methods are reliable but often difficult to perform. They are recommended depending on the patients' cooperativeness and the facility's competence and are suited for facilities specializing in hypertension.

2) Estimation of the Na excretion from the Na/Cr ratio in spot urine: This method is less reliable, but it is easy to perform and is considered to be practical. Since the daily Cr excretion of Japanese is about 1 g (about 10 mmol) (*10*), salt intake is estimated to be about 6 g if the Na excretion per gram of Cr is 100 mmol. Therefore, this method is considered to be useful for salt reduction guidance. However, the urinary Cr excretion varies considerably according to the physique, age, and gender of patients. Therefore, note that true salt intake is lower in small females and higher in large males than the value estimated from the Na/Cr ratio. Overnight urine (nighttime urine) may also be used. The reliability can be increased by the use of a calculation formula (Tables 2–4).

3) Estimation using an electronic salt sensor equipped with a calculation formula in early morning urine (overnight urine): Although this method is less reliable, it can be recommended, because it is simple and can be performed by the patients themselves. However, the patient must purchase a salt sensor, or the medical facility must lend one to the patient, for home monitoring.

According to the guidelines for lifestyle modifications in the management of hypertension, the patient is considered to be compliant with the salt restriction regimen if the salt intake measured by whichever method is less than 6 g (100 mmol Na)/day and not if it is higher.

Conclusions

Although there are several methods for the assessment of salt intake, the precise determination of salt intake in individual patients is difficult. Reliable methods are difficult to perform, and simpler methods are less reliable. However, the assessment of salt intake is strongly recommended, because it is useful for informing patients of their salt intake and conducting salt restriction.

In the management of hypertension, it is desirable to assess salt intake by one of the following three methods whenever possible: 1) Measurement of the Na excretion in 24-h pooled urine or a nutritionist's analysis of the dietary contents: Although these are desirable methods because of their reliability, they are difficult to perform and are suited for facilities specializing in hypertension. 2) Estimation of the Na excretion from the Na/Cr ratio in spot urine: This is a less reliable but practical method and is suited for general medical facilities. Overnight urine (nighttime urine) may also be used, and the reliability is increased by the use of a calculation formula. 3) Estimation using an electronic salt sensor equipped with a calculation formula in overnight urine: While this method is less reliable, it can be recommended, because it is simple and can be performed by the patients themselves. The patient is judged to be compliant with the salt restriction regimen if salt intake (excretion) estimated by any of the methods is less than 6 g (100 mmol)/day but not if it is higher.

References

1. Japanese Society of Hypertension: Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2004). *Hypertens Res* 2006; **29** (Suppl): S1–S105.
2. Lifestyle-Related Disease Control Section, General Affairs Division, Health Bureau, Ministry of Health, Labor and Welfare: An Outline of the Results of the 2004 National Health and Nutrition Survey (in Japanese). http://www.mhlw.go.jp/houdou/2006/05/h0508-1a.html
3. Guidelines Subcommittee: 1999 World Health Organization–International Society of Hypertension Guidelines for the management of hypertension. *J Hypertens* 1999; **17**: 151–183.
4. Kawano Y, Tsuchihashi T, Matsuura H, Ando K, Fujita T, Ueshima H: Assessment of salt intake in the management of hypertension, in Report of the Working Group for Dietary Salt Reduction of the Japanese Society of Hypertension. Tokyo, Japanese Society of Hypertension, 2006, pp 13–23 (in Japanese).
5. Liu K, Cooper R, McKeever J, *et al*: Assessment of the association between habitual salt intake and high blood pressure: methodological problems. *Am J Epidemiol* 1979; **110**: 219–226.
6. Saito T, Shimizu A, Hokimoto S, *et al*: Salt intake of the Japanese. *J Kyoto Women's Univ Food Sci Soc* 1995; **50**: 20–27 (in Japanese).
7. Yoshita K, Miura K, Okayama A, *et al*: A validation study on food composition tables for the international cooperative INTERMAP study in Japan. *Environ Health Prev Med* 2005; **10**: 150–156.
8. Takagi H, Kaneko S, Saeki K, *et al*: Estimation of salt intake using a questionnaire. *J Health Human Ecol* 1993; **59**: 113–122 (in Japanese).
9. Fukumoto T, Ueno M, Haga K, *et al*: Estimation of salt intake at home. Lecture Summaries at the 55th Conference of the Japanese Society of Nutrition and Food Science. Tokyo, Japanese Society of Nutrition and Food Science, 2001, p 212 (in Japanese).
10. Intersalt Cooperative Research Group: Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *BMJ* 1998; **297**: 319–328.
11. Yamori Y, Nara Y, Kihara M, *et al*: Simple method for sampling consecutive 24-hour urine for epidemiological and clinical studies. *Clin Exp Hypertens* 1984; **6**: 1161–1167.
12. Luft FC, Fineberg NS, Sloan RS: Estimating dietary sodium intake in individuals receiving a randomly fluctuating intake. *Hypertension* 1982; **4**: 805–808.
13. Sakai H, Ikeda T, Saito H: Effects of seasons and perspiration on Na and protein intake and their daily urinary excretion. *Jpn J Public Health* 1989; **36**: 309–315 (in Japanese).
14. Liu K, Dyer AR, Cooper RS, *et al*: Can overnight urine replace 24-hour urine collection to assess salt intake?

Hypertension 1979; **1**: 529–536.
15. Tsai TJ, Chen YM, Hsieh BS, *et al*: Comparison between spot urine and overnight urine in the estimation of 24-hour excretion of urine protein, sodium and kallikrein. *J Formos Med Assoc* 1991; **90**: 755–759.
16. Kawano Y, Kawasaki T, Kawazoe N, *et al*: Circadian variations of urinary dopamine, norepinephrine, epinephrine and sodium in normotensive and hypertensive subjects. *Nephron* 1990; **55**: 277–282.
17. Staessen J, Broughton PMG, Fletcher AE, *et al*: The assessment of the relationship between blood pressure and sodium intake using whole-day, daytime and overnight urine collections. *J Hypertens* 1991; **9**: 1035–1040.
18. Kamata K, Tochikubo O: Estimation of 24-h urinary sodium excretion using lean body mass and overnight urine collected by a pipe-sