Table 1. Age-adjusted prevalence and mean values of risk factors in 1974, 1988 and 2002 by sex

	Men				Women			
	1974 n = 911	1988 n = 1165	2002 n = 1414	P for trend	1974 n = 1207	1988 n = 1576	2002 n = 1883	P for trend
Age, years	56 ± 11	59 ± 12	61 ± 12	<0.001	57 ± 12	60 ± 12	62 ± 13	<0.001
Systolic blood pressure, mmHg	139 ± 21	136 ± 21	134 ± 21	<0.01	141 ± 21	134 ± 21	129 ± 21	<0.01
Diastolic blood pressure, mmHg	79 ± 12	81 ± 12	81 ± 12	<0.01	78 ± 12	76 ± 12	76 ± 12	<0.01
Hypertension, %	42.0 (39.0–46.0)	44.4 (40.6–48.2)	42.5 (39.0–46.0)	0.90	42.0 (38.4–45.6)	34.7 (31.9–37.5)	31.3 (28.9–33.7)	<0.001
Treated, %	9.2 (7.2–11.2)	13.8 (11.7–15.9)	19.4 (17.2–21.6)	<0.001	7.9 (6.4–9.4)	13.3 (11.6–15.0)	16.8 (15.1–18.5)	<0.001
Untreated, %	32.8 (29.1–36.5)	30.6 (27.4–33.8)	23.1 (20.4–25.8)	<0.001	34.1 (30.9–37.3)	21.3 (19.0–23.6)	14.5 (12.7–16.3)	<0.001
Diabetes mellitus, %	2.5 (1.5–3.5)	14.3 (12.1–16.5)	20.6 (18.2–23.0)	<0.001	2.0 (1.2–2.8)	9.0 (7.6–10.4)	11.5 (10.0–13.0)	<0.001
Treated, %	–	2.7 (1.8–3.6)	5.6 (4.4–6.8)	<0.001	–	2.6 (1.8–3.4)	2.8 (2.1–3.5)	0.23
Untreated, %	–	11.5 (9.5–13.5)	14.9 (12.8–17.0)	0.002	–	6.4 (5.2–7.6)	8.7 (7.3–10.1)	0.01
Hypercholesterolaemia, %	12.4 (10.1–14.7)	27.1 (24.0–30.2)	26.9 (23.9–29.9)	<0.001	20.3 (17.8–22.8)	41.4 (38.2–44.6)	41.0 (38.0–44.0)	<0.001
Treated, %	–	–	6.3 (5.0–7.6)	–	–	–	8.9 (7.7–10.1)	–
Untreated, %	–	–	20.6 (17.9–23.3)	–	–	–	32.1 (29.3–34.9)	–
Obesity, %	11.3 (9.1–13.5)	24.4 (21.4–27.4)	29.4 (26.2–32.6)	<0.001	21.3 (18.6–24.0)	23.9 (21.4–26.4)	23.8 (21.4–26.2)	0.004
Metabolic syndrome, %	–	8.1 (6.4–9.8)	13.4 (11.3–15.5)	<0.001	–	16.5 (14.5–18.5)	18.6 (16.7–20.5)	<0.01
Smoking habits, %	72.2 (66.6–77.8)	50.6 (46.4–54.8)	46.7 (42.6–50.8)	<0.001	10.2 (8.4–12.0)	6.9 (5.5–8.3)	8.6 (7.0–10.2)	0.002
Alcohol intake, %	63.6 (58.4–68.8)	61.9 (57.2–66.6)	71.2 (66.2–76.2)	<0.001	5.4 (4.1–6.7)	9.8 (8.1–11.5)	29.5 (26.6–32.4)	<0.001

Age is not age-adjusted. Values are means ± standard deviations or frequencies. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or current use of antihypertensive agents. Diabetes mellitus was defined by fasting glucose concentrations ≥126 mg/dl (7.0 mmol/L) or postprandial glucose concentrations ≥200 mg/dl (11.1 mmol/L) in 1974 and by a 75-g oral glucose tolerance test in 1988 and 2002 in addition to a medical history of diabetes according to the recommendations of the American Diabetes Association. Hypercholesterolaemia was defined as serum total cholesterol ≥220 mg/dl (5.7 mmol/L) or current use of a lipid-modifying agent. Obesity was defined as body mass index ≥25 kg/m². Treated or untreated statuses were defined as the presence or absence of use of any medication for the treatment. Metabolic syndrome was defined by using criteria recommended in a joint interim statement of five major scientific organizations.
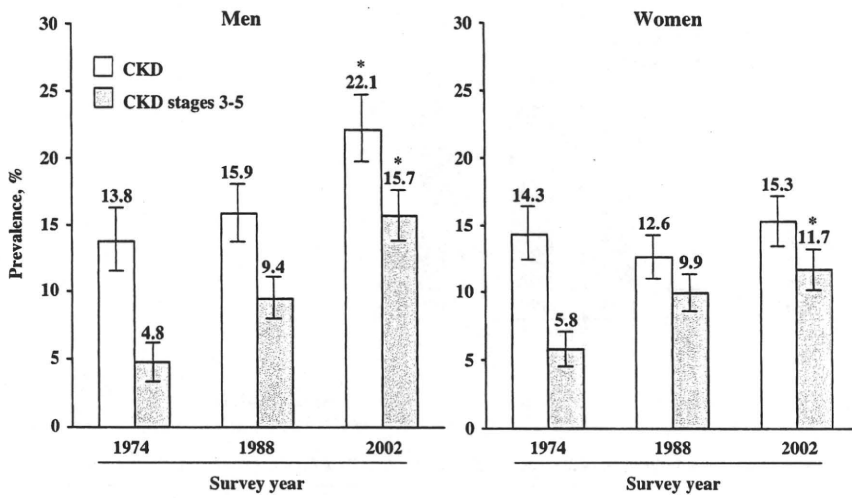
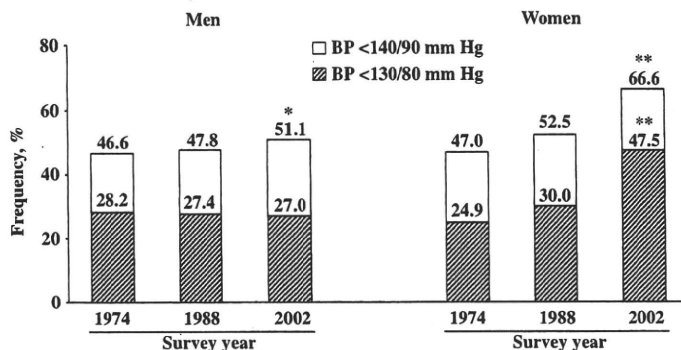**Fig. 1.** Trends in the age-adjusted prevalence of CKD in 1974, 1988 and 2002 by sex.

*P for trend < 0.01

Fig. 2. Trends in the prevalence of CKD by age and sex.
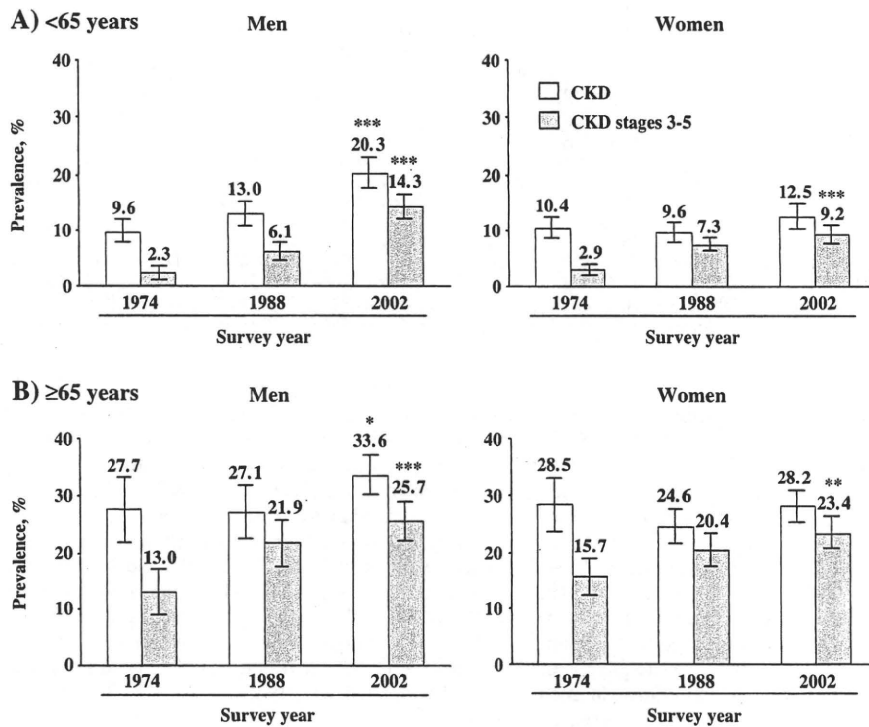
*P for trend < 0.05, **P for trend < 0.01, ***P for trend 0.001

CKD Stages 4–5, but the number of subjects with this stage of CKD was too small to assess reliably according to age or sex [eight subjects (0.4%) in 1974, seven subjects (0.3%) in 1988, 33 subjects (1.0%) in 2002 overall].

The number of subjects undergoing dialysis was zero in 1974, one in 1988 and 10 in 2002. The age-adjusted proportion of subjects with proteinuria did not change across the surveys in men (10.7% in 1974, 7.6% in 1988 and

Fig. 3. Age-adjusted frequencies of well-controlled blood pressure in subjects with CKD in 1974, 1988 and 2002 by sex.

