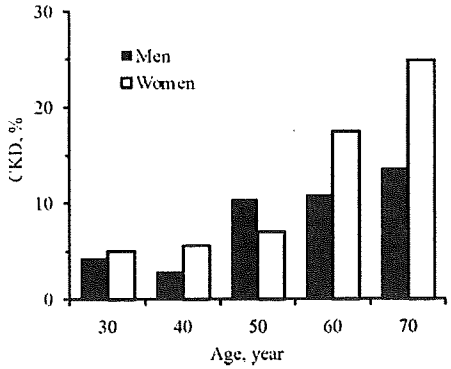


**Figure 1.** Frequencies of CKD according to sex and age.

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 GFR ≥90 mL/min/1.73m$^2$ the multivariable HRs (95% confidence intervals; CIs) for CVD incidence were 1.75 (1.22 to 2.50) in GFR=50 to 59 mL/min/1.73m$^2$ and 2.48 (1.56 to 3.94) in <50 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 GFR=50 to 59 mL/min/1.73m$^2$ and 2.19 (1.18 to 4.06) in the GFR <50 mL/min/1.73m$^2$ compared with subjects for GFR ≥90 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 GFR=50 to 59 mL/min/1.73m$^2$ and 2.52 (0.72 to 8.80) in the GFR <50 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**

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| Variables | 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$^2$ | 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 ≥5.7 mmol/L (220 mg/dl), diabetes; antihyperglycemic drug use or fasting blood sugar ≥7.0 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**

| | Glomerular Filtration Rate, ml/min/1.73m$^2$ | | | | |
|---|---|---|---|---|---|
| Variables | ≥90 | 60 to 89 | 50 to 59 | <50 | *P* for Trend |
| Men and Women | | | | | |
| 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 |
| Men | | | | | |
| 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 |
| Women | | | | | |
| 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.[6] 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.[5] In contrast, NHANES I did not provide relationships between mortality and moderately higher serum creatinine levels.[4] The Framingham Heart Study and Offspring cohorts have shown no significant association between the presence of kidney disease and CVD incidence.[3]

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.[8] The Ohasama study indicated that decreased kidney function increased the risk of first symptomatic stroke events.[19] 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[7] but did not examine the risk of GFR 50 to 59 mL/min/1.73m$^2$ The NIPPON DATA 90 indicated that CKD was an independent risk factor for cardiovascular death in a community-dwelling Japanese population.[20] 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**

| | Glomerular Filtration Rate, ml/min/1.73m$^2$ | | | | |
|---|---|---|---|---|---|
| Variables | ≥90 | 60 to 89 | 50 to 59 | <50 | *P* for Trend |
| Person-years | 28 258 | 28 690 | 4528 | 1446 | |
| All strokes | | | | | |
| 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 |
| Cerebral infarction | | | | | |
| 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 |
| Myocardial infarction | | | | | |
| 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).

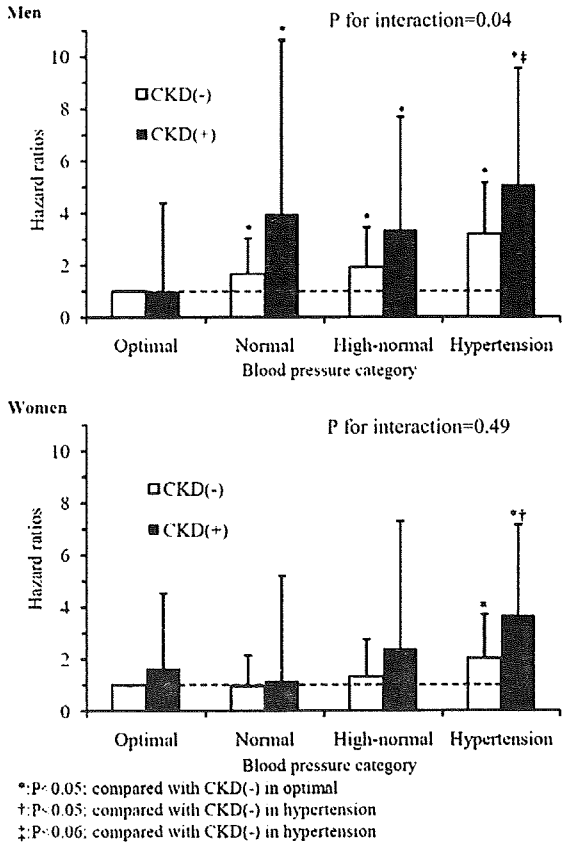


**Figure 2.** The combination of CKD and BP categories on multivariable hazard ratios for CVD. Data for men and women are presented separately. Multivariable analyses are adjusted age in 5-year increments as stratified variables and other potential confounding factors of hypercholesterolemia, diabetes, 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.[10] 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.[7,8,19]

