

definite MI. As seen in Figure 1, even with a follow-up period of  $\geq 8.41$  years, median s-CK level among subjects with definite MI was higher than among those without definite MI. This suggested that an association between s-CK levels and MI in a specific period did not influence the present results. Therefore, the possibility that any obviously biased data collection for MI events affected the present results seemed to be low.

The present study had several limitations except for the above-mentioned creatine kinase isozyme. First, since we did not measure thyroid function including thyroid-stimulating hormone at baseline, we could not estimate the involvement of hypothyroidism with the present results. Secondly, we did not collect medical history, such as muscle diseases, thyroid diseases, medications causing rhabdomyolysis or recent history of severe physical training, all of which might affect s-CK levels. Therefore, we could not completely rule out the existence of unknown confounding factors. Thirdly, the single s-CK measurement at baseline may have underestimated the relationship between s-CK levels and CVD due to regression dilution bias.<sup>25</sup> Fourthly, the participation rate at baseline was not so high, but those of other population-based cohort studies in Japan with baseline clinical laboratory data were similar,<sup>26–29</sup> except for large cohort studies with baseline survey by questionnaires.<sup>30,31</sup> Especially in urban areas, the participation rates in cohort studies tend to be lower because the urban residents generally have more opportunities for health check-up than the rural population.<sup>29</sup> That is, the present participation rate was not always low. Moreover, previous reports in the present cohort showed reasonable causal relationship among blood pressure, low-density lipoprotein cholesterol or metabolic syndrome and

CVD,<sup>9–11</sup> which were similar with previous findings from the Japanese cohort studies.<sup>23</sup> This indirectly suggested that the results from the present cohort and analysis were not extremely biased by participation rates or loss to follow-up.

In conclusion, the present study suggested that screening for elevated s-CK levels in initially healthy Japanese subjects was useful to predict first-ever MI in the future, especially in subjects with dyslipidaemia. However, since the present study was conducted in a limited Japanese population with a relatively low MI incidence rate and had several limitations, these findings should be interpreted carefully and examined by further studies in various populations, races and areas.

## Funding

Japanese Ministry of Health, Labor and Welfare grant-in-aids (H20-SeiShu-013, H20-SeiShu-021 and H18-SeiShu-012); Research Grant for Cardiovascular Disease from the Ministry of Health, Labour and Welfare (18S-2).

## Acknowledgements

We would like to express our gratitude to all members of Suita City Health Center and the Suita Medical Association. We also thank all researchers and the staff of the Division of Preventive Cardiology for performing medical examinations and the follow-up. We also thank Satsuki-Junyukai, the volunteers for the administration of the Suita study.

**Conflict of interest:** None declared.

### KEY MESSAGES

- Slight elevation in s-CK is associated with increased risk of MI, not stroke, in population-based cohort study of a Japanese urban area.
- Especially in individuals with dyslipidaemia, measurement of s-CK might be more useful to predict MI.
- However, since present findings seemed to be the first report, they should be examined by further studies in various populations, races and areas.

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# Relationship Between Blood Pressure Category and Incidence of Stroke and Myocardial Infarction in an Urban Japanese Population With and Without Chronic Kidney Disease

## The Suita Study

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**Background and Purpose**—Chronic kidney disease (CKD) is increasingly recognized as an independent risk factor for stroke and myocardial infarction (MI). Few studies, however, have examined the relationship between blood pressure (BP) category and these diseases in subjects with and without CKD.

**Methods**—We studied 5494 Japanese individuals (ages 30 to 79, without stroke or MI at baseline) who completed a baseline survey and received follow-up through December 2005. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease study equation modified by the Japanese coefficient. CKD was defined as an estimated GFR  $<60$  mL/min/1.73m<sup>2</sup>. BP categories were defined by the European Society of Hypertension and European Society of Cardiology 2007 criteria.

**Results**—In 64 395 person-years of follow-up, we documented 346 incidences of cardiovascular diseases (CVD; 213 strokes and 133 MI events). Compared with the GFR ( $\geq 90$  mL/min/1.73m<sup>2</sup>) group, the hazard ratios (95% confidential intervals) for stroke were 1.9 (1.3 to 3.0) in the GFR 50 to 59 mL/min/1.73m<sup>2</sup> group and 2.2 (1.2 to 4.1) in the GFR  $<50$  mL/min/1.73m<sup>2</sup> group. Results for cerebral infarction were similar. Compared with the optimal BP subjects without CKD, the normal BP, high-normal BP, and hypertensive subjects without CKD showed increased risks of CVD and stroke; however the impact of each BP category on CVD (*P* for interaction: 0.04 in men, 0.49 in women) and stroke (0.03 in men, 0.90 in women) was more evident in men with CKD.

**Conclusions**—CKD may increase the association of BP and CVD in a Japanese urban population. (*Stroke*. 2009;40:2674-2679.)

**Key Words:** chronic kidney disease ■ blood pressure category ■ stroke ■ myocardial infarction ■ epidemiology ■ prospective studies ■ general population

Recently, chronic kidney disease (CKD) has become a major public health problem and a risk factor for all-causes mortality, stroke, and myocardial infarction (MI).<sup>1</sup> In end-stage renal disease, the cardiovascular disease (CVD) mortality rate is more than 10 times as high as that in the general population.<sup>2</sup> In asymptomatic general populations or outpatients, a severely or moderately decreased glomerular filtration rate (GFR) has been shown by most but not all studies to be an independent risk factor for stroke and MI.<sup>1</sup> However, in low-risk or general populations, the relationship between levels of kidney function and clinical outcomes has

not been as clear. Some studies have demonstrated no association between CKD and CVD,<sup>3,4</sup> whereas others have shown CKD as an independent risk factor for CVD.<sup>5-8</sup> These inconsistencies may be attributable to differences between the selected study populations as well as the severity of the CKD.

The frequency of hypertension is relatively higher in Japanese than in Western countries.<sup>9</sup> Hypertension is one of the major risk factors for both CVD and CKD. Recently, a larger prospective study has indicated that CKD increased the association between blood pressure (BP) categories and CVD, although the relevant data were gathered from 10 rural areas with different methods

Received February 19, 2009; final revision received March 25, 2009; accepted April 14, 2009.

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DOI: 10.1161/STROKEAHA.109.550707

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for the measurement of creatinine.<sup>10</sup> A few studies in general population have demonstrated a stronger association between BP and CVD in subjects with CKD.<sup>5,10</sup> We examined the association between BP category and incidence of stroke and MI subjects with and without CKD in a Japanese urban population.

## Methods

### Study Subjects

Suita city is located adjacent to Osaka city, which is the second largest metropolitan area in Japan. The Suita Study,<sup>11–13</sup> an epidemiological study of cerebrovascular and cardiovascular diseases, was based on a random sampling of 12 200 Japanese urban residents. As a baseline, participants (aged 30 to 79 years) were randomly selected from the municipality population registry and stratified into groups by sex and age in 10-year increments in 1989. Of these, 6485 people underwent regular health checkups between September 1989 and March 1994.

Cohort members in the study population were excluded from these analyses if they had a past or present history of CVD at baseline (n=208), were missing data (n=170), attended health checkups after April 1994 (n=79), or failed to complete the follow-up health surveys or questionnaires after the baseline examination (n=534). After applying these exclusions, a total of 5494 participants aged 30 to 79 years old were selected. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the National Cardiovascular Center.

### Measurement of Blood Pressure and Covariates

Well-trained physicians measured BP 3 times using a mercury column sphygmomanometer, an appropriate-size cuff, and a standard protocol. Before the initial BP reading was obtained, participants were seated at rest for at least 5 minutes. First, systolic blood pressure (SBP) was measured for the purpose of obtaining approximate SBP levels. SBP and diastolic blood pressures (DBP) were taken as the average of the second and third measurements, which were recorded more than 1 minute apart.

At the time of the baseline examination, subjects were classified into 1 of the 5 BP categories based on the European Society of Hypertension and European Society of Cardiology (ESH-ESC) 2007 criteria<sup>14</sup>: optimal (SBP <120 mm Hg and DBP <80 mm Hg), normal (SBP 120 to 129 mm Hg or DBP 80 to 84 mm Hg), high-normal BP (SBP 130 to 139 mm Hg or DBP 85 to 89 mm Hg), and hypertensive (SBP ≥140 mm Hg or DBP ≥90 mm Hg). Antihypertensive drug users were classified according to their BP levels at the baseline survey. If the SBP and DBP readings for a subject were in different categories, the subjects were categorized into the higher of the two BP categories.

At the baseline examination, we performed routine blood tests that included serum total cholesterol, HDL cholesterol, and glucose levels. Physicians or nurses administered questionnaires covering personal habits and present illness. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Hypercholesterolemia was defined as total cholesterol levels ≥5.7 mmol/L or current use of antihyperlipidemic medications. Diabetes was defined as a fasting plasma glucose level ≥7.0 mmol/L, a nonfasting plasma glucose level ≥11.0 mmol/L, or current use of antidiabetic medications.

### Definition of CKD

Serum creatinine (Cre) was measured by noncompensated kinetic Jaffé methods. The glomerular filtration rate (GFR) of each participant was calculated from the Cre value and the age, using the MDRD equation modified by the Japanese coefficient (0.881), as follows<sup>15</sup>:

$$\text{GFR (ml/min/1.73 m}^2\text{)} = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \text{ (for men)}$$

$$\text{and GFR (ml/min/1.73 m}^2\text{)} = 0.881 \times 186 \times \text{age}^{-0.203}$$

$$\times \text{Cre}^{-1.154} \times 0.742 \text{ (for women).}$$

CKD was defined as an estimated GFR <60 mL/min/1.73m<sup>2</sup>.

### Confirmation of Stroke and MI and End Point Determination

The confirmation of stroke and MI in the Suita Study has been described elsewhere.<sup>11–13</sup> In brief, the 5 hospitals in this area, where acute stroke and MI patients were admitted, were all capable of performing computed tomographic scans or MRI. Medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline data. Strokes were defined according to the U.S. National Survey of Stroke criteria.<sup>16</sup> For each stroke subtype (ie, cerebral infarction [thrombotic or embolic infarction], intracerebral hemorrhage, and subarachnoid hemorrhage), a definite diagnosis was established based on examination of computed tomographic scans, magnetic resonance images, or autopsies. Definite and probable MIs were defined according to the criteria set out by the MONICA project.<sup>17</sup> Sudden deaths of unknown origin were deaths that occurred within 24 hours from the onset of symptoms, and were also classified as MI. In this study CVD was defined as stroke or MI.

