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Risk of Smoking and Metabolic Syndrome for Incidence of Cardiovascular Disease

—— Comparison of Relative Contribution in Urban Japanese Population: The Suita Study ——

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Background: Risk factor clustering, the so-called metabolic syndrome (MetS), is an important risk factor for cardiovascular disease (CVD). Smoking is also an important CVD risk factor with still a high prevalence. However, few previous studies have compared the risk for CVD or the population-attributable fraction (PAF) of smoking, MetS, and both.

Methods and Results: The present study was an 11.9-year cohort study of 1,822 men and 2,089 women, aged 40–74 years, selected randomly from an urban general population in Japan. MetS was defined according to the National Cholesterol Education Program on Adult Treatment Panel III (NCEP-ATPIII) guideline modified by the Asian criteria for waist circumference. The prevalence of smoking was 49.5% in men and 11.1% in women, and that of MetS was 19.8% and 23.5%, respectively. In men, the multivariate-adjusted hazard ratio for CVD incidence, compared with non-smoking participants without MetS, was 2.07 (1.26–3.40) in those who smoked, 2.09 (1.08–4.04) in those with MetS, and 3.56 (1.89–6.72) in those with both. In men the PAF for CVD incidence was 21.8% because of smoking, 7.5% because of MetS, and 11.9% because of both.

Conclusions: Although countermeasures for MetS are important, smoking should continue to be considered an important public health problem and antismoking campaigns should be promoted, especially for men, to prevent CVD. (*Circ J* 2009; **73:** 2258–2263)

Key Words: Cohort; Hazard ratio; Metabolic syndrome; Smoking

Risk factor clustering, the so-called metabolic syndrome (MetS), is an important risk factor for cardiovascular disease (CVD), and previous studies have shown the risk of MetS for CVD in the Japanese population.[1–4] In addition, health guidance for people aged 40–74 years who fulfill the Japanese MetS criteria[5] began in April 2008 and countermeasures for MetS has become a national project.[6]

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However, cigarette smoking is a widely accepted risk factor for CVD,[7–9] and the prevalence of smoking is still high in Japan compared with Western developed countries.[10] Accordingly, in Japan, countermeasures for MetS are being applied with a still high prevalence of smoking, which might be different from the situation in Western developed countries with a lower prevalence of smoking.[10] To improve this situation, it is important to examine and show the combined risk of MetS and smoking, and compare the impact of each risk factor and both for CVD from the viewpoint of the impact not only on the individual but also on the population using indicators such as population-attributable fraction (PAF). In addition, such an assessment could be useful for motivating individuals with MetS, smoking, or both because both MetS and smoking are targets of lifestyle modification. However, few studies have compared the risk of smoking, MetS, and both for CVD.

Our a priori hypothesis was that the coexistence of smoking and MetS worsens the CVD risk, and that the PAF of smoking in Japanese men is larger than that of MetS because of their high prevalence of smoking. To examine this hypothesis, we performed a 11.9 year (mean length) cohort study in an urban general Japanese population to compare the effects of smoking, MetS and both on CVD risk.

Methods

Population

The Suita study,[2,11–14] a cohort study of CVD, was established in 1989 in Suita City, Osaka. In that study, 6,485 participants who were randomly selected from the municipal population registry participated in a baseline survey at the National Cardiovascular Center (NCVC) between

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Table 1. Baseline Characteristics of the Participants According to the Combination of Smoking and MetS

	MetS (−)		MetS (+)	
	Non-smoker	Smoker	Non-smoker	Smoker
Men				
n	732	730	189	171
Age (years)	58.9±9.9	56.1±9.4	59.3±8.4	57.4±9.1
Waist (m)	0.82±0.07	0.81±0.07	0.89±0.07	0.89±0.07
BMI (kg/m^2)	22.7±2.7	22.1±2.5	24.9±2.5	24.8±2.5
Total cholesterol (mmol/L)	5.26±0.88	5.08±0.85	5.49±0.88	5.44±0.98
Non-HDL-cholesterol (mmol/L)	3.90±0.88	3.79±0.88	4.41±0.85	4.41±0.98
High blood pressure (%)	48.6	39.3	86.8	84.8
High triglycerides (%)	19.3	22.5	83.1	80.7
Low HDL-cholesterol (%)	13.5	19.6	63.0	69.6
High blood glucose (%)	9.6	10.0	47.1	42.1
Abdominal obesity (%)	13.9	7.5	56.1	59.1
Medication				
For hypertension (%)	32.7	33.7	36.8	39.3
For hypercholesterolemia (%)	1.0	0.5	4.8	4.1
For hypertriglyceridemia (%)	0.5	0.4	2.1	1.2
For diabetes (%)	14.9	12.9	26.9	14.3
Smoking				
Never (%)	37.8	0.0	32.3	0.0
Ex (%)	62.2	0.0	67.7	0.0
Current (%)	0.0	100.0	0.0	100.0
Alcohol drinking				
Never (%)	20.9	19.6	20.6	22.8
Ex (%)	4.2	2.5	5.8	3.5
Current (%)	74.9	77.9	73.5	73.7
Women				
n	1,424	174	433	58
Age (years)	55.3±9.4	52.6±9.1	60.3±8.7	59.3±8.6
Waist (m)	0.77±0.09	0.75±0.09	0.88±0.09	0.87±0.09
BMI (kg/m^2)	21.8±2.8	21.4±3.0	24.8±3.3	24.7±3.2
Total cholesterol (mmol/L)	5.57±0.90	5.39±0.98	5.93±1.00	5.83±0.98
Non-HDL-cholesterol (mmol/L)	4.02±0.90	3.97±1.03	4.75±1.01	4.77±0.95
High blood pressure (%)	35.1	20.1	85.2	70.7
High triglycerides (%)	6.6	6.3	58.0	81.0
Low HDL-cholesterol (%)	18.3	34.5	82.0	87.9
High blood glucose (%)	4.3	1.7	30.5	24.1
Abdominal obesity (%)	30.1	27.6	86.6	79.3
Medication				
For hypertension (%)	33.7	17.4	43.6	44.4
For hypercholesterolemia (%)	1.6	0.0	6.5	3.4
For hypertriglyceridemia (%)	0.1	0.0	1.4	1.7
For diabetes (%)	16.7	0.0	17.5	30.0
Smoking				
Never (%)	97.1	0.0	94.2	0.0
Ex (%)	2.9	0.0	5.8	0.0
Current (%)	0.0	100.0	0.0	100.0
Alcohol drinking				
Never (%)	67.4	50.6	75.5	65.5
Ex (%)	1.0	5.7	1.6	0.0
Current (%)	31.6	43.7	22.9	34.5

Data are value±indicate standard deviation.
MetS = presence of 3 or more of the following: (1) abdominal obesity defined as a waist circumference ≥90 cm in men and ≥80 cm in women; (2) high blood pressure defined as average systolic/diastolic blood pressures of ≥130/85 mmHg and/or current medication for hypertension; (3) high triglycerides defined as serum level ≥1.68 mmol/L; (4) low HDL-cholesterol defined as serum level <1.03 mmol/L in men and <1.29 mmol/L in women; (5) high blood glucose defined as fasting blood glucose ≥6.10 mmol/L and/or current use of insulin or oral medication for diabetes.
MetS, metabolic syndrome; BMI, body mass index; HDL, high-density lipoprotein.

September 1989 and February 1994. Of the 4,285 participants who were aged 40–74 years at baseline, a total of 374 were excluded for the following reasons: past history of CVD (ischemic heart disease and stroke: n=127), non-fasting visit (n=155), and missing information at the time of the baseline survey or lost to follow-up (n=92). The data for the remaining 3,911 participants (1,822 men and 2,089 women) were then analyzed. Informed consent was given by all participants. The present cohort study was approved by the Institutional Review Board of the NCVC.

Baseline Examination

Well-trained nurses obtained information on smoking (never, ex-, or current smoker), alcohol drinking (never, ex-, or current drinker), and the medical history of each participant. If the participant answered yes to "current smoker", information was obtained for how many cigarettes per day were smoked.

Table 2. HRs and 95% CIs of Smoking for Incidence of CVD (Stroke+MI), Stroke, Ischemic Stroke, and MI

	Never-smoker	Ex-smoker	Current-smoker	
			≤20 cigarettes/day	>20 cigarettes/day
Men (n)	338	583	524	373
Person-years	4,147	6,837	5,965	4,343
CVD (stroke+MI)				
Cases (n)	11	29	40	16
Incidence (/1,000 person-years)	2.65	4.24	6.71	3.68
Multivariate-adjusted HR (95%CI)	1.00	1.34 (0.67–2.69)	2.65 (1.35–5.21)	2.31 (1.06–5.05)
Stroke				
Cases (n)	8	18	30	12
Incidence (/1,000 person-years)	1.93	2.63	5.03	2.76
Multivariate-adjusted HR (95%CI)	1.00	1.07 (0.46–2.48)	2.47 (1.12–5.45)	2.48 (1.00–6.20)
Ischemic stroke				
Cases (n)	4	16	24	8
Incidence (/1,000 person-years)	0.96	2.34	4.02	1.84
Multivariate-adjusted HR (95%CI)	1.00	1.94 (0.64–5.86)	4.06 (1.40–11.83)	3.37 (1.00–11.41)
MI				
Cases (n)	3	11	10	4
Incidence (/1,000 person-years)	0.72	1.61	1.68	0.92
Multivariate-adjusted HR (95%CI)	1.00	2.21 (0.61–8.00)	2.74 (0.80–10.90)	1.89 (0.41–8.70)
Women (n)	1,790	67	209	23
Person-years	21,881	727	2,363	240
CVD (stroke+MI)				
Cases (n)	45	0	10	1
Incidence (/1,000 person-years)	0.21	–	4.23	4.17
Multivariate-adjusted HR (95%CI)	1.00	–	2.70 (1.34–5.45)	2.80 (0.36–21.55)
Stroke				
Cases (n)	37	0	5	1
Incidence (/1,000 person-years)	1.69	–	2.12	4.17
Multivariate-adjusted HR (95%CI)	1.00	–	1.60 (0.62–4.16)	2.70 (0.34–21.68)
Ischemic stroke				
Cases (n)	19	0	4	1
Incidence (/1,000 person-years)	0.87	–	1.69	4.17
Multivariate-adjusted HR (95%CI)	1.00	–	3.00 (1.00–8.97)	7.15 (0.84–60.64)
MI				
Cases (n)	8	0	5	0
Incidence (/1,000 person-years)	0.37	–	2.12	–
Multivariate-adjusted HR (95%CI)	1.00	–	8.35 (2.64–26.48)	–

Multivariate-adjusted HR (95%CI): age, BMI, systolic blood pressure, blood glucose, non-HDL-cholesterol, glomerular filtration rate, and alcohol drinking were adjusted.
HRs, hazard ratios; CIs, confidence intervals; CVD, cardiovascular disease; MI, myocardial infarction. Other abbreviations see in Table 1.

Well-trained physicians measured blood pressure (BP) 3 times in the right arm using a standard mercury sphygmomanometer while the participant was seated after a 5-min rest. The average of the 2nd and 3rd measurements was used in the analyses. Height in stockings and weight in light clothing were measured. Trained public health nurses or technicians measured waist circumference at the umbilical level while the participant was standing.

Blood samples were collected at the NCVC after the participants had fasted for at least 12 h. The samples were centrifuged immediately, and a routine blood examination, which included serum total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), triglycerides and glucose levels, was then carried out. Non-HDLC was calculated by subtracting the HDL from the TC. Serum creatinine (Cre) was measured by the non-compensated kinetic Jaffe method. The glomerular filtration rate (GFR: $ml \cdot min^{-1} \cdot 1.73\,m^{-2}$) was calculated using the MDRD equation modified by the Japanese coefficient (0.881): $186 \times (\text{Cre (mg/dl)})^{-1.154} \times (\text{age (years)})^{-0.203} \times 0.881 \times (0.742 \text{ if female})$.[15,16]

Definition of MetS

In the present study, MetS was defined using the criteria recommended in the National Cholesterol Education Program on Adult Treatment Panel III guideline with a modification (modified NCEP-ATP III criteria).[17,18] Specifically, abdominal obesity was defined as a waist circumference ≥90 cm in men and ≥80 cm in women according to the International Obesity Task Force central obesity criteria for Asia.[17] High BP was defined as average systolic/diastolic BPs ≥130/85 mmHg and/or current medication for hypertension. High triglyceride was defined as a serum level ≥1.68 mmol/L. Low HDLC was defined as a serum level <1.03 mmol/L in men and <1.29 mmol/L in women. High blood glucose was defined as fasting blood glucose (FBG) ≥6.10 mmol/L and/or current use of insulin or oral medication for diabetes. MetS was defined as the presence of 3 or more of these components.

Follow-up and Endpoints

The method of follow-up has been described elsewhere.[2,11–14] Briefly, the participants were followed until December 31, 2005. The first step in the survey involved checking the health status of all participants by repeat visits to NCVC every 2 years and yearly questionnaires conducted by mail or telephone interview. The in-hospital medical records of the participants who were suspected of having had a myocardial infarction (MI) or stroke were reviewed

Table 3. Risk of Smoking and MetS for CVD (Stroke+MI)

	MetS (−)		MetS (+)	
	Non-smoker	Smoker	Non-smoker	Smoker
Men				
n	732	730	189	171
Person-years	8,721	8,506	2,263	1,835
CVD (stroke+MI) cases (n)	26	41	14	16
CVD incidence (/1,000 person-years)	2.98	4.82	6.19	8.72
Multivariate-adjusted HR (95%CI)†	Reference	2.03(1.24–3.33)	2.11(1.10–4.04)	3.39(1.81–6.33)
Multivariate-adjusted HR (95%CI)‡	Reference	2.07(1.26–3.40)	2.09(1.08–4.04)	3.56(1.89–6.72)
PAF		21.8	7.5	11.9
Women				
n	1,424	174	433	58
Person-years	17,684	2,027	4,925	577
CVD (stroke+MI) cases (n)	23	6	22	5
CVD incidence (/1,000 person-years)	1.30	2.96	4.47	8.67
Multivariate-adjusted HR (95%CI)†	Reference	2.64(1.07–6.51)	2.58(1.42–4.69)	5.40(2.04–14.25)
Multivariate-adjusted HR (95%CI)‡	Reference	2.67(1.07–6.65)	2.33(1.25–4.34)	4.84(1.81–12.97)
PAF		6.7	22.4	7.1

Multivariate-adjusted HR (95%CI): †adjusted for age.
Multivariate-adjusted HR (95%CI): ‡adjusted for age, alcohol drinking (never-, ex-, current-), glomerular filtration rate and non-HDL-cholesterol.
PAF, population attributable fraction. Other abbreviations see in Tables 1,2.

by registered hospital physicians or research physicians who were unaware of the baseline information.

The criteria for definite and probable MI were defined according to the criteria of the Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) project,[19] which requires evidence from an ECG, cardiac enzymes, and/or autopsy. Stroke was defined according to the National Survey of Stroke criteria,[20] which require rapid onset of a constellation of neurological deficits lasting at least 24 h or until death. Strokes were classified as ischemic stroke (thrombotic or embolic), intracerebral hemorrhage, subarachnoid hemorrhage, or undetermined type. A definite stroke was defined by autopsy or diagnostic imaging, such as computed tomography or magnetic resonance imaging. In the present study, cases of definite MI or stroke were used in the analysis.

Statistical Analysis

To compare baseline risk characteristics among the 4 groups classified by the combination of MetS and smoking status, analysis of variance was used for continuous variables, and the chi-squared test was used for dichotomous variables. In this analysis, ex-smoker and never-smoker were classified as non-smokers.

Sex-specific analyses were performed. First, the Cox proportional hazards model was used to estimate the hazard ratios (HR) of smoking status for the incidence of CVD (stroke+MI) and its subtypes. Smoking status was classified as never-, ex-, or current smoker (≤20 cigarettes/day and >20 cigarettes/day). In this analysis, age, body mass index (BMI), systolic BP, FBG, non-HDLC,[11] GFR, and alcohol drinking (never-, ex-, and current drinker) were included as confounding factors.

Second, the source population was divided into 4 groups according to the combination of smoking and the presence of MetS. In this analysis, ex-smoker and never-smoker were also classified as non-smokers. The 2 models were used for estimating the HRs of the combinations for CVD incidence. To adjust for the confounding factors, only age was included in model 1, and alcohol drinking (never, ex-, and current drinker), GFR and non-HDLC were also included in model 2. To express the impact of smoking on CVD incidence in the participants, the PAF (%) was estimated as $Pe \times (HR-1)/HR$, in which Pe is the proportion of incident cases in each category.[21]

All statistical analyses were performed using SPSS statistical software, version 15.0 J (SPSS, Tokyo, Japan). $P<0.05$ (2-tailed) was considered statistically significant.

Results

Baseline Characteristics

Among the participants, 901 of the 1,822 men and 232 of 2,089 women were current smokers (smoking rate: men, 49.5%; women, 11.1%). Similarly, 360 men and 491 women had MetS (prevalence: men, 19.8%; women, 23.5%). **Table 1** summarizes the baseline characteristics of the participants classified into 4 groups according to the combination of current smoking and MetS by sex. All variables, except for alcohol drinking in men, were significantly different among the 4 groups.

Risk of Smoking for CVD Incidence

In the present study, the mean follow-up period was 11.9 years, and 42 definite cases of MI and 111 of definite stroke occurred.

Table 2 shows the multivariate-adjusted HRs and 95% confidence intervals (CI) of smoking status for the incidence of CVD and its subtypes. In men, the HR of current smokers who were smoking ≤20 cigarettes/day compared with never smokers was 2.65 (95%CI 1.35–5.21) for CVD, 2.47 (95%CI 1.12–5.45) for stroke, 4.06 (95%CI 1.40–11.83) for ischemic stroke, and 2.74 (95%CI 0.80–10.90) for MI. Similarly in women, the HR was 2.70 (95%CI 1.34–5.45) for CVD, 1.60 (95%CI 0.62–4.16) for stroke, 3.00 (95%CI 1.00–8.97) for ischemic stroke, and 8.35 (95%CI 2.64–26.48) for MI. Among the participants who were smoking >20 cigarettes/day, the HRs for CVD incidence were similar to those who were smoking ≤20 cigarettes/day, although in both men and women most of them did not reach to statistical significance because of the small sample size.

Among the ex-smokers, the HR was 1.34 (95%CI 0.67–

2.69) for CVD incidence, 1.07 (95%CI 0.46–2.48) for stroke, 1.94 (95%CI 0.64–5.86) for ischemic stroke, and 2.21 (95%CI 0.61–8.00) for MI in men. In women, there was no case of CVD among ex-smokers.

Risk of Smoking and MetS for CVD Incidence

Table 3 shows the multivariate-adjusted HRs of the combination of smoking and MetS for CVD incidence.

In men, the multivariate-adjusted HRs were 2.07 (95%CI 1.26–3.40) for participants with smoking without MetS, 2.09 (95%CI 1.08–4.04) for those with MetS without smoking, and 3.56 (95%CI 1.89–6.72) for those with both, compared with those both smoking and MetS. In women, the multivariate-adjusted HRs were 2.67 (95%CI 1.07–6.65) for participants with smoking without MetS, 2.33 (95%CI 1.25–4.34) for those with MetS without smoking, and 4.84 (95%CI 1.81–12.97) for those with both, compared with those without both smoking and MetS. When we excluded the ex-smokers among women in this analysis, the HRs were almost similar to the results shown in **Table 3**. And these results were not substantially affected when TC instead of non-HDLC was included as a confounding factor in the Cox proportional hazard models.

In men the PAF for CVD incidence was 21.8% because of smoking, 7.5% because of MetS, and 11.9% because of both. In women, the respective PAFs were 6.7%, 22.4%, and 7.1%.

Discussion

To our knowledge, this is the first report of a comparison of the CVD risk of smoking, MetS, and both. The magnitude of the HR of smoking or MetS was almost equal. As expected, the risk for the participants with both was the highest. The PAF for CVD incidence among men with smoking alone was much higher than that among those with MetS alone. In women, the PAF among those with MetS was higher than that among those with smoking.

Furthermore, this is also the first report to show the risk of smoking for CVD in an urban area of Japan. In the present study, the prevalence of smoking was 49.5% in men and 11.1% in women. Compared with the data from the National Health and Nutrition Survey conducted in 1989 (men aged 40–69 years in 1989, 50.4–59.5%; women aged 40–69 years in 1988, 6.8–10.6%)[22] and several large collaborative cohort studies in Japan,[8,9,23,24] the prevalence of smoking in the present study was lower in men and higher in women, but is most consistent with the current Japanese prevalence of smoking (men: 39.9%; women: 10.0%). The present study might reflect the prevalence of smoking in urban Japanese communities around the 1990s. In addition, the high smoking prevalence in women and low prevalence in men in the present study is consistent with that in most of the Asia-Pacific region.

Our study showed that smoking is a prominent risk factor for CVD in an urban Japanese cohort, as shown in previous studies in Japanese rural populations.[9,23,24] Similarly, as previously reported,[1,25–27] MetS was a risk factor for CVD in our cohort.[2] The association between MetS and CVD has been reported in several Japanese cohort studies; however, the number of participants was fewer than in the present study,[1] or non-fasting blood samples and BMI were used instead of waist circumference for the analysis.[25] These points are another important strength of our study.

MetS has been reported as associated with high percent plaque volume and abnormal plaque quality in coronary arteries,[28] and chronic subclinical inflammation.[29] As for smoking, Howard et al reported that smoking is associated with progression of an index of atherosclerosis expressed as the intima–medial thickness of the carotid artery.[30] Antoniades et al also stated that smoking induces both functional and structural abnormalities in the vascular wall, by mechanisms involving endothelial dysfunction and impairment of vascular smooth muscle cells in the human arterial tree.[31] They also stated that smoking must be approached within the context of the overall lifestyle: smoking coexists with a pro-atherogenic metabolic profile.[31] The reason for the elevated CVD risk among the present participants with both MetS and smoking is unclear, but the concurrent effect on plaque formation by MetS and smoking, and the additional abnormality in function of vascular smooth muscle cells because of smoking, might be associated with the highest CVD risk among the participants with both risk factors in the present study. Individuals with both smoking and MetS are inevitably in the highest risk group for CVD and should be targeted for intervention.

We compared the HRs of these important CVD risk factors, and the HRs of smoking or MetS for CVD incidence were almost consistent. Accordingly, we calculated the PAF, which shows the impact on CVD incidence. As the result, the PAF of smoking was higher than that of MetS in men, and that of MetS was higher than that of smoking in women, a result that may reflect the higher smoking rate in men. Our study results offer a simple key to solving the problem of "which risk factor should we intervene on first for the population to improve their health outcome". Recently, the smoking rate has been decreasing in Japanese men; however, compared with the United States for example,[10] it remains still high. As well as countermeasures against MetS, we need to continue considering smoking as an important public health problem and promoting antismoking campaigns in Japan.

In Western developed countries such as the United States, evaluating the risk of MetS under a high prevalence of smoking is difficult because the prevalence of smoking is much lower[10] than in Japan. Although the data of the present study are limited to 1 city in Japan, it might offer evidence of the risk of MetS under a high prevalence of smoking.

There has been controversy about defining the optimal diagnostic criteria for MetS. We have already compared the predictive value between the Japanese criteria and the modified NCEP-ATPIII criteria.[2] The results suggested that the modified NCEP-ATPIII criteria are suitable for predicting CVD in the Japanese community setting, as well as in the Hisayama study.[1] Accordingly, in the present study MetS was defined using the modified NCEP-ATPIII criteria[17,18] Some investigators consider that MetS is an adipose tissue disease different from obesity. If it is an adipose tissue disease, it would be characterized by inflammation detected through high-sensitivity C-reactive protein (hs-CRP) and insulin resistance, reflecting histological changes in adipose tissue.[32] Thus, inflammation-related factors such as hs-CRP might be a candidate for 1 of the components of MetS.[33] Furthermore, according to the Japanese MetS criteria, the prevalence of MetS tends to be very low in women because obesity is a required component and the definition of obesity is waist circumference ≥90 cm. In addition, because some previous studies showed that the prevalence of non-obese individuals with several metabolic risk factors is high

and their CVD risk is also high, the simple exclusion of non-obese participants from the diagnosis of MetS may overlook their potential risk for CVD.[25–27] We might misclassify participants with a high risk for CVD if we adopt the Japanese MetS criteria.

Study Limitations

First, we could not assess the risk of smoking on the incidence of hemorrhagic stroke because of the small number of cases. Second, the measurement of single MetS components and the questionnaire for smoking in the baseline survey may have underestimated the relationship between these risk factors and CVD because of a regression dilution bias.

In conclusion, smoking is still an important risk factor for CVD in urban areas of Japan, and the combination of smoking and MetS worsens the risk for CVD. Lifestyle modification for not only MetS but also smoking continues to be important in populations with a high PAF for CVD because of a high prevalence of smoking.

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Disclosure

None.

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Genetic Polymorphisms of L-Type Calcium Channel α1C and α1D Subunit Genes are Associated With Sensitivity to the Antihypertensive Effects of L-Type Dihydropyridine Calcium-Channel Blockers

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Background: The response of blood pressure (BP) to L-type dihydropyridine calcium-channel blockers (dCCBs) differs among individuals.

Methods and Results: A pharmacogenomic analysis was undertaken in 161 patients with essential hypertension who were treated with dCCBs to study whether genetic polymorphisms of the calcium channel α1C and α1D subunit genes, *CACNA1C* and *CACNA1D*, are associated with the antihypertensive effects of dCCBs. Responders were defined as those in whom systolic BP (SBP) was lowered by more than 20 mmHg or diastolic BP (DBP) was lowered by more than 10 mmHg after treatment with dCCBs. Eleven sequence-proven polymorphisms of *CACNA1C* and 5 common polymorphisms of *CACNA1D* chosen from a public database were subjected to genotypic analysis. The comparison of polymorphism prevalence between responders and nonresponders showed significant differences in *CACNA1D* rs312481G>A and rs3774426C>T, and in *CACNA1C* 527974G>A. There were significant differences in SBP or DBP between alleles in these single nucleotide polymorphisms (SNPs). A much more significant reduction in BP was observed for the combined presence of these SNPs.

Conclusions: Three SNPs in *CACNA1D* or *CACNA1C* are genetic polymorphisms conferring sensitivity to the antihypertensive effects of L-type dCCBs in patients with hypertension. The BP reduction by L-type dCCBs might be predicted by evaluating these polymorphisms. (*Circ J* 2009; **73:** 732–740)

Key Words: Essential hypertension; Genetic polymorphism; L-type calcium channel gene; L-type dihydropyridine channel blockers

Calcium-channel blockers (CCBs), especially those of the L-type dihydropyridine (DHP) subclass, are widely used to treat hypertension because they are better able to lower blood pressure (BP) than are other types of antihypertensive agents[1] The DHP CCBs (dCCBs) complement the BP-lowering ability in both salt-sensitive and salt-resistant forms of hypertension (HT), and elderly patients generally respond well to dCCBs[2] Recently, a number of trials, including VALUE[3] ALLHAT[4] ASCOT[5] Syst-Eur[6] and STONE[7] and most correctly performed meta-analyses have demonstrated that dCCBs effectively reduce the incidence of stroke events in older patients with HT[6,7] and could be the preferred agents for treating HT in patients with ischemia heart diseases because of their vasodilatory effects on the coronary arteries[3,8,9] Moreover, dCCBs demonstrate additive effects on BP reduction by most other kinds of antihypertensive agents, especially angiotensin-I-converting enzyme inhibitors, angiotensin-II-receptor blockers, β-blockers, and thiazide diuretics, with few side-effects[2,10] Because of these advantages of dCCBs, several groups that establish international guidelines have recently endorsed them as an initial therapy option in patients with essential HT (EHT), and as an important component of most multidrug regimens for BP control according to a Japanese guideline (JSH2004)[11] However, the response of BP to dCCBs differs among individuals, so, to lower BP more effectively, determining an individual's sensitivity to a dCCB before prescribing it would be useful.

Recent studies indicate that the heterogeneity of a patient's responses to antihypertensive treatment is, at least in part, genetically determined[12] This finding underscores the role of pharmacogenetic research to identify either functional genetic variations or variations inherited in linkage disequilibrium (LD) with these variations as markers to enable more individualized evaluation and selection of agents for treating HT in each drug class[13]

In Japan, more than a dozen dCCBs, particularly DHP derivatives, are available for clinical use. These DHP derivatives bind to receptors on the α1 subunits of DHP-sensitive voltage-gated (L-type) calcium channels to exert their antihypertensive effects and are believed to play a central role in the excitation–contraction coupling for cardiac and

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Table 1. Comparison of Characteristics of Responders and Nonresponders to L-Type CCBs

	R=ΔSBP>20 mmHg			R=ΔDBP>10 mmHg		
	Responder (±SD)	Nonresponder (±SD)	P value	Responder (±SD)	Nonresponder (±SD)	P value
n	48	113		56	105	
Age (years)	62.7±10.8	66.7±9.0	0.016	63.9±11.3	66.4±8.6	0.124
Sex (M/F)	23/25	62/51	0.419	34/22	51/54	0.140
BMI (kg/m^2)	21.1±7.9	21.4±8.6	0.849	22.5±7.0	20.7±9.0	0.201
Pre-SBP (mmHg)	169.7±18.8	151.2±18.5	<0.001	161.8±23.8	154.0±17.8	0.020
Pre-DBP (mmHg)	102.9±11.5	93.6±10.0	<0.001	102.4±12.1	93.2±9.4	<0.001
Pre-MBP (mmHg)	125.2±12.2	112.8±9.2	<0.001	122.2±13.0	113.4±9.6	<0.001
Pre-HR (beats/min)	68.6±9.3	69.6±10.9	0.585	68.6±9.3	69.6±10.9	0.585
Post-SBP (mmHg)	137.6±14.2	146.3±15.1	<0.001	137.7±14.2	146.9±15.0	<0.001
Post-DBP (mmHg)	86.4±9.3	88.4±9.9	0.243	84.4±10.4	89.6±8.9	0.001
Post-MBP (mmHg)	103.5±9.3	107.7±9.7	0.012	102.2±9.3	108.7±9.2	<0.001
Post-HR (beats/min)	72.6±9.8	71.8±12.8	0.708	72.6±9.8	71.8±12.8	0.708
Monotherapy (%)	37.5	21.2	0.035	30.4	23.8	0.371
Type of CCB (%)						
Amlodipine	39.6	44.2	0.584	42.9	42.9	1.000
Nifedipine	22.9	18.6	0.533	23.2	18.1	0.442
Nicardipine	10.4	11.5	0.840	5.4	14.3	0.071
Manidipine	8.3	9.7	0.778	10.7	8.6	0.659
Nilvadipine	6.3	8	0.700	8.9	6.7	0.607
Benidipine	2.1	2.7	0.829	1.8	2.9	0.669
Nitrendipine	4.2	1.8	0.392	1.8	2.9	0.669
Bamidipine	0.0	1.8	0.232	0.0	1.9	0.189
Cilnidipine	2.1	0.9	0.548	1.8	0.9	0.657
Efonidipine	2.1	0.0	0.119	1.8	0.0	0.145

Responder defined as SBP reduction (ΔSBP)>20 mmHg or DBP reduction (ΔDBP)>10 mmHg, respectively, after taking L-type CCB.

CCBs, calcium-channel blockers; R, response; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; Pre-SBP, SBP before treatment; Pre-DBP, DBP before treatment; Pre-MBP, mean blood pressure before treatment; Pre-HR, heart rate before treatment; Post-SBP, SBP after treatment; Post-DBP, DBP after treatment; Post MBP, mean blood pressure after treatment; Post-HR, heart rate after treatment; Monotherapy, prevalence of monotherapy.

smooth muscle[14] L-type calcium channels are formed by 1 of 4 principle pore-forming α1 subunits [α1S (Cav1.1), α1C (Cav1.2), α1D (Cav1.3), and α1F (Cav1.4)], which are encoded by different individual genes, in association with several auxiliary subunits[15] Expression of α1S and of α1F is restricted to skeletal muscle and retina, respectively, but α1C and α1D are widely expressed in neuronal and (neuro)endocrine cells and in electrically excitable cells in the cardiovascular system, including cardiac muscle and vascular smooth muscle[16] In most cases, both channel types are found in the same cells, with α1C usually being the predominant isoform[17] Although previous studies have shown that the effects of dCCBs on the contractility of ventricular muscle and aortic smooth muscle are exclusively mediated by α1C (not by α1D),[18] and that α1D might control physiological processes, such as diastolic depolarization in sinoatrial node cells and neurotransmitter release and neuronal excitability,[19] the physiological effects of these subunits are largely unknown. Considering their expression patterns, the central role of α1C on the contractility of heart muscle and of vascular smooth muscle, and the important role of the neuroendocrine system in the pathophysiology of HT,[20] genes encoding α1C (*CACNA1C*) or α1D (*CACNA1D*) might be candidates for influencing the antihypertensive effects of L-type dCCBs.

The aim of the present study was to evaluate *CACNA1C* and *CACNA1D*, which encode L-type calcium-channel subunits α1C and α1D, respectively, in relation to the responsiveness of patients with EHT to treatment with L-type dCCBs. We focused on evaluating the effects of the α1C subunit. First, we screened for possible genetic polymorphisms in the promoter region, all exon regions, and a small part of the intron regions of *CACNA1C* in 48 patients with HT. Next, we performed genotyping of the missense mutations and representative common polymorphisms of *CACNA1C* found with direct sequencing or common single nucleotide polymorphisms (SNPs) of *CACNA1D* chosen from a public database in 161 patients with EHT who were treated with L-type dCCBs. Finally, we examined the association of these genetic polymorphisms with the responsiveness of patients with EHT to treatment with L-type dCCBs.

Methods

Study Subjects

Peripheral blood samples for genetic analysis were collected after written informed consent was given by Japanese patients with EHT at an outpatient clinic of the Division of Hypertension and Nephrology, National Cardiovascular Center, Suita, Japan. The study protocol was approved by the Ethics Committee of the National Cardiovascular Center. A total of 161 patients (85 men, 76 women), for whom L-type dCCBs had been newly prescribed as monotherapy or in addition to other antihypertensive agents and for whom BP data could be obtained from records of 3 consecutive outpatient visits before and after the start of treatment with L-type dCCBs, were retrospectively enrolled. BP was measured in the subjects after they had rested while seated for at least 10 min. Systolic BP (SBP) and diastolic BP (DBP) values were the means of 3 physician-obtained measurements. All subjects visited the outpatient clinic every month. The L-type dCCBs prescribed were amlodipine (43.5%), nifedipine (19.9%), nicardipine (11.8%), manidipine (9.3%), nilvadipine (7.5%), benidipine (2.5%),

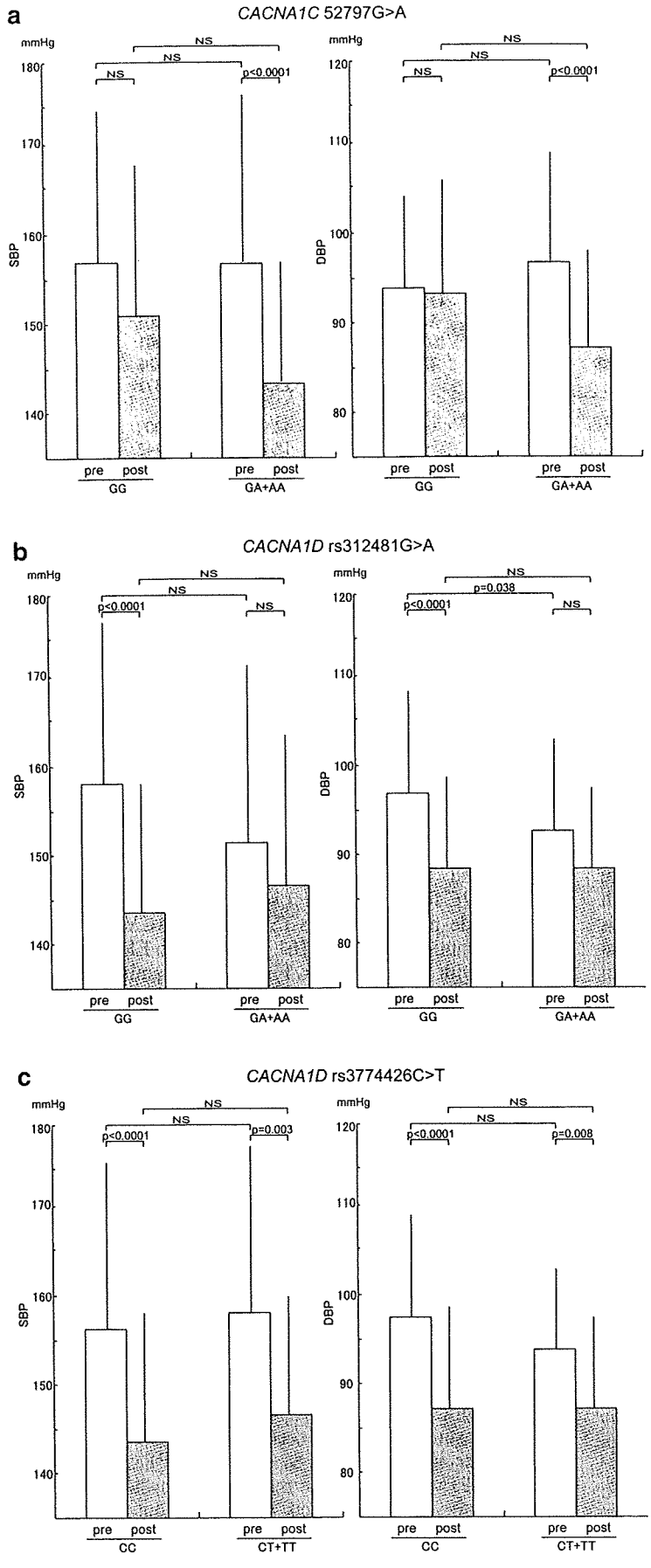

Figure. (a) Comparison of blood pressure (BP) between pre- and post-administration of CCBs in each genotype of dominant model in patients with *CACNA1C* 527974G>A. (b) Comparison of BP between pre- and post-administration of CCBs in each genotype of recessive model in patients with *CACNA1D* rs312481G>A. (c) Comparison of BP between pre- and post-administration of CCBs in each genotype of recessive model in patients with *CACNA1D* rs3774426C>T. CCBs, calcium-channel blockers; SBP, systolic BP; DBP, diastolic BP.

Table 2. Characteristics of Genetic Polymorphisms Identified by Direct Sequencing or Genotyping With TaqMan PCR

Gene, location	SNPs	LD	aa. Info	Region	Allele 1	Hetero	Allele 2	Total	Allele 1 freq.	Allele 2 freq.	TaqMan
*CACNA1C**	395458G>A		A174A	Exon 4	43	5	0	48	0.948	0.052	
12p 13.3	395570G>A	a		Intron 4	29 (102)	17 (48)	2 (11)	48 (161)	0.781 (0.783)	0.219 (0.217)	OK
	395572T>C	a		Intron 4	29	17	2	48	0.781	0.219	
	439886C>A			Intron 7	45	3	0	48	0.969	0.031	
	459184G>A	b		Intron 8	35 (137)	13 (22)	0 (1)	48 (160)	0.865 (0.919)	0.135 (0.081)	OK
	496317delG	c		Intron 9	30	14	0	44	0.841	0.159	
	496354G>T	c		Intron 9	30 (101)	18 (55)	0 (5)	48 (161)	0.813 (0.798)	0.187 (0.202)	OK
	513074G>A	b, c		Intron 12	32	13	0	45	0.856	0.144	
	513955C>T	d		Intron 12	0	2	46	48	0.021	0.979	
	527974 G>A	c, e		Intron 13	4 (8)	20 (62)	24 (91)	48 (161)	0.292 (0.242)	0.708 (0.758)	OK
	529458T>G	e, f		Intron 15	1	22	23	46	0.261	0.739	
	530778G>C			Intron 15	47	1	0	48	0.990	0.010	
	531126A>G			Intron 16	47	1	0	48	0.990	0.010	
	531910C>T	f	D812D	Exon 17	18	24	3	45	0.667	0.333	
	539757G>A		A879A	Exon 19	47	1	0	48	0.990	0.010	
	542532G>T	g		Intron 20	47	1	0	48	0.990	0.010	
	551409T>C	h		Intron 22	47	1	0	48	0.990	0.010	
	552959A>G	d		Intron 24	46	2	0	48	0.979	0.021	
	554886G>A			Intron 26	47	1	0	48	0.990	0.010	
	557206C>T	d		Intron 28	46	2	0	48	0.979	0.021	
	557231C>T			Intron 28	47	1	0	48	0.990	0.010	
	558260T>C	f		Intron 28	14	27	6	47	0.585	0.415	
	558409 C>T	f	F1262F	Exon 29	14 (58)	27 (79)	6 (24)	47 (161)	0.585 (0.606)	0.415 (0.394)	OK
	594891C>G			Intron 30	45	3	0	48	0.969	0.031	
	595028 T>C	i		Intron 31	8 (9)	18 (60)	22 (92)	48 (161)	0.354 (0.242)	0.646 (0.758)	OK
	595041T>C	i		Intron 31	8	18	22	48	0.354	0.646	
	595054C>T	i		Intron 31	8	18	22	48	0.354	0.646	
	597980 G>A	j		Intron 31	4 (14)	17 (39)	23 (108)	44 (161)	0.284 (0.208)	0.716 (0.792)	OK
	598239delA	j		Intron 32	4	17	23	44	0.284	0.716	
	615494delT	g		Intron 38	47	1	0	48	0.990	0.010	
	615546–615547insC	k		Intron 38	32	15	0	47	0.840	0.160	
	624139G>A	h		Intron 40	43	1	0	44	0.989	0.011	
	624330 C>T	k		Intron 41	33 (105)	15 (46)	0 (10)	48 (161)	0.844 (0.795)	0.156 (0.205)	OK
	626151G>A		T1787T	Exon 43	8	17	16	41	0.402	0.598	
	632652 G>A		R1910Q	Exon 45	45 (159)	3 (2)	0 (0)	48 (161)	0.969 (0.994)	0.031 (0.006)	OK
	635110 G>A		G2004S	Exon 46	35 (160)	1 (0)	0 (0)	36 (160)	0.986 (1.000)	0.014 (0.000)	OK
	637259C>T	j, l		Intron 46	28	17	3	48	0.760	0.240	
	638741–638742insT	l		3'-UTR	28	17	3	31	0.875	0.125	Failed
*CACNA1D***	rs3774414 C>T			Intron 2	64	78	18	160	0.644	0.356	OK
3p 14.3	rs219847 G>A			Intron 2	40	82	38	160	0.506	0.494	OK
	rs312481 G>A			Intron 3	131	26	3	160	0.900	0.100	OK
	rs3774425 G>A			Intron 3	73	72	16	161	0.677	0.323	OK
	rs3774426 C>T			Intron 3	118	35	7	160	0.847	0.153	OK

*Genetic polymorphisms of *CACNA1C* were firstly screened in 48 randomly chosen hypertensive subjects, and then representative polymorphisms were genotyped in 161 patients with essential hypertension who were treated with L-type CCBs.
Data in parentheses ( ) based on genotyping results for *CACNA1C*.
Based on the sequencing result, the apparent LD, defined by $r^2>0.5$, was indicated by a-l.
The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (Hum. Mut., 11, 1–3, 1998).
The nucleotide number was according to the reference sequences GenBank Accession ID: NT_009759.15.
Sequence for promoter region, exon 44, and exon 47 of *CACNA1C* was abortive.
**Common SNPs of *CACNA1D* were chosen from JSNP database and genotyped in 161 patients with essential hypertension who were treated with L-type CCBs.
PCR, polymerase chain reaction; LD, linkage disequilibrium; SNPs, single nucleotide polymorphisms. Other abbreviation see in Table 1.

nitrendipine (2.5%), barnidipine (1.2%), cilnidipine (1.2%), and efonidipine (0.6%) (**Table 1**). Patients who could achieve a SBP reduction greater than 20 mmHg or a DBP reduction greater than 10 mmHg after taking L-type dCCBs were defined as responders, and those who could not were defined as nonresponders. These criteria are often used in the clinical trial of new antihypertensive drugs in Japan.

DNA Studies

Direct Sequencing for Detection of Polymorphisms in CACNA1C Genomic DNA was extracted using an NA-3000 nucleic acid isolation system (Kurabo, Osaka, Japan) and stored at –80°C until use. Human *CACNA1C*, located on chromosome 12 at p13.3, consists of 47 exons. We sequenced 48 samples from Japanese patients with HT, using a direct sequencing method described previously[21] Briefly, all exons with their flanking sequences and approximately 1,000 bp of the upstream region of exon 1, which would include promoter regions of *CACNA1C*, were individually amplified with the polymerase chain reaction (PCR) and sequenced with a ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). We failed to sequence the promoter region and exons 44 and 47 of *CACNA1C* because of amplification problems by the PCR. Information on the primers and the PCR conditions is available on request. The polymorphisms were identified with Sequencer software (Gene Codes Corporation, Ann Arbor, MI, USA), followed by visual inspection.

Genotyping of Polymorphisms The TaqMan-PCR method was used for genotyping sequence-proven genetic

Table 3. Genotype Distribution Between Responders and Nonresponders Treated With L-Type CCBs

Gene	SNP	R=ΔDBP >10 mmHg					R=ΔSBP >20 mmHg				
		Genotype	R	NR	χ^2	P value	Genotype	R	NR	χ^2	P value
CACNA1C	527974G>A	GG	0	8	4.501	0.105	GG	0	8	4.418	0.110
		GA	23	39			GA	22	40		
		AA	33	58			AA	26	65		
		GG	0	8	4.490	0.034	GG	0	8	3.576	0.059
		GA+AA	56	97			GA+AA	48	105		
		GG+GA	23	47	0.202	0.653	GG+GA	22	48	0.154	0.694
		AA	33	58			AA	26	65		
		OR 1.163, 95%CI 0.603–2.242					OR 0.873, 95%CI 0.442–1.722				
CACNA1D	rs312481G>A	GG	51	80	5.291	0.071	GG	45	86	11.571	0.003
		GA	4	22			GA	1	25		
		AA	1	2			AA	2	1		
		GG	51	80	4.910	0.027	GG	45	86	6.516	0.011
		GA+AA	5	24			GA+AA	3	26		
		OR 0.327, 95%CI 0.117–0.911					OR 0.221, 95%CI 0.063–0.768				
		GG+GA	55	102	0.004	0.951	GG+GA	46	111	1.957	0.162
		AA	1	2			AA	2	1		
		OR 0.927, 95%CI 0.082–10.457					OR 4.826, 95%CI 0.427–54.544				
	rs3774426C>T	CC	48	70	6.705	0.035	CC	40	78	3.616	0.164
		CT	6	29			CT	6	29		
		TT	2	5			TT	2	5		
		CC	48	70	6.370	0.012	CC	40	78	3.253	0.071
		CT+TT	8	34			CT+TT	8	34		
		OR 0.343, 95%CI 0.146–0.805					OR 0.459, 95%CI 0.194–1.084				
		CC+CT	54	99	0.133	0.715	CC+CT	46	107	0.007	0.933
		TT	2	5			TT	2	5		
		OR 0.733, 95%CI 0.138–3.907					OR 0.930, 95%CI 0.174–4.972				

ΔDBP=DBP (before treatment)-DBP (after treatment); ΔSBP=SBP (before treatment)-SBP (after treatment). Other abbreviations see in Tables 1,2.

polymorphisms of *CACNA1C* and common SNPs of *CACNA1D* chosen from the db SNP database (http://www.ncbi.nlm.nih.gov/SNP/). For sequence-proven genetic polymorphisms, polymorphisms with a minor allele frequency greater than 5% (common polymorphism) were considered candidates for genotyping. We chose a representative common SNP for genotyping among SNPs showing strong LD with an r-square greater than 0.5. Because a missense mutation may cause a direct functional change of the α1c subunit, 2 missense mutations of *CACNA1C* with a minor allele frequency less than 5% were also subjected to genotype analysis. For genetic polymorphisms of *CACNA1D* chosen from the db SNP database, 5 common SNPs (rs219847 G>A, rs312481 G>A, rs3774414 C>T, rs3774425 G>A, rs3774426 C>T) with a minor allelic frequency greater than 5% were chosen for genotyping. There was no tight LD with an r-square greater than 0.5 among these 5 SNPs in *CACNA1D*. As a consequence, 11 SNPs for *CACNA1C* and 5 SNPs for *CACNA1D* in 161 Japanese patients with HT treated with L-type dCCBs were subjected to genotype analysis. We did not perform haplotype analysis because of the study design. We evaluated the synergistic effects of SNPs associated with the effect of CCBs.

Statistical Analysis

Values are expressed as means±SD. Hardy-Weinberg equilibrium was assessed with χ^2 analysis. The overall distribution of alleles was analyzed with χ^2 analysis. The distribution of genotypes between responders and nonresponders was analyzed with 2×2 contingency tables and a 2-sided Fisher exact probability test. The statistical significance was established at $P<0.05$. Comparison of BP reduction between allelic variants was performed with ANOVA followed by the Fisher protected least-significant difference test using Stat-View version 5.0 (SAS Institute Inc, Cary, NC, USA).

Results

Group Characteristics

Overall, both SBP and DBP were significantly reduced after treatment with L-type dCCBs (**Figure**). **Table 1** shows the characteristics of responders and nonresponders. When responder was defined as a SBP reduction >20 mmHg, 48 patients were defined as responders and 113 as nonresponders. When responder was defined as a DBP reduction >10 mmHg, 56 patients were responders and 105 were nonresponders. Neither sex nor body mass index showed a significant difference between responders and nonresponders. Average age and the percentage receiving monotherapy differed significantly between responders and nonresponders when responder was defined as a SBP reduction >20 mmHg. The BP before treatment with dCCBs was significantly higher in responders than in nonresponders. After treatment with dCCBs, the average BP in responders was markedly decreased; however, the average BP in nonresponders was significantly higher than that in responders. Heart rate did not differ significantly between responders and nonresponders before or after treatment with dCCBs. No significant difference in the types of L-type dCCB was found between responders and nonresponders.

Detection of Genetic Polymorphisms

First, we screened for genetic polymorphisms of *CACNA1C* in 48 randomly chosen patients with HT by means of direct sequencing. As shown in **Table 2**, we identified 2 missense mutations in *CACNA1C*. Three of 48 patients had a G-to-A substitution at nucleotide 632652 in

Table 4. Selected Genotype Interactions on the Effects of L-Type CCBs

Comparison	Positively-related polymorphisms			Number	ΔSBP	ΔDBP	P1	P2
	CACNA1C 527974G>A	*CACNA1D* rs312481G>A	*CACNA1D* rs3774426C>T					
2-way interaction								
1	AG+AA	GG	Any	124	15.2±21.1	9.9±9.9	0.0109	0.0007
	Any others			36	5.4±15.9	3.9±6.3		
2	AG+AA	Any	GG	112	13.6±22.3	10.1±10.2	0.5651	0.0031
	Any others			48	11.6±15.2	5.3±6.9		
3	Any	GG	GG	113	14.6±21.0	9.8±10.1	0.1098	0.0136
	Any others			46	8.9±18.7	5.7±7.3		
3-way interaction								
4	AG+AA	GG	GG	107	14.9±21.5	10.3±10.1	0.0801	0.0013
	Any others			52	8.9±17.6	5.2±7.2		

P1, comparison of ΔSBP between genotype groups; P2, comparison of ΔDBP between genotype groups. Other abbreviations see in Tables 1,2.

exon 45, leading to an Arg-to-Gln substitution at position 1910 (R1910Q). One patient had a G-to-A substitution at nucleotide 635110 in exon 46, leading to a Gly-to-Ser substitution at position 2004 (G2004S). Both missense mutations were found in heterozygous form. In addition, we identified 5 synonymous variations (395458G>A in exon 4, 531910C>T in exon 17, 539757G>A in exon 19, 558409C>T in exon 29, 626151G>A in exon 43) encoded for A174 (minor allelic frequency, 0.052), for D812 (0.333), for A879 (0.010), for F1262 (0.415), and for T1787 (0.402). Thirty-one additional variations in the intron and 3'-untranslated regions were also detected. As described in the Methods section, we finally chose 11 genetic polymorphisms of *CACNA1C* and 5 common SNPs of *CACNA1D* for genotype analysis in 161 patients with EHT who were treated with L-type dCCBs (**Table 2**). We failed to genotype 638741-638742insT of CACNA1C because of incomplete discrimination of the genotyping signals. We did not identify 635110G>A (G2004S) of *CACNA1C* in the 161 samples. The allelic frequencies of another 8 SNPs of *CACNA1C* determined with genotyping were similar to those identified with direct sequencing.

Association Study for the Effect of L-Type dCCBs

The clinical characteristics of patients with the 632652G>A (R1910Q) mutation did not show any specific clinical features after treatment with L-type dCCBs (data not shown). Thus, 8 common SNPs of *CACNA1C* and 5 of *CACNA1D* subjected to genotype analysis were used to study their relationship to the effects of L-type dCCBs. Control for deviation from Hardy-Weinberg equilibrium yielded nonsignificant results in all SNPs examined in this study. On basis of a comparison of each allele frequency between responders and nonresponders, 1 of *CACNA1C*, 527974G>A, and 2 SNPs of *CACNA1D*, rs312481G>A and rs3774426C>T, showed significant correlations with the effects of L-type dCCBs (**Table 3**). When a response was defined as a DBP reduction >10 mmHg, the prevalence of *CACNA1C* 527974G>A differed significantly in the dominant model, in that *CACNA1D* rs3774426C>T differed in the additive and recessive models, and that of *CACNA1D* rs312481G>A differed only in the recessive model. When a response was defined as a SBP reduction >20 mmHg, the prevalence of *CACNA1D* rs312481G>A significantly differed in the additive and recessive models. *CACNA1C* 527974G>A and *CACNA1D* rs3774426C>T showed a marginal relation to the effects of L-type dCCBs.

Figure show the comparison of BP in the dominant or recessive model in 3 SNPs that were significantly associated with the effect of L-type dCCBs shown in **Table 3**. The basal SBP and DBP were significantly reduced by treatment with L-type dCCBs in patients with GG carriers in *CACNA1D* rs312481G>A or CC carriers in rs3774426C>T, with GA+AA carriers in *CACNA1C* 527974G>A, and also with CT+TT carriers in *CACNA1D* rs3774426C>T. After treatment with dCCBs, DBP in patients with GG in rs312481G>A, with CC in rs3774426C>T, and with GA+AA in *CACNA1C* 527974G>A was significantly reduced when compared with patients with other allele carriers (P=0.0126 for rs312481G>A, 0.0283 for rs3774426C>T, and 0.0108 for 527974G>A) (**Figure**). Patients with GG carrier in rs312481G>A also showed a significant reduction in SBP after treatment with L-type dCCBs when compared with patients with GA+AA carrier (P=0.0101). Both SBP and DBP were significantly decreased by treatment with dCCBs in patients with GG carrier in *CACNA1D* rs312481G>A, but there was no significant reduction in BP in GA+AA in *CACNA1D* rs312481G>A. In contrast, significant differences in the antihypertensive effect on either SBP or DBP of treatment with dCCBs between alleles were not seen in *CACNA1D* rs3774426C>T or *CACNA1C* 527974G>A.

The genotype interactions on the effects of L-type dCCBs are shown in **Table 4**. When interactions between 2 polymorphisms were analyzed, a much greater reduction in DBP after treatment with dCCBs was observed for the simultaneous presence of *CACNA1C* 527974 GA+AA–*CACNA1D* rs312481 GG or *CACNA1C* 529874 GA+AA–*CACNA1D* rs3774426 CC. The 3-way interaction models also showed a much greater reduction in DBP for the simultaneous presence of *CACNA1C* 527974 GA+AA–*CACNA1D* rs312481 GG–*CACNA1D* rs3774426 CC.

Discussion

The present study has demonstrated that *CACNA1C* 527974G>A, *CACNA1D* rs312481G>A, and *CACNA1D* rs3774426C>T are associated with the antihypertensive effects of L-type dCCBs in Japanese patients with EHT. In particular, the greatest sensitivity to the effects of dCCBs was observed with *CACNA1D* rs312481G>A, which showed a significant association with the effects of L-type dCCBs in the reduction of both SBP and DBP. A patient with HT and GA+AA in *CACNA1D* rs312481G>A or with GG in *CACNA1C* 527974G>A is predicted to be a nonresponder to

L-type dCCBs (**Table 3**, **Figure**). In addition, there was a synergistic effect between the genetic polymorphisms of *CACNA1C* and *CACNA1D* on the lowering BP by L-type dCCBs (**Table 4**). The L-type α1c subunit plays a central role in regulating cardiac function and BP[22,23] and is a target of the L-type dCCBs widely used in the treatment of HT.[24] Therefore, we speculated that genetic polymorphisms of *CACNA1C* might be related to the effects of L-type dCCBs. In this study, we demonstrated that 527974G>A of *CACNA1C* has a significant association with the effects of L-type dCCBs. While we were preparing this report, Bremer et al reported that *CACNA1C* polymorphisms are associated with the efficacy of dCCBs in the treatment of HT in white subjects.[25] The results of both studies suggest that genetic polymorphisms of *CACNA1C* influence the effects of L-type dCCBs in patients with HT; however, how these genetic polymorphisms affect the effects of L-type dCCBs is still unknown. Because 527974G>A is located in intron 13, this SNP itself might not influence α1c function. Although we could not find functional polymorphisms linked with 527974G>A in our results or in HapMap data for Japanese, there may be functional polymorphisms in the promoter region (which we failed to sequence) or genes adjacent to *CACNA1C*. In addition, human *CACNA1C*, spanning >500kb, maps to chromosome 12p11.2 and undergoes extensive mRNA splicing, leading to numerous isoforms with different functions in altering electrophysiology properties,[26–28] affinity to DHPs,[29,30] and loss of channel functions.[31] Alternative splicing is regulated by multiple factors, including the 5' splice site, the 3' splice site, the branch site and the Py tract, as well as the intronic or exonic splicing enhancer and silencer.[31] Identifying genetic polymorphisms that affect splicing has proven difficult, as they can be located not just in the splice regions but anywhere in the large intron. Therefore, we could not rule out the possibility that 527974G>A, as well as polymorphisms linked with it in intron regions, might influence *CACNA1C* mRNA splicing.

The present study is the first to demonstrate that genetic polymorphisms of *CACNA1D* might be associated with the effects of L-type dCCBs in patients with EHT. Of the 3 SNPs that were identified to be associated with the effects of L-type dCCBs in the present study, *CACNA1D* rs312481G>A was the most strongly associated. Patients with GG homozygous for rs312481G>A were more sensitive to the effects of L-type dCCBs for reducing DBP and SBP than were patients with the GA+AA genotype. *CACNA1D* rs3774426C>T also showed a significant association with the effects of L-type dCCBs for reducing DBP. A previous study has shown that α1D does not mediate the contractility of ventricular muscle or aortic smooth muscle.[18] In addition, all L-type calcium channels studied to date are sensitive to L-type dCCBs. However, α1D-containing L-type calcium channels appear to be significantly less sensitive to L-type dCCBs.[19,32] Therefore, how the genetic polymorphisms of *CACNA1D* affect the L-type dCCBs reduction of BP would be very interesting to know. Importantly, recent studies have shown that the lower sensitivity of α1D-containing L-type calcium channels to L-type dCCBs becomes even more significant when membrane potentials are hyperpolarized and α1c-containing L-type calcium channels are not open. The α1D-containing L-type calcium-channel current that remains in the presence of DHPs takes on the profile of an inactivating current with barium as the charge carrier.[32] This is consistent with the state-dependent nature of the blockade by DHPs.[33,34] In the presence of L-type dCCBs, α1D-containing L-type calcium channels generate low-threshold, drug-resistant, inactivating currents that resemble the R-type current of many neurons or the T-type current of sinoatrial node cells and control physiological processes, such as diastolic depolarization in sinoatrial node cells and neurotransmitter release and neuronal excitability. Because the neuroendocrine system and pacemaking may play important roles in regulating BP, variations of *CACNA1D* may influence the effects of L-type dCCBs through a change in the sensitivity of α1D-containing L-type calcium channels to L-type dCCBs. *CACNA1D* rs312481G>A and rs3774426C>T are both in intron regions. We did not find functional polymorphisms linked with them in HapMap data for Japanese (data not shown). The α1D subunit also undergoes extensive mRNA splicing, which may lead to numerous isoforms with different functions.[35,36] Whether *CACNA1D* rs312481G>A and rs3774426C>T or polymorphisms linked with them in intron regions influence *CACNA1D* mRNA splicing needs to be clarified.

Our data also show a possible synergistic effect of genetic polymorphisms of *CACNA1C* and those of *CACNA1D* on L-type dCCBs treatment in patients with EHT. This result suggests that α1D-containing and α1c-containing L-type calcium channels might coordinate the regulation of BP under physiological conditions or the responsiveness to treatment with L-type dCCBs under pathological conditions. Further functional studies are needed to clarify this point.

There is a question as to whether the contributions of *CACNA1D* rs312481G>A and rs3774426C>T and of *CACNA1C* 527974G>A to the effects of L-type dCCBs are an L-type CCB-specific finding. We speculate that the contribution of these 3 SNPs to the antihypertensive effects of L-type dCCBs is in fact dCCB-specific, because these SNPs also showed a significant association with the effects of L-type dCCBs in a study of patients who received only L-type dCCB monotherapy, despite a small sample size (data not shown).

Study Limitations

The present study was retrospective design and had a small sample size. The study subjects included not only patients receiving monotherapy with L-type dCCBs, but also those receiving combined therapy with L-type dCCBs and other antihypertensive drugs. We do not believe that this issue greatly affects the relationship between the 3 SNPs and the effects of L-type dCCBs, because the percentages of patients receiving monotherapy with L-type dCCBs and of patients receiving different L-type dCCBs, such as amlodipine and nifedipine, did not differ significantly between each allele of these SNPs. In addition, the SNPs also showed a significant association with the effects of L-type dCCBs in a study that examined only patients who had received amlodipine therapy (data not shown). However, a large-scale, prospective, controlled study of L-type dCCBs is needed to confirm the importance of these SNPs in the antihypertensive effects of L-type dCCBs. Furthermore, the BP before treatment is an important factor in the effects of antihypertensive drugs. In the present study, both SBP and DBP before treatment with L-type dCCBs were significantly higher in responders than in nonresponders. However, the BP before treatment with L-type dCCBs did not differ significantly between dCCB-sensitive and dCCB-insensitive genotypes in *CACNA1D* rs312481G

>A and rs3774426C>T and in *CACNA1C* 527974G>A when a response was defined as a change in SBP>20 mmHg or in *CACNA1D* rs3774426C>T and *CACNA1C* 527974G>A when a response was defined as a change in DBP>10 mmHg (**Table 3**). In addition, age and aging may influence the effects of antihypertensive drugs because of higher SBP and slower metabolism of dCCBs (compared with younger patients)? However, there was no significant difference in the average age of patients with dCCB-sensitive or -insensitive genotypes. Finally, regarding the statistical approach, the Bonferroni method was not performed, although multiple SNPs were investigated in the present study. No SNPs were significantly associated with the effects of L-type dCCBs according to Bonferroni criteria (P=0.05/13 SNPs, P<0.005). Although this correlation might be considered weak for this type of genetic research, we consider these 3 SNPs to be prominent candidates related to the effectiveness of L-type dCCBs, because both *CACNA1C* and *CACNA1D* have been suggested to play important roles in the effectiveness of L-type dCCBs in patients with EHT, as mentioned earlier.

In summary, rs312481G>A and rs3774426C>T of *CACNA1D* and 527974G>A of *CACNA1C* are believed to be genetic polymorphisms that confer sensitivity to the antihypertensive effects of L-type dCCBs in patients with EHT. Because association studies are not consistently reproducible, as a result of false-positive and false-negative results;[37] the association of these polymorphisms with the effects of L-type dCCBs should be re-examined in other populations. These genetic polymorphisms may be useful for predicting the sensitivity of patients to treatment with L-type dCCBs and may lead to individualized therapies for HT based on genetic background.

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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^2. 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 (≥90 mL/min/1.73m^2) 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^2 group and 2.2 (1.2 to 4.1) in the GFR <50 mL/min/1.73m^2 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).[1] In end-stage renal disease, the cardiovascular disease (CVD) mortality rate is more than 10 times as high as that in the general population.[2] 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.[1] 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,[3,4] whereas others have shown CKD as an independent risk factor for CVD.[5–8] 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.[9] 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

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for the measurement of creatinine.[10] A few studies in general population have demonstrated a stronger association between BP and CVD in subjects with CKD.[5,10] 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,[11–13] 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[14]: 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[15]:

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

$$\text{and GFR (ml/min/1.73 m}^2\text{)} = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \times 0.742 \text{ (for women).}$$

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

Confirmation of Stroke and MI and End Point Determination

The confirmation of stroke and MI in the Suita Study has been described elsewhere.[11–13] 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.[16] 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.[17] 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 χ^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.[18] 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)

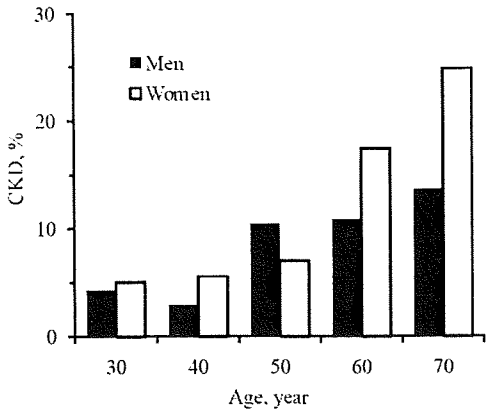

Figure 1. Frequencies of CKD according to sex and age.

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 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).

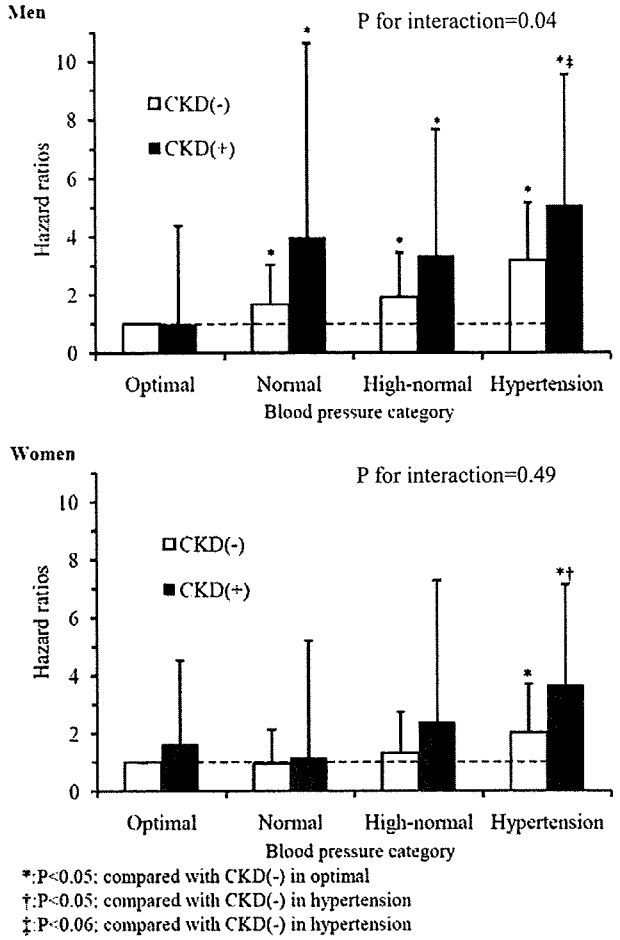

Figure 2. The combination of CKD and BP categories on multivariable hazard ratios for CVD. Data for men and women are presented separately. Multivariable analyses are adjusted age in 5-year increments as stratified variables and other potential confounding factors of hypercholesterolemia, diabetes, and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