reanalyzed the association of ARR with stroke after changing the ARR cutoff for exclusion of patients with primary aldosteronism from 20 to 30 ng/dl per ng/ml/h, the significant association of logARR with stroke remained in subjects with high sodium intake (hazard ratio: 1.49, $P = 0.03$, $n = 458$).

DISCUSSION

The key novel finding of our study was that higher ARR, even within normal ranges, can be a predictor for stroke in a general population with high sodium intake, independent of sex, age, BMI, and systolic blood pressure. It is noteworthy that this finding was observed in a general population in whom primary aldosteronism had been ruled out by a standardized criterion of ARR ≥20 ng/dl per ng/ml/h. It has been reported that patients with primary aldosteronism have an increased prevalence of stroke compared with those with essential hypertension.[3] Our findings extend the validation of the relationship between aldosterone levels and cerebrovascular disease from primary aldosteronism patients to a general population of an Asian cohort.

Several studies have investigated the association of aldosterone alone[4] or renin alone[20] with stroke or cardiovascular disease. However, in terms of the relation with ARR, only the Ludwigshafen Risk and Cardiovascular Health (LURIC) study has reported this in terms of prognostic significance of cardiovascular mortality.[4] Their results indicated an inverse association between ARR and cardiovascular mortality; among 3,153 Caucasian patients with New York Heart Association I–IV heart failure who were referred for coronary angiography, patients who died from cardiovascular disease had a lower ARR than survivors at baseline.[4] Unlike their study, we observed a positive association between ARR and stroke incidence in subjects with high sodium intake. The inconsistency in the results between the LURIC study and our present study could be explained by differences in study populations (patients with heart failure vs. general population), antihypertensive treatment use (with antihypertensive treatment vs. without), or salt intake. Salt intake is higher in East Asia, including Japan, compared with Western countries[21] and is considered to strengthen the association of ARR with cardiovascular disease, which might lead to the positive relation of ARR with stroke that we found.

Our results regarding the adverse prognostic value of high ARR in combination with high sodium intake suggest an involvement of salt-sensitivity hypertension. So far, previous studies reported that salt sensitivity is caused by insulin resistance,[22] nonmodulating,[23] circadian clock-deficient,[24] or relative aldosterone excess.[7,25,26] Among them, relative aldosterone excess is most likely to be accountable for our present results, as relative aldosterone excess is a clinical entity of hypertension characterized by low renin combined with "normal" aldosterone level, leading to high ARR. In this clinical condition, aldosterone does not decrease despite low renin suppressed by sodium-volume overload, which is responsible for salt-sensitive hypertension due to inappropriate sodium and fluid retention.[7,18,25,26] In fact, our group previously demonstrated the possible contribution of relative aldosterone excess to salt-sensitivity in the general population.[7,18] In addition, we recently reported that relative aldosterone excess was related to a nondipping pattern, especially in subjects with high sodium excretion. The nondipping pattern is also considered an independent risk factor for cardiovascular events in both hypertensive and normotensive subjects.[27] In line with our previous studies,[7,18,27] our present results raise the hypothesis that relative aldosterone excess may adversely affect the cardiovascular system under chronic high-salt dietary conditions. Although some studies indicate that high ARR individuals are more common among hypertensive patients,[7,18,28,29] results of the present study highlight that relative aldosterone excess in a general population, including normotensive subjects, should not be ignored. However, the association of logARR with stroke was slightly weakened after adjustment for hypertensive status during follow-up. Thus, the hypertensive condition during follow-up might represent the pathogenetic link between an increased ARR and stroke.

Experimental studies also have reported direct adverse effects of aldosterone on the cardiovascular system such as cardiac hypertrophy, fibrosis, or inflammation,[30] which can be increased under conditions of high sodium intake.[1] Recently, Rigsby *et al.* reported that a mineralocorticoid receptor antagonist improved cerebrovascular structure after remodeling developed in rats by preventing fibrosis or inflammation in spite of no change in blood pressure.[31,32] Thus, treatment with a mineralocorticoid receptor antagonist might be potentially effective for preventing cardiovascular disease in a population with high sodium intake. However, before initiation of antihypertensive treatment, lifestyle modifications, including restricting salt intake, should be considered first. Salt reduction is an important component of standard therapy for hypertension, has antihypertensive effects without any side effects, and is more cost-effective than antihypertensive medications.[33] Reducing salt intake should be a target not only for hypertensive patients but also for the general population. A series of our studies based on a general population[7,18] provides further evidence of the importance of salt reduction for public health.

In **Table 1**, more women were seen in higher tertiles of ARR. The Framingham study also showed higher ARR in women than in men.[29] Estrogen is one of the regulators of angiotensinogen synthesis, which can increase aldosterone synthesis and suppress renin secretion.[34] Thus, sex differences or sex hormone balances might affect the association of ARR with stroke. In the present study, subjects with higher ARR had a relatively

high serum potassium level (**Table 1**). In typical patients with primary aldosteronism, aldosterone excess induces hypokalemia. However, most patients with a mild form of primary aldosteronism do not show typical clinical symptoms or hypokalemia. High serum potassium in a general population without apparent primary aldosteronism may be affected by not only ARR but also other factors. In contrast, elevated serum potassium levels might stimulate aldosterone excretion,[35] which can cause a high level of ARR. However, the results shown in **Table 1** were based on a cross-sectional analysis, and it is thus difficult to discuss any cause–effect relationship.

The present study has several limitations. First, the study population predominantly included middle-aged, elderly, and female individuals. These imbalances might, to some extent, limit the external validity of the findings. Second, subjects underwent blood sampling between 9 and 11 AM or between 1 and 3 PM and most often had not fasted. The nonstandardized conditions of blood sampling could affect both PRA and PAC levels through circadian variations and dietary salt intake before the health checkup. However, it is also important to emphasize that, even when measured under nonstandardized conditions, ARR levels are clinically useful indices for stroke.[36] Third, we collected data on nondipping patterns in only a small fraction of study subjects; thus, we could not take into account the effect of nondipping patterns. Fourth, we obtained data on sodium intake from the 1-year food-frequency questionnaire, which was previously validated against a 3-day diet record method as a gold standard method of dietary assessment.[14,15] However, the validation was not confirmed by direct comparison with urinary sodium or potassium excretion levels. This might weaken the interaction between logARR and sodium intake on stroke incidence observed in this study. Fifth, the number of stroke events was too few to allow definitive conclusions to be drawn. The limited number of events might lead to a nonsignificant interaction between sodium intake and logARR ($P = 0.2$). Further prospective studies including a larger sample size are needed to support the associations among ARR, salt intake, and the future risk of stroke.

In conclusion, the present study provides perhaps the first prospective evidence that high ARR, even within normal ranges, may predict stroke in a general population with high sodium intake. These results raise the hypothesis that relative aldosterone excess may have a deleterious effect on stroke mediated by salt-sensitivity and suggest the possibility that salt reduction may relieve the deleterious effects of relative aldosterone excess.

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Original Article

Plasma renin activity and the aldosterone-to-renin ratio are associated with the development of chronic kidney disease: the Ohasama Study

Shiho Terata[a], Masahiro Kikuya[a], Michihiro Satoh[a], Takayoshi Ohkubo[a,b], Takanao Hashimoto[a], Azusa Hara[c], Takuo Hirose[a], Taku Obara[a], Hirohito Metoki[a], Ryusuke Inoue[a], Kei Asayama[a,f], Atsuhiro Kanno[a], Kazuhito Totsune[a,d], Haruhisa Hoshi[e], Hiroshi Satoh[g], Hiroshi Sato[a], and Yutaka Imai[a]

Background: The aldosterone-to-renin ratio (ARR) is used to screen for primary aldosteronism and could be an index for salt sensitivity. The association between ARR and the development of chronic kidney disease (CKD) is completely unknown.

Method: A longitudinal observational study involving 689 participants from a general Japanese population (mean age 58.2 years; 68.5% women) who did not have CKD and were not receiving antihypertensive medication at baseline was conducted. The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine levels, and CKD was defined as eGFR less than 60 ml/min per 1.73 m^2 and/or dipstick-positive proteinuria. The associations of baseline plasma renin activity (PRA), plasma aldosterone concentration, and ARR with the development of CKD were examined using Cox proportional hazard regression analysis adjusted for sex, age, BMI, smoking, drinking, history of hypercholesterolemia, diabetes mellitus, and cardiovascular disease, SBP, and baseline eGFR.

Results: During a mean 9.1-year follow-up, 118 participants developed CKD. A 1 standard deviation increment in the natural log-transformed (ln) ARR was positively associated with the incidence of CKD (hazard ratio 1.29, $P=0.012$). LnPRA showed an inverse association (hazard ratio 0.76, $P=0.007$). Meanwhile, plasma aldosterone concentration was not associated with CKD. Individuals who developed CKD had significantly lower baseline PRA (0.97 vs. 1.14 ng/ml per h; $P=0.03$) and higher baseline ARR levels [66.6 vs. 56.8 (pg/ml)/(ng/ml per h); $P=0.02$] than those who did not.

Conclusions: Lower PRA and higher ARR were associated with the development of CKD in a general population, suggesting that they are independent predictors of CKD.

Keywords: aldosterone excess, aldosterone-to-renin ratio, chronic kidney disease, general population, prospective cohort study

Abbreviations: ANCOVA, analysis of covariance; ARR, aldosterone-to-renin ratio; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SD, standard deviation

INTRODUCTION

Chronic kidney disease (CKD) is an independent risk factor for cardiovascular disease events [1–3], stroke events [4], end-stage renal disease [3,5], and all-cause mortality [1–4] in the general population. CKD has been recognized as a major public health issue. The prevalence of CKD has been increasing in Japan, caused by aging of the population and an increase in the number of people with metabolic syndrome [6]. A recent study estimated that approximately 13% of the Japanese adult population, approximately 13.3 million people, has CKD [7]. Traditional risk factors for CKD include older age, diabetes mellitus, hypertension, low high-density lipoprotein cholesterol, and metabolic syndrome [8–10]. Meanwhile, nontraditional risk factors include homocysteine, lipoprotein, oxidative stress, inflammation, electrolyte imbalance, and thrombogenic factors [11,12].

The aldosterone-to-renin ratio (ARR) is believed to be a more robust screening test than aldosterone levels for primary aldosteronism, and it could be an index for inappropriate aldosterone activity and salt sensitivity [13–15]. It has been reported that patients with primary aldosteronism have renal damage more frequently than hypertensive controls [16]. Recently, in a community-based longitudinal

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[a]Tohoku University Graduate School of Medicine and Pharmaceutical Sciences, Sendai, [b]Shiga University of Medical Science, Otsu City, [c]Research Center for Cancer Prevention and Screening National Cancer Center, Tokyo, [d]Tohoku Fukushi University, Sendai, [e]Ohasama Hospital, Iwate, Japan, [f]University of Leuven, Leuven, Belgium and [g]National Institute for Environmental Studies, Japan

Correspondence to Masahiro Kikuya, MD, PhD, Department of Planning for Drug Development and Clinical Evaluation, Graduate School of Medicine and Pharmaceutical Sciences, Tohoku University, 6-3 Aramaki-aza-aoba, Sendai 980-8578, Japan. Tel: +81 22 795 4528; fax: +81 22 795 4532; e-mail: kikuyam@med.tohoku.ac.jp

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study in USA, aldosterone was associated with incident CKD and microalbuminuria independent of traditional risk factors [17]. Renin and aldosterone are known to be affected by salt intake [18], which is higher in east Asia, including Japan, than in western countries. Therefore, the associations among CKD, renin, and aldosterone could potentially differ according to cultural or ethnic background. Additionally, the influence of ARR on the development of renal damage has not been previously reported. Therefore, a longitudinal study was conducted to examine the associations of plasma renin activity (PRA), plasma aldosterone concentration (PAC) and ARR with the development of CKD in a general Japanese population.

METHODS

Study design

The investigation was part of the Ohasama study. The socio-economic and demographic characteristics of this region and the full details of the project have been described elsewhere [19]. The study protocol was approved by the Institutional Review Board of Tohoku University School of Medicine, Sendai, Japan, and by the Department of Health of the Ohasama Town Government.

Participants

In Japan, annual health check-ups are available for farmers, the self-employed, pensioners, and dependents aged at least 35 years. Of the residents of Ohasama, 2719 were eligible for annual health check-ups in 1997, which is the baseline in this study. Of the 1831 individuals who participated in the check-ups that year, 1624 gave informed consent and participated in the present study. Individuals who lacked data for blood tests ($n=278$) and for the dipstick test for spot urine ($n=19$), had insufficient data on salt intake ($n=33$), and were treated with antihypertensive drugs ($n=362$) were excluded. In addition, three individuals were excluded who met the clinical criteria for primary aldosteronism, that is ARR greater than 200 (pg/ml)/(ng/ml per h) and PAC greater than 150 pg/ml. Furthermore, 66 individuals with CKD at baseline were excluded to examine the incidence of new CKD, and 174 individuals who did not have health check-ups for follow-up from 2002 to 2010 were also excluded. Therefore, the statistical analysis included data from 689 individuals.

Data collection

Blood samples were collected with participants in a sitting position after an approximately 30-min rest interval, between 0900 and 1100 h or between 1300 and 1500 h; most participants had not fasted. Measurements of PRA (ng/ml per h) and PAC have been validated previously [20]. At the same time, serum creatinine, total cholesterol, glucose, glycosylated hemoglobin (HbA1c), serum sodium, and potassium levels were measured.

Information on smoking and drinking status, use of antihypertensive medication, and history of hypercholesterolemia, diabetes mellitus, and cardiovascular disease was verified on the basis of the medical records and a questionnaire that was administered. Hypercholesterolemia was defined as total cholesterol at least 5.68 mmol/l ($\geq$220 mg/dl), use of medication for hypercholesterolemia, and/or a history of hypercholesterolemia. Diabetes mellitus was defined as a random blood glucose level at least 11.11 mmol/l ($\geq$200 mg/dl), HbA1c level at least 6.5%, use of medication for diabetes, and/or a history of diabetes mellitus. Blood pressure was measured twice consecutively in the sitting position, after a minimum 2 min rest interval, by nurses or technicians using an automatic USM-700F sphygmomanometer [21] (UEDA Electronic Works, Tokyo, Japan) based on the Korotkoff sound technique. The average of the two readings was used. For calculating salt intake, a standardized method was used from data obtained in a Japanese version of the food-frequency questionnaire, which asked about the average frequency of consumption of each food during the previous year [22,23]. Estimated salt intake was adjusted for total energy by the residual method [23,24].

Measurement of estimated glomerular filtration rate and proteinuria

Serum creatinine was measured using the Jaffe assay at baseline and the enzymatic assay at follow-up examinations. Kidney function was calculated by the estimated glomerular filtration rate (eGFR) using a modified three-variable equation based on insulin clearance for Japanese individuals as follows: eGFR (ml/min per 1.73 m^2) = 194 $\times$ (serum creatinine in enzymatic method)$^{-1.094}$ $\times$ age$^{-0.287}$ ($\times$0.739, if female) [25]. When eGFR was calculated, serum creatinine levels obtained using the Jaffe assay were calibrated to the enzymatic assay by subtracting 0.2 mg/dl [26,27]. Proteinuria was diagnosed with a dipstick test (Urohemabonbix 5G08C; Bayer Medical, Tokyo, Japan). Proteinuria was considered to be present with a dipstick result of 1+ or more, which corresponds to a urinary protein level above 30 mg/dl. CKD was defined as eGFR less than 60 ml/min per 1.73 m^2 and/or positive proteinuria [28].

Follow-up and outcome

The outcome was defined as new-onset CKD at the time of the annual check-ups from 2002 to 2010. If a participant experienced the outcome more than once during follow-up, only the first outcome contributed to the analysis. The date of incidence was defined as the midpoint between the examination date before the development of CKD and the examination date when the participant was first noted to have CKD. The observation period was from the baseline to the date of incidence for participants who developed CKD, and to the date of the final check-up for others.

Data analysis

To analyze the relationships between tertiles of ARR and participant characteristics, means and proportions were compared using analysis of variance (ANOVA) and the chi-square test for univariate analysis. The hazard ratios for CKD development were calculated for a 1-standard deviation (SD) increase of PRA, PAC, and ARR using Cox proportional hazards regression analysis. For the analysis, three models were examined. Model 1 was adjusted for sex and age. Model 2 was adjusted for risk factors including

BMI, ever smoking, ever drinking, a history of hypercholesterolemia, diabetes mellitus, and cardiovascular disease, SBP, and eGFR at baseline, in addition to the adjustments for model 1. Model 3 was adjusted for the other confounding factors, including serum sodium, serum potassium, dietary salt intake (greater than or equal to median vs. less than median), and the number of follow-up visits, in addition to the adjustments for model 2. A sensitivity analysis excluding participants who started antihypertensive treatment during the follow-up period was also performed. Missing BMI values ($n=5$) were interpolated from the regression slope on age by sex. In the Cox proportional hazards analysis and ANCOVA, PRA, PAC, and ARR were natural log-transformed because of their positively skewed distributions. All statistical analyses were conducted using SAS software, version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). The values are expressed as means ± SD unless otherwise noted. P less than 0.05 was considered significant.

RESULTS

Baseline characteristics

Of the 689 participants (mean age 58.2 ± 10.5 years), 472 (68.5%) were women, 163 (23.7%) were ever smokers, 292 (42.4%) were ever drinkers, and 173 (25.1%), 46 (6.7%), and 30 (4.4%) were classified as having a history of hypercholesterolemia, diabetes mellitus, and cardiovascular disease, respectively. SBP/DBP levels were 127.9 ± 13.2/71.9 ± 8.7 mmHg.