To detect MI and stroke occurrences, each participant's health status was checked at clinical visits to the National Cardiovascular Center every 2 years. Yearly questionnaires by mail or telephone were also completed for all participants. In addition, to complete our surveillance for fatal strokes and MIs, we conducted a systematic search for death certificates. All the data (health check-ups, questionnaires, and death certificates) were checked against medical records to confirm the incidence of CVD. We identified possible strokes or MIs using data from (1) the health examination and questionnaires from the stroke and MI registries without informed consent for medical records survey; and (2) death certificates bearing a diagnosis of probable stroke or MI without registration of CVD incidence.

The end points of the current follow-up study were (1) date of the first MI or stroke event (2); date of death (3); date of leaving Suita; and (4) December 31, 2005 (censored).

### Statistical Analysis

Analyses of variances and  $\chi^2$  tests were used to compare mean values and frequencies. The Cox proportional-hazard ratios (HRs) were fitted to the GFR categories and CKD after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors at the baseline survey: namely, present illness of hypertension, hypercholesterolemia and diabetes, smoking status (never, quit, and current smoker), and drinking status (never, quit, and current drinker). The Cox proportional HRs were fitted to the combination of the BP categories and CKD (positive or negative) after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors including an interactive term for CKD and BP categories. The fit of the proportional hazards model was evaluated by examining discrete regression models and by permitting the proportionality assumption to vary with time, and assessments of nonlinearity involving associations with blood pressure and GFR categories were made. The probability values for the model of interaction between CVD incidence and log (person year) were 0.38 in men and 0.81 in women. Proportionality was also checked by log-log survival plot.

To express the impact of CKD on CVD occurrence in the participants, we estimated the population attributable fraction (PAF, %). PAF was estimated as follows:

$$Pe \times (HR - 1) / HR,$$

in which *Pe* is the proportion of incident cases in CKD, and *HR* is the multiple-adjusted hazard ratio.<sup>18</sup> All statistical analyses were conducted using the SAS statistical package software (release version 8.2, SAS Institute Inc).

## Results

Figure 1 shows that the frequency of CKD increases with age in both men and women. At the baseline survey, both men and women with CKD (8.9% for men and 11.3% for women)

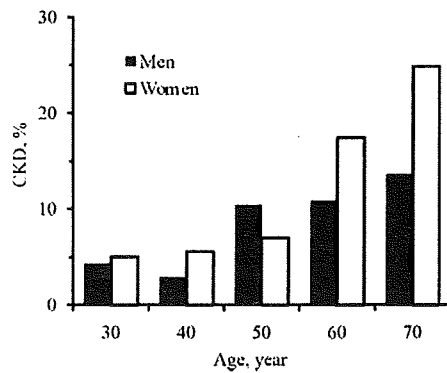


Figure 1. Frequencies of CKD according to sex and age.

were older, had higher prevalence of hypertension and hypercholesterolemia, and had a lower frequency of current drinking than those without CKD (Table 1).

During an average 11.7-year follow-up period, we documented 213 strokes and 133 MIs. In men and women combined, compared with subjects for  $\text{GFR} \geq 90 \text{ mL/min/1.73m}^2$  the multivariable HRs (95% confidence intervals; CIs) for CVD incidence were 1.75 (1.22 to 2.50) in  $\text{GFR} = 50$  to  $59 \text{ mL/min/1.73m}^2$  and 2.48 (1.56 to 3.94) in  $< 50 \text{ mL/min/1.73m}^2$  (Table 2). In addition, the risks of CVD for each GFR category in men and women separately were similar to the risks for all participants. The multivariable HR (95% CIs) of CVD incidence for CKD was 1.70 (1.30 to 2.23) in all subjects (data not shown).

In Table 3, the multivariable HRs (95% CIs) for strokes were 1.94 (1.26 to 2.98) in the  $\text{GFR} = 50$  to  $59 \text{ mL/min/1.73m}^2$  and 2.19 (1.18 to 4.06) in the  $\text{GFR} < 50 \text{ mL/min/1.73m}^2$  compared with subjects for  $\text{GFR} \geq 90 \text{ mL/min/1.73m}^2$ . Results for cerebral infarction were similar to strokes. Age-adjusted HRs (95% CIs) for intracerebral hemorrhage were 1.93 (0.77 to 4.85) in the  $\text{GFR} = 50$  to  $59 \text{ mL/min/1.73m}^2$  and 2.52 (0.72 to 8.80) in the  $\text{GFR} < 50 \text{ mL/min/1.73m}^2$  (supplemental Table I, available online at <http://stroke.ahajournals.org>).

In Figure 2, compared with the optimal BP subjects without CKD, the normal BP, high-normal BP, and hypertensive subjects without CKD showed increased risks of CVD, whereas the impact of each BP category on CVD was more evident in subjects with CKD (probability values for interaction between CKD and BP category were 0.04 in men, 0.49 in women, and 0.06 in all subjects). Results of stroke were similar (probability values for the interaction were 0.03 in men and 0.90 in women, data not shown). Supplemental Table II shows the hazard ratios for the association between 10 mm Hg of SBP and the risk of CVD in subjects with or without CKD.

Using the HRs, we estimated the population attributable fraction of CVD to exposure for CKD at baseline by sex. We found that 8.3% in men and 17.6% in men with CVD incidences could be described as excessive incidence attributable to CKD.

## Discussion

In this cohort study of a general urban Japanese population, CKD was a risk factor for CVD and its subtypes. A stronger association between BP and the incidence of CVD was

Table 1. Baseline Characteristics of Study Subjects According to Chronic Kidney Disease

Variables	Men			Women		
	CKD (-)	CKD (+)	P Value	CKD (-)	CKD (+)	P Value
No. of subjects	2341	229		2593	331	
Age at baseline, y	55±13	61±12	<0.001	53±13	62±12	<0.001
Body mass index, kg/m <sup>2</sup>	22±3	23±3	<0.001	22±3	22±3	0.332
Blood pressure category, %			0.005			<0.001
Optimal	31.7	24.0		43.9	27.2	
Normal	19.2	14.4		16.6	15.4	
High-normal blood pressure	16.2	20.5		14.0	14.8	
Hypertension	32.9	41.1		25.5	42.6	
Present illness, %*						
Hypercholesterolemia	28.1	35.8	0.014	40.7	54.7	<0.001
Diabetes	6.1	6.6	0.791	3.2	5.4	0.036
Smoking status, %			0.007			0.713
Current	51	42		12	12	
Quit	30	40		4	4	
Never	19	18		84	83	
Drinking status, %			0.024			0.017
Current	76	68		34	26	
Quit	3	6		2	3	
Never	21	26		65	71	

\*Hypercholesterolemia; antilipidemic drug use or total cholesterol  $\geq 5.7 \text{ mmol/L}$  (220 mg/dl), diabetes; antihyperglycemic drug use or fasting blood sugar  $\geq 7.0 \text{ mmol/L}$  (126 mg/dl).

Plus-minus values are means±SD.

**Table 2. Age and Multivariable Adjusted Hazard Ratios (95% CIs) for Incidence of Cardiovascular Disease† According to Category of Glomerular Filtration Rate by Sex**

Variables	Glomerular Filtration Rate, ml/min/1.73m <sup>2</sup>				P for Trend
	≥90	60 to 89	50 to 59	<50	
<b>Men and Women</b>					
Cases, n	94	176	51	25	
Person-years	28 736	29 336	4764	1558	
Age-adjusted	1	1.22 (0.94–1.58)	1.71 (1.20–2.42)	2.49 (1.59–3.90)	<0.001
Multivariable adjusted*	1	1.21 (0.93–1.58)	1.75 (1.22–2.50)	2.48 (1.56–3.94)	<0.001
<b>Men</b>					
Cases, n	50	124	24	11	
Person-years	12 092	14 835	1928	522	
Age-adjusted	1	1.20 (0.85–1.70)	1.63 (1.00–2.68)	2.17 (1.11–4.23)	0.008
Multivariable adjusted*	1	1.21 (0.85–1.70)	1.78 (1.08–2.94)	2.38 (1.21–4.68)	0.004
<b>Women</b>					
Cases, n	44	52	27	14	
Person-years	16 644	14 502	2836	1036	
Age-adjusted	1	1.22 (0.81–1.83)	1.79 (1.09–2.92)	2.81 (1.53–5.18)	<0.001
Multivariable adjusted*	1	1.21 (0.80–1.84)	1.76 (1.05–2.93)	2.31 (1.20–4.43)	0.002

\*Multivariable adjusted for age, BMI, smoking, drinking, and present illness (hypertension, diabetes, and hypercholesterolemia).  
†Cardiovascular disease includes both stroke and MI.

observed in the presence of CKD. Furthermore, we found that 8% in men and 18% in women of CVD incidence may be derived from CKD cases.

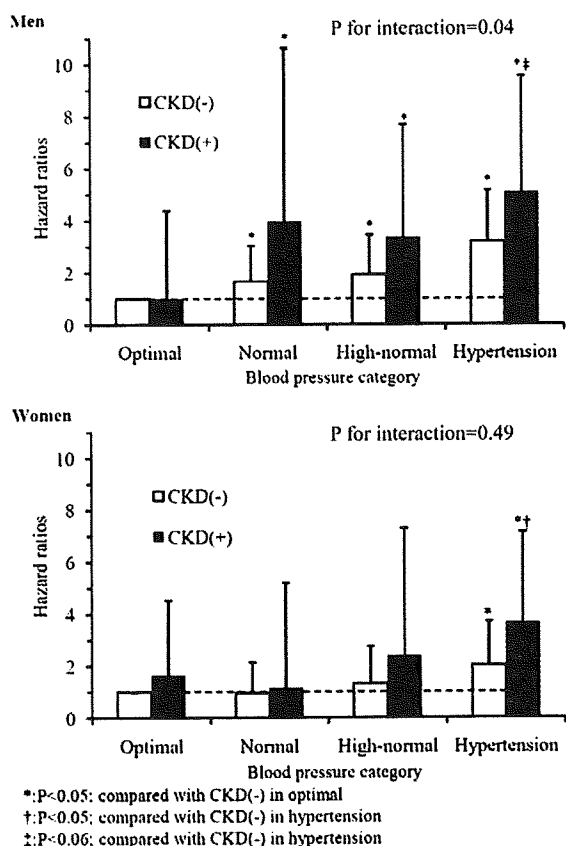
Go et al reported that both severe and moderate renal diseases were risk factors for CVD incidence.<sup>6</sup> A pooled analysis of community-based studies demonstrated that CKD is an independent risk factor for the composite of all-cause mortality in blacks and whites and CVD incidence in blacks.<sup>5</sup> In contrast, NHANES I did not provide relationships between mortality and moderately higher serum creatinine levels.<sup>4</sup> The Framingham Heart Study and Offspring cohorts have shown no significant association between the presence of kidney disease and CVD incidence.<sup>3</sup>