*P for trend < 0.05, **P for trend < 0.001

Table 2. Age-adjusted prevalence of CKD according to hypertension status in 1974, 1988 and 2002 by sex

	Men				Women			
	1974	1988	2002	P for trend	1974	1988	2002	P for trend
Non-hypertension								
Prevalence	10.9	11.2	15.5		11.4	8.6	12.6	
(95% CI)[a], %	(7.6–14.2)	(8.5–13.9)	(12.7–18.3)	0.008	(8.4–14.4)	(6.6–10.6)	(10.5–14.7)	0.20
RR (95% CI)[a]	1.00	1.11	1.53		1.00	0.79	1.13	
	(reference)	(0.76–1.61)	(1.09–2.17)		(reference)	(0.57–1.11)	(0.84–1.53)	
Treated hypertension								
Prevalence	18.8	23.8	36.1		28.8	19.8	22.5	
(95% CI)[a], %	(10.7–26.9)	(16.7–30.9)	(23.7–48.5)	0.48	(15.8–41.8)	(13.3–26.3)	(10.8–34.2)	0.11
RR (95% CI)[a]	1.00	1.10	1.16		1.00	0.79	0.72	
	(reference)	(0.70–1.77)	(0.78–1.81)		(reference)	(0.54–1.19)	(0.50–1.07)	
Untreated hypertension								
Prevalence	16.6	17.5	28.8		15.8	16.7	19.8	
(95% CI)[a], %	(11.8–21.4)	(13.0–22.0)	(22.6–35.0)	0.001	(11.9–19.7)	(11.9–21.5)	(12.5–27.1)	0.66
RR (95% CI)[a]	1.00	1.00	1.65		1.00	0.93	0.93	
	(reference)	(0.70–1.43)	(1.19–2.30)		(reference)	(0.69–1.27)	(0.68–1.28)	

[a]Adjusted for age.

9.6% in 2002; P for trend = 0.65), but decreased significantly with time in women (10.2% in 1974, 3.8% in 1988 and 5.3% in 2002; P for trend < 0.001).

Next, we estimated the frequencies of well-controlled blood pressure in men and women with CKD in each of the three surveys (Figure 3). Among subjects with CKD, the proportion with blood pressure levels of <140/90 mmHg increased from 46.6% in 1974 to 51.1% in 2002 for men and from 47.0% to 66.6% for women, in parallel with the increment in the proportion of subjects taking antihypertensive agents. The frequency of blood pressure of <130/80 mmHg was <30% in men with CKD in all three surveys, whereas it increased from 24.9% in 1974 to 47.5% in 2002 in women. Among CKD subjects taking antihypertensive agents in 2002, 36.3% of men and 26.3% of women had a blood pressure level <140/90 mmHg, and only 11.1% and 12.8%, respectively, had a blood pressure level <130/80 mmHg. Table 2 shows the age-adjusted prevalence and RR of CKD by the status of hypertension treatment among the three surveys by sex. For men, the RR of presence of CKD increased with time in subjects with untreated hypertension (P for trend = 0.001), but not in subjects with treated hypertension (P for trend = 0.48). For women, there was no evidence of significant differences in the prevalence of CKD over time in any of the hypertension treatment statuses.

Finally, we assessed the relationship between metabolic syndrome and the risk of CKD in 1988 and 2002. Metabolic syndrome was associated with an increased risk of prevalent CKD in either sex (Figure 4). The strength of the relationship did not change over time for men (P for heterogeneity = 0.99), whereas it was attenuated significantly in 2002 compared with 1988 for women (P for heterogeneity = 0.01).

Discussion

In the present study, we demonstrated that the prevalence of CKD increased significantly in men, but not in women from the 1970s to the 2000s in a general Japanese population, whereas CKD Stages 3–5 increased progressively with time in both sexes. Importantly, more than half of

individuals with CKD did not reach the optimal target levels of blood pressure recommended by the current guidelines [23,24], despite an increment in the proportion of subjects taking antihypertensive agents over the last three decades. Furthermore, our findings implied that the recent increment in the number of subjects with metabolic disorders is linked to the increasing prevalence of CKD. These analyses, therefore, would seem to highlight the importance of the comprehensive management of metabolic disorders in addition to the strict control of blood pressure in order to reduce the burden of CKD in the general Japanese population.

The prevalences of CKD have been reported for several countries. The National Health and Nutrition Examination Surveys reported that the age-adjusted prevalence of CKD Stages 1–4 among subjects aged 20 years or older in the United States increased from 10.0% in 1988–1994 to 13.1% in 1999–2004 [8]. In Nord-Trondelag, Norway, the prevalence of CKD Stages 3–5 was 4.4% [9]. CKD may be more prevalent in Asian countries than in developed Western countries. A cross-sectional study conducted in 574 024 Japanese subjects over 20 years old demonstrated that the prevalence of CKD Stages 3–5 was 10.6 % in Japan [11]. Data from the screenings in Okinawa, Japan showed that the unadjusted prevalence of CKD Stages 3–5 among subjects aged 20 years or older increased between 1993 (10.4%) and 2003 (12.2%) in men, but decreased in women (19.5% in 1993, 17.4% in 2003), although the average serum creatinine levels increased in all age categories during this period in either sex [25]. An increasing trend in the prevalence of CKD in men was thus observed both in our study and Okinawa's study. The discrepancy observed in women between the two studies may have arisen from a self-selection bias caused by the low participation rate (<20%) in Okinawa's study, with subjects having an underlying disease (e.g. advanced kidney disease) being less likely to participate in the examination. Importantly, the prevalences of CKD in these studies were estimated on the basis of different eGFR equations, the direct comparison of which might be inappropriate. A nationwide examination will be needed to estimate the burden of CKD in Japan more reliably.

In the present study, the prevalence of metabolic disorders, such as diabetes, hypercholesterolaemia and obesity, was found to have increased dramatically over the last three decades, probably due to the westernization of lifestyle in Japan [26]. In the 2002 survey, diabetes was significantly associated with the likelihood of CKD for both sexes. Diabetes is an especially serious problem in the prevention strategy for CKD because it has been the leading cause of end-stage renal disease since 1998 in Japan [13]. Likewise, hypercholesterolaemia and obesity have been shown to be independent risk factors for CKD [27,28]. Our findings showed a jump in the prevalence of metabolic disorder from 1974 to 1988 ahead of the increment in the prevalence in CKD, possibly suggesting a causal association of metabolic disorder with the risk of CKD. In this study, furthermore, metabolic syndrome, which is defined as the accumulation of three or more risk factors such as elevated blood pressure, glucose intolerance, central obesity and dyslipidemia, was associated with an increased

risk of CKD. Our previous longitudinal study has demonstrated that individuals with metabolic syndrome have 2.1-fold greater risk than those without it [29]. It has also been reported that clusters of multiple metabolic disorders tended to cause CKD in the several epidemiological studies [30,31]. Therefore, it is reasonable to suppose that the increasing prevalence of metabolic disorders has contributed to the increasing trend in CKD, especially CKD Stages 3–5, in our subjects.

Hypertension is well-established as a powerful risk factor for not only cardiovascular disease, but also CKD [32]. In this study, blood pressure levels significantly declined in both sexes over the last three decades, probably because of the widespread use of antihypertensive medication. Nevertheless, about 70% of men with CKD and 50% of women with CKD did not reach the optimal blood pressure levels of <130/80 mmHg even in the latest survey. Several clinical trials have demonstrated that blood pressure lowering was beneficial for the prevention of progressive kidney disease [33,34] and cardiovascular disease in individuals with CKD [35–38]. A recent meta-analysis of Japanese cohort studies also revealed that lower blood pressure level is linearly associated with a lower risk of cardiovascular disease and death in subjects with CKD [39]. These findings, therefore, suggest that blood pressure should be controlled more strictly in individuals with CKD, using the recommendations in the current guidelines [23,24].

Our study showed that the prevalence of CKD Stages 1–2 decreased over the last three decades in both sexes. Importantly, the frequency of women with CKD Stages 1–2 was halved over time, and therefore, the overall prevalence of CKD did not change. In the 2002 survey, blood pressure was well-controlled in women, compared with men (Table 1). It has been established that blood pressure-lowering therapy, particularly the use of renin–angiotensin system inhibitors, reduces the risk of the development of proteinuria and subsequent kidney dysfunction [40–45]. Furthermore, the relationship between metabolic syndrome and the likelihood of CKD for women tended to be attenuated from the 1988 survey to the 2002 survey, possibly due to early interventions, including lifestyle modification or medications against metabolic disorder. Thus, our findings imply that optimal management of blood pressure and metabolic disorder may reduce the prevalence of CKD in women in the next decade.

Several limitations of our study should be noted. First, it is well-known that eGFR values calculated using the MDRD study equation with a single measure of serum creatinine are not fully accurate. In addition, measurement of serum creatinine was not repeated after an interval of at least 3 months. Additionally, the values of serum creatinine were not calibrated using the values from the Cleveland Clinic, although they were calibrated across the three surveys. These matters may have caused some degree of misclassification of eGFR levels. Nevertheless, these limitations may have had little effect on our conclusions because the extent of misclassification of eGFR levels would be similar across the surveys. Second, the method for measuring serum cholesterol could not be calibrated across the surveys in this study. However, we believe that our findings with regard to the trend in the propor-

tion of hypercholesterolaemia over time are likely to be real because the proportion of obesity showed a similar pattern. Third, a 75-g oral glucose tolerance test was not performed in 1974. Thus, the prevalence of diabetes in 1974 was likely to be underestimated because the glucose tolerance test is a more sensitive method to diagnose diabetes. Fourth, the blood pressure levels were estimated with office blood pressure measurement, but not with home blood pressure monitoring, likely attenuating the accuracy of the information about blood pressure control. Fifth, we were unable to obtain information regarding the cause of CKD or the type of antihypertensive drugs, including renin–angiotensin system inhibitors. This information would have enabled a deeper understanding of our results. Finally, this is a cross-sectional study, and thus, the data are of limited use in inferring causality between risk factors and CKD.

Conclusion

In conclusion, the prevalence of CKD increased significantly in men, but not in women from the 1970s to the 2000s in a general Japanese population. Despite the popularization of antihypertensive medication, blood pressure was not sufficiently controlled over time to meet the optimal level recommended by the current guidelines for patients with CKD. Additionally, the increasing prevalence of metabolic disorders would be expected to play a role in the increasing trend in CKD. Our findings support the requirement for a comprehensive treatment for hypertension and metabolic disorders in order to reduce the burden of CKD.