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.[9,21] 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.[15]

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

Subjects with GFR levels of 50 to 59 mL/min/1.73m$^2$ 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$^2$) and less severe kidney disease (50 to 59 mL/min/1.73m$^2$), although an accelerated decline in GFR occurred for the subjects whose initial GFR <50 mL/min/1.73m$^2$.[25]

Hypertension is a strong risk factor for early decline in kidney function; hypertensive patients (BP ≥160/95 mm Hg) have a 5-fold greater decline in GFR (2.7 mL/min/1.73m$^2$/yr) compared with patients with BP <140/90 mm Hg.[26] 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.[27] 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,[28] 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.[15] 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

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## 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 ≥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.[1] 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.[2–4] 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.[5] 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)[6]; 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.[6] 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.[7] 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.[8] The aim of the present article is to derive a better definition from the existing MetS

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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.[9] A detailed description of this survey was published previously.[9] Briefly, a total of 2736 residents aged ≥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 ≥24 hours.[2] 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.[10] 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).[2] 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 ≥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 ≥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[5] 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 ≥130/85 mm Hg and/or current use of antihypertensive agents), elevated fasting plasma glucose (≥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 (≥1.68 mmol/L).

In the IDF criteria,[7] 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 ≥90 cm for men and ≥80 cm for women; blood pressure ≥130/85 mm Hg and/or current use of antihypertensive agents; fasting plasma glucose ≥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 ≥1.68 mmol/L.

Unlike the other criteria, the Japanese criteria[6] 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 (≥85 cm in men and ≥90 cm in women) plus any 2 of the following: (1) blood pressures of ≥130/85 mm Hg and/or current use of antihypertensive medicine; (2) fasting plasma glucose ≥6.1 mmol/L and/or current use of antidiabetic medication; and (3) triglycerides ≥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 ≥90 cm in men and ≥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.[11] 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$^2$ | 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 ≥130/85 mm Hg and/or current use of antihypertensive medicine; elevated fasting plasma glucose: fasting plasma glucose ≥6.1 mmol/L and/or current use of antidiabetic medication; reduced HDL cholesterol: HDL cholesterol <1.03 mmol/L; elevated triglycerides: triglycerides ≥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×(HR−1)/[prevalence×(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 ≥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 ≥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 Europids, 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 |
|---|---|---|---|---|---|
| Men | | | | | |
| 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 |
| Women | | | | | |
| 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.[12] 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 |
|---|---|---|---|---|---|---|---|
| Men | | | | | | | |
| NCEP | | | | | | | |
| 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 |
| IDF for Asians | | | | | | | |
| 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 |
| Japanese | | | | | | | |
| 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 |
| Modified NCEP | | | | | | | |
| 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 |
| Modified Japanese | | | | | | | |
| 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 |
| Women | | | | | | | |
| NCEP | | | | | | | |
| 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 |
| IDF for Asians | | | | | | | |
| 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 |
| Japanese | | | | | | | |
| 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 |
| Modified NCEP | | | | | | | |
| 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 |
| Modified Japanese | | | | | | | |
| 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.[13] Similar analyses reported that 90 cm in men and 84 cm in women was optimal in Japanese American[14] and 85 cm in men and 80 cm in women in Chinese populations.[15,16] 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 |
|---|---|---|---|---|---|
| Cardiovascular disease | | | | | |
| Central obesity (−) | 0 | 509 | 31 | 1 (referent) | |
| | 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) | 0.58 |
| 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) | <0.001 |
| Ischemic stroke | | | | | |
| Central obesity (−) | 0 | 509 | 19 | 1 (referent) | |
| | 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) | 0.76 |
| 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) | <0.001 |
| Coronary heart disease | | | | | |
| Central obesity (−) | 0 | 509 | 16 | 1 (referent) | |
| | 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) | 0.56 |
| 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) | 0.001 |

*Note:* Central obesity was defined by waist circumference of ≥90 cm in men and ≥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.[8] 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.[17] 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.[18] 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**

| | | Ischemic Stroke | | Coronary Heart Disease | |
|---|---|---|---|---|---|
| | Population at Risk, n | No. of Events | Multivariate-Adjusted HR (95% CI) | No. of Events | Multivariate-Adjusted HR (95% CI) |
| Diabetes | | | | | |
| 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)†‡ |
| Hypertension | | | | | |
| 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.[19,20] 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.[21] 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.[22] 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.[2,23] 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.[24] On the other hand, hyperglycemia in diabetes itself directly affects the progression of atherosclerosis through the increase in nonenzymatic glycation of proteins and lipids,[25] the production of reactive oxygen species,[26] and the activation of protein kinase $C^{27}$ isoform and the hexosamine biosynthetic pathway.[28] 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.

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## 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[1,2] 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.[3] 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.[4–18] 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

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CEI, none replicated the original findings.[4–7] Among the 14 studies that examined at least SNP45,[4–17] only one found a nominal association with combined ATI and CEI.[6] Other groups reported significant associations between different phenotypes and different SNPs in the 1.5-Mb region of the gene.[6–13,19] 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.[20] 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.[21] 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.[22] 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).[23]

Another case–control sample was selected from the BioBank Japan project.[24] 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.[25] In this cohort, 2634 Hisayama residents aged ≥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 ≥140 mm Hg or diastolic blood pressure of ≥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[26] (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.[27]

## 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 | SNP | Allele 1 | Allele 2 | Sample | Case AF | Case 11/12/22 | Control AF | Control 11/12/22 | *P* Value | OR (95% CI) | Meta-Analysis *P* Value | Meta-Analysis 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) | | |
| | | | | BioBank | 0.13 | 7/79/269 | 0.15 | 42/436/1308 | 0.32 | 0.88 (0.70–1.12) | 0.14 | 0.87 (0.73–1.05) |
| | | | | 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 | | | | |
| | | | | 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 | | | | |
| | | | | 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) | | |
| | | | | BioBank | 0.56 | 169/290/102 | 0.56 | 554/860/352 | 0.88 | 1.01 (0.88–1.16) | 0.11 | 1.09 (0.98–1.21) |
| | | | | 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) | | |
| | | | | BioBank | 0.11 | 7/116/445 | 0.13 | 32/412/1340 | 0.10 | 0.84 (0.68–1.03) | 0.21 | 0.91 (0.78–1.06) |
| | | | | 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) | | |
| | | | | BioBank | 0.95 | 516/50/2 | 0.95 | 1619/153/8 | 0.99 | 1.00 (0.73–1.37) | 0.27 | 0.87 (0.67–1.12) |
| | | | | 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**

| | | | Hypertension | | | | Without Hypertension | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Frequency, % | | | | Frequency, % | | | |
| Ischemic Stroke Subtype | SNP | RA | Case (n=572) | Control (n=942) | *P* Value | OR (95% CI) | Case (n=130) | Control (n=842) | *P* Value | OR (95% CI) |
| 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) |
| | | | Hypertension | | | | Without Hypertension | | | |
| | | | Frequency, % | | | | Frequency, % | | | |
| Ischemic Stroke Subtype | SNP | RA | Case (n=822) | Control (n=1017) | *P* Value | OR (95% CI) | Case (n=219) | Control (n=903) | *P* Value | OR (95% CI) |
| 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.[11,17] 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

**Table 3. Continued**

| Frequency, % | | | Haplotype in Block C | | | | | Frequency, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Case | Control | *P* Value | rs35387 | rs40512 | rs26954 | rs26950 | rs26948 | Case | Control | *P* Value |
| 41.5 | 40.9 | 0.63 | G | C | C | A | G | 34.4 | 31.7 | 0.03 |
| 14.5 | 16.0 | 0.11 | C | T | T | G | G | 26.0 | 27.3 | 0.26 |
| 8.1 | 7.2 | 0.17 | G | C | C | A | A | 23.1 | 24.6 | 0.16 |
| 4.7 | 5.7 | 0.10 | G | T | C | A | A | 7.5 | 7.0 | 0.51 |
| 4.1 | 4.7 | 0.33 | G | C | T | G | G | 2.7 | 2.5 | 0.56 |
| 4.1 | 3.6 | 0.41 | | | | | | | | |
| 3.1 | 3.1 | 0.87 | | | | | | | | |
| 3.5 | 2.7 | 0.10 | | | | | | | | |
| 2.9 | 2.2 | 0.08 | | | | | | | | |
| 1.9 | 2.6 | 0.07 | | | | | | | | |
| 2.1 | 2.1 | 0.82 | | | | | | | | |