Serum creatinine by the Jaffe assay and eGFR were 0.82 ± 0.13 (range 0.5–1.2) mg/dl and 87.3 ± 17.5 (range 60.8–167.9) ml/min per 1.73 m^2, respectively. The median (25–75th percentiles) values were 1.1 (0.6–1.9) ng/ml per h for PRA, 65 (50–81) pg/ml for PAC, and 56 (35–97) (pg/ml)/(ng/ml per h) for ARR. The proportion of women, BMI greater than 25 kg/m^2, and the levels of SBP/DBP increased significantly with increases in ARR after adjustment for sex and age (Table 1). Serum creatinine and eGFR showed no significant differences between ARR groups at baseline. The proportion of women, BMI at least 25 kg/m^2, diabetes, age, BMI, blood pressure, and serum sodium decreased significantly with increase in PRA (Supplemental Digital Content 1, http://links.lww.com/HJH/A179).

Follow-up and development of chronic kidney disease

The number of follow-up visits was 1, 2, 3, 4, or 5 in 130, 135, 238, 117, and 69 participants, respectively. The observation period was 9.1 ± 2.9 years (median 9.7 years, range 2.2–13.0 years). During follow-up, 118 participants (69.5% women) developed CKD, of whom 97 had eGFR less than 60 ml/min per 1.73 m^2, 17 had proteinuria, and 4 had both simultaneously.

The Kaplan–Meier survival estimates for the cumulative incidence rate of CKD are shown in Fig. 1. With adjustments for sex and age, the incidence rate of CKD increased significantly with decreases in PRA and with increases in ARR during the follow-up period. Table 2 shows the hazard ratios of PRA, PAC, and ARR for development of CKD in the Cox regression analysis. The natural log-transformed (ln)PRA was significantly and negatively associated with the risk of CKD, and this relationship was independent from traditional risk factors (hazard ratio 0.76, $P=0.007$, model 2). Elevated lnARR was positively associated with the incidence of CKD (hazard ratio 1.29, $P=0.012$, model 2). However, lnPAC was not associated with CKD ($P\geq 0.41$). These results did not change even after the additional adjustment for serum sodium and potassium, salt intake, and the number of follow-up visits (model 3). During the follow-up period, 146 participants initiated antihypertensive treatment. When excluding these 146 participants,

TABLE 1. Baseline characteristics of groups classified by ARR tertiles

	ARR tertiles (ng/dl)/(ng/ml per h)				
	<4.2 ($n=229$)	4.2–7.9 ($n=230$)	≥7.9 ($n=230$)	P	Sex and age-adjusted P
Women, *n* (%)	116 (50.7)	169 (73.5)	187 (81.3)	<0.0001	NA
Age (years)	58.3 ± 10.7	58.6 ± 10.4	57.6 ± 10.4	0.57	NA
BMI (kg/m^2) ($n=684$)	23.1 ± 3.0	23.6 ± 3.0	23.7 ± 3.0	0.046	0.054
BMI ≥25 kg/m^2, *n* (%) ($n=684$)	49 (21.6)	73 (31.7)	74 (32.6)	0.02	0.01
Ever smoker, *n* (%)	82 (35.8)	47 (20.4)	34 (14.8)	<0.0001	0.75
Ever drinker, *n* (%)	113 (49.3)	96 (41.7)	83 (36.1)	0.02	0.63
Hypercholesterolemia, *n* (%)	59 (25.8)	60 (26.1)	54 (23.5)	0.78	0.30
Diabetes, *n* (%)	15 (6.6)	13 (5.7)	18 (7.8)	0.64	0.52
Cardiovascular disease, *n* (%)	11 (4.8)	13 (5.7)	6 (2.6)	0.26	0.37
SBP (mmHg)	126.9 ± 13.9	128.0 ± 12.5	128.9 ± 13.2	0.28	0.01
DBP (mmHg)	71.2 ± 9.2	72.0 ± 7.8	72.5 ± 9.2	0.28	0.02
Serum sodium (mEg/l)	141.6 ± 1.9	142.0 ± 1.7	142.1 ± 1.8	0.01	0.07
Serum potassium (mEg/l)	4.30 ± 0.38	4.30 ± 0.38	4.32 ± 0.34	0.84	0.50
Serum uric acid (mg/dl) ($n=688$)	4.4 ± 1.3	4.0 ± 1.1	3.9 ± 1.0	<0.0001	0.09
Serum creatinine (mg/dl)	0.85 ± 0.13	0.81 ± 0.13	0.79 ± 0.11	<0.0001	0.54
Hematuria, *n* (%)	8 (3.5)	3 (1.3)	6 (2.6)	0.31	0.80
eGFR (ml/min per 1.73 m^2)	87.1 ± 17.4	86.5 ± 17.8	88.2 ± 17.4	0.58	0.79
Salt intake (g/day)	11.3 ± 8.9	11.4 ± 7.8	11.1 ± 5.3	0.91	0.63

ARR, aldosterone-to-renin ratio; BP, blood pressure; eGFR, estimated glomerular filtration rate; NA, not applicable.
Data are presented as means ± SD for continuous variables and percentages for dichotomous variables. Serum creatinine was measured using the Jaffe assay. eGFR was estimated from the serum creatinine value using a Japanese equation: eGFR (ml/min per 1.73 m^2) = 194 × (serum creatinine in enzymatic method)$^{-1.094}$ × age$^{-0.287}$ (×0.739, if women) [25]. Hematuria was considered to be present with a dipstick result of 1+ or more.

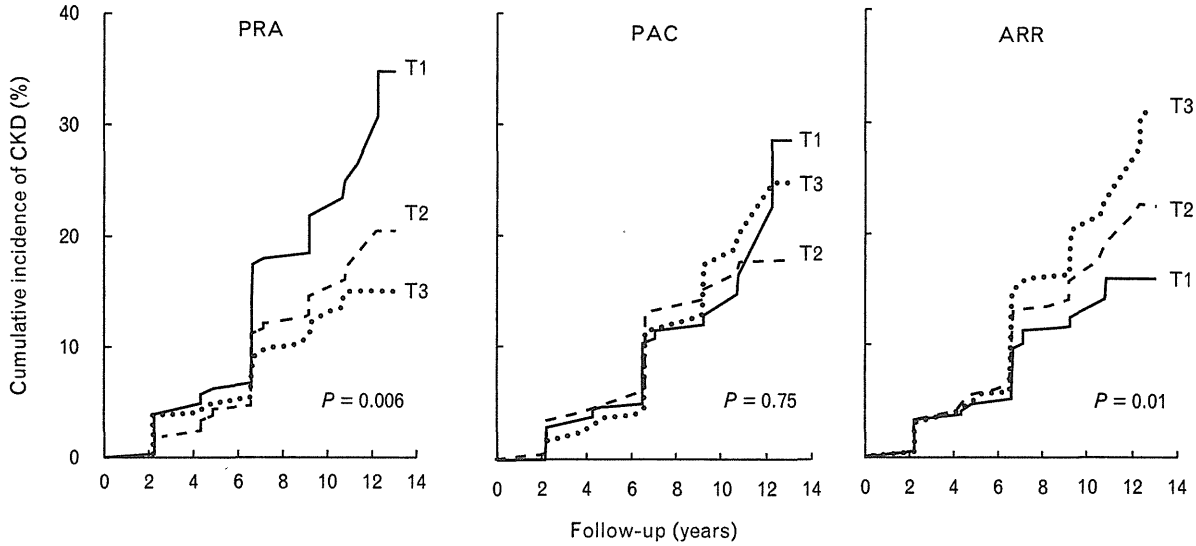

FIGURE 1 Kaplan–Meier survival function estimates for the cumulative incidence rate of chronic kidney disease (CKD) across tertiles of plasma renin activity (PRA), plasma aldosterone concentration (PAC), and the aldosterone-to-renin ratio (ARR). T1 to T3 indicate ascending tertiles; T1 below 0.8 ($n=213$), T2 0.8–1.4 ($n=229$), T3 above 1.4 (ng/ml per h) ($n=247$) for PRA; T1 below 5.4 ($n=221$), T2 5.4–7.4 ($n=231$), T3 above 7.4 (ng/dl) ($n=237$) for PAC; T1 below 4.2 ($n=229$), T2 4.2–7.9 ($n=230$), T3 above 7.9 (ng/ml)/(ng/ml per h) ($n=230$) for ARR. *P* values are for trends across the tertiles. Incidence rates and *P* values are adjusted for sex and age.

hazard ratios for development of CKD were 0.68 for lnPRA [95% confidence interval (CI) 0.53–0.87, $P=0.003$, in model 2) and 1.50 for ARR (95% CI 1.17–1.91, $P=0.001$, in model 2). When the participants with only one follow-up visit ($n=130$) were excluded, similar and significant results were observed (hazard ratio 0.72, $P=0.003$ for lnPRA; hazard ratio 1.39, $P=0.002$ for lnARR in model 2). Stratified analyses were also performed. Sex (men/women), age (<65/≥65 years), BMI (<25/≥25 kg/m^2), diabetes (with/without), hypertension [(<140/90)/(≥140/90) mmHg], or salt intake (below or above the median of 10.2 g/day) did not significantly affect the associations of lnARR with the incidence of CKD ($P>0.05$ for all interactions). Notably, the *P* value for the interaction term between sexes was 0.76. Individuals with CKD ($n=118$) had significantly lower PRA and higher ARR levels at baseline than those without CKD ($n=571$) after adjustment for sex and age (Fig. 2).

TABLE 2. Hazard ratios for the incidence of chronic kidney disease according to baseline PRA, PAC, and ARR levels

Independent variables	HRs	95% CIs	*P*
lnPRA			
Model 1	0.81	0.67–0.97	0.025
Model 2	0.76	0.63–0.93	0.007
Model 3	0.76	0.62–0.93	0.007
lnPAC			
Model 1	0.99	0.82–1.20	0.93
Model 2	0.94	0.78–1.14	0.52
Model 3	0.92	0.76–1.12	0.41
lnARR			
Model 1	1.24	1.03–1.48	0.023
Model 2	1.29	1.06–1.57	0.012
Model 3	1.28	1.05–1.57	0.017

Abbreviations: ARR, aldosterone-to-renin ratio; CI, confidence interval; HR, hazard ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity.
Hazard ratios (95% CIs) reflect risk associated with an increase in independent variables of 1 between-participant SD in each model. Model 1 is adjusted for sex and age. Model 2 is adjusted for BMI, ever smoking, ever drinking, a history of hypercholesterolemia, diabetes mellitus, and cardiovascular disease, SBP, and estimated glomerular filtration rate, in addition to the adjustments to model 1. Model 3 is adjusted for serum sodium, serum potassium, dietary salt intake (greater than or equal to median vs. less than median), and the number of follow-up visits in addition to the adjustments to model 2.

DISCUSSION

In the present longitudinal study, low PRA and high ARR levels were significantly and independently associated with the incidence of CKD in a community-based population with relatively high salt intake, after adjustment for sex, age, BMI, smoking, drinking, history of hypercholesterolemia, diabetes mellitus, and cardiovascular disease, and baseline eGFR. Individuals who developed CKD had significantly lower baseline levels of PRA and higher baseline levels of ARR than those without CKD, whereas there was no difference in PAC levels between them.

Few studies have examined the relationship between ARR and kidney dysfunction. One cross-sectional study showed an inverse relationship between the ARR level and the creatinine level in 28 patients with renal damage, whereas this relationship was not observed in 22 healthy individuals [29]. The present study demonstrated for the first time that not only elevated ARR but also reduced PRA were independent predictors of CKD in participants with normal baseline kidney function (eGFR ≥60 ml/min per 1.73 m^2). The present participants who developed CKD had lower baseline PRA levels than those without CKD. Theoretically, in normal individuals, it is expected that suppressed PRA mediates a low PAC. However, in the present study, PAC was not decreased in participants who developed CKD (Fig. 2). Such a form of low PRA and 'normal' PAC is called a relative aldosterone excess, which might increase the salt sensitivity of blood pressure due to inappropriate sodium and fluid retention or due to impaired nitric oxide-mediated

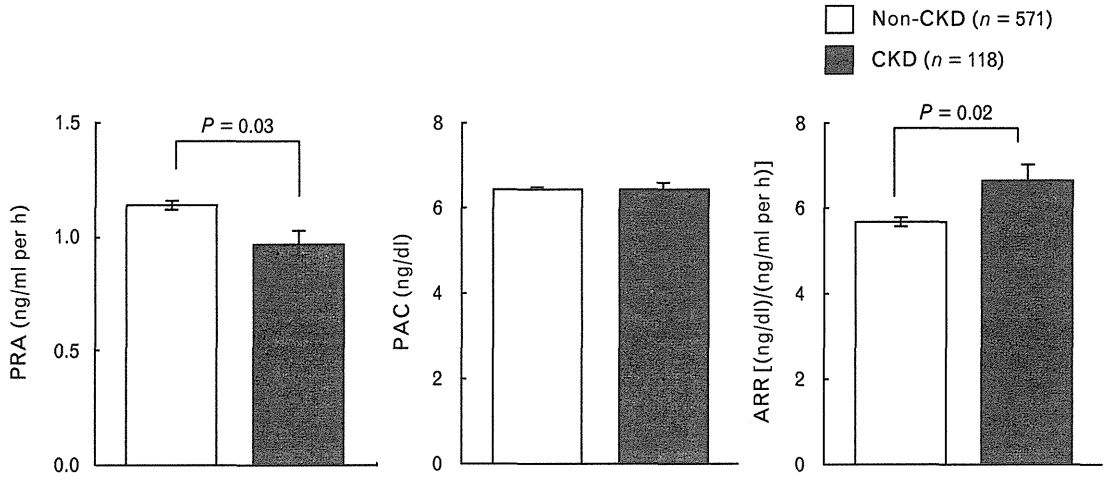

FIGURE 2 Baseline plasma renin activity (PRA), plasma aldosterone concentration (PAC), and aldosterone-to-renin ratio (ARR) levels in participants who did and did not develop chronic kidney disease (CKD). Means (standard error) and *P* values are adjusted for sex and age.

vasodilation [30,31]. Our group previously demonstrated that, even in the general population, relative aldosterone excess may be associated with salt-sensitive hypertension [20]. In salt-insensitive patients, high dietary sodium intakes suppress the renin-angiotensin system, which causes preferential vasodilatation of the efferent renal arteriole and mildly decreases intraglomerular pressure [32]. By contrast, in salt-sensitive patients, whose circulating renin is basically suppressed, intraglomerular pressure is known to be elevated during high sodium intake [32]. Therefore, an elevated ARR may cause kidney dysfunction mediated by the elevation of intraglomerular pressure with an increase in salt sensitivity. Patients with primary aldosteronism are known to be more likely to develop renal damage than patients with essential hypertension [16]. However, it is unlikely that primary aldosteronism patients were included as participants in the present study, because individuals who met the screening criteria [ARR >200 (pg/ml)/(ng/ml per h) and PAC >150 pg/ml] and who were taking antihypertensive drugs were excluded. The present results demonstrated that high ARR is a risk for CKD even in patients without primary aldosteronism.

A recent study by our group demonstrated that individuals with high ARR show a nondipper pattern of circadian blood pressure variation, with a diminished nocturnal decline in blood pressure [33]. This is explained by assuming that sodium retention during the daytime from relative aldosterone excess causes nocturnal elevation of blood pressure to enhance pressure natriuresis at night [34]. Previous clinical studies have reported that a nondipper status was a risk factor for progression of kidney dysfunction, independent of 24-h blood pressure and other risk factors [35,36]. Therefore, a high ARR level might cause CKD through a nondipper pattern with high nocturnal blood pressure. On the contrary, it has been postulated that reduced renal capacity of urine sodium excretion accompanied by diminished kidney function causes nocturnal blood pressure elevation [37]. Thus, the decline in kidney function, increase in salt sensitivity, and the nondipper pattern of blood pressure could influence each other and lead to a vicious circle.

It has been reported that a high serum aldosterone concentration was weakly but significantly associated with the development of eGFR less than 60 ml/min per 1.73 m^2 [adjusted odds ratio (OR) per a 1-SD increment 1.17, $P = 0.047$) in the Framingham Offspring Study – a population-based study [17]. However, this association was not observed in the present study (Table 2). The different results may be partly attributable to a difference in salt intake. Because the average salt intake in the Japanese population is higher than in western populations, renin and aldosterone secretion may be inhibited by high sodium dietary conditions in the present study [18]. In fact, the PAC level in the present participants (median 65, 25–75th percentile 50–81 pg/ml) was lower than that in the Framingham participants (median 100, 25–75th percentile 70–140 pg/ml) [17], although the measurement conditions were different. Because PAC was suppressed, it could be not associated with CKD development in the present study. Many previous clinical studies of aldosterone-induced renal injury reported that a mineralocorticoid receptor inhibitor reduced albuminuria independent of blood pressure reduction [38–40]. However, the participants in these studies were patients with type 2 diabetes [38], hypertension [39], or CKD [40]. Thus, the states of the renin–angiotensin–aldosterone system in these patients [38–40] may differ from that in the present study population. The present result may suggest that volume expansion by relative aldosterone excess may be more important as a pathway for CKD pathogenesis than direct organ damage caused by aldosterone in people with high salt intake. Further studies in various populations are needed to clarify the pathophysiological effects of renin and aldosterone in renal damage. So far, only extremely high ARR has been considered in terms of the differential diagnosis of primary aldosteronism. However, physicians should be aware that even moderately high ARR

levels are linked to not only salt sensitivity but also organ damage.

There were some limitations to the present study. First, participants underwent blood sampling between 0900 and 1100 h or between 1300 and 1500 h, and most often they had not fasted. These nonstandardized conditions of blood sampling could affect both PRA and PAC levels through circadian variations and dietary salt intake before the health check-up [41] and lead to a bias towards the null. ARR would reduce those variations, because various clinical situations that would change PRA levels would also proportionately affect PAC levels. The findings in the present study might imply that, even when measured under nonstandardized conditions, PRA and ARR are clinically useful indices for CKD. Second, the study population predominantly included middle-aged, elderly, and female individuals with relatively high salt intakes. These imbalances might, to some extent, limit the external validity of the findings. Especially, in Table 1, the highest tertile of ARR was constructed by mainly female participants (81.3%). However, this was consistent with previous observations [13,33,42], and the associations of lnPRA and lnARR with the development of CKD did not differ between female and male participants. Third, we did not have any information of drugs other than antihypertensive medications, which could potentially reduce their GFR, such as NSAIDs.