Supplementary data

Supplementary data is available online at http://ndt. oxfordjournals.org.

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Ethnic disparities in prevalence and impact of risk factors of chronic kidney disease

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Abstract

Background. There is substantial heterogeneity in literature regarding the epidemiology for chronic kidney disease (CKD) in different Asian populations. We aimed to assess the prevalence and risk factors of CKD in a multi-ethnic Asian population in Singapore.

Methods. We examined 4499 participants of Chinese, Malay and Indian ethnicity, aged 24–95 years, who

Prevalence and Systemic Risk Factors for Retinal Vein Occlusion in a General Japanese Population: The Hisayama Study

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PURPOSE. To examine the prevalence of retinal vein occlusion (RVO) and its systemic relevant factors in a general Japanese population aged 40 years or older.

METHODS. In 1998, 1775 Hisayama residents consented to participate in the study. Each participant underwent a comprehensive examination that included ophthalmic testing. RVO was determined by grading color fundus photographs. Logistic regression analysis was performed to determine risk factors for RVO.

RESULTS. Of the 1775 subjects examined, 38 had RVO. The prevalence of RVO was 2.1% (2.0% for branch RVO and 0.2% for central RVO). After adjustment for age and sex, it was found that systolic and diastolic blood pressures, hypertension, and hematocrit were significantly associated with RVO. In multivariate analysis, age (per 10 years; odds ratio [OR], 1.47; 95% confidence interval [CI], 1.04–2.08), hypertension (OR, 4.25; 95% CI, 1.82–9.94), and hematocrit (per 10%; OR, 3.09; 95% CI, 1.10–1.22) remained independently significant risk factors for RVO. Both high-normal blood pressure and hypertension were significantly associated with RVO. Furthermore, compared with normotensive subjects without high hematocrit, the likelihood of RVO was markedly high in subjects having both high blood pressure and high hematocrit (age- and sex-adjusted OR, 36.0; 95% CI, 4.43–292).

CONCLUSIONS. The findings suggest that the prevalence of RVO is higher in the Japanese than in other Asians or Caucasians and that older age, higher hematocrit, and both hypertension and high-normal blood pressure are significant risk factors for RVO in the Japanese. (*Invest Ophthalmol Vis Sci.* 2010;51:3205–3209) DOI:10.1167/iovs.09-4453

R etinal vein occlusion (RVO) is a cause of significant loss of vision in elderly populations in developed countries.[1] Despite the magnitude of this problem, the available treatment options remain limited.[2,3] Furthermore, RVO has also been associated with increased risk of cardiovascular disease.[4–6] In developing measures to prevent this disease, it is thus very important to determine the prevalence of RVO and to identify its systemic risk factors. To date, several population-based studies,[6–11] mostly in Caucasian populations, have provided valuable information on the prevalence and systemic risk factors for RVO. These include hypertension,[6–11] diabetes,[10] smoking habits,[10] dyslipidemia,[7,9] and a history of angina.[9] However, there have been only a limited number of population-based epidemiologic studies on RVO in Japanese and other Asians.[9,11,12]

The purpose of this article was to examine the prevalence of RVO and its systemic relevant factors in a cross-sectional study of a general Japanese population.

METHODS

Study Population

The Hisayama Study is an ongoing long-term prospective cohort study on cardiovascular disease and its risk factors in Hisayama, a town adjoining Fukuoka City, a metropolitan area in southern Japan.[13,14] As a part of the follow-up survey, we performed a cross-sectional examination, including an eye examination, of Hisayama residents aged 40 years or older in 1998.[15] Among 4187 residents in that age group, 1775 (42.4%; 688 men and 1087 women) were enrolled in the present study.

Ophthalmic Examination and Definition of RVO

The methods used for the ophthalmic examination have been published in detail.[15] Briefly, each participant underwent a comprehensive ophthalmic examination, including a stereoscopic fundus examination with indirect ophthalmoscopy and examination with a slit-lamp biomicroscope with a superfield lens (Volk, Mentor, OH), after pupil dilation with 1.0% tropicamide and 5% phenylephrine. Fundus photographs (45°) were taken of both eyes of each participant with a nonmydriatic fundus camera (TRC NW-5; Topcon, Tokyo, Japan) and slide film (Fujichrome Sensia II; Fujifilm, Tokyo, Japan). We photographed one field, centered at a point midway between the temporal edge of the optic disc and the fovea in both eyes. The presence of RVO was determined based on the grading of fundus examinations by indirect ophthalmoscopy and slit lamp and the color fundus photographs. All photographs were evaluated by retinal specialists (MY and TI) who were masked to the participants' data. The presence or absence of central or branch RVO was defined according to a standardized protocol.[6,10,16] Recent central RVO was characterized by retinal edema, optic disc hyperemia or edema, scattered superficial and deep retinal hemorrhages, and venous dilation. Old central RVOs were characterized by occluded and sheathed retinal veins or vascular anastomosis at the optic disc. Branch RVOs involved a more localized area of the retina in the sector of the obstructed venule and were characterized by scattered superficial and deep retinal hemorrhages, venous dilation, intraretinal microvascular abnormalities, and occluded and sheathed

TABLE 1. Mean Values or Frequencies of Risk Factors by Status of Retinal Vein Occlusion

Variable	Non-RVO (n = 1736)	RVO (n = 38)	P
Age, y	62 ± 11	67 ± 7	0.002
Sex (men), %	38.5	50.0	0.15
Systolic blood pressure, mm Hg	133 ± 21	147 ± 18	<0.0001
Diastolic blood pressure, mm Hg	77 ± 11	81 ± 10	0.02
Hypertension, %	43.7	81.6	<0.0001
Total cholesterol, mmol/L	5.3 ± 0.9	5.4 ± 1.1	0.73
High-density lipoprotein cholesterol, mmol/L	1.5 ± 0.4	1.6 ± 0.4	0.16
Triglycerides, mmol/L	1.20 (0.59–2.98)	1.02 (0.51–2.19)	0.14
Body mass index, kg/m^2	23.1 ± 3.1	23.2 ± 3.5	0.77
Diabetes, %	12.6	10.5	0.70
White blood cells, ×10^3/mm^3	5.8 ± 1.5	6.1 ± 1.6	0.15
Platelets, ×10^4/mm^3	21.9 ± 5.2	19.8 ± 6.0	0.02
Hematocrit, %	40.1 ± 4.1	41.6 ± 3.6	0.03
ECG abnormalities, %	17.1	29.0	0.06
History of cardiovascular disease, %	2.7	5.3	0.42
Smoking habit (yes), %	17.5	18.4	0.88
Alcohol intake (yes), %	36.5	44.7	0.30
Regular exercise (yes), %	16.7	23.7	0.51

Data are expressed as the mean ± SD or percentages. Geometric mean value and 95% prediction interval of triglycerides are shown because of the skewed distribution. RVO, retinal vein occlusion.

retinal venules. The presence of any RVO was defined as the presence of branch or central RVO in either eye.

Data Collection

Information on smoking habits, alcohol intake, and regular exercise during leisure time was obtained by trained interviewers using a standard questionnaire. Smoking habits and alcohol intake were classified as either current use or not. Those subjects who engaged in sports or other forms of exertion three or more times per week during their leisure time were designated as the regular exercise group. The questionnaire also ·investigated history of cardiovascular disease, including stroke and coronary heart disease.

Blood pressure was measured three times in the sitting position after the subject had rested for at least 5 minutes. The average of the three measurements was used for the analysis. According to the 2007 European Society of Hypertension (ESH) and European Society of Cardiology (ESC) Practice Guidelines,[17] blood pressure levels were categorized as follows: optimal (systolic <120 mm Hg and diastolic <80 mm Hg), normal (120–129/80 to 84 mm Hg), high-normal (130–139/85 to 89 mm Hg), and hypertension (≥140/≥90 mm Hg or current use of antihypertensive medication).

Plasma glucose levels were determined by the glucose-oxidase method, and diabetes mellitus was defined by a 75-g oral glucose tolerance test or by fasting (≥7.0 mM) or postprandial (≥11.1 mM) blood glucose level, or by the use of hypoglycemic agents. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycer-

ide levels were determined enzymatically by using an autoanalyzer (TBA-80S; Toshiba Inc., Tokyo, Japan). White blood cell (WBC) and platelet counts and hematocrit levels were determined with a cell counter (STKS; Coulter Inc., Hialeah, FL). ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code,[18] 3-1) or ST depression (4-1, 2, 3). Body height and weight were measured in light clothing without shoes, and the body mass index (in kilograms per square meter) was calculated.

Statistical Methods

We considered the following 18 possible risk factors for RVO: age, sex, systolic and diastolic blood pressures, hypertension, total cholesterol, HDL cholesterol, triglycerides, body mass index, diabetes mellitus, WBC count, platelet count, hematocrit, ECG abnormalities, history of cardiovascular disease, smoking habits, alcohol intake, and regular exercise. Mean values were compared by Student's t-test, and frequencies by the χ^2 test. We estimated the age- and sex-adjusted and multivariate-adjusted odds ratio (OR) and 95% confidence interval (CI) for each potential risk factor by using logistic regression analysis (SAS software; SAS Institute, Cary, NC[19]). A two-sided $P < 0.05$ was considered statistically significant.

Ethical Considerations

This study was approved by the Human Ethics Review Committee of Kyushu University Graduate School of Medical Sciences and was per-

TABLE 2. Age-Specific Prevalence of RVO by Sex

Age Range, y	Men			Women			All Subjects			
	Subjects, n	Branch RVO, n (%)	Central RVO, n (%)	Subjects, n	Branch RVO, n (%)	Central RVO, n (%)	Subjects, n	Branch RVO, n (%)	Central RVO, n (%)	All RVO, n (%)
40–49	92	0 (0.0)	0 (0.0)	201	0 (0.0)	0 (0.0)	293	0 (0.0)	0 (0.0)	0 (0.0)
50–59	154	2 (1.3)	0 (0.0)	284	5 (1.8)	0 (0.0)	438	7 (1.6)	0 (0.0)	7 (1.6)
60–69	231	5 (2.2)	3 (1.3)	335	10 (3.0)	0 (0.0)	566	15 (2.7)	3 (0.5)	18 (3.2)
70–79	178	7 (3.9)	0 (0.0)	212	2 (0.9)	0 (0.0)	390	9 (2.3)	0 (0.0)	9 (2.3)
80+	33	2 (6.1)	0 (0.0)	55	2 (3.6)	0 (0.0)	88	4 (4.6)	0 (0.0)	4 (4.6)
Total	688	16 (2.3)	3 (0.4)	1087	19 (1.8)	0 (0.0)	1775	35 (2.0)	3 (0.2)	38 (2.1)
P_{trend}		0.01	0.87		0.15			0.005	0.66	0.005

RVO, retinal vein occlusion.

TABLE 3. Age- and Sex-Adjusted and Multivariate-Adjusted OR of Relevant Factors of RVO

Association	Age- and Sex-Adjusted		Multivariate-Adjusted	
	OR	95% CI	OR	95% CI
Age, per 10 years			1.47*	1.04-2.08
Sex (men), %			0.93	0.42-2.07
Systolic blood pressure, per 10 mm Hg	1.23†	1.07-1.41		
Diastolic blood pressure, per 10 mm Hg	1.46*	1.09-1.97		
Hypertension	4.53†	1.94-10.6	4.25†	1.82-9.94
Total cholesterol, per 1 mmol/L	1.20	0.83-1.74		
High-density lipoprotein cholesterol, per 1 mmol/L	2.22	0.94-5.25		
Triglycerides, per 1 mmol/L	0.63	0.36-1.10		
Body mass index, per 1 kg/m²	1.04	0.94-1.15		
Diabetes	0.65	0.23-1.87		
White blood cells, per 10^3/mm³	1.15	0.94-1.40		
Platelets, per 10^4/mm³	0.94	0.88-1.01		
Hematocrit, per 10 %	3.09*	1.13-8.46	1.10*	1.00-1.22
ECG abnormalities	1.57	0.76-3.26		
History of cardiovascular disease	0.91	0.21-3.91		
Smoking habit	0.95	0.39-2.34		
Alcohol intake	1.42	0.67-3.01		
Regular exercise	1.24	0.58-2.68		

RVO, retinal vein occlusion; OR, odds ratio; CI confidence interval.
* $P < 0.05$.
† $P < 0.01$.

formed in accordance with the Declaration of Helsinki. The study subjects provided written informed consent to participate in the study.

RESULTS

Table 1 shows the mean values or frequencies of potential risk factors according to the presence or absence of RVO. The geometric mean values and 95% prediction intervals of triglycerides are shown because of the skewed distribution. Subjects with RVO were older than those without RVO. Subjects with RVO had higher mean systolic and diastolic blood pressures and hematocrits, as well as a higher frequency of hypertension, whereas those without RVO had a lower mean platelet count.

The age-specific prevalences of RVO are shown by sex in Table 2. Of the 1775 subjects examined, 38 (2.1%) had RVO. Of the subjects with RVO, 35 (92.1%) had branch RVO. The prevalence of branch RVO was slightly but not significantly higher in the men than in the women (2.3% vs. 1.8%). Central RVO was observed only in the men (0.4%). The prevalence of all RVO significantly increased with advancing age in all the

subjects ($P_{trend} = 0.005$), whereas the prevalence of branch RVO significantly increased with advancing age only in the men ($P_{trend} = 0.01$).

The results of age- and sex-adjusted and multivariate-adjusted logistic regression analyses of relevant factors for RVO are presented in Table 3. After adjusting for age and sex, we found that systolic and diastolic blood pressures, hypertension, and hematocrit were significantly associated with RVO. In multivariate analysis, age (per 10 years; OR, 1.47; 95% CI, 1.04-2.08), hypertension (OR, 4.25; 95% CI, 1.82- 9.94), and hematocrit (per 10%) (OR, 3.09; 95% CI, 1.10-1.22) remained independently significant relevant factors for RVO.

Table 4 demonstrates the age- and sex-adjusted OR of RVO according to blood pressure levels and quartiles of hematocrit. The age- and sex-adjusted OR of RVO significantly increased with elevated blood pressure levels ($P_{trend} < 0.001$). Compared with those with optimal or normal blood pressure, the OR of RVO was significantly higher, not only in the subjects with hypertension (age- and sex-adjusted OR, 11.9; 95% CI, 2.78-50.9), but also in the subjects with high-normal blood

TABLE 4. Age- and Sex-Adjusted OR of RVO According to Blood Pressure Levels and Quartiles of Hematocrit

Risk Factor Level	Subjects, n	Cases, n	Age- and Sex-Adjusted OR (95% CI)	P_{trend}
Blood pressure level				
Optimal	469	1	1.00 (reference)	<0.001
Normal	276	1		
High-normal	240	5	6.81 (1.30-35.6)*	
Hypertension	790	31	11.9 (2.78-50.9)†	
Hematocrit				
First quartile, <37.7	436	5	1.00 (reference)	0.004
Second quartile, 37.7-39.9	447	7	1.40 (0.44-4.46)	
Third quartile, 40.0-42.6	445	8	1.81 (0.58-5.70)	
Fourth quartile, ≥42.7	446	18	6.03 (1.85-19.7)*	

RVO, retinal vein occlusion; OR, odds ratio; CI, confidence interval.
* $P < 0.05$.
† $P < 0.01$.

Table 5. Age- and Sex-Adjusted OR of RVO According to the Presence or Absence of High Blood Pressure and High Hematocrit

	Subjects, n	Cases, n	Age- and Sex-Adjusted OR (95% CI)	P
Normal blood pressure + low hematocrit	595	1	1.00 (reference)	
Normal blood pressure + high hematocrit	150	1	4.81 (0.28-82.2)	0.28
High blood pressure + low hematocrit	742	20	11.9 (1.57-90.9)	0.02
High blood pressure + high hematocrit	288	16	36.0 (4.43-292)	<0.01

Normal blood pressure: optimal + normal; high blood pressure: high normal + hypertension; low hematocrit: first to third quartiles (<42.7%); high hematocrit: fourth quartiles (≥42.7%).

pressure (age- and sex-adjusted OR, 6.81; 95% CI, 1.30-35.6). The age- and sex-adjusted OR of RVO also significantly increased with rising hematocrit levels (P_{trend} = 0.003): the likelihood of RVO was significantly higher in the fourth quartile than in the first (age- and sex-adjusted OR, 6.03; 95% CI, 1.85-19.7).

Further, we examined both the combined and separate effects of high blood pressure and elevated hematocrit levels on RVO in the groups according to the presence or absence of high blood pressure (high normal blood pressure or hypertension) and high hematocrit level (fourth quartile, ≥42.7%). As shown in Table 5, compared with normotensive subjects without high hematocrit, the OR of RVO was significantly increased in subjects with high blood pressure alone (age- and sex-adjusted OR, 11.9; 95% CI, 1.57-90.9), whereas the OR of RVO was slightly but not significantly increased in subjects with high hematocrit alone (age- and sex-adjusted OR, 4.81; 95% CI, 0.28-82.2). Furthermore, the OR of RVO was markedly high in subjects having both high blood pressure and high hematocrit (age- and sex-adjusted OR, 36.0; 95% CI, 4.43-292). However, the interaction between high blood pressure and high hematocrit level was not significant (P = 0.35).

DISCUSSION

In a cross-sectional examination of a general Japanese population, we demonstrated that the prevalence of RVO was 2.1% and that age, high blood pressure, and elevation of hematocrit levels were independent relevant risk factors for RVO. In addition, the likelihood of RVO increased significantly in subjects having both high blood pressure and high hematocrit.

The prevalence of RVO has also been estimated in several other population-based studies (Table 6). The disease prevalence was reported to be 1.6% in the Blue Mountains Eye Study in Australia[16] and 1.1% in the Multiethnic Study of Atherosclerosis in the United States.[7] A study on a Chinese population, the Beijing Eye Study, reported an RVO prevalence of 1.2%,[12] and a study of a Malay population, the Singapore Malay Eye Study, reported a prevalence of 0.7%.[9] The prevalence of RVO in the present study (2.1%) seemed to be somewhat higher than those in the previous studies. Although the variation in disease prevalence among these studies could be due to differences in the characteristics of subjects and in the methodologies, our findings of a higher prevalence suggest that RVO is

more common among the Japanese population than among other Asian or Western populations, since the same grading protocols and RVO definitions were used in most of those studies.[7,9,12,16] Indeed, some studies have shown racial differences in the prevalence of RVO.[9,10] The reason for such differences remains uncertain, although genetic or environmental factors could contribute to the discrepancy.

In the present study, we found that the prevalence of RVO increased significantly with advancing age. The etiology and pathogenesis of RVO are largely unknown. The consistent association with increasing age found in this study is in accordance with the findings in many others,[6,7,9] confirming the age-related nature of the disease.

Our data indicated a clear association between hypertension and RVO, which is consistent with clinical knowledge and the findings of other population-based studies.[6-8,10-12] Our results also showed that not only hypertension but also high-normal blood pressure was significantly associated with RVO. The Framingham Heart Study indicated that the risk of cardiovascular disease is significantly increased in patients with high-normal blood pressure and higher blood pressure levels.[20] Based on these findings, it may be reasonable to suppose that high-normal blood pressure promotes systemic arteriosclerosis, including retinal vascular changes, and thereby causes RVO. Therefore, subjects with high-normal blood pressure should be considered at high risk for RVO. Strict control of elevated blood pressure may be important in preventing the disease.