The results of our study are essentially compatible with previous cohort studies in Japan. The Hisayama study demonstrated that CKD was a risk factor for incidence of coronary heart disease in men and ischemic stroke in women.<sup>8</sup> The Ohasama study indicated that decreased kidney function increased the risk of first symptomatic stroke events.<sup>19</sup> This study used creatinine clearance rather than estimated GFR. Irie et al showed that subjects with GFR <60 had a higher risk of CVD mortality<sup>7</sup> but did not examine the risk of GFR 50 to 59 mL/min/1.73m<sup>2</sup>. The NIPPON DATA 90 indicated that CKD was an independent risk factor for cardiovascular death in a community-dwelling Japanese population.<sup>20</sup> The end point of these studies was also mortality. Ninomiya et al

**Table 3. Age-Sex and Multivariable Adjusted Hazard Ratios (95% CIs) for Incidence of All Strokes, Cerebral Infarction, and Myocardial Infarction According to Category of Glomerular Filtration Rate**

Variables	Glomerular Filtration Rate, ml/min/1.73m <sup>2</sup>				P for Trend
	≥90	60 to 89	50 to 59	<50	
Person-years	28 258	28 690	4528	1446	
<b>All strokes</b>					
Cases, n	65	99	36	13	
Age and sex adjusted	1	1.02 (0.73–1.41)	1.78 (1.17–2.70)	1.93 (1.05–3.54)	0.004
Multivariable adjusted*	1	1.04 (0.74–1.45)	1.94 (1.26–2.98)	2.19 (1.18–4.06)	<0.001
<b>Cerebral infarction</b>					
Cases, n	42	66	24	9	
Age and sex adjusted	1	0.99 (0.66–1.49)	1.72 (1.03–4.19)	2.01 (0.97–4.19)	0.020
Multivariable adjusted*	1	0.98 (0.65–1.49)	1.81 (1.07–3.07)	2.26 (1.07–4.78)	0.008
<b>Myocardial infarction</b>					
Cases, n	29	77	15	12	
Age and sex adjusted	1	1.68 (1.08–2.61)	1.64 (0.87–3.09)	4.26 (2.14–8.45)	<0.001
Multivariable adjusted*	1	1.60 (1.03–2.49)	1.51 (0.80–2.88)	3.56 (1.73–7.30)	0.002

\*Multivariable adjusted for age, sex, BMI, smoking, drinking, and present illness (hypertension, diabetes, and hypercholesterolemia).



**Figure 2.** The combination of CKD and BP categories on multivariable hazard ratios for CVD. Data for men and women are presented separately. Multivariable analyses are adjusted age in 5-year increments as stratified variables and other potential confounding factors of hypercholesterolemia, diabetes, and smoking and drinking status.

has recently reported that CKD was risk factors for CVD and stroke in women and that CKD increased the association between BP category and CVD in all subjects from 10 combined different cohort studies using different methods of creatinine measurement.<sup>10</sup> All of our samples were measured using the same analyzer at one laboratory.

Compared with the previous studies, our study has several methodological strengths. First, we could perform subanalysis by age and CVD subtype, because we evaluated a large cohort of participants. Second, each participant's health status was checked during a clinical visit at the National Cardiovascular Center every 2 years. In addition, each year, a health questionnaire was given to each participant via mail or telephone. We could evaluate the registry of CVD incidence with the data obtained from clinical visits, annual questionnaires, or death certificates. Finally, our cohort population was selected at random from an urban population, in contrast to most other cohort studies in Japan, which have relied on rural populations.<sup>7,8,19</sup>

There may be some reasons why CKD is more positively associated with CVD in blacks or Japanese than in whites. Blacks and Japanese are more likely to have hypertension at

an earlier age.<sup>9,21</sup> Therefore, the period of hypertension exposure tends to be longer in blacks and Japanese than in whites. The GFR estimation has been adjusted by a factor suitable for Japanese populations.<sup>15</sup>

Reduced kidney function is associated with increased levels of inflammatory factors,<sup>22,23</sup> abnormal apolipoprotein levels,<sup>22</sup> elevated plasma homocysteine,<sup>22</sup> enhanced coagulability,<sup>23</sup> anemia, left ventricular hypertrophy, increased arterial calcification, endothelial dysfunction, and arterial stiffness.<sup>2,24</sup> How these and other factors interact to increase the risk of adverse outcomes remains unclear but is the focus of ongoing investigations.<sup>24</sup>

Subjects with GFR levels of 50 to 59 mL/min/1.73m<sup>2</sup> were observed to be at risk for stroke. It is desirable to prevent CVD in subjects with both high-risk (<50 mL/min/1.73m<sup>2</sup>) and less severe kidney disease (50 to 59 mL/min/1.73m<sup>2</sup>), although an accelerated decline in GFR occurred for the subjects whose initial GFR <50 mL/min/1.73m<sup>2</sup>.<sup>25</sup>

Hypertension is a strong risk factor for early decline in kidney function; hypertensive patients (BP  $\geq$ 160/95 mm Hg) have a 5-fold greater decline in GFR (2.7 mL/min/1.73m<sup>2</sup>/yr) compared with patients with BP <140/90 mm Hg.<sup>26</sup> Furthermore, in this study, the association between BP and the incidence of CVD were evident by CKD. The risk of CVD was higher in CKD subjects with normal and high-normal BP than in non-CKD subjects in the same BP categories. Using the combination of BP and CKD, it could be possible to screen more efficiently for higher risk of stroke and MI. This is compatible with the CKD clinical guidelines, which state that the preferable BP for subjects with CKD is 130/80 mm Hg.<sup>27</sup> For the prevention of CVD incidence for all hypertensive subjects in health check-ups, it might be desirable to measure serum creatinine levels and to intervene in lifestyle modification such as reducing salt intake, more frequent exercise, or quit smoking.

Our study has several limitations. The primary limitation is dilution bias,<sup>28</sup> in that the current study was based on single-day measurement of creatinine levels. The creatinine levels might have been misclassified, despite the fact that measurements of creatinine levels on a single day have been found to be accurate in other epidemiological studies. Second, we did not perform a creatinine clearance test or 2 measurements of serum creatinine at least 3 months apart. Although our definition of CKD is based on a single assessment of serum creatinine, the equation provides an accurate estimated GFR value.<sup>15</sup> Third, even with the moderate sample size (n=5494) and 12-year duration, the numbers of end points were limited, especially when the data were stratified by 2 variables, such as sex and glomerular filtration rates. A study with more participants with the same protocol is required to validate to the association between BP category and CVD by CKD.

In conclusion, CKD was associated with an increased risk for stroke and MI in a general urban Japanese population. Furthermore, the association between BP and CVD may be evident by CKD. To prevent the incidence of stroke and MI, it is necessary for subjects with CKD to control their BP by lifestyle modification and proper clinical treatment.

### Acknowledgments

We express our deepest gratitude to Dr Yasushi Kotani, all members of the Suita City Health Center, and the Suita Medical Association. We also thank all researchers and the staff of the Division of Preventive Cardiology for performing medical examinations and the follow-up. We also thank *Satsuki-Junyukai*, the volunteers for the administration of the Suita study.

### Sources of Funding

This study was supported by Grant-in-Aid from the Ministry of Health, Labor, and Welfare of Japan (H20-SeiShu-013, H19-SeiShu-017, and H18-SeiShu-012), and by a Research Grant for Cardiovascular Disease from the Ministry of Health, Labor, and Welfare (19S-6, 18S-1).

### Disclosures

None.

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# Proposed Criteria for Metabolic Syndrome in Japanese Based on Prospective Evidence

## The Hisayama Study

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**Background and Purpose**—The current criteria of metabolic syndrome (MetS) are not based on evidence derived from prospective studies on cardiovascular disease (CVD).

**Methods**—In a 14-year follow-up study of 2452 community-dwelling Japanese individuals aged  $\geq 40$  years, we examined which of the MetS criteria are most predictive for the development of CVD. During the follow-up, 246 first-ever CVD events occurred.

**Results**—An optimal cutoff point of waist circumference for predicting CVD was 90 cm in men (age-adjusted hazard ratio=1.81; 95% CI, 1.19 to 2.74;  $P=0.005$ ) and 80 cm in women (age-adjusted hazard ratio=1.46; 95% CI, 0.99 to 2.16;  $P=0.05$ ). A comparison of MetS criteria showed that the modified Japanese criteria using this cutoff point instead of the original definition were the strongest predictor of CVD events in both sexes (men: age-adjusted hazard ratio=2.58; 95% CI, 1.65 to 4.02;  $P<0.001$ ; women: age-adjusted hazard ratio=2.39; 95% CI, 1.65 to 3.48;  $P<0.001$ ). These observations remained robust even after adjustment for other confounding factors. According to this criteria set, only in the presence of central obesity, the hazard ratios for future CVD increased significantly as the number of MetS components increased, and a significant relationship was identified from 2 or more MetS components compared with individuals who had no MetS component.

**Conclusions**—Our findings suggest that the optimal cutoff point of waist circumference is 90 cm in men and 80 cm in women and that the modified Japanese criteria of MetS with this cutoff point as an essential component better predict CVD in the general Japanese population. (*Stroke*. 2009;40:1187-1194.)