In conclusion, low PRA and high ARR were associated with the development of CKD in a general population with high salt intake. The present results suggest that PRA and ARR may be independent predictors of CKD, and that salt sensitivity indicated by relative aldosterone excess may contribute to an increased risk of CKD.

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Conflicts of interest

Financial Disclosure: None of the authors declares a conflict of interest.

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ORIGINAL CONTRIBUTIONS

Home Blood Pressure Level, Blood Pressure Variability, Smoking, and Stroke Risk in Japanese Men: The Ohasama Study

Takanao Hashimoto[1–3], Masahiro Kikuya[2], Takayoshi Ohkubo[2,4], Michihiro Satoh[2], Hirohito Metoki[5], Ryusuke Inoue[6], Kei Asayama[2,7], Atsuhiro Kanno[1,2], Taku Obara[8], Takuo Hirose[9–12], Azusa Hara[13], Haruhisa Hoshi[14], Kazuhito Totsune[2,15], Hiroshi Satoh[16], Hiroshi Sato[1] and Yutaka Imai[2]

BACKGROUND
Hypertension and smoking independently contribute to the risk of stroke. Our objective was to investigate home blood pressure (HBP) levels, day-by-day BP variability, and smoking in the prediction of stroke in Japanese men.

METHODS
In this study, 902 men (mean age, 58.6 years) without a past history of stroke were evaluated. HBP was measured once every morning for 4 weeks. Day-by-day BP variability was defined as within-subject standard deviations (SD) of HBP. Smoking history was obtained from a standardized questionnaire. Hazard ratios (HRs) for stroke were examined by Cox regression model, with adjustment for possible confounders.

RESULTS
During 13.1 years (median) of follow-up, 89 cerebral infarctions, 28 intracranial hemorrhages, and six other strokes occurred. Systolic HBP levels (HR = 1.59 per 14.6 mm Hg increase, $P < 0.0001$) and variability (HR = 1.26 per 3.1 mm Hg increase, $P = 0.03$) of +1 between-subject SD were significantly associated with cerebral infarction. The relationship between HBP and cerebral infarction differed with smoking status (interaction $P = 0.021$ and 0.017 for systolic level and variability, respectively). In analyses stratified according to smoking, systolic level (HR = 1.78, $P < 0.0001$) and variability (HR = 1.38, $P = 0.006$) were significantly associated with cerebral infarction in ever smokers ($N = 511$), but not in never smokers ($N = 391$; $P \geq 0.6$ for both). No significant association was found between smoking and the risk of intracranial hemorrhage.

CONCLUSIONS
In ever smokers, both HBP levels and variability are significantly associated with the risk of cerebral infarction. Our findings further validate the benefit of smoking cessation in preventing cardiovascular disease (CVD), especially cerebral infarction.

Keywords: blood pressure; epidemiology; home blood pressure monitoring; hypertension; prognosis; smoking; stroke

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[1]Department of Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [2]Department of Planning for Drug Development and Clinical Evaluation, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [3]Tohoku University Institute for International Advanced Research and Education, Sendai, Japan; [4]Department of Health Science, Shiga University of Medical Science, Otsu, Japan; [5]Department of Gynecology and Obstetrics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [6]Department of Medical Informatics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [7]Katholieke Universiteit Leuven, Leuven, Belgium; [8]Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan; [9]Department of Nephrology, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [10]Department of Endocrinology, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [11]Department of Vascular Medicine, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan; [12]Center for Interdisciplinary Research in Biology, College de France, Paris, France; [13]Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan; [14]Ohasama Hospital, Iwate, Japan; [15]Tohoku Fukushi University, Sendai, Japan; [16]Department of Environmental Health Science, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, Japan. Correspondence: Masahiro Kikuya (kikuyam@med.tohoku.ac.jp)

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Hypertension and smoking are major risk factors for the development of cardiovascular diseases (CVDs).[1,2] Indeed, high blood pressure (BP) and smoking may act synergistically to elevate the risk of increased carotid intima-media thickness,[3] coronary heart disease[4], and stroke.[5] Nevertheless, this synergistic effect of smoking and hypertension on CVD risk has not been universally supported.[6,7] The limitations inherent to conventional BP evaluation performed in prior studies,[2–7] including a white coat effect and observer and regression dilution biases,[8,9] may explain these discrepant findings.

We previously reported that in contrast to conventional BP, self-measurement of BP at home (HBP) more accurately reflects an individual's BP and is able to better predict CVD risk.[8,10,11] Moreover, we recently found that subjects with passive exposure to cigarette smoke had a significantly higher level of HBP than nonexposed subjects.[12] This latter finding suggests that HBP measurements can detect the relatively small effect of smoking on BP.

The clinical significance of BP variability is gradually becoming understood.[13] We previously reported the utility of day-by-day BP variability provided by multiple HBP measurements in the prediction of CVD risk.[14] However, although ambulatory BP variability and smoking have been shown to significantly and independently predict CVD events, including fatal or nonfatal stroke,[15] the clinical value of day-by-day BP variability in the prediction of stroke risk, as stratified by smoking, has never been examined. Thus, in this study, we examined the hypothesis that HBP parameters (level and variability) and smoking are interactively associated with the first onset of stroke. Additionally, we examined the influence of smoking on the relationship between HBP parameters and mortality from CVDs.

METHODS

Design. This report was based on longitudinal observations of subjects who have been participating in a BP measurement project in Ohasama, Iwate, Japan, since 1987. Socioeconomic and demographic characteristics of this region and details of the study project have been described previously.[10,16] The institutional review boards of the Tohoku University School of Medicine and the Ohasama Municipal Government Department of Health approved the study. All subjects provided written informed consent for participation in the study.

Study population. From 1988 until 1995, we contacted 4,969 subjects who were at least 35 years old and who lived in four districts of Ohasama.[17–19] Of these, 1,057 subjects who were not at home (e.g., working outside of Ohasama) during the normal working hours of the study nurses were excluded, as were subjects who were hospitalized ($N = 166$) or incapacitated ($N = 94$). Of the remaining 3,652 residents, 2,917 (80%) participated in baseline and follow-up examinations. We further excluded 478 subjects from whom either HBP measurements were not obtained ($N = 53$) or the number of HBP measurements were too low (<10) to calculate HBP variability ($N = 425$).[14] In order to examine the risk of onset of the first stroke, 114 individuals who had a past history of stroke were excluded. Due to the low percentage of female smokers (2.1%), women ($N = 1423$) were excluded from the analysis. Finally, 902 men were included in the present study.

HBP level and variability. Trained nurses obtained measurements of anthropometric variables at public health centers in Ohasama. Physicians and/or public health nurses instructed subjects on how to perform HBP measurements. Over a period of 4 weeks, subjects were asked to measure their BP each morning using an oscillometric device (HEM 401C; Omron Healthcare, Kyoto, Japan). Measurements were taken in the sitting position after a minimum of 2 min of rest and within 1 h of waking.[20,21] The HBP level was calculated as the average of within-subject readings. HBP variability was calculated as the standard deviation (SD) of more than 10 HBP measurements.[14,16] This method has been previously validated.[14]

Data collection including smoking. Well-trained study nurses ascertained the cigarette smoking habit of each subject using a standardized questionnaire. Other data, including medical history, medications, and alcohol consumption of each subject were also obtained. Risk factors for CVD were obtained from questionnaires and medical records at Ohasama Hospital. Total cholesterol and blood glucose levels were analyzed using standard automated enzymatic methods of venous blood sampling. According to published criteria,[22] diabetes mellitus was defined as a fasting glucose level ≥7.0 mmol/l (126 mg/dl), nonfasting glucose level ≥11.1 mmol/l (200 mg/dl), and/or use of antidiabetic medications.

Hypercholesterolemia was defined as serum total cholesterol ≥5.68 mmol/l (220 mg/dl) and/or the use of medications for hypercholesterolemia. Obesity was defined as a body mass index of ≥25 kg/m^2.

Follow-up and risk ascertainment. We collected follow-up data until 31 May 2007. Past history, mortality from CVDs and stroke incidence were investigated via the Stroke Registration System of Iwate Prefecture, death certificates from the Ohasama Health Department, and questionnaires sent to each household. Events were subsequently confirmed by checking subjects' medical records at Ohasama Hospital, the only local hospital. The cause of death was classified according to the recommendations of the 10th revision of the World Health Organization's International Classification of Diseases. The specific cause of death was attributed to the underlying cause initiating the sequence of events leading to mortality. Computed tomography and magnetic resonance imaging of the brain were available, and >90% of the subjects had their regular checkups at this facility. The end points evaluated were incidence of CVD and death from all causes, including cardiovascular (International Classification of Diseases, 10th Revision code "I"), stroke (I6), cerebral infarction (I63), intracranial hemorrhage (I61), subarachnoid hemorrhage (I60), and cardiac disorders (I05, I11, I20–I25, I34, I35, I38, I46–I50, I71, I74, I77, and I99). The present analysis considered only the first stroke event. Follow-up of each subject was continued until the occurrence of one of the following censoring events: onset of stroke, death, loss to follow-up because of moving away from Ohasama town, or the end of our follow-up period. The Stroke Registration System of Iwate Prefecture was previously validated in another of our reports,[23] ensuring that misclassification of stroke subtypes would be minimum.