We found that a higher hematocrit level was associated with RVO, independent of age, sex, and hypertension. A previous case-control study also indicated that hematocrit was significantly higher in a branch RVO group than in the control subjects.[21] Moreover, another study reported a significantly higher prevalence of elevated hematocrit in subjects with central RVO than in control subjects.[22] RVO is caused by thrombosis of the vein, but the role played by various hematologic abnormalities in its etiology and pathogenesis remains unclear and controversial. It is known that elevated hematocrit increases blood viscosity.[22] Therefore, increased hematocrit may augment the risk of RVO through the increase in blood viscosity.

The present study showed an extremely increased likelihood of RVO in subjects who had both hypertension and a higher hematocrit level. Although the mechanism underly-

Table 6. Prevalence of RVO in the Hisyama Study and Other Population-Based Studies

Study	Country	Subjects, n	Age	n (Prevalence %)
Blue Mountains Eye Study[16]	Australia	3654	49	59 (1.6)
Multiethnic Study of Atherosclerosis[7]	United States	6147	45	65 (1.1)
Beijing Eye Study[12]	China	4439	40	58 (1.3)
Singapore Malay Eye Study[9]	Singapore	3280	40	22 (0.7)
Hisayama Study[15]	Japan	1775	40	38 (2.1)

ing this phenomenon is not clearly understood, a possible explanation is that hypertension is a strong risk factor for systemic arteriosclerosis, including retinal arteriosclerosis,[5,8] and sclerotic arteriolar walls in the retina may compress the underlying veins at arteriovenous crossings, leading to reduced blood flow, which in turn could facilitate the development of a thrombus and downstream venous occlusion. It is therefore speculated that increased hematocrit levels markedly enhance the likelihood of RVO by hyperviscosity in people whose retinal vessel walls have already been damaged by hypertension.

This study has several limitations. First, we ascertained RVO cases by using one photographic field per eye, whereas in most previous population-based studies, two to six photographic fields were taken per eye. This difference could have resulted in underestimation of the prevalence of RVO if peripheral lesions were overlooked. Second, the number of our RVO cases is relatively small, and therefore the CIs around the prevalence and ORs are very wide. It might be misleading to compare the prevalence in this study with that in other population-based studies, and there is a possibility that the ORs are inflated due to the small samples. The estimates of our study should be interpreted with caution. Third, because of the cross-sectional design of this study, it is still unclear how risk factors are related to the onset of RVO. Further prospective investigation would help to clarify this issue.

In conclusion, the results of this study suggest that RVO is more common among the Japanese than among other Asians or Caucasians and that older age, higher hematocrit, and not only hypertension but also high-normal blood pressure are risk factors for RVO in the Japanese. In addition, among subjects who have both high blood pressure and higher hematocrit, the likelihood of RVO was substantially increased. Therefore, patients having both high blood pressure and higher hematocrit should be considered a population at high risk for RVO and continued preventive efforts should be made in these patients to reduce the burden of the disease.

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Circulating resistin is increased with decreasing renal function in a general Japanese population: the Hisayama Study

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Abstract

Background. The purpose of this study is to investigate the relationship between serum resistin levels and chronic kidney disease (CKD).
Methods. A total of 3192 community-dwelling subjects (1377 men, 1815 women), aged ≥40 years and without renal failure, were divided into four groups according to quartiles of serum resistin concentrations: ≤7.1, 7.2–9.9, 10.0–14.7 and ≥14.8 ng/mL. The associations of resistin levels with renal function status were examined cross-sectionally. The estimated glomerular filtration rate (eGFR) was calculated using the equation from the Modification of Diet in Renal Disease Study, and CKD was defined as an eGFR of <60 mL/min/1.73 m^2.
Results. The age- and sex-adjusted mean values of eGFR decreased significantly with elevating quartiles of resistin (P for trend <0.001). The age- and sex-adjusted odds ratios (ORs) for the presence of CKD increased progressively with higher quartiles of resistin. This trend remained robust even after controlling for age, sex, body mass index, diabetes, homeostasis model assessment of insulin resistance (HOMA-IR), high-sensitivity C-reactive protein (hs-CRP), triglycerides, high-density lipoprotein and total cholesterol, hypertension, current smoking, current drinking, and regular exercise [second quartile: OR 1.44, 95% confidence interval (CI) 1.05–1.99; third quartile: OR 2.15, 95% CI 1.58–2.92; fourth quartile: OR 2.32, 95% CI 1.71–3.16; P for trend <0.001]. In stratified analyses, high resistin level (≥7.2 ng/mL) was a significant relevant factor in CKD, independent of HOMA-IR or hs-CRP level.
Conclusion. Our findings suggest that elevated resistin level is significantly associated with the likelihood of CKD in the general Japanese population.

Keywords: chronic kidney disease; cross-sectional study; epidemiology; resistin

Introduction

Chronic kidney disease (CKD) is a worldwide public health concern and a major risk factor for end-stage renal disease, cardiovascular disease and premature death [1]. Identifying and treating risk factors for mild CKD may be the best approach to prevent and delay advanced outcomes [1]. Several epidemiological studies associated age, high blood pressure, diabetes, proteinuria, dyslipidaemia and smoking with the subsequent decline in estimated glomerular filtration rate (eGFR) [2,3]. It was also reported that insulin resistance and inflammation were emerging risk factors for the occurrence of CKD [4,5]. However, regardless of the treatment and prevention of these factors, patients with renal failure are increasing in number [6], suggesting the presence of other risk factors.

Resistin belongs to a family of cysteine-rich secretory proteins called resistin-like molecules [7]. In rodents, resistin is derived almost exclusively from fat tissue, and its serum levels are elevated in animal models of obesity and insulin resistance [8]. In humans, on the other hand, resistin is highly expressed in monocytes and macrophages [9]; thus, its pathophysiological role may differ between species. In vitro, resistin activated human endothelial cells, leading to increased expression of adhesion molecules, and induced human aortic muscle cell proliferation [10]. Furthermore, some clinical and epidemiological studies revealed positive correlations between plasma resistin levels and pro-inflammatory cytokines [11,12]. A few recent clinical studies also showed an inverse correlation between resistin level and eGFR in CKD patients [13–15]. To date, however, there have been no investigations into the link between serum resistin levels and CKD in large general populations excluding patients with renal failure. The aim of the present study is to examine the relationship between serum resistin levels and CKD in a cross-sectional study of a general Japanese population.

- 49 -

Table 1. Age- and sex-adjusted mean values or frequencies of risk factors according to quartiles of serum resistin concentrations

Variable	Serum resistin levels (ng/mL)				P-value for trend
	1.5–7.1 (n = 788)	7.2–9.9 (n = 803)	10.0–14.7 (n = 805)	14.8–90.2 (n = 796)	
Age (years)	59 ± 11	61 ± 12	62 ± 13	64 ± 13	<0.001
Men (%)	38.1	40.4	44.6	49.5	<0.001
BMI (kg/m^2)	23.0 ± 3.4	23.1 ± 3.4	23.0 ± 3.4	23.2 ± 3.4	0.50
Serum creatinine (μmol/L)	59 ± 15	62 ± 15	64 ± 15	65 ± 15	<0.001
Fasting plasma glucose (mmol/L)	6.1 ± 1.3	6.1 ± 1.3	6.0 ± 1.3	6.1 ± 1.3	0.70
Fasting insulin (pmol/L)	44.6 (13.8–144.5)	46.6 (14.5–150.0)	46.3 (14.4–148.7)	49.5 (15.3–160.5)	0.001
Diabetes (%)	16.5	16.8	18.0	18.9	0.45
HOMA-IR	1.66 (0.44–6.29)	1.72 (0.46–6.48)	1.71 (0.45–6.42)	1.83 (0.48–6.94)	0.007
Hs-CRP (mg/L)	0.44 (0.04–4.53)	0.51 (0.05–5.28)	0.54 (0.05–5.49)	0.66 (0.06–6.88)	<0.001
Triglycerides (mmol/L)	1.12 (0.39–3.18)	1.16 (0.41–3.29)	1.14 (0.40–3.23)	1.17 (0.41–3.35)	0.13
HDL-cholesterol (mmol/L)	1.70 ± 0.40	1.61 ± 0.40	1.62 ± 0.40	1.55 ± 0.40	<0.001
Total cholesterol (mmol/L)	5.30 ± 0.90	5.27 ± 0.89	5.31 ± 0.89	5.23 ± 0.90	0.15
Systolic blood pressure (mmHg)	132 ± 20	132 ± 20	132 ± 20	132 ± 20	0.96
Diastolic blood pressure (mmHg)	79 ± 12	79 ± 12	78 ± 12	78 ± 12	0.66
Hypertension (%)	45.0	42.9	43.2	46.3	0.98
Current smoking (%)	20.7	21.5	21.9	22.8	0.29
Current drinking (%)	48.8	44.4	43.4	38.3	<0.001
Regular exercise (%)	11.1	10.4	9.8	8.7	0.04

Values are given as means ± SD or frequencies. Age and percentage of men are not adjusted. Fasting insulin, HOMA-IR, hs-CRP and triglycerides are shown by geometric means and 95% CIs due to the skewed distribution. BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein.

Materials and methods

Study population

A population-based prospective study of cardiovascular disease has been under way since 1961 in the town of Hisayama, a suburb of Fukuoka City in southern Japan. As part of this study, in 2002, we conducted a cross-sectional examination among residents of the town. A detailed description of this survey was published previously [16]. Briefly, of all residents aged 40 years or over, 3328 underwent the examination (participation rate, 77.6%). After excluding 30 subjects who did not consent to participate in the study, 13 subjects with renal failure (eGFR <15 mL/min/1.73 m^2 or treated by dialysis), 82 subjects who had already eaten breakfast on the day serum samples were to be taken and 11 subjects without serum samples for resistin measurement, the remaining 3192 subjects (1377 men, 1815 women) were enrolled in this study.

This study was conducted with the approval of the Ethics Committee of the Faculty of Medicine, Kyushu University. Written informed consent was obtained from all participants.