**Key Words:** brain infarction ■ coronary artery disease ■ epidemiology ■ metabolic syndrome

Metabolic syndrome (MetS) consists of a clustering of cardiovascular risk factors, and individuals with this condition have an elevated risk of developing cardiovascular diseases and type 2 diabetes.<sup>1</sup> Practical and valuable criteria must be established promptly, because the prevalence of metabolic disorders has been increasing rapidly in recent years in Japan and other countries.<sup>2-4</sup> Over the past decade, several institutions have proposed various criteria in attempts to define MetS as a diagnostic category. Among these, the criteria of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP) has most often been used in the literature.<sup>5</sup> In this criteria set, the cutoff points of waist circumference were 102 cm in men and 88 cm in women; this parameter comprised a component of this syndrome but not a prerequisite for its diagnosis. However, this cutoff level may be unsuitable for Asian populations. For Japanese, 2 sets of diagnostic criteria of MetS exist at the present time, resulting

in a great deal of confusion in clinical practice. One set is proposed by the Japanese Society of Internal Medicine (Japanese criteria)<sup>6</sup>; in these criteria, waist circumference is defined as an essential component, and its cutoff value is 85 cm for men and 90 cm for women.<sup>6</sup> The other criteria set is offered by the International Diabetes Federation (IDF), in which ethnic-specific waist circumference cutoff points are used as a requirement of diagnosis.<sup>7</sup> The IDF recommended cutoff levels of 90 cm in men and 80 cm in women for central obesity in Japanese individuals. In the current knowledge, it remains unclear which of these criteria or cutoff points of waist circumference are a better predictor of the development of cardiovascular disease (CVD) in the general population of Japanese. There has also been controversy as to whether the component of waist circumference should be considered a prerequisite for a diagnosis of MetS.<sup>8</sup> The aim of the present article is to derive a better definition from the existing MetS

Received July 12, 2008; final revision received September 30, 2008; accepted October 22, 2008.

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DOI: 10.1161/STROKEAHA.108.531319

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criteria for predicting CVD in a prospective study of a defined general population of Japanese.

## Materials and Methods

### Study Population

In 1988, a screening survey for the present study was performed in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. The age and occupational distributions and nutritional intake of the population were almost identical to those of Japan as a whole based on data from the national census and nutrition survey.<sup>9</sup> A detailed description of this survey was published previously.<sup>9</sup> Briefly, a total of 2736 residents aged  $\geq 40$  years (80.7% of the total population of this age group) consented to participate in the examination and underwent a comprehensive assessment. After the exclusion of 102 subjects who had a history of coronary heart disease or stroke, as determined by a questionnaire and medical records, one subject for whom no blood sample was obtained, 120 subjects who had already eaten breakfast, and 61 subjects for whom waist circumference was not measured, the remaining 2452 subjects (1050 men and 1402 women) were enrolled in this study.

### Follow-Up Survey

The subjects were followed prospectively from December 1988 to November 2002 by repeated health examinations. Health status was checked yearly by mail or telephone for any subjects who did not undergo a regular examination or who had moved out of town. We also established a daily monitoring system among the study team and local physicians or members of the town's Health and Welfare Office. When a subject died, an autopsy was performed at the Departments of Pathology of Kyushu University. During the follow-up period, 479 subjects died, of whom 362 (75.6%) underwent autopsy. Only one subject was lost to follow-up.

### Definition of Cardiovascular Events

CVD was defined as the development of ischemic stroke or coronary heart disease. Each CVD case was coded according to the International Classification of Disease, Ninth Revision (ICD-9) from 1988 to 1996 and Tenth Revision (ICD-10) from 1997 to 2002. Stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for  $\geq 24$  hours.<sup>2</sup> Each diagnosis of ischemic stroke (ICD-9: 434, ICD-10: I63) was made by 2 neurologists (Y.K. and Y.T.) separately using collected clinical and pathological information including brain CT/MRI and autopsy findings based on the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke.<sup>10</sup> Coronary heart disease included acute myocardial infarction (ICD-9: 410, ICD-10: I21), silent myocardial infarction (ICD-9: 412, ICD-10: I25.2), sudden cardiac death within 1 hour after the onset of acute illness (ICD-9: 798.1, ICD-10: I96.0), or coronary artery disease followed by coronary artery bypass surgery (ICD-9: E878.2, ICD-10: Z95.1) or angioplasty (ICD-9: E879.0, ICD-10: Z95.5).<sup>2</sup> Acute myocardial infarction was diagnosed when a subject met at least 2 of the following criteria: (1) typical symptoms, including prolonged severe anterior chest pain; (2) cardiac enzyme levels more than twice the upper limit of the normal range; (3) evolving diagnostic electrocardiographic changes; and (4) morphological changes, including local asynergy of cardiac wall motion on echocardiography, persistent perfusion defect on cardiac scintigraphy, or myocardial necrosis or scars  $\geq 1$  cm long accompanied by coronary atherosclerosis at autopsy. Silent myocardial infarction was defined as myocardial scarring without any historical indication of clinical symptoms or abnormal cardiac enzyme changes. During the 14-year follow-up, 246 first-ever cardiovascular events (131 men and 115 women) occurred. Of these, there were 145 ischemic strokes (66 men and 79 women) and 125 cases of coronary heart disease (78 men and 47 women).

### Risk Factor Measurements

At the baseline examination, waist circumference was measured by a trained staff member at the umbilical level with the subject standing. Body height and weight were measured in light clothing without shoes, and body mass index (BMI) was calculated.

To measure blood glucose and lipid levels, blood samples were collected from an antecubital vein between 8:00 and 10:30 AM after an overnight fast of at least 12 hours. Blood for glucose assay was obtained by venipuncture into tubes containing sodium fluoride (NaF), and plasma glucose levels were determined by the glucose-oxidase method. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were determined enzymatically. Blood pressure was measured 3 times using a standard mercury sphygmomanometer in the sitting position after the subject rested for at least 5 minutes. Freshly voided urine samples were collected at the screening, and proteinuria was defined as 1+ or more using a reagent strip. Electrocardiographic abnormalities were defined as left ventricular hypertrophy (Minnesota Code 3 to 1) and/or ST depression (Minnesota code 4-1, 2, 3).

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

### Definition of Metabolic Syndrome

The Third Report of the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults criteria<sup>5</sup> of MetS include the presence of at least 3 of 5 factors: central obesity (waist circumference  $>102$  cm in men,  $>88$  cm in women), elevated blood pressure (blood pressure  $\geq 130/85$  mm Hg and/or current use of antihypertensive agents), elevated fasting plasma glucose ( $\geq 6.1$  mmol/L and/or current use of antidiabetic medication), reduced HDL cholesterol ( $<1.03$  mmol/L for men,  $<1.29$  mmol/L for women), and elevated triglycerides ( $\geq 1.68$  mmol/L).

In the IDF criteria,<sup>7</sup> central obesity must be present for a diagnosis of MetS in addition to at least 2 of the other 4 factors. The IDF categories for Asians are: waist circumference  $\geq 90$  cm for men and  $\geq 80$  cm for women; blood pressure  $\geq 130/85$  mm Hg and/or current use of antihypertensive agents; fasting plasma glucose  $\geq 5.6$  mmol/L and/or current use of antidiabetic medication; HDL cholesterol  $<1.03$  mmol/L in men and  $<1.29$  mmol/L in women; and triglycerides  $\geq 1.68$  mmol/L.

Unlike the other criteria, the Japanese criteria<sup>6</sup> consist of 4 factors, because they deal with HDL cholesterol and triglycerides together and the cutoff value of waist circumference is larger in women than in men. MetS in the Japanese criteria was diagnosed in individuals who had a high waist circumference ( $\geq 85$  cm in men and  $\geq 90$  cm in women) plus any 2 of the following: (1) blood pressures of  $\geq 130/85$  mm Hg and/or current use of antihypertensive medicine; (2) fasting plasma glucose  $\geq 6.1$  mmol/L and/or current use of antidiabetic medication; and (3) triglycerides  $\geq 1.68$  mmol/L and/or HDL cholesterol  $<1.03$  mmol/L in men and women. Additionally, we created 2 new criteria. The modified NCEP and Japanese criteria used a waist circumference of  $\geq 90$  cm in men and  $\geq 80$  cm in women instead of the original cutoff points.

### Statistical Analysis

The SAS software package Version 8.2 (SAS Institute, Cary, NC) was used to perform all statistical analyses. Serum triglycerides were transformed into logarithms to improve the skewed distribution. The age- and multivariate-adjusted hazard ratios (HRs) and 95% CIs were estimated with the use of the Cox proportional hazards model. To find the cutoff value of abdominal obesity, we also plotted receiver operating characteristic curves. In this method, the optimal cutoff value of abdominal obesity was defined by maximizing the sensitivity and specificity to the development of CVD.<sup>11</sup> In addition, population-attributable risk percent was estimated for various MetS

Table 1. Characteristics of Subjects by Sex, 1988

	Men (n=1050)	Women (n=1402)	P
Age, years	58 (11)	59 (11)	0.05
Prevalence of MetS			
NCEP	16.8	22.3	<0.001
IDF for Asians	13.4	34.5	<0.001
Japanese	21.4	8.1	<0.001
Modified NCEP	21.6	31.3	<0.001
Modified Japanese	10.0	18.5	<0.001
Waist circumference, cm	82.0 (8.2)	81.1 (10.1)	0.01
BMI, kg/m <sup>2</sup>	22.8 (2.9)	23.0 (3.2)	0.37
Systolic blood pressure, mm Hg	134 (20)	132 (21)	0.002
Diastolic blood pressure, mm Hg	81 (11)	76 (11)	<0.001
Elevated blood pressure, %	60.0	51.6	<0.001
Fasting plasma glucose, mmol/L	5.9 (1.3)	5.7 (1.3)	<0.001
Elevated fasting plasma glucose, %	27.2	17.9	<0.001
Total cholesterol, mmol/L	5.11 (1.07)	5.56 (1.07)	<0.001
HDL cholesterol, mmol/L	1.26 (0.31)	1.34 (0.29)	<0.001
Reduced HDL cholesterol, %	22.7	12.9	<0.001
Triglycerides, mmol/L	1.32 (0.41–4.22)	1.06 (0.41–2.72)	<0.001
Elevated triglycerides, %	29.3	16.4	<0.001
Proteinuria, %	7.9	4.1	<0.001
Electrocardiogram abnormalities, %	19.0	13.1	<0.001
Current drinking, %	61.5	8.9	<0.001
Current smoking, %	50.4	6.7	<0.001
Regular exercise, %	11.6	9.2	0.06

Note: All values are given as means (SD) or as percentages except for triglycerides. Triglycerides are shown by geometric means and 95% prediction intervals due to the skewed distribution. Elevated blood pressure: blood pressures of  $\geq 130/85$  mm Hg and/or current use of antihypertensive medicine; elevated fasting plasma glucose: fasting plasma glucose  $\geq 6.1$  mmol/L and/or current use of antidiabetic medication; reduced HDL cholesterol: HDL cholesterol  $< 1.03$  mmol/L; elevated triglycerides: triglycerides  $\geq 1.68$  mmol/L; electrocardiogram abnormalities: left ventricular hypertrophy (Minnesota Code 3–1) and/or ST depression (Minnesota Code 4–1, 2, 3).

criteria sets with the following formula: prevalence $\times$ (HR–1)/[prevalence $\times$ (HR–1)+1].