Statistical analysis. Means and proportions of baseline characteristics according to smoking were compared using the analysis of variance (ANOVA) and χ^2 tests, respectively. Stroke incidence per 1,000 person-years was plotted according to quartiles of HBP levels and variability. Stroke hazard ratios (HRs) equal to an increase of 1 between-subject SD in HBP level were calculated by multiple Cox proportional hazard model, adjusting for age, smoking, alcohol drinking, CVD, diabetes mellitus, hypercholesterolemia, and use of antihypertensive medication. HRs for an increase of 1 between-subject SD in BP variability were

further adjusted for systolic HBP level. [14] When performing stratified analyses according to smoking, we categorized smoking as ever (current or former) vs. never. We assessed the interaction between HBP and smoking for predicting stroke by adding interaction terms to the Cox proportional hazard models. Baseline characteristics of subjects are presented as mean ± SD or as percentages. All statistical analyses were performed using SAS version 9.1 software (SAS Institute, Cary, NC). Two-tailed values of $P < 0.05$ were considered statistically significant.

RESULTS

Baseline characteristics of the subjects

Mean age was 59 years (range: 35–92 years). **Table 1** shows the baseline characteristics of all study subjects according to smoking (never, former, or current). Age (P for ANOVA <0.0001) and the proportion of subjects receiving antihypertensive treatment (chi-square $P = 0.006$) were significantly different between the three smoking groups, whereas groups were similar in all other baseline characteristics, including HBP values.

Analysis of stroke incidence and cardiovascular mortality

Of the 902 subjects, 29 (3%) were lost to follow-up, while 267 (30%) died. During a median 13.1 years of follow-up, first strokes were observed in 123 subjects, including 89 (72%) cerebral infarctions, 28 (23%) intracranial hemorrhages, 4 (3%) subarachnoid hemorrhages, and 2 (2%) of unknown origin. The 267 deaths included 75 (28.1%) cardiovascular deaths, with 38 (14.2%) strokes and 37 (13.9%) cardiac deaths.

Table 2 shows the HRs of stroke incidence and mortality for an increase of 1 between-subject SD in systolic HBP parameters, because in a previous study we showed that systolic HBP has the strongest association with stroke incidence as compared to diastolic BP, mean BP, or pulse pressure.[23] Systolic HBP parameters were significantly predictive of the incidence of cerebral infarction and stroke mortality.

Next, we performed stratified analyses according to smoking. A significant interaction was observed between systolic HBP parameters and smoking in predicting cerebral infarction incidence (P for interaction =0.021 and 0.017 for systolic BP level and variability, respectively). **Figure 1** shows the incidence of stroke per 1,000 person-years across quartiles of HBP parameters. In the ever smoking group ($N = 511$), a significant linear association was observed between incidence of cerebral infarction and BP level and variability (P for trend <0.0001 for both), whereas no association existed between HBP parameters and intracranial hemorrhage (P for trend ≥0.1). **Figures 2** and **3** show HRs of stroke for an increase of 1 between-subject SD in systolic HBP parameters according to smoking. In the ever smoking group, both systolic HBP level and variability were significantly predictive of cerebral infarction. In untreated subjects ($N = 667$), the systolic HBP level, but not the variability, was significantly predictive of cerebral infarction: HR (95% confidence interval) = 1.61 (1.171–2.21), $P = 0.003$, and HR (95% confidence interval) = 1.01 (0.70–1.45), $P = 0.97$, respectively.

Tables 3 and **4** show the HRs of mortality for an increase of 1 between-subject SD in systolic HBP parameters according to smoking. No significant interactions were observed between systolic HBP parameters and smoking ($P \geq 0.08$).

We also performed sensitivity analyses. First, we examined stroke risk relative to elevated HBP parameters in current smokers ($N = 417$). Because the number of subjects categorized as former smokers ($N = 94$) was small, we could not calculate HRs and linear trends for them. We therefore calculated

Table 1 | Baseline characteristics of 902 Japanese men according to smoking status

		Smoking status			
	All subjects	Never	Former	Current	*P*
No. of subjects	902	391	94	417	
Age (years)	58.6 ± 12.2	60.5 ± 12.1	62.3 ± 11.4	56.1 ± 12.0	<0.0001
BMI (kg/m^2)	23.2 ± 2.6	23.5 ± 2.5	22.8 ± 2.8	23.1 ± 2.7	0.05
Alcohol drinking (%)	67.1	63.8	69.4	63.8	0.3
Diabetes mellitus (%)	10.1	10.7	11.7	9.1	0.6
Hypercholesterolemia (%)	18.9	17.9	19.2	19.7	0.8
History of heart diseases (%)	0.7	0.5	0.0	1.0	0.5
Antihypertensive treatment (%)	26.1	31.0	27.7	21.1	0.006
Home BP level					
Systolic BP (mm Hg)	127.8 ± 14.6	128.2 ± 14.7	127.8 ± 16.5	127.3 ± 14.2	0.7
Diastolic BP (mm Hg)	78.8 ± 9.6	79.2 ± 9.3	78.6 ± 10.6	78.5 ± 9.5	0.5
Home BP variability					
Systolic BP variability (mm Hg)	8.4 ± 3.1	8.5 ± 3.1	8.8 ± 3.2	8.3 ± 3.2	0.4
Diastolic BP variability (mm Hg)	6.4 ± 2.2	6.4 ± 2.3	6.2 ± 2.0	6.4 ± 2.2	0.6

Values are expressed as mean ± s.d. *P* values were calculated using analysis of variance (ANOVA) for continuous variables or chi-square test for categorical variables.
BMI, body mass index; BP, blood pressure.

Table 2 | Hazard ratios for stroke and cardiovascular mortality in relation to home blood pressure parameters in Japanese men (*N* = 902)

		Systolic level			Systolic variability		
Events	Event, *N*	HR	95% CI	*P*	HR	95% CI	*P*
Incidence							
Total stroke	123	1.59	1.32–1.92	<0.0001	1.15	0.96–1.38	0.1
Cerebral infarction	89	1.59	1.28–1.98	<0.0001	1.26	1.02–1.55	0.03
Intracranial hemorrhage	28	1.39	0.91–2.13	0.1	0.97	0.65–1.46	0.9
Mortality							
Total	267	1.16	1.02–1.32	0.03	1.06	0.93–1.20	0.4
Cardiovascular	75	1.43	1.13–1.83	0.004	1.13	0.90–1.40	0.3
Stroke	38	1.52	1.08–2.13	0.02	1.47	1.11–1.95	0.007
Cerebral infarction	21	1.55	0.99–2.42	0.06	1.88	1.31–2.69	0.0006
Intracranial hemorrhage	14	1.44	0.81–2.56	0.2	1.34	0.83–2.14	0.2
Cardiac	37	1.42	1.00–2.01	0.050	0.84	0.59–1.18	0.3
Myocardial infarction	14	1.21	0.68–2.14	0.5	0.84	0.45–1.55	0.6
Ischemic heart disease	17	1.50	0.89–2.51	0.1	0.69	0.40–1.20	0.2

HRs of a 1 between-subject SD increase were adjusted for age, smoking, alcohol drinking, history of cardiovascular disease, diabetes mellitus, hypercholesterolemia, and the use of antihypertensive medication. HRs of home systolic variability were further adjusted for home systolic level. Values of a 1 between-subject SD increase were 14.6 and 3.1 mm Hg for systolic level and variability, respectively. The following deaths were not analyzed because of low event size: four for subarachnoid hemorrhage (incidence, also 4), seven for heart failure.
CI, confidence interval; HR, hazard ratio; SD, standard deviation.

linear trends for cerebral infarction only in the current smoking group (N = 417). The trends for cerebral infarction of the quartiles of HBP parameters showed similarly significant linearity (P for trend <0.0001 for both systolic BP level and variability). Multivariate-adjusted HRs for cerebral infarction were also significant (HR = 2.10, P < 0.0001 of systolic BP level; HR = 1.35, P = 0.02 of systolic BP variability). Second, we examined HRs of HBP variability, defined by coefficients of variation, which were calculated as HBP variability divided by the mean value of HBP level. Similar results were observed with HBP variability defined by coefficients of variation and SD (data not shown).

DISCUSSION

In this study, higher systolic HBP parameters were significantly associated with cerebral infarction. The interactions between HBP parameters and smoking for predicting cerebral infarction were significant. In analyses stratified according to smoking, systolic HBP level and variability were significantly associated with cerebral infarction only in ever smokers. Systolic HBP parameters were also significantly associated with stroke death, although no significant interactions with smoking were observed.

The present study illustrated the significance of HBP parameters, as well as smoking, in the prediction of stroke incidence, especially for cerebral infarction. It is well established that BP and smoking are both crucial risk factors for CVD.[1,2] However, whether an interaction between BP and smoking exists in the prediction of CVD risk requires further discussion. A previous investigation of the Atherosclerosis Risk in Communities (ARIC) study suggested that both smoking and hypertension may increase carotid intima-media thickness, reflecting subclinical atherosclerosis.[24] In contrast, a subanalysis by the Asia Pacific Cohort Studies Collaboration (APCSC) observed no relevant difference in stroke risk between smokers and nonsmokers with elevated BP (measured conventionally).[2] A prospective study of 1,700 Danish men and women without major CVD reported that the 10-year absolute risk of CVD mortality according to 24-h ambulatory BP was greater among smokers compared with nonsmokers.[25]

The significant associations between systolic HBP parameters and stroke death observed in this study were consistent with our previous findings in the Ohasama study.[8,14] However, stratified analyses according to smoking did not detect significant associations between HBP parameters and mortality risks, because of the smaller number of deaths. Furthermore, in this study, the case fatality rate for intracranial hemorrhage was 50.0%, which was higher than that of cerebral infarction (23.6%). Therefore, the significant associations between systolic HBP level and cardiovascular and stroke deaths observed in the never smoking group could have been affected by the higher percentage of deaths from intracranial hemorrhage in the never (22.9% and 53.8% cardiovascular and stroke deaths, respectively) compared with the ever smoking group (16.3% and 28.0% cardiovascular and stroke deaths, respectively) (**Table 3**). A previous report on the stratified analyses of men, which examined 9,638 Japanese subjects to assess the influence of smoking on stroke death, showed that smoking was not significantly associated with intracranial hemorrhage, and its HRs were below 1.0 regardless of number of cigarettes smoked, although it was a significant predictor of cerebral infarction.[26] Systolic HBP variability remained a significant predictor of stroke mortality in the ever smoking group. However, in this study, no difference was observed in the association between

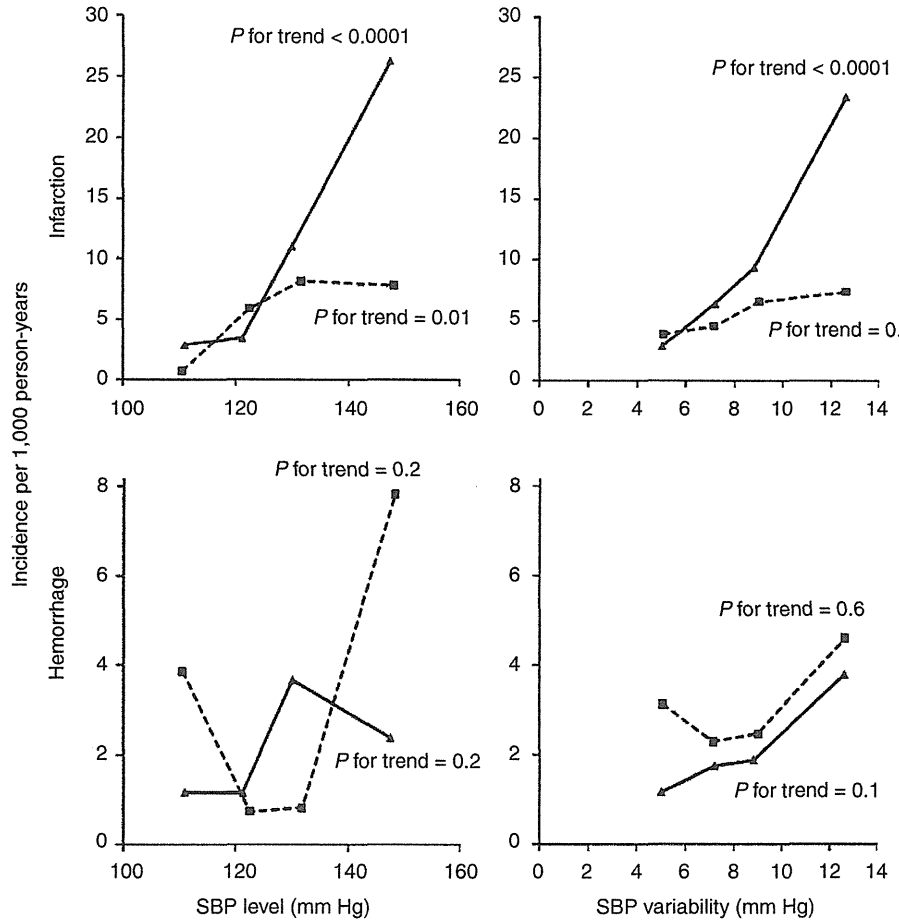

Figure 1 | Stroke incidence per 1,000 person-years according to quartiles of systolic blood pressure (SBP) level and variability. The values plotted along the horizontal axis are within-quartile means of home blood pressure level and variability. Solid and dashed lines represent ever and never smoking groups, respectively. The ever smoking group includes current and former smokers.

systolic HBP variability and mortality from cerebral infarction in terms of smoking status (**Table 4**). Furthermore, interactions between HBP parameters and smoking were not significant. Thus, to fully investigate the influence of smoking on the association between HBP parameters and mortality, a larger sample size and longer follow-up period would be needed.

The observation that smoking interacts with HBP parameters and contributes to the incidence of cerebral infarction could be explained by several potential mechanisms. First, Polish investigators found that smoking was independently associated with a chronic increase in resting muscle sympathetic nerve activity in essential hypertensive patients.[27] Previous reports have mentioned that sympathetic nerve activity could play a role in the pathogenesis of hypertension and related diseases.[28,29] Previous studies that examined the significance of ambulatory BP variability as a risk for hypertension and CVD also suggested that the sympathetic nervous system may be positively associated with the morning BP surge,[30] and the daytime variability and sleep-morning BP difference.[31] Although the prognostic value of HBP variability may not be equal to that of ambulatory BP variability, smoking, by inducing activation of the sympathetic nervous system, may partially contribute to the increase in HBP level and variability, as well as the increased risk of cerebral infarction. Second, prior investigations also examined the relationship between smoking and endothelial dysfunction. *In vitro* analyses of human coronary artery endothelial cells revealed that human coronary artery endothelial cells exposed to serum from a smoker showed decreased nitric oxide synthesis, representative of reduced endothelial-dependent vasodilatation.[32] Moreover, a large-scale epidemiological study including ~6,600 healthy Japanese subjects reported that BP and smoking were significantly and interactively associated with the augmentation index in men.[33] Therefore, the synergistic effect of HBP and smoking on cerebral infarction risk might result from atherosclerosis and arteriosclerosis secondary to the endothelial dysfunction induced by chronic smoking.

Alcohol consumption might affect HBP parameters. From cross-sectional analyses, including the Ohasama study[34] and the Finn-home study,[35] alcohol consumption was recognized as a determinant of increased day-by-day BP variability. However, in our present study, HBP parameters were significant predictors of stroke incidence, especially cerebral infarction, even after adjustment for alcohol drinking. We observed no interaction between

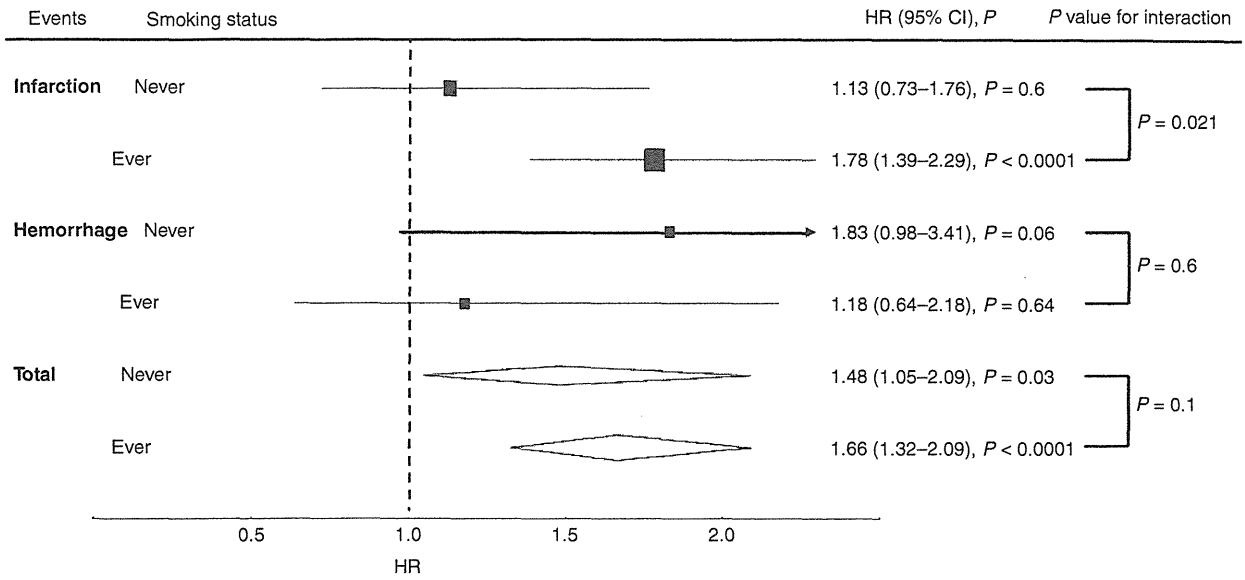

Figure 2 | Stroke risks in relation to elevated home systolic blood pressure level according to smoking. Hazard ratios (HRs) and 95% confidence intervals (CIs) reflect the risk associated with an increase in home blood pressure level of 1 between-subject standard deviation (14.6 mm Hg). Boxes represent HRs, whereas the horizontal lines depict 95% CIs. Diamonds show HRs and 95% CIs for all kinds of stroke. HRs were adjusted for age, obesity, alcohol drinking, diabetes mellitus, hypercholesterolemia, history of CVD and the use of antihypertensive medications. The ever smoking group includes current and former smokers. CVD, cardiovascular disease.

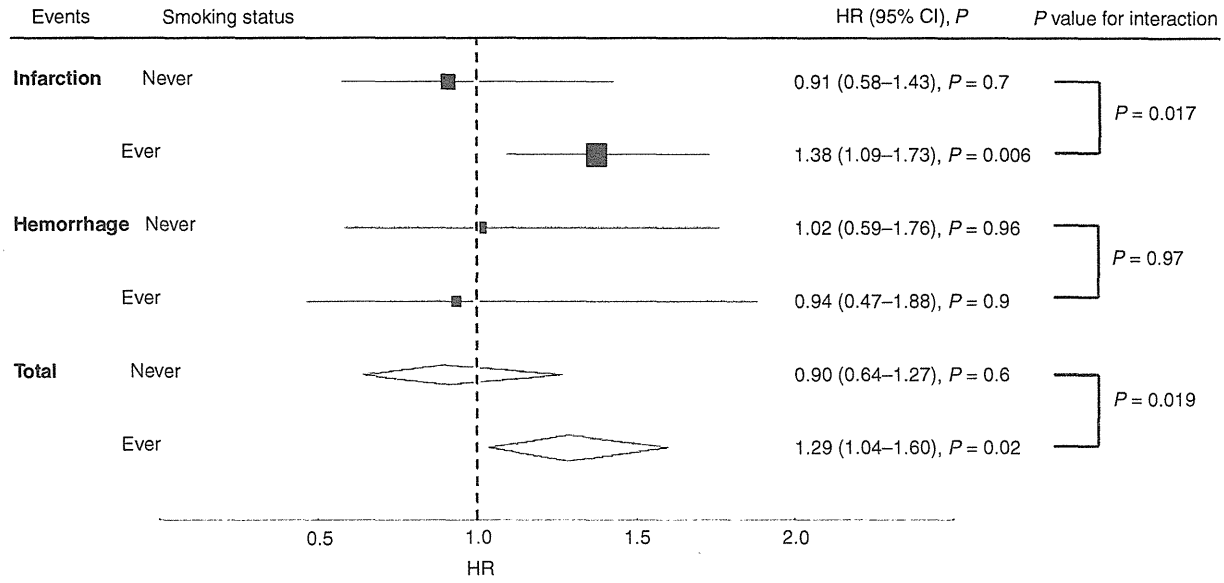

Figure 3 | Stroke risks in relation to increased home systolic blood pressure variability according to smoking. Hazard ratios (HRs) and 95% confidence intervals (CIs) reflect the risk associated with an increase in home blood pressure variability of 1 between-subject standard deviation (3.1 mm Hg). Boxes represent HRs, whereas the horizontal lines depict 95% CIs. Diamonds depict HRs and 95% CIs for all kinds of stroke. HRs were adjusted for age, obesity, alcohol drinking, diabetes mellitus, hypercholesterolemia, history of CVD, the use of antihypertensive medications and home systolic blood pressure level. The ever smoking group includes current and former smokers. CVD, cardiovascular disease.

HBP parameters and alcohol drinking ($P > 0.3$). Therefore, although we do not have detailed information on the amount or types of alcohol consumed by our study subjects, the influence of alcohol drinking on our results might not be large.

The present study had several limitations that warrant mention. First, day-by-day BP variability could be influenced by methodological problems[36] or environmental conditions (e.g., mental and physical stress).[14] Second, although several

Table 3 | Mortality risks in relation to elevated home systolic blood pressure levels according to smoking status in Japanese men (*N* = 902)

			Systolic level			
Mortality	Smoking status	Events, *N*	HR	95% CI	*P*	*P* for interaction
Total	Never	107	1.33	1.07–1.66	0.01	0.2
	Ever	160	1.07	0.91–1.26	0.4	
Cardiovascular	Never	32	2.03	1.34–3.08	0.0009	0.1
	Ever	43	1.17	0.86–1.59	0.3	
Stroke	Never	13	2.58	1.32–5.04	0.006	0.6
	Ever	25	1.29	0.86–1.95	0.2	
Cerebral infarction	Never	5	2.25	0.83–6.10	0.1	0.8
	Ever	16	1.45	0.88–2.40	0.1	
Intracranial hemorrhage	Never	7	2.50	0.96–6.46	0.06	0.95
	Ever	7	1.11	0.50–2.47	0.8	
Cardiac	Never	19	1.83	1.07–3.14	0.03	0.08
	Ever	18	1.13	0.71–1.81	0.6	
Ischemic heart disease	Never	9	1.89	0.84–4.26	0.1	0.4
	Ever	8	1.10	0.56–2.20	0.8	

Number of never and ever smokers were 391 and 511, respectively. The ever smoking group includes current and former smokers. HRs of a 1 between-subject standard deviation increase (14.6 mm Hg) were adjusted for age, smoking, alcohol drinking, history of cardiovascular disease, diabetes mellitus, hypercholesterolemia, and the use of antihypertensive medication.
CI, confidence interval; HR, hazard ratios.

Table 4 | Mortality risks in relation to elevated home systolic blood pressure variability according to smoking status in Japanese men (*N* = 902)

			Systolic variability			
Mortality	Smoking status	Events, *N*	HR	95% CI	*P*	*P* for interaction
Total	Never	107	1.11	0.89–1.38	0.3	0.5
	Ever	160	1.06	0.89–1.26	0.5	
Cardiovascular	Never	32	1.15	0.80–1.66	0.5	0.9
	Ever	43	1.19	0.89–1.59	0.2	
Stroke	Never	13	1.28	0.72–2.28	0.4	0.4
	Ever	25	1.59	1.14–2.22	0.007	
Cerebral infarction	Never	5	2.62	1.03–6.67	0.04	0.8
	Ever	16	1.85	1.11–2.79	0.004	
Intracranial hemorrhage	Never	7	1.09	0.48–2.47	0.8	0.4
	Ever	7	1.55	0.79–3.04	0.2	
Cardiac	Never	19	1.01	0.63–1.64	0.95	0.1
	Ever	18	0.73	0.40–1.31	0.3	
Ischemic heart disease	Never	9	0.77	0.35–1.69	0.5	0.4
	Ever	8	0.54	0.21–1.37	0.2	

Number of never and ever smokers were 391 and 511, respectively. The ever smoking group includes current and former smokers. HRs of a 1 between-subject standard deviation increase (3.1 mm Hg) were adjusted for home systolic level, age, smoking, drinking, history of cardiovascular disease, diabetes mellitus, hypercholesterolemia, and the use of antihypertensive medication.
CI, confidence interval; HR, hazard ratios.

previous studies demonstrated a dose–response relationship between tobacco smoking and risk of CVD,[37,38] we did not collect data regarding smoking duration or the numbers of cigarettes smoked per day. Third, baseline characteristics such as age and antihypertensive treatment might have been confounding factors affecting our results. In our previous report[39] that examined the predictive power of four ambulatory BP indices (systolic, diastolic, mean BP, and pulse pressure) for stroke, systolic BP was the strongest predictor for stroke, although this association was weakened by age as a covariate. It is suggested that the age-dependent decrease in diastolic BP might contribute to the weaker age-adjusted association of

systolic BP and CVD. In addition, the Ohasama study showed that the predictive power of HBP for stroke could be independent of the influence of anti-hypertensive treatment.[19] In the present study, after adjustment for these confounders, elevated systolic HBP parameters were still significantly predictive of stroke, and then these effects might be partial. Fourth, the present study included a small sample of Japanese men with a low stroke incidence, especially for hemorrhagic stroke. Furthermore, since women were excluded in this study, our findings cannot to be generalized to women. The limited generalizability of this study should prompt further examinations in other populations with a larger sample size.

In the present study of Japanese men over 35 years of age, HBP parameters (level and variability) were significantly associated with the risk of cerebral infarction, especially in ever smokers. The fact that smoking contributes to both increased BP and a risk of CVD is well known. Therefore, the results of this study further substantiate the utility of smoking cessation in preventing CVD.

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