Clinical evaluation and laboratory measurements

At the screening examination, blood samples were collected from an antecubital vein between 8:00 and 10:30 a.m. after at least a 12-h overnight fast. A portion of each serum specimen was stored at −80°C for 5 years, until 2007, when it was used for the measurement of resistin concentrations by a human resistin enzyme-linked immunosorbent assay kit supplied by R&D Systems (Minneapolis, MN) following the manufacturer's protocol. Linearity was maintained <0.16 ng/mL, and both intra- and inter-assay coefficient variations were comparable to those specified by the manufacturer (2.6–10.5%). Using fresh blood samples, serum creatinine was measured by the enzymatic method. Levels of triglycerides, high-density lipoprotein (HDL) and total cholesterol were determined enzymatically. Blood for the glucose assay was obtained by venipuncture into tubes containing sodium fluoride, and plasma glucose concentrations were determined by the glucose oxidase method. Subjects were considered to have diabetes mellitus if they had a fasting plasma glucose level of ≥7.0 mmol/L, had a 2-h post-load glucose level of ≥11.1 mmol/L or were taking antidiabetic medications. Serum insulin values were measured by a chemiluminescent enzyme immunoassay. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with the formula fasting plasma glucose (mmol/L) × fasting serum insulin (μU/mL) / 22.5 [17], and subjects in the top quartile of HOMA-IR distribution were defined

as having insulin resistance [18]. High-sensitivity C-reactive protein (hs-CRP) levels were quantified using a modification of the Behring latex-enhanced CRP assay on a Behring nephelometer BN-100 (Behring Diagnostics, Westwood, MA). High CRP values were defined as ≥1.0 mg/L, according to our previous report [19].

Blood pressure was obtained three times using an automated sphygmomanometer (BP-203RV III; Colin, Tokyo, Japan) with the subjects in a sitting position; the average of the three measurements was used in the present analysis. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or current treatment with antihypertensive agents. Height and weight were measured with the subject wearing light clothes without shoes, and body mass index [BMI (kg/m^2)] was calculated.

Each participant completed a self-administered questionnaire covering medical history, smoking habit, alcohol intake and exercise. The questionnaire was checked by trained interviewers at the screening. Smoking habit and alcohol intake were classified as either current habitual use or not. Those subjects who engaged in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group.

Definition of CKD

GFR was estimated by using the following modified equation of the Modification of Diet in Renal Disease (MDRD) Study for Japanese [20]: eGFR (mL/min/1.73 m^2) = 175 × [serum creatinine (mg/dL)]$^{-1.154}$ × [age (years)]$^{-0.203}$ × [0.741 (Japanese coefficient)] × (0.742 if female). Based on the National Kidney Foundation Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines [1], we divided kidney function levels into four categories according to eGFR: normal and CKD stage 1 (eGFR ≥90 mL/min/1.73 m^2), CKD stage 2 (eGFR 60–89 mL/min/1.73 m^2), CKD stage 3 (eGFR 30–59 mL/min/1.73 m^2) and CKD stage 4 (eGFR 15–29 mL/min/1.73 m^2). We also determined CKD as a dichotomized category when eGFR was <60 mL/min/1.73 m^2.

Statistical analysis

SAS software package version 8.2 (SAS Institute, Cary, NC) was used to perform all statistical analyses. Because the distributions of fasting insulin, HOMA-IR, hs-CRP and triglycerides were skewed, these variables were natural log-transformed for statistical analysis. The subjects were divided into quartiles of resistin concentrations: ≤7.1, 7.2–9.9, 10.0–14.7 and ≥14.8 ng/mL. The mean values of possible risk factors were adjusted for
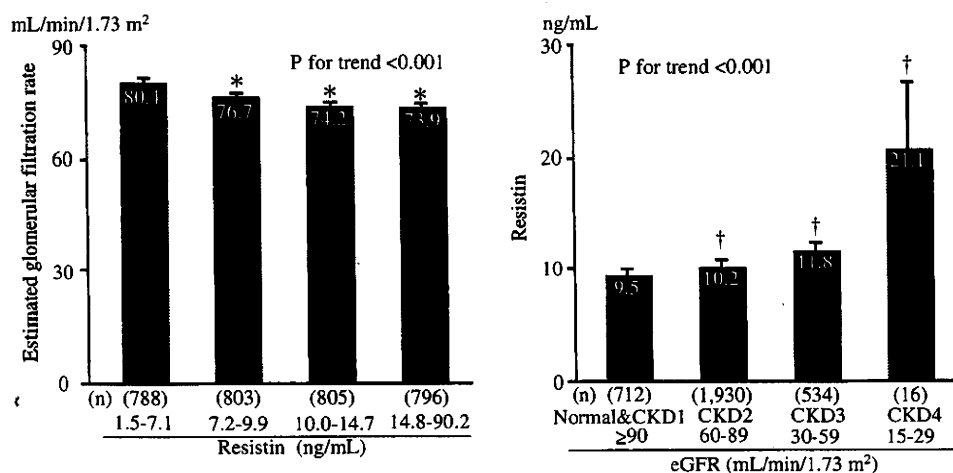
Fig. 1. The age- and sex-adjusted mean values of estimated glomerular filtration rate (eGFR) according to quartiles of serum resistin concentrations (left panel), and the age- and sex-adjusted mean values of serum resistin levels according to eGFR (right panel). Values are given as means ± standard error. *P < 0.001 *vs* the first quartile, †P < 0.001 *vs* eGFR of ≥90 mL/min/1.73 m².

age and sex using the analysis of covariance and were compared among the resistin quartiles according to the linear regression model. Age- and sex-adjusted means of eGFR among the quartiles were determined by the same method. The frequencies of risk factors were adjusted for age and sex by the direct method using all subjects as a standard population and were tested for trends using logistic regression analysis. The age- and sex- or multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for CKD were also determined by logistic regression analysis.

Results

Table 1 shows the age- and sex-adjusted means or frequencies of potential risk factors according to the quartiles of serum resistin concentrations. The mean values of age, serum creatinine, fasting insulin, HOMA-IR and hs-CRP, as well as the percentage of men, increased with the quartiles of resistin values, while the mean HDL cholesterol concentration and the frequencies of alcohol intake and regular exercise were negatively correlated with the quartiles. The other variables were not significantly associated with the quartiles.

The left panel in Figure 1 shows the age- and sex-adjusted mean values of eGFR according to the quartiles of serum resistin values. The mean values of eGFR decreased signif-

icantly with the quartiles of resistin values (80.4, 76.7, 74.2 and 73.9 mL/min/1.73 m², respectively; P for trend <0.001). Meanwhile, the age- and sex-adjusted mean values of serum resistin according to eGFR are shown in the right panel in Figure 1. The age- and sex-adjusted geometric mean values of serum resistin increased significantly as eGFR decreased (9.5 ng/mL in the normal and CKD stage 1, 10.2 ng/mL in CKD stage 2, 11.8 ng/mL in CKD stage 3 and 21.1 ng/mL in CKD stage 4; P for trend <0.001): the differences were significant between normal and CKD stage 1 and CKD stages 2–4 (all P < 0.001).

Table 2 shows the age- and sex-adjusted or multivariate-adjusted ORs and 95% CIs for the presence of CKD according to the resistin quartiles. The age- and sex-adjusted OR for CKD significantly increased with elevating quartiles (P for trend <0.001); compared to the first quartile, OR was greater in the second to fourth quartiles. Such associations were substantially unchanged after adjustment for age, sex, BMI, diabetes, HOMA-IR, hs-CRP, triglycerides, HDL and total cholesterol, hypertension, current smoking, current drinking, and regular exercise (second quartile: OR 1.44, 95% CI 1.05–1.99; third quartile: OR 2.15, 95% CI 1.58–2.92; fourth quartile: OR 2.32, 95% CI 1.71–3.16).

Table 2. Age- and sex- or multivariate-adjusted ORs and their 95% CIs for the presence of chronic kidney disease according to quartiles of serum resistin concentrations

	Serum resistin levels (ng/mL)				
	1.5–7.1	7.2–9.9	10.0–14.7	14.8–90.2	P-value for trend
Subjects (n)	788	803	805	796	
CKD cases (n)	75	117	166	192	
Age- and sex-adjusted OR (95% CI)	1 (reference)	1.44 (1.06–1.98)	2.15 (1.59–2.90)	2.33 (1.73–3.14)	<0.001
Multivariate-adjusted OR (95% CI)	1 (reference)	1.44 (1.05–1.99)	2.15 (1.58–2.92)	2.32 (1.71–3.16)	<0.001

Multivariate adjustment was made for age, sex, body mass index, diabetes, homeostasis model assessment of insulin resistance, high-sensitivity C-reactive protein, triglycerides, high-density lipoprotein and total cholesterol, hypertension, current smoking, current drinking, and regular exercise.CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval.

Table 3. Age- and sex- or multivariate-adjusted ORs and their 95% CIs for chronic kidney disease according to the presence or absence of high resistin levels and high HOMA-IR as well as high hs-CRP values

	Subjects, n	CKD cases, n	Age- and sex-adjusted OR (95% CI)	P-value	Multivariate-adjusted OR (95% CI)	P-value
HOMA-IR						
Low + low resistin	602	57	1 (reference)		1 (reference)	
Low + high resistin	1773	340	1.84 (1.35–2.49)	<0.001	1.85 (1.35–2.52)	<0.001
High + low resistin	178	17	1.00 (0.56–1.78)	0.99	0.93 (0.51–1.68)	0.80
High + high resistin	613	131	2.34 (1.66–3.29)	<0.001	2.14 (1.47–3.12)	<0.001
hs-CRP						
Low + low resistin	645	53	1 (reference)		1 (reference)	
Low + high resistin	1717	309	2.13 (1.56–2.91)	<0.001	2.12 (1.54–2.91)	<0.001
High + low resistin	143	22	1.69 (0.98–2.92)	0.06	1.62 (0.92–2.84)	0.09
High + high resistin	687	166	2.45 (1.74–3.45)	<0.001	2.46 (1.72–3.50)	<0.001

High resistin levels were defined as the second or higher quartiles of its values; low resistin levels were the first quartile of its values.HOMA-IR: 'high' indicates ≥75th percentile (HOMA-IR ≥2.6); 'low' <75th percentile.Hs-CRP: 'high' indicates ≥1.0 mg/L; 'low' <1.0 mg/L. Multivariate adjustment was made for age, sex, body mass index, diabetes, HOMA-IR, hs-CRP, triglycerides, high-density lipoprotein and total cholesterol, hypertension, current smoking, current drinking, and regular exercise, but each risk factor that had been used for categorization was excluded from the confounding factors.CKD, chronic kidney disease; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval.