### Ethical Considerations

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

### Results

Table 1 shows the subjects' baseline clinical characteristics by sex. The prevalence of MetS defined by the NCEP, IDF for Asians, modified NCEP, and modified Japanese criteria was significantly higher in women than in men, whereas the prevalence of MetS by the Japanese criteria was higher in men. Mean values of waist circumference, systolic and

diastolic blood pressures, fasting plasma glucose and triglyceride levels, and frequencies of elevated blood pressure, fasting plasma glucose, and triglycerides, reduced HDL cholesterol, proteinuria, electrocardiographic abnormalities, alcohol intake, and smoking habits were significantly higher in men than in women, whereas women had higher total and HDL cholesterol concentrations. Mean age and BMI and frequency of regular exercise did not differ between the sexes.

To compare the ability to predict CVD at each published cutoff level of waist circumference among the NCEP, IDF, and Japanese MetS criteria, we estimated the age-adjusted HRs and 95% CIs by sex (Table 2). In men, the age-adjusted HR of incident CVD was significantly higher in subjects with a waist of  $\geq 90$  cm (IDF criteria for Asians) than in those with a smaller waist (age-adjusted HR=1.81; 95% CI, 1.19 to 2.74;  $P=0.005$ ), whereas in women, this association was marginally significant at the cutoff level of  $\geq 80$  cm (age-adjusted HR=1.46; 95% CI, 0.99 to 2.16;  $P=0.05$ ). The levels of central obesity determined by the cutoff levels of waist circumference proposed by the NCEP, IDF for Europeans, and Japanese criteria were not significant predictors of CVD in either sex.

In the analysis with the receiver operating characteristic curve method, the cutoff point defined as the maximum combination of sensitivity and specificity was 80.2 cm for men and 81.5 cm for women. This cutoff point significantly predicted CVD in women but did not in men (men: age-adjusted HR=1.30; 95% CI, 0.91 to 1.85;  $P=0.15$ ; women: age-adjusted HR=1.61; 95% CI, 1.11 to 2.35;  $P=0.01$ ).

Age- and multivariate-adjusted HRs and population-attributable risk percents of various MetS criteria for the development of CVD were estimated by sex (Table 3). The age-adjusted analyses showed that MetS defined by all of the criteria sets, except for the Japanese one in men, was a significant risk factor for CVD. Among these, MetS as determined by the modified Japanese criteria was the strongest predictor for the development of CVD in both sexes (men: age-adjusted HR=2.58; 95% CI, 1.65 to 4.02;  $P<0.001$ ; women: age-adjusted HR=2.39; 95% CI, 1.65 to 3.48;  $P<0.001$ ). These findings remained substantially unchanged even after adjustment for the following confounding factors: age, serum total cholesterol, proteinuria, electrocardiographic abnormalities, alcohol intake, smoking habits, and regular exercise. When we divided CVD into ischemic stroke and coronary heart disease, the age-adjusted incidence of ischemic stroke was significantly higher in subjects with MetS defined by the modified Japanese criteria than those without MetS for both sexes (men: 18.0 versus 5.2 per 1000 person-years.  $P<0.001$ ; women: 9.2 versus 4.0,  $P<0.001$ ). The same was true incidence of coronary heart disease in both sexes (mean: 10.4 versus 6.4,  $P=0.003$ ; women: 6.7 versus 2.0,  $P<0.001$ ). These associations remained significant even after adjustment for the previously mentioned confounding factors (ischemic stroke: HR=3.07; 95% CI, 1.68 to 5.61;  $P<0.001$ , in men; HR=2.21; 95% CI, 1.39 to 3.51;  $P<0.001$ , in women; coronary heart disease: HR=2.37; 95% CI, 1.28 to 4.39;  $P=0.006$ , in men; HR=2.91; 95% CI, 1.62 to 5.22;  $P<0.001$ , in women). On the other hand, the multivariate-

**Table 2. Age-Adjusted HRs for the Development of CVD According to the Cutoff Points of Waist Circumference Among Various Criteria of MetS**

MetS Criteria	Waist Cutoff, cm	No. of Subjects	No. of Events	Age-Adjusted HR (95% CI)	P Value
<b>Men</b>					
NCEP	≤102	1042	131	1 (referent)	
	>102	8	0	...	...
IDF (Europids)	<94	972	120	1 (referent)	
	≥94	78	11	1.54 (0.83–2.87)	0.17
IDF (Asians)	<90	873	102	1 (referent)	
	≥90	177	29	1.81 (1.19–2.74)	0.005
Japanese	<85	621	77	1 (referent)	
	≥85	429	54	1.22 (0.86–1.73)	0.28
<b>Women</b>					
NCEP	<88	1069	82	1 (referent)	
	≥88	333	33	1.22 (0.81–1.82)	0.34
IDF (Asians)	<80	601	38	1 (referent)	
	≥80	801	77	1.46 (0.99–2.16)	0.05
Japanese	<90	1113	89	1 (referent)	
	≥90	289	26	1.05 (0.68–1.62)	0.83

adjusted population-attributable risk percents for MetS defined by the IDF, modified NCEP, and modified Japanese criteria were comparably higher than those for MetS defined by the other criteria in both sexes, and all of the population-attributable risk percents were larger in women than in men.

To investigate the necessity of central obesity defined as a waist circumference of ≥90 cm in men and ≥80 cm in women for predicting CVD in the modified Japanese criteria, the previously mentioned risk factor-adjusted HR according to the number of MetS components other than waist circumference were estimated by the presence or absence of central obesity (Table 4). In the subjects who had central obesity, the HR of CVD increased significantly as the number of MetS components increased, whereas this trend was not observed in the subjects without central obesity. In the subjects with central obesity, the risk of CVD significantly increased if subjects had 2 or more MetS components compared with individuals who had no MetS component (one component: adjusted HR=1.13; 95% CI, 0.53 to 2.40;  $P=0.74$ ; 2 components: adjusted HR=2.47; 95% CI, 1.21 to 5.04;  $P=0.01$ ; 3 components: adjusted HR=3.09; 95% CI, 1.40 to 6.79;  $P=0.005$ ). Similar relationships were found when CVD was stratified into ischemic stroke and coronary heart disease.

Because diabetes and hypertension are strong risk factors for CVD, we examined both the combined and separate effects of MetS and diabetes or hypertension on the development of CVD. As shown in Table 5, compared with nondiabetic subjects without MetS, nondiabetic subjects with MetS had significantly higher multivariate-adjusted HR of ischemic stroke (adjusted HR=1.65; 95% CI, 1.04 to 2.62;  $P=0.03$ ); HR was markedly higher than that in diabetic subjects with MetS (adjusted HR=5.35; 95% CI, 3.28 to 8.73;  $P<0.001$ ). However, no elevation was found in diabetic subjects without MetS. Similar associations were observed for coronary heart disease. Likewise, the multivariate-adjusted HR of ischemic stroke was significantly higher in normotensive subjects with

MetS (adjusted HR=2.13; 95% CI, 1.03 to 4.39;  $P=0.04$ ) and in hypertensive subjects with MetS (adjusted HR=3.17; 95% CI, 2.01 to 5.02;  $P<0.001$ ) but was not significant in hypertensive subjects without MetS. Similar patterns were seen for coronary heart disease. Significant interactions between MetS and diabetes were revealed in the risk of ischemic stroke and coronary heart disease ( $P<0.01$ ), whereas the interactions between MetS and hypertension were not significant.

## Discussion

Using data from a 14-year follow-up study of a general Japanese population, we demonstrated that the optimal cutoff point of waist circumference for predicting CVD in Japanese was 90 cm in men and 80 cm in women. In the comparison of various MetS criteria, the modified Japanese criteria set, which uses this cutoff point instead of the original one, was a better predictor for incident CVD in both sexes. According to this criteria set, in subjects with central obesity only, the HR of future CVD increased as the number of MetS components increased, and a significantly elevated risk was identified in subjects who had ≥2 MetS components compared with those who had no MetS component. Furthermore, the significant effects of MetS on the development of ischemic stroke and coronary heart disease were independent of hypertension and diabetes. These findings suggest that the modified Japanese criteria are better for predicting CVD in Japanese.

The existence of different criteria sets for MetS has caused a great deal of confusion in routine practice in Japan. Whereas the IDF criteria are recommended internationally, the Japanese criteria are commonly used in Japan. The established MetS criteria are based mainly on "expert" opinions, and the evidence derived from prospective studies is scarce.<sup>12</sup> Thus, it remains uncertain whether the threshold at which each MetS component is defined as positive or negative is optimal or even useful for predicting the risk of

**Table 3. Age- or Multivariate-Adjusted HRs and Population-Attributable Risk Percents of MetS Defined by Various Criteria for the Development of CVD**

MetS Criteria	Population at Risk, n	No. of Events	Age-Adjusted HR (95% CI)	P Value	Multivariate-Adjusted HR (95% CI)	P Value	Population-Attributable Risk Percents
<b>Men</b>							
<b>NCEP</b>							
Mets (-)	874	100	1 (referent)		1 (referent)		
Mets (+)	176	31	1.63 (1.09–2.44)	0.01	1.55 (1.03–2.33)	0.03	8.4
<b>IDF for Asians</b>							
Mets (-)	909	106	1 (referent)		1 (referent)		
Mets (+)	141	25	1.95 (1.26–3.02)	0.003	1.96 (1.25–3.08)	0.003	11.4
<b>Japanese</b>							
Mets (-)	825	97	1 (referent)		1 (referent)		
Mets (+)	225	34	1.40 (0.95–2.07)	0.09	1.28 (0.86–1.91)	0.21	5.7
<b>Modified NCEP</b>							
Mets (-)	823	91	1 (referent)		1 (referent)		
Mets (+)	227	40	1.74 (1.20–2.52)	0.003	1.66 (1.14–2.43)	0.008	12.5
<b>Modified Japanese</b>							
Mets (-)	945	107	1 (referent)		1 (referent)		
Mets (+)	105	24	2.58 (1.65–4.02)	<0.001	2.49 (1.57–3.94)	<0.001	13.0
<b>Women</b>							
<b>NCEP</b>							
Mets (-)	1,090	71	1 (referent)		1 (referent)		
Mets (+)	312	44	1.74 (1.19–2.54)	0.004	1.65 (1.13–2.43)	0.01	12.6
<b>IDF for Asians</b>							
Mets (-)	918	53	1 (referent)		1 (referent)		
Mets (+)	484	62	1.82 (1.26–2.63)	0.001	1.79 (1.23–2.60)	0.002	21.4
<b>Japanese</b>							
Mets (-)	1,289	96	1 (referent)		1 (referent)		
Mets (+)	113	19	1.96 (1.20–3.21)	0.007	1.89 (1.15–3.10)	0.01	6.7
<b>Modified NCEP</b>							
Mets (-)	963	53	1 (referent)		1 (referent)		
Mets (+)	439	62	1.96 (1.36–2.84)	<0.001	1.88 (1.30–2.74)	<0.001	21.6
<b>Modified Japanese</b>							
Mets (-)	1,142	68	1 (referent)		1 (referent)		
Mets (+)	260	47	2.39 (1.65–3.48)	<0.001	2.27 (1.55–3.32)	<0.001	19.1

Note: Multivariate adjustment was made for age, serum total cholesterol, proteinuria, electrocardiogram abnormalities, alcohol intake, smoking habits, and regular exercise.