missed true associations of modest effect. Assuming our sample size, the allele frequencies of the SNPs in our control subjects, and the relative risks of the SNPs in the initial study, the power to detect associations at a significance level of 0.05 would be greater than 99% for SNP83 and SNP56, 98.3% for SNP87, and 69.7% for SNP89 in the case–control samples. In contrast, the statistical power of the prospective cohort was <30% for the 6 SNPs. However, a meta-analysis of these samples should increase the statistical power to detect the association. Therefore, if a true association exists, our study could detect the association between SNPs or haplotypes in *PDE4D* and ischemic stroke with high probability. A recent meta-analysis of 5216 cases and 6615 control subjects also showed that allele 0 of AC008818 and haplotype G0 carriers were associated with increased risk of ischemic stroke, but these associations become nonsignificant after exclusion of the initial study.[28] These results indicate that the effect size of *PDE4D* variants on ischemic stroke, if it exists, may be small.

Because the initial study could not determine a causative SNP or haplotype in *PDE4D*, many replication studies have reported positive associations between different SNPs in *PDE4D* and various ischemic stroke subtypes.[19] This indicates the possibility that hidden causative SNPs for ischemic stroke might exist in *PDE4D*. We analyzed a total of 198 tag-SNPs that covered the 2.2-Mb region, including *PDE4D*, but none of the SNPs were significant after adjustment for multiple testing. Because we selected tag-SNPs according to strict criteria, this analysis was able to capture the most common SNPs in *PDE4D*. Therefore, the previous positive findings of different SNPs may be attributable to chance.

One possible reason for the lack of association between *PDE4D* and ischemic stroke in our study was the difference in the ethnic background. Indeed, SNP45 and SNP41, which showed the most significant association with the combined ATI and CEI phenotypes in an Icelandic population, were monomorphic and all of the Japanese populations studied were homozygotes of the risk alleles in both SNPs. If SNP45 or SNP41 or absolutely linked variations are causative, we cannot estimate the effects of these variations on ischemic stroke, because all causative variations are homozygotes of risk alleles in both cases and control subjects.

Several limitations of this study should be discussed. First, we did not genotype the microsatellite marker, AC008818-1, in this study. However, we genotyped 16 tag-SNPs selected

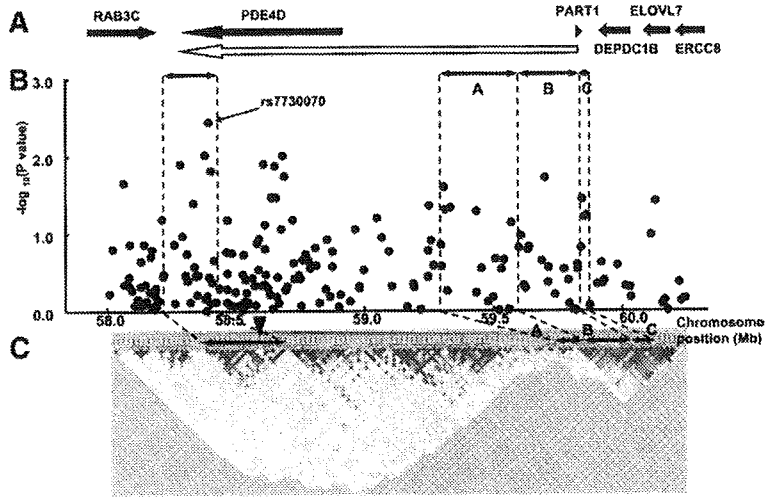


**Figure.** Genomic structure, case–control results, and linkage disequilibrium map of the 2.2-Mb region, including *PDE4D*. A, Genomic structure around *PDE4D*. The white arrow indicates *PDE4D* reported by the initial study. B, Case–control association results for ischemic stroke among Japanese. The log 10-transformed probability values calculated by the Cochran-Armitage trend test are plotted on the y axis. "A" indicates block A; "B," block B; "C," block C in the initial study. C, Pairwise linkage disequilibrium map between SNPs. The strength of the linkage disequilibrium increases from white to black. A black inverse triangle indicates the location of rs7730070 in the map.

from the regions of blocks B and C according to strict criteria. Therefore, we believe that the effect of AC008818-1 could be sufficiently covered by haplotype analysis using tag-SNPs. Second, we could use only 1656 of 2634 subjects in the prospective cohort. Subjects who developed ischemic stroke would have a higher mortality rate than subjects who did not, and this may have resulted in the lower participation rate in this study. There is a possibility that the results of the prospective cohort might have been distorted by a survivorship bias. Third, the criteria used for classifying ischemic stroke were different between the initial study and ours. For classification of ischemic stroke, the initial study used the Trial of Org 10172 in Acute Stroke Treatment research criteria[29] and we used NINDS-III.[23] However, these 2 classifications are similar to each other, and we diagnosed the subtypes of ischemic stroke by adequate laboratory examinations. We believe that there is no large difference in the phenotype definition.

In conclusion, although we performed a replication study between the variations of *PDE4D* and ischemic stroke risk using 2 independent large case–control samples and a population-based prospective cohort, we failed to replicate the associations. We suggest that variations of *PDE4D* do not confer risk for ischemic stroke in the Japanese population.

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## Disclosures

None.

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# Impact of Metabolic Syndrome Compared With Impaired Fasting Glucose on the Development of Type 2 Diabetes in a General Japanese Population

## The Hisayama study

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**OBJECTIVE** — We examined whether metabolic syndrome predicts incident type 2 diabetes more effectively than impaired fasting glucose (IFG) in a general Japanese population.

**RESEARCH DESIGN AND METHODS** — A total of 1,935 nondiabetic subjects aged 40–79 years were followed-up prospectively for a mean of 11.8 years.

**RESULTS** — During the follow-up, 286 subjects developed type 2 diabetes. Compared with those without metabolic syndrome, the multivariate-adjusted hazard ratio (HR) for incident type 2 diabetes was significantly higher in subjects of both sexes with metabolic syndrome, even after adjustment for confounding factors, age, family history of diabetes, total cholesterol, alcohol intake, smoking habits, and regular exercise (men: HR 2.58 [95% CI 1.85–3.59]; women: 3.69 [2.58–5.27]). The multivariate-adjusted HR of metabolic syndrome for type 2 diabetes was slightly lower in men and similar in women compared with that of IFG. The multivariate-adjusted HR for type 2 diabetes rose progressively as the number of metabolic syndrome components increased in both subjects with and without IFG. In stratified analysis, the multivariate-adjusted risk of type 2 diabetes was significantly higher in subjects with metabolic syndrome alone (2.37 [1.45–3.88]) or IFG alone (3.49 [2.57–4.74]) and markedly increased in subjects with both metabolic syndrome and IFG (6.76 [4.75–9.61]) than in subjects with neither metabolic syndrome nor IFG. Furthermore, the multivariate-adjusted risk for type 2 diabetes was also significantly higher in subjects with both metabolic syndrome and IFG than in those with either one alone (both $P < 0.001$).

**CONCLUSIONS** — Our findings suggest that metabolic syndrome significantly increases the risk of incident type 2 diabetes, independent of IFG, and is therefore a valuable tool to identify individuals at high risk of type 2 diabetes.

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Metabolic syndrome consists of a clustering of cardiovascular risk factors, such as central obesity, elevated blood pressure, glucose intolerance, and dyslipidemia, and individuals with this condition have an elevated risk of developing cardiovascular diseases (1–5) and type 2 diabetes in different ethnic populations (1–4,6–11). Thus, the concept of metabolic syndrome could be used to reduce the incidence of these diseases worldwide. However, a number of experts in the field of diabetes have questioned whether the idea of metabolic syndrome is useful and valuable (12–14). Because all of the criteria sets for metabolic syndrome have included the component of impaired fasting glucose (IFG), which is a powerful predictor of type 2 diabetes, detractors have questioned whether the more complex definition of metabolic syndrome is better than a simple measurement of fasting plasma glucose (FPG). However, reported findings concerning this issue are controversial: a cohort study has shown that the ability of metabolic syndrome to predict type 2 diabetes was superior to that of IFG alone (3), whereas in other studies, the value of metabolic syndrome was comparable or inferior to that of IFG alone (2,6,7). Furthermore, most of these epidemiological studies were performed in Western populations, and this subject has not been assessed sufficiently in Asian populations.

The purpose of the present study was to investigate the association between metabolic syndrome and the development of type 2 diabetes in a prospective study of a defined Japanese population, taking into account comprehensive risk factors. In addition, we compared which of the two measures, metabolic syndrome or IFG, better predicted incident type 2 diabetes.

## RESEARCH DESIGN AND METHODS

### Study population and follow-up survey

A population-based prospective study of cardiovascular disease and its risk factors has been underway since 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. In 1988, a screening survey for the present study was performed in the town. A detailed description of this survey was published previously (15). In brief, of the total of 3,227 residents aged 40–79 years based on the town registry, 2,587 residents (participation rate,

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