Finally, we examined the combined as well as separate effects of resistin and HOMA-IR or hs-CRP levels on the presence of CKD (Table 3). When a high resistin level was in the second or higher quartile (≥7.2 ng/mL), the age- and sex-adjusted ORs of CKD were significantly higher in subjects with high resistin and low HOMA-IR (<75th percentile) and in subjects with high resistin and high HOMA-IR (≥75th percentile) compared to the reference group, who had low resistin and low HOMA-IR. Similarly, the age- and sex-adjusted risks of CKD were significantly higher in subjects with high resistin levels, independent of hs-CRP levels. These relationships remained robust even after adjusting for the confounding factors named above.

Discussion

Using the large cross-sectional data of a general Japanese population, we demonstrated that serum resistin levels were negatively associated with eGFR, and that the mean values of serum resistin increased even in CKD stage 2. Furthermore, the elevated levels of serum resistin were an independent relevant factor for CKD after controlling for age, sex, BMI, diabetes, HOMA-IR, hs-CRP, triglycerides, HDL and total cholesterol, hypertension, current smoking, current drinking, and regular exercise. In stratified analyses, high resistin levels were associated significantly with the likelihood of CKD in those with low HOMA-IR (<75th percentile) as well as in those with low hs-CRP levels (<1.0 mg/L). These findings suggest that the measurement of resistin values provides additional information on the risk factors for CKD.

In a few clinical studies of CKD patients, resistin levels have been shown to be inversely correlated with eGFR [13–15]. Although polypeptides that have molecular weights comparable to those of resistin are thought to be freely filtered at the normal glomerulus [21], subjects with advanced renal impairment might have serum resistin accumulations due to reduced renal clearance: that is, renal dysfunction might cause elevated serum resistin levels. However, the present study showed that resistin levels were significantly raised even in subjects with CKD stage 2 (eGFR of 60–89 mL/min/1.73 m^2), in which polypeptides would be filtered almost normally. In a clinical study of patients with immunoglobulin A glomerulonephritis, serum resistin levels were also significantly higher in subjects with mild renal dysfunction who had a mean GFR of 76 mL/min/1.73 m^2 than in those who had a mean GFR of 114 mL/min/1.73 m^2 [13]. These observations support the hypothesis that resistin potentially plays an important role in the development of CKD.

It has been assumed that the effect of resistin is mediated via insulin resistance or inflammation. In our study, serum resistin levels were positively associated with HOMA-IR, but the association between circulating resistin levels and the likelihood of CKD was independent of HOMA-IR, which is in accord with the results of other clinical studies [13,14,22]. Thus, insulin resistance may not be a major mediator of the association between resistin levels and the risk of CKD. Meanwhile, the present study also indicated that the association between resistin levels and the likelihood of CKD was unrelated to hs-CRP, though resistin levels were closely associated with those levels. Resistin directly stimulated the expression of pro-inflammatory cytokines such as tumour necrosis factor-α and interleukin-6 in human peripheral blood mononuclear cells [23], and increased the expression of vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 genes in vascular endothelial cells [10]. Furthermore, a recent clinical study provided evidence that the association between plasma resistin levels and plasma monocyte chemoattractant protein-1 concentrations was independent of hs-CRP levels [24]. Since it has become apparent that CKD is a state of chronic glomerular inflammation [5], resistin may play an important role in potentiating inflammation in the kidney, irrespective of hs-CRP and other confounding factors.

There are a variety of methods for estimating GFR. Although inulin clearance is the gold standard among these

methods, it is difficult to perform in routine practice. During the past decade, a great deal of effort has been devoted to establishing an equation for estimating GFR. There is a debate about which equation is optimal for Japanese; the original MDRD Study equation is widely accepted by clinical practitioners, but it may be unsuitable for Asian populations [20]. When some existing equations, such as the original MDRD Study equations [25] or the Cockcroft–Gault formula [26], were used instead of the modified MDRD Study equation for Japanese in our subjects, we also found significant associations between elevated resistin levels and CKD (data not shown). These findings imply a robust association between serum resistin levels and CKD.

A limitation of our study should be discussed. Due to the study's cross-sectional design, we cannot exclude the possibility that hyperresistinaemia is a consequence of CKD, which is a condition of low urine excretion from serum. However, we found significantly increased resistin levels even when eGFR was 60–89 mL/min/1.73 m². Thus, we believe that elevated resistin levels are a potential risk factor for the development of CKD.

In conclusion, an elevated resistin level was a significant relevant factor for CKD in a general Japanese population after taking into account other risk factors, including HOMA-IR and hs-CRP. Because of the cross-sectional design of this study, it is still unclear whether or not hyperresistinaemia is a cause of CKD. Further prospective studies are needed to clarify the causative relationship between serum resistin concentrations and CKD.

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SHORT COMMUNICATION

Association study of the polymorphisms on chromosome 12p13 with atherothrombotic stroke in the Japanese population

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Recent genome-wide association study using four prospective population-based cohorts identified two single-nucleotide polymorphisms (SNPs) on chromosome 12p13, rs12425791 and rs11833579, to be significantly associated with the incidence of atherothrombotic stroke. To examine the association of these SNPs with atherothrombotic stroke in the Japanese population, we carried out a case–control association study using a total of 3784 cases and 3102 controls. We also examined the effect of these SNPs on the subtypes of ischemic stroke. Association analysis was carried out using logistic regression model after adjustment of age, sex and cardiovascular risk factors. Rs12425791 was significantly associated with atherothrombotic stroke (P=0.0084, odds ratio (OR)=1.15). When we analyzed effects of rs12425791 on ischemic stroke subtypes, rs12425791 was significantly associated with both small-artery occlusion (P=0.015, OR=1.15) and large-artery atherosclerosis (P=0.024, OR=1.19). Rs11833579 showed no association with atherothrombotic stroke or its subtypes in our population. Our data suggest that rs12425791 on chromosome 12p13 is a genetic marker for atherothrombotic stroke in multiethnic population.
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Keywords: atherothrombotic stroke; replication study; SNP

INTRODUCTION

Genome-wide association study (GWAS) has emerged as a powerful new approach to identify many susceptibility variants with moderate genetic risk on various common diseases, such as diabetes[1] and coronary heart diseases.[2,3] As for ischemic stroke, few GWASs have been carried out and genetic components of common forms of ischemic stroke are still largely unknown. Recently, the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium reported two single-nucleotide polymorphisms (SNPs), rs11833579 and rs12425791, to be significantly associated with the incidence of ischemic stroke in a GWAS of four population-based cohorts, which included 19 602 white persons with an average of 11 years of follow-up data.[4] These SNPs were located in close proximity to ninjurin 2 (*NINJ2*) gene on chromosome 12p13. Both SNPs showed genome-wide significance, however, only rs12425791 was replicated in both the African-American cohort and the white case–control sample.[4] Although this study has an advantage of a prospective study design, these SNPs were merely the marker and the true causative variant(s) have not been identified yet. Moreover, this study did not analyze the effects of these SNPs on ischemic stroke subtypes probably because of small number of events.

As the association of these SNPs in Asian population remains unknown, we examined the association of these SNPs with atherothrombotic stroke using two Japanese case–control sets with a sufficient sample size. We also examined the effect of these SNPs on the subtypes of ischemic stroke.

MATERIALS AND METHODS

We used two independent Japanese case–control sets for this study. One case–control set (set-1) is consisted of 860 cases of atherothrombotic stroke and 860 age- and sex-matched controls. Details of the registration and case ascertainment were previously described.[5] We selected 860 cases of atherothrombotic stroke on the basis of the classification as in the CHARGE study[4] and subdivided them into 491 small-artery occlusion (SAO) and 369 large-artery atherosclerosis (LAA) according to the TOAST criteria.[6] Age- (within 5 years) and sex-matched controls were selected from the 3196 participants of the

Hisayama screening survey between 2002 and 2003. Another case–control set (set-2) consisted of 2924 atherothrombotic stroke and 2242 controls. Cases were selected from the BioBank Japan Project[7] based on the similar criteria as in the set-1 cases. These cases were classified into 2256 SAO and 668 LAA. The Hisayama participants who did not have ischemic stroke and did not enrolled in the set-1 were used as controls in the set-2. Clinical characteristics of the study populations are shown in Table 1.

Written informed consent was obtained from all study subjects, and this study was approved by the ethics committees of the Graduate School of Medical Sciences, Kyushu University and RIKEN Yokohama Institute.

We genotyped SNPs using the multiplex PCR-based Invader assay (Third Wave Technologies, Madison, WI, USA).[8] Crude association analysis was carried out using χ^2-test under allele model. We also assessed the association after adjustment of age, sex, body mass index, hypertension (yes/no), diabetes (yes/no) and dyslipidemia (yes/no) using logistic regression analysis under additive model. In a combined analysis, pooled estimates of the odds ratio (OR) for two case–control sets were obtained using inverse-variance-weighting analysis.[4] Heterogeneities across the population were estimated formally using Cochran's Q-test.[9]

RESULTS

We carried out association analysis for atherothrombotic stroke using two case–control sets (Table 2). In the crude analysis, we found a weak association of rs12425791 with atherothrombotic stroke in the combined sample ($P=0.041$), although each case–control set did not show significant association. This association became stronger after adjusted for various cardiovascular risk factors ($P=0.0084$, OR=1.15, 95% confidence interval=1.04–1.27). In contrast, rs11833579 showed no association with atherothrombotic stroke even in the combined sample set ($P=0.58$).

When we examined these associations by ischemic stroke subtypes, rs12425791 showed no association with SAO ($P=0.072$) or LAA ($P=0.13$) in the crude analysis. However, after adjustment of cardiovascular risk factors, rs12425791 was significantly associated with SAO ($P=0.015$, OR=1.15, 95% confidence interval=1.03–1.28) and LAA ($P=0.024$, OR=1.19, 95% confidence interval=1.02–1.39) in the combined sample set (Table 3). We found no significant association of rs11833579 with either SAO or LAA.

We also carried out the association analysis stratified by sex. After adjustment of cardiovascular risk factors, rs12425791 did not show significant association with atherothrombotic stroke in men ($P=0.086$, OR=1.14), whereas it showed a weak association in women ($P=0.027$, OR=1.17). When we examined these associations by ischemic stroke subtypes, rs12425791 was associated with SAO ($P=0.022$, OR=1.19), but not with LAA ($P=0.080$, OR=1.25), in women. Rs12425791 did not show any association with SAO ($P=0.19$, OR=1.11) or LAA ($P=0.075$, OR=1.20) in men. Rs11833579 showed no association with

Table 1 Clinical characteristics of the study population

	Set-1		Set-2	
	Case	*Control*	*Case*	*Control*
N	860	860	2924	2242
Male sex (%)	60.7	60.7	64.0	36.3
Age (years)	70.3 ± 9.9	70.2 ± 10.0	69.1 ± 9.2	58.2 ± 11.7
Body mass index (kg m^{-2})	22.0 ± 3.9	22.7 ± 3.3	23.5 ± 3.4	23.2 ± 3.4
Ischemic stroke subtype				
Small-artery occlusion	491		2256	
Large-artery atherosclerosis	369		668	
Cardiovascular risk factors				
Hypertension (%)	82.6	53.8	72.2	39.8
Diabetes mellitus (%)	32.8	20.6	15.6	16.2
Dyslipidemia (%)	50.9	41.1	21.9	47.5

Data are shown in mean ± s.d. or percentage except for ischemic stroke subtypes.

Table 2 Association between the SNPs reported in the CHARGE study and atherothrombotic stroke among Japanese

		Case					Control					Crude			Adjusted		
SNP (allele 1/2)	*Set*	*11*	*12*	*22*	*Total*	*MAF*	*11*	*12*	*22*	*Total*	*MAF*	*P-value*	*OR*	*(95% CI)*	*P-value*	*OR*	*(95% CI)*
rs12425791 (G/A)	Set-1	342	419	93	854	0.35	392	360	107	859	0.33	0.22	1.09	(0.95–1.26)	0.69	1.07	(0.76–1.51)
	Set-2	1200	1353	361	2914	0.36	976	999	262	2237	0.34	0.099	1.07	(0.99–1.16)	0.0084	1.15	(1.04–1.28)
	Combined											0.041	1.08	(1.00–1.16)	0.0084	1.15	(1.04–1.27)
rs11833579 (G/A)	Set-1	264	455	136	855	0.43	292	422	146	860	0.42	0.55	1.04	(0.91–1.19)	0.98	1.00	(0.71–1.40)
	Set-2	942	1469	507	2918	0.43	749	1082	403	2234	0.42	0.77	1.01	(0.94–1.09)	0.58	1.03	(0.93–1.14)
	Combined											0.58	1.02	(0.95–1.09)	0.60	1.03	(0.93–1.13)

Abbreviations: CHARGE study, the Cohorts for Heart and Aging Research in Genomic Epidemiology study; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.
Alleles for the SNPs on the forward strand of the human genome reference sequence (NCBI build 36.3) are shown. Crude analysis was carried out using χ^2-test under allele model. Adjusted analysis was carried out using logistic regression model after adjustment of cardiovascular risk factors.

Table 3 Association between the SNPs reported in the CHARGE study and the subtypes of ischemic stroke among Japanese

Subtype	SNP (allele 1/2)	Set	Case					Control					Crude			Adjusted		
			11	12	22	Total	MAF	11	12	22	Total	MAF	P-value	OR	(95% CI)	P-value	OR	(95% CI)
SAO																		
	rs12425791 (G/A)	Set-1	197	238	54	489	0.35	230	204	56	490	0.32	0.14	1.15	(0.95–1.39)	0.58	1.13	(0.74–1.72)
		Set-2	931	1046	272	2249	0.35	976	999	262	2237	0.34	0.19	1.06	(0.97–1.16)	0.017	1.15	(1.02–1.28)
		Combined											0.072	1.07	(0.99–1.16)	0.015	1.15	(1.03–1.28)
	rs11833579 (G/A)	Set-1	153	256	80	489	0.43	162	252	77	491	0.41	0.59	1.05	(0.88–1.26)	0.77	0.94	(0.61–1.44)
		Set-2	728	1142	380	2250	0.42	749	1082	403	2234	0.42	0.99	1.00	(0.92–1.09)	0.73	1.02	(0.91–1.14)
		Combined											0.81	1.01	(0.94–1.09)	0.79	1.01	(0.91–1.13)
LAA																		
	rs12425791 (G/A)	Set-1	145	181	39	365	0.35	162	156	51	369	0.35	0.83	1.02	(0.83–1.27)	0.97	0.99	(0.54–1.82)
		Set-2	269	307	89	665	0.36	976	999	262	2237	0.34	0.10	1.11	(0.98–1.26)	0.019	1.21	(1.03–1.42)
		Combined											0.13	1.09	(0.98–1.21)	0.024	1.19	(1.02–1.39)
	rs11833579 (G/A)	Set-1	111	199	56	366	0.42	130	170	69	369	0.42	0.77	1.03	(0.84–1.27)	0.80	1.08	(0.60–1.93)
		Set-2	214	327	127	668	0.43	749	1082	403	2234	0.42	0.42	1.05	(0.93–1.19)	0.26	1.09	(0.94–1.27)
		Combined											0.40	1.05	(0.94–1.16)	0.25	1.09	(0.94–1.27)

Abbreviations: CHARGE study, the Cohorts for Heart and Aging Research in Genomic Epidemiology study; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SAO, small-artery occlusion; LAA, large-artery atherosclerosis; SNP, single-nucleotide polymorphism.
Alleles for the SNPs on the forward strand of the human genome reference sequence (NCBI build 36.3) are shown. Crude analysis was carried out using χ^2-test under allele model.
Adjusted analysis was carried out using logistic regression model after adjustment of cardiovascular risk factors.

atherothrombotic stroke or ischemic stroke subtypes in both sexes (data not shown).

DISCUSSION

Using two independent Japanese case–control sets, we examined the association of two SNPs on chromosome 12p13 recently identified by a Caucasian GWAS of stroke. Rs12425791 was significantly associated with atherothrombotic stroke, whereas rs11833579 was not. Rs12425791 was also associated with both SAO and LAA, and its effect on the risk of SAO and LAA were similar. These results suggest that rs12425791 is a genetic marker for the incidence of atherothrombotic stroke in multiethnic populations including Japanese and might equally affect the risk of both SAO and LAA.

Similar ORs of rs12425791 on both SAO and LAA indicate that this SNP may be a marker for common pathogenesis of both ischemic stroke subtypes, probably for atherosclerosis. Rs12425791 is located at ~10 kb proximal from the 5′ untranslated region of the NINJ2 gene. On the basis of the Hapmap JPT data, rs12425791 is linked to the promoter region of NINJ2. Although fine mapping is needed, SNPs linked to rs12425791 might regulate the expression level of NINJ2. Ninjurin2, a gene product of NINJ2, is a cell surface adhesion molecule and is highly expressed in the bone marrow, peripheral leukocyte, lung and lymph node in human.[10] Although ninjurin2 is reported to be upregulated after nerve injury in Schwann cells and promotes neurite outgrowth,[10] the function of ninjurin2 on the ischemic stroke is largely unknown. Further functional studies are needed to clarify this issue.

Assuming the sample size of our study population using the allele frequencies in our controls and the hazard ratios in the CHARGE study, the statistical power to detect the associations at a significance level of 0.05 would be >99% for both SNPs. However, we found a significant association of atherothrombotic stroke only in rs12425791. Similarly, the CHARGE consortium showed that the association of ischemic stroke for rs12425791 was replicated in the African-American cohort, but the association for rs11833579 was not significant. This might be due to the difference in the linkage disequilibrium between the two SNPs and true causative variant among different populations.

On the basis of the Hapmap data, linkage disequilibrium between the two SNPs was different among populations (r^2=0.69 for JPT, r^2=0.34 for YRI and r^2=0.75 for CEU). There is a possibility that rs12425791 is closely linked to the true causative variant of atherothrombotic stroke among different populations. In contrast, the linkage disequilibrium between true causative variant and rs11833579 will be strong in Caucasian population, but it may be weak in Japanese and African-American populations.

The association between rs12425791 and ischemic stroke in this study was much weaker than that in the CHARGE study. The relationship of rs12425791 with atherothrombotic stroke or stroke subtypes was observed in the case–control set-2 but not in the case–control set-1. Furthermore, the relationship of rs12425791 with atherothrombotic stroke or stroke subtypes in the set-2 was not detected by the χ^2-test of allele frequencies. These results suggest that the impact of rs12425791 to atherothrombotic stroke or stroke subtypes in Japanese individuals is relatively low as compared with Caucasian population. Another possible explanation is that the effect size obtained from GWAS overestimates the true effect (winner's course). Indeed, CHARGE study showed that the genetic risk of rs12425791 in the replication study is lower than that in GWAS: in the GWAS, rs12425791 showed the strong association with atherothrombotic stroke (P=3.3×10^{-8}, hazard ratio=1.37), whereas it showed the P-value of 0.0052 and OR of 1.29 in the Dutch case–control study using 652 cases and 3613 controls.

In conclusion, our study suggests that rs12425791 or linked variations would be the true causative variant(s) for the genetic risk of atherothrombotic stroke in multiethnic population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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