CVD. The findings of our study indicate that the definition of MetS by the modified Japanese criteria confers greater accuracy in predicting CVD events compared with the other ones. There are some possible explanations for this superiority. First, this criteria set adopted the optimal cutoff value of waist circumference for predicting vascular events in the present cohort. An optimal cutoff point of waist circumference for having cardiovascular risk factors has been discussed extensively in several cross-sectional studies of Asian populations. Hara et al showed in a receiver operating characteristic analysis that 85 cm for men and 78 cm for women were the best values for predicting other MetS features in a Japanese population.<sup>13</sup> Similar analyses reported that 90 cm in men and 84 cm in women was optimal in Japanese American<sup>14</sup> and 85 cm in men and 80 cm in women in

Chinese populations.<sup>15,16</sup> However, no studies showed an optimal cutoff value of waist circumference for CVD risk in a prospective cohort design. Our finding is the first evidence that the optimal cutoff point of waist circumference for predicting CVD was 90 cm in men and 80 cm in women in a general Japanese population. This evidence might be extrapolated to other Asian populations having similar physiques and genetics.

Second, when we used our modified Japanese criteria, the HR of cardiovascular events rose obviously as the number of MetS components increased only in subjects with central obesity. Thus, to treat waist circumference as an essential component would likely improve the precision of the prediction of cardiovascular events in the current subjects. There has been controversy over the necessity of central obesity for

**Table 4. Multivariate-Adjusted HRs for the Development of CVD According to the No. of MetS Components by the Presence or Absence of Central Obesity**

	No. of MetS Components	Population at risk, n	No. of Events	Multivariate-Adjusted HR (95% CI)	P for Trend
<b>Cardiovascular disease</b>					
Central obesity (–)	0	509	31	1 (referent)	0.58
	1	563	64	1.32 (0.85–2.04)	
	2	311	32	1.07 (0.64–1.78)	
	3	91	13	1.39 (0.71–2.73)	
Central obesity (+)	0	259	10	1 (referent)	
	1	354	25	1.13 (0.53–2.40)	
	2	261	47	2.47 (1.21–5.04)	
	3	104	24	3.09 (1.40–6.79)	
<b>Ischemic stroke</b>					
Central obesity (–)	0	509	19	1 (referent)	0.76
	1	563	39	1.37 (0.78–2.40)	
	2	311	16	1.02 (0.51–2.01)	
	3	91	4	0.84 (0.28–2.52)	
Central obesity (+)	0	259	6	1 (referent)	
	1	354	15	1.31 (0.50–3.42)	
	2	261	30	2.95 (1.18–7.34)	
	3	104	16	3.99 (1.47–10.84)	
<b>Coronary heart disease</b>					
Central obesity (–)	0	509	16	1 (referent)	0.56
	1	563	31	1.13 (0.61–2.09)	
	2	311	16	0.84 (0.41–1.73)	
	3	91	10	1.76 (0.77–4.02)	
Central obesity (+)	0	259	4	1 (referent)	
	1	354	11	1.10 (0.34–3.57)	
	2	261	24	2.85 (0.95–8.56)	
	3	104	13	3.50 (1.07–11.49)	

*Note:* Central obesity was defined by waist circumference of  $\geq 90$  cm in men and  $\geq 80$  cm in women. Multivariate adjustment was made for age, serum total cholesterol, proteinuria, electrocardiogram abnormalities, alcohol intake, smoking habits, and regular exercise.

the diagnosis of MetS. In NIPPON DATA90, a Japanese cohort study, the risk of CVD death increased significantly as the number of MetS components rose both in nonobese participants and obese ones.<sup>8</sup> On the other hand, in the present study, a clear trend in the risk of CVD occurrence was observed only in the subjects with central obesity. This inconsistency in findings might be caused by the difference in populations and the definition of obesity. In the NIPPON DATA90 study, BMI was substituted for waist circumference in the MetS definition. However, there is often remarkable heterogeneity of waist circumference among individuals with similar BMI values. It has been also shown that, among obese individuals, waist circumference indicates an increased risk of CVD, and this association is independent of the risk predicted by increased BMI.<sup>17</sup> Thus, the use of BMI instead of waist circumference may lead to a misdiagnosis of MetS.

In the present study, the risk of CVD occurrence was higher for the modified Japanese MetS criteria than for the IDF criteria despite the identical condition regarding central obesity. One possibility for this is that the definitions of

hyperglycemia and dyslipidemia are different between the 2 sets of MetS criteria. In our subjects, the definitions of hyperglycemia and dyslipidemia in Japanese criteria were superior to those in the other criteria for the prediction of the development of CVD (data not shown). These facts may explain why the modified Japanese criteria had a higher HR. Further studies are needed to optimize the cutoff points of fasting plasma glucose and lipid levels for predicting cardiovascular events.

In our study, there was no large difference in waist circumference between our men and women (82.0 cm versus 81.1 cm). On the other hand, the optimal cutoff point of waist circumference for predicting CVD was lower in women (80 cm) than in men (90 cm). It is known that men are prone to intra-abdominal fat accumulation, whereas women are prone to subcutaneous fat accumulation.<sup>18</sup> Because men would have more intra-abdominal fat than women at a given waist circumference, it may be valid to select a lower cutpoint of waist circumference for men than for women. However, recent epidemiological studies using CT revealed that women

**Table 5. Multivariate-Adjusted HRs for the Development of Ischemic Stroke and Coronary Heart Disease According to the Presence or Absence of MetS and Diabetes as well as Hypertension**

	Population at Risk, n	Ischemic Stroke		Coronary Heart Disease	
		No. of Events	Multivariate-Adjusted HR (95% CI)	No. of Events	Multivariate-Adjusted HR (95% CI)
<b>Diabetes</b>					
DM (-)+MetS (-)	1956	93	1 (referent)	79	1 (referent)
DM (-)+MetS (+)	274	25	1.65 (1.04–2.62)*	22	2.01 (1.22–3.32)†
DM (+)+MetS (-)	131	6	0.77 (0.33–1.77)	9	1.18 (0.59–2.38)
DM (+)+MetS (+)	91	21	5.35 (3.28–8.73)†‡	15	5.13 (2.89–9.11)†‡
<b>Hypertension</b>					
HT (-)+MetS (-)	1355	48	1 (referent)	39	1 (referent)
HT (-)+MetS (+)	114	9	2.13 (1.03–4.39)*	8	2.43 (1.11–5.30)*
HT (+)+MetS (-)	732	51	1.36 (0.90–2.06)	49	1.39 (0.89–2.17)
HT (+)+MetS (+)	251	37	3.17 (2.01–5.02)†‡	29	3.45 (2.06–5.80)†‡

Note: Multivariate adjustment was made for age, serum total cholesterol, proteinuria, electrocardiogram abnormalities, alcohol intake, smoking habits, and regular exercise.

\* $P < 0.05$ , † $P < 0.01$  versus reference.

‡ $P < 0.01$  versus DM (+)+MetS (-) or HT (+)+MetS (-). DM indicates diabetes; HT, hypertension.

who had more visceral fat tended to have more metabolic risk factors for CVD compared with men.<sup>19,20</sup> The cause of this sex difference is uncertain but may be related to a higher amount of hepatic free fatty acid delivery derived from visceral fat in women than in men.<sup>21</sup> These findings imply that it is reasonable to choose the lower cutoff point of waist circumference for women than for men. Furthermore, the population-attributable risk percents for any MetS criteria sets were larger in women than in men. These findings also suggest that MetS has a stronger influence on women than on men.

The American Diabetes Association/European Association for the Study of Diabetes says that MetS has been imprecisely defined, that its pathogenesis is uncertain, and that its value as a CVD risk marker is doubtful. Furthermore, it recommends that clinicians should evaluate and manage all CVD risk factors without regard to whether a patient meets the criteria for a diagnosis of MetS. Certainly, Sone et al documented that the diagnosis of MetS using the modified NCEP criteria was not useful for predicting CVD in patients with diabetes.<sup>22</sup> However, our stratified analysis indicated that MetS is a significant risk factor for CVD in both nondiabetic and normotensive individuals. Moreover, the present study revealed that the risk of CVD was higher in subjects with MetS than in those with diabetes or hypertension. These results imply that MetS plays a main role in the development of CVD in the general population, including patients with mild diabetes and hypertension. In the general Japanese population, blood pressure levels decreased significantly with time due to the increment in the use of antihypertensive medication, whereas metabolic disorders greatly increased in recent periods.<sup>2,23</sup> Even with advances in therapeutic agents, it is difficult to treat MetS and diabetes because lifestyle modifications are also needed. These disorders remain large problems for the prevention of CVD, especially in developed countries.

Additionally, our subjects showed a synergistic effect between MetS and diabetes for the development of CVD. The conditions of MetS are accompanied by adipokine disorders, inducing inflammatory cytokines and immune response, and endothelial dysfunction, which promotes the development of atherosclerosis.<sup>24</sup> On the other hand, hyperglycemia in diabetes itself directly affects the progression of atherosclerosis through the increase in nonenzymatic glycation of proteins and lipids,<sup>25</sup> the production of reactive oxygen species,<sup>26</sup> and the activation of protein kinase C<sup>27</sup> isoform and the hexosamine biosynthetic pathway.<sup>28</sup> It is therefore speculated that MetS and diabetes mutually enhance the risk of CVD by distinct mechanisms.

In our men, the cutoff value of waist circumference derived from the receiver operating characteristic analysis (80.2 cm) was much lower than that derived from the cohort study (90 cm), and the former was not a significant predictor of incident CVD in the follow-up study. This suggests that a value defined by maximizing the sensitivity and specificity would be not always best.

The strengths of our study include its longitudinal population-based design, the long duration of follow-up, the sufficient number of CVD events, and the almost perfect follow-up of subjects. However, 2 limitations of the present study should be discussed. One is that the diagnosis of MetS was based on a single measurement of its components at baseline as was the case in other epidemiological studies. During the follow-up, risk factor levels were changed due to modifications in lifestyle or medication, and misclassification of MetS was possible. This would weaken the association found in this study, biasing the results toward the null hypothesis. Therefore, the true association may be stronger than that shown in our study. The other limitation is that the present study lacked information on drugs, fibrates, and nicotinic acid, affecting the metabolism of HDL cholesterol and triglycerides. However, these medications were rarely

used in our country at this study's 1988 baseline. This suggests that such a bias did not invalidate the present findings.

In conclusion, the present analysis has clearly demonstrated that the optimal cutoff point of waist circumference is 90 cm in men and 80 cm in women and that the modified Japanese criteria of MetS with this cutoff point as an essential component better predicted CVD in the general Japanese population than did the other criteria sets. Furthermore, the increasing effects of MetS on the development of ischemic stroke and coronary heart disease were independent of hypertension and diabetes. High-risk strategies using this criteria set offer additional protection against CVD.

### Acknowledgments

We thank the staff of the Division of Health and Welfare of Hisayama for their cooperation in this study.

### Sources of Funding

This study was supported in part by a Grant-in-Aid for Scientific Research C (No. 20591063) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

### Disclosures

None.

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# Lack of Association Between Variations of *PDE4D* and Ischemic Stroke in the Japanese Population

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**Background and Purpose**—After the first genomewide association study of ischemic stroke identified *PDE4D* as a susceptible gene, many replication studies have been conducted. However, the validity of the association has remained controversial because of the heterogeneity of both genetic markers and phenotypes.

**Methods**—We investigated the association between variations of *PDE4D* and ischemic stroke by 3 methods: single-marker, haplotype, and tag-single nucleotide polymorphism (SNP) analyses. In the single-marker analysis, we evaluated the association using 2 large case-control samples (1112 cases and 1112 control subjects in a sample obtained from Kyushu, Japan, and 1711 cases and 1786 control subjects in BioBank Japan) and a prospective cohort with 14 years of follow-up. These samples were analyzed both separately and pooled. Haplotype and tag-SNP analyses were performed using the 2 case-control samples together.

**Results**—In single-marker association tests, we found no significant association in the same direction among the 6 SNP reported in the initial study and ischemic stroke subtypes. Haplotype analysis revealed no significant association between the region around the 5'-end of the gene and combined atherothrombotic and cardioembolic infarction. Rs7730070, a SNP located around the 3'-end of *PDE4D*, showed the lowest nominal probability value by tag-SNP analysis but was not significant after adjustment for multiple testing (adjusted probability value = 0.36).

**Conclusions**—These results suggest that variations in *PDE4D* are not associated with ischemic stroke risk in the Japanese population. (*Stroke*. 2009;40:1245-1251.)

**Key Words:** cerebral infarct ■ genetics ■ *PDE4D*

Stroke is one of the most common causes of death and long-term disability around the world. Ischemic stroke is the most common form of stroke and is further subdivided into lacunar, atherothrombotic (ATI), and cardioembolic infarction (CEI). As for genetic contributions to the pathogenesis of ischemic stroke, twin and family studies<sup>1,2</sup> suggested that stroke risk was mediated by both environmental and genetic factors. The first genomewide association study of ischemic stroke reported the phosphodiesterase 4D gene (*PDE4D*) as a susceptible gene using 864 cases and 908 control subjects in an Icelandic population.<sup>3</sup> This study showed that the microsatellite marker AC008818-1 and 6 single nucleotide polymorphisms (SNPs) located in the 5'-end of the gene (SNP41, SNP45, SNP56, SNP87, SNP89, and SNP83) were significantly associated with ATI or with the combined ATI and CEI phenotype. Haplotype blocks B and

C, which covered 260 kb around the 5'-end of the gene, were also associated, and the combination of the G allele of SNP45, the 0 allele of AC008818-1, and a common haplotype in block C led to the classification of individuals into at-risk, wild-type, and protective groups. Although the authors of the study showed that the affected individuals with the G0 haplotype had lower expression levels of some *PDE4D* isoforms, they could not find causative SNPs or haplotypes. Moreover, the biological role of *PDE4D* in ischemic stroke or the underlying atherosclerosis remained uncertain.

To our knowledge, 15 replication studies have been published on the association between SNPs in *PDE4D* and ischemic stroke.<sup>4-18</sup> However, the results are still controversial. Of the 4 studies that examined associations between the 2 markers (AC008818-1 and SNP45) and combined ATI and

Received June 1, 2008; final revision received September 8, 2008; accepted October 22, 2008.

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*Stroke* is available at <http://stroke.ahajournals.org>

DOI: 10.1161/STROKEAHA.108.527408

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CEI, none replicated the original findings.<sup>4–7</sup> Among the 14 studies that examined at least SNP45,<sup>4–17</sup> only one found a nominal association with combined ATI and CEI.<sup>6</sup> Other groups reported significant associations between different phenotypes and different SNPs in the 1.5-Mb region of the gene.<sup>6–13,19</sup> There are thought to be several reasons for these inconsistencies among the results. The sample sizes in most studies were too small and had insufficient power to detect associations.<sup>20</sup> Sampling biases of cases and controls may have distorted true associations. Several positive findings in different SNPs might reflect associations among hidden causative variants linked to the SNPs or to the G0 haplotype. The association between variants in *PDE4D* and ischemic stroke risk might differ among ethnic groups.

According to the recent published criteria, replication studies should examine the same SNP or a SNP in perfect or very high linkage disequilibrium with the prior SNP on the same or a very similar phenotype. They also should show similar magnitude of effect and significance in the same direction.<sup>21</sup> Therefore, we performed single-marker association tests between the 6 SNPs and the same subtypes of ischemic stroke as in the initial study and used a sufficient sample size. We also performed haplotype analyses in blocks B and C using tag-SNPs selected from the same regions. To examine the possibility of hidden causative SNPs, we additionally genotyped 190 tag-SNPs that covered a 2.2-Mb region, including *PDE4D*, and performed association analyses.

## Materials and Methods

### Study Populations

We used 2 independent Japanese case-control samples and a prospective cohort for this study. One is a Kyushu sample consisting of 1112 cases of ischemic stroke and 1112 age- and sex-matched control subjects. Details on this population were described previously.<sup>22</sup> Briefly, patients with ischemic stroke were recruited from 7 medical centers in and around Fukuoka City, Japan, in 2004. These included 491 cases of lacunar infarction, 369 of ATI, 136 of CEI, and 116 of undetermined subtype. Age (within 5 years) and sex-matched control subjects were selected from the 3328 participants of the Hisayama screening survey between 2002 and 2003. All case subjects were diagnosed by stroke neurologists on the basis of detailed clinical features and ancillary laboratory examinations such as brain imaging. The subtypes of ischemic stroke were determined on the basis of the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke (NINDS-III).<sup>23</sup>

Another case-control sample was selected from the BioBank Japan project.<sup>24</sup> This project was started in 2003 to collect a total of 300 000 cases who have at least one of 47 diseases by a collaborative network of 66 hospitals located throughout Japan. The registration of cases was based on diagnoses made by physicians at the affiliated hospitals. From June 2003 to March 2006, 7974 cases with ischemic stroke were registered. We selected 1711 cases diagnosed with ischemic stroke subtypes by brain imaging, the same as with the Kyushu sample. The subtypes included 1143 with lacunar infarction, 355 with ATI, and 213 with CEI. Control subjects were randomly selected from the subjects who were registered with BioBank Japan for other diseases.

For the prospective cohort study, we used a cohort population of the Hisayama study established in 1988.<sup>25</sup> In this cohort, 2634 Hisayama residents aged  $\geq 40$  years and who had no history of stroke

or coronary heart disease were enrolled in 1988 and continuously followed up for 14 years until the occurrence of cardiovascular disease or death. Among them, 1656 subjects participated in the examination between 2002 and 2003 and were used in the present study. During the 14-year follow-up, 67 events of first-ever ischemic stroke were observed.

Written informed consent was obtained from all study subjects. The study was approved by the ethics committees of the Graduate School of Medical Sciences at Kyushu University and the Institute of Physical and Chemical Research.

Clinical characteristics of 2 case-control samples are shown in Supplemental Table I, available online at <http://stroke.ahajournals.org>. In both samples, hypertension was defined as systolic blood pressure of  $\geq 140$  mm Hg or diastolic blood pressure of  $\geq 90$  mm Hg or current treatment with hypertensive medication.

### SNP Selection and Genotyping

For the association study, we selected 6 SNPs that were significantly associated with ischemic stroke in the initial study: SNP41 (rs12153798), SNP45 (rs12188950), SNP56 (rs702553), SNP83 (rs966221), SNP87 (rs2910829), and SNP89 (rs1396476). In the haplotype analysis, we selected 16 additional tag-SNPs from the regions of blocks B and C defined in the initial study. For tag-SNP analysis, we selected 190 tag-SNPs from the 2.2-Mb region, including *PDE4D*. Tag-SNPs were selected from the Hapmap JPT data by the pairwise tagging method with the following criteria:  $r^2 > 0.8$ , minor allele frequency  $> 5\%$ , and call rate  $> 75\%$ .

Genomic DNA was extracted from peripheral blood leukocytes by a standard method. We genotyped SNPs using the multiplex polymerase chain reaction-based Invader assay<sup>26</sup> (Third Wave Technologies) or TaqMan assays (Applied Biosystems) in a blind fashion to the clinical information of study samples. All genotypes were called by visual inspection, and we determined genotype success as  $< 10$  undetermined samples in a 384-well plate. When we failed to genotype more than one 384-well plate in a total of 16 plates, we excluded the SNP from further analyses. To validate the genotyping data, we genotyped 10 SNPs in 48 subjects using direct sequencing, and the concordance rate was 99.6%.

### Statistical Analysis

We examined the association both by each population and by meta-analysis. We assessed case-control association analysis and Hardy-Weinberg equilibrium by  $\chi^2$  test or Fisher exact test, as appropriate. In the association analysis, we mainly used an additive model and also referenced dominant and recessive models. For an easy understanding of the risk direction, we calculated the OR and 95% CI of each SNP according to the risk allele in the initial study. In a meta-analysis of the single-marker association test, pooled estimates of the ORs for 2 case-control studies and one prospective study were obtained using a fixed-effect model. Heterogeneities across the population were estimated formally using Cochran's Q test and the  $I^2$  statistic. Haplotype analysis was performed using Haploview version 4.0 (Broad Institute). For the adjustment for multiple testing, we performed a random permutation test with 10 000 replications. Linkage disequilibrium was calculated as  $D'$ , and haplotype blocks were defined by Gabriel's criteria.<sup>27</sup>

## Results

### Single-Marker Association Test

We initially performed single-marker association tests between the 6 SNPs reported in the initial study and the same ischemic stroke subtypes (Table 1). SNP45 and SNP41, which showed the most significant association in

Table 1. Association Between SNPs Reported in the Initial Study and the Subtypes of Ischemic Stroke Among Japanese

Ischemic Stroke Subtype	Allele		Sample	Case		Control		P Value	OR (95% CI)	Meta-Analysis	
	SNP	1 2		AF	11/12/22	AF	11/12/22			P Value	OR (95% CI)
ATI	SNP83	C T	Kyushu	0.13	4/84/279	0.14	7/91/269	0.31	0.86 (0.64–1.16)	0.14	0.87 (0.73–1.05)
			BioBank	0.13	7/79/269	0.15	42/436/1308	0.32	0.88 (0.70–1.12)		
			Prospective	0.12	0/4/13	0.14	36/383/1157	0.66	0.79 (0.28–2.25)		
Combined ATI and CEI	SNP41	T C	Kyushu	1.00	502/0/0	1.00	501/0/0			0.21	0.91 (0.78–1.06)
			BioBank	1.00	568/0/0	1.00	1779/0/0				
			Prospective	1.00	24/0/0	1.00	1573/0/0				
	SNP45	C T	Kyushu	1.00	502/0/0	1.00	501/0/0			0.21	0.91 (0.78–1.06)
			BioBank	1.00	568/0/0	1.00	1779/0/0				
			Prospective	1.00	24/0/0	1.00	1573/0/0				
	SNP56	A T	Kyushu	0.58	163/252/81	0.54	146/246/104	0.07	1.18 (0.99–1.41)	0.11	1.09 (0.98–1.21)
			BioBank	0.56	169/290/102	0.56	554/860/352	0.88	1.01 (0.88–1.16)		
			Prospective	0.73	14/7/3	0.55	485/766/315	0.02	2.17 (1.14–4.11)		
SNP87	T C	Kyushu	0.15	10/126/364	0.15	2/144/352	0.87	0.98 (0.76–1.25)	0.21	0.91 (0.78–1.06)	
		BioBank	0.11	7/116/445	0.13	32/412/1340	0.10	0.84 (0.68–1.03)			
		Prospective	0.17	1/6/17	0.14	23/394/1148	0.60	1.22 (0.57–2.63)			
SNP89	T G	Kyushu	0.95	450/52/0	0.97	470/33/0	0.03	0.62 (0.40–0.97)	0.27	0.87 (0.67–1.12)	
		BioBank	0.95	516/50/2	0.95	1619/153/8	0.99	1.00 (0.73–1.37)			
		Prospective	0.98	23/1/0	0.95	1430/143/3	0.39	2.33 (0.32–17.0)			

Allele 1 indicates the risk allele in the initial study; AF, allele frequency of allele 1; Meta-analysis was performed using a fixed-effect model.

the initial study, were monomorphic, and all individuals were homozygotes of the risk alleles in our population. In all samples, SNP83 showed no significant association with ATI. For the combined ATI and CEI subtypes, we found SNP56 to be significantly associated in the prospective cohort ( $P=0.02$ ; OR, 2.17; 95% CI, 1.14 to 4.11), but it was not associated in the 2 case–control samples. In the

meta-analysis, we could not find a significant association between SNP56 and the combined ATI and CEI phenotypes. SNP89 showed a significant association in the Kyushu sample, but its risk was in the opposite direction of the effect ( $P=0.03$ ; OR, 0.62; 95% CI, 0.40 to 0.97). SNP89 was not significantly associated in the BioBank Japan sample and the prospective cohort, and we found no

Table 2. Association Between SNPs Reported in the Initial Study and Subtypes of Ischemic Stroke Among Combined Samples After Stratification by Hypertension

Ischemic Stroke Subtype	SNP	RA	Hypertension				Without Hypertension			
			Frequency, %		P Value	OR (95% CI)	Frequency, %		P Value	OR (95% CI)
			Case (n=572)	Control (n=942)			Case (n=130)	Control (n=842)		
ATI	SNP83	C	12.9	13.8	0.50	0.93 (0.75–1.15)	11.9	15.6	0.13	0.73 (0.49–1.09)

Ischemic Stroke Subtype	SNP	RA	Hypertension				Without Hypertension			
			Frequency, %		P Value	OR (95% CI)	Frequency, %		P Value	OR (95% CI)
			Case (n=822)	Control (n=1017)			Case (n=219)	Control (n=903)		
Combined ATI and CEI	SNP41	T	100	100			100	100		
	SNP45	C	100	100			100	100		
	SNP56	A	56.5	55.1	0.38	1.06 (0.93–1.21)	57.4	55.4	0.45	1.08 (0.88–1.34)
	SNP87	T	12.7	13.6	0.45	0.93 (0.77–1.13)	13.1	14.0	0.62	0.93 (0.68–1.26)
	SNP89	T	95.0	95.8	0.23	0.82 (0.60–1.12)	94.7	95.0	0.83	0.95 (0.59–1.52)

RA indicates risk allele in the initial study; Frequency, risk allele frequency; Due to the lack of hypertension status data, 22 ATI cases, 10 CEI cases, and 371 control subjects were excluded in the stratified analysis.

**Table 3. Haplotype Analysis of SNPs Selected From the Region of Blocks B and C Among Combined Samples**

Haplotype in Block B													
rs4502776	rs13172481	rs6869495	rs1423246	rs1345782	rs6860887	rs10514896	SNP56	rs27222	rs7712662	rs1423473	SNP45	rs153031	SNP41
A	G	A	A	C	C	A	A	C	T	C	C	A	T
G	C	G	G	C	T	G	T	T	T	C	C	G	T
A	G	A	A	C	C	A	A	T	C	T	C	G	T
A	G	A	G	A	T	A	T	T	C	T	C	G	T
G	C	A	G	C	T	G	T	T	T	C	C	G	T
A	G	A	A	C	T	G	T	T	T	C	C	G	T
G	C	A	G	A	T	A	T	T	C	T	C	G	T
G	C	A	A	C	C	A	A	C	T	C	C	A	T
G	C	A	G	C	T	G	T	C	T	C	C	A	T
A	G	A	G	C	C	A	A	C	T	C	C	A	T
A	G	A	A	C	C	G	T	T	T	C	C	G	T

Haplotypes with frequency >2% are shown.

significant association with SNP89 in the meta-analysis. SNP87 was not associated with the combined ATI and CEI phenotypes in any of the samples. We also examined the associations of these SNPs with ischemic stroke or other subtypes in the 2 case-control samples (Supplemental Table II, available online at <http://stroke.ahajournals.org>). SNP56 showed nominal association with ATI in the Kyushu sample ( $P=0.02$ ; OR, 1.27; 95% CI, 1.03 to 1.57) but was not associated in the BioBank Japan sample. The meta-analysis showed no significant association between ATI and SNP56. No other SNPs showed a significant association with any phenotype in the same direction as the initial study.

#### Stratified Analysis by Hypertension Status

Some replication studies showed significant associations between the SNPs in *PDE4D* and ischemic stroke in subjects without hypertension.<sup>11,17</sup> Thus, we evaluated the association between the 6 SNPs and the subtypes of ischemic stroke among the combined samples stratified by hypertension status (Table 2). However, none of the SNPs were associated with ATI or the combined ATI and CEI phenotypes even in the subjects without hypertension.

#### Haplotype Analysis

Because SNP45 and SNP41, which are key SNPs for haplotype construction in block B, were monomorphic in our population, we constructed haplotypes using SNP56 and 16 additional tag SNPs selected from the regions of blocks B and C (Table 3). In block B, none of the haplotypes were significantly associated with the combined ATI and CEI phenotypes. In block C, the most common haplotype, G-C-C-A-G, showed the lowest probability value, but the association was not significant after adjustment for multiple testing (adjusted  $P=0.33$ ). There was no significant haplotype in the combined region of blocks B and C (data not shown).

#### Tag-SNP Analysis

To determine the possibility of a hidden causative SNP, we attempted to examine the associations between tag-SNPs

in *PDE4D* and ischemic stroke. We selected 190 additional tag-SNPs from the 2.2-Mb region that included *PDE4D* and genotyped in combined samples of 2823 cases and 2898 control subjects. Because 14 SNPs did not pass our criteria, we finally analyzed 198 SNPs (the 6 reported in the initial study and 192 tag-SNPs). The genomic structure, case-control results, and linkage disequilibrium map of the 2.2-Mb region are shown in the Figure. Although the initial study showed a strong association around the region of blocks A to C, none of the SNPs in this region showed any association. The rs7730070 SNP, located around the 3'-end of *PDE4D*, showed the lowest probability value (OR, 1.21; 95% CI, 1.06 to 1.37;  $P=0.0037$ ). However, this SNP was not linked to the 5'-end of the gene that was the causative region in the initial study (Figure, C). Moreover, this association was not significant after adjustment for multiple testing (adjusted  $P=0.36$ ).

#### Discussion

We examined the association between variations of *PDE4D* and ischemic stroke using 2 independent large case-control samples and a population-based cohort. Using these samples, we tried to replicate the previous reports in 3 ways: a single-marker association test, haplotype analysis in blocks B and C, and tag-SNP analysis, which covered the entire *PDE4D* gene region. Using 2 case-control samples consisting of 2823 cases and 2898 control subjects and a prospective cohort consisting of 1656 subjects, we found no significant association between the same SNPs and the same ischemic stroke subtypes in the single-marker tests. Similarly, no haplotypes in blocks B and C were found to be associated with the combined ATI and CEI phenotypes. Tag-SNP analysis could not find the hidden causative SNP in *PDE4D*. From these results, we suggest that the common variants of *PDE4D* did not confer risk for ischemic stroke, at least in the Japanese population.

Among the replication studies that examined variations of *PDE4D* and ischemic stroke, the most probable reason for the inconsistent findings is that the small sample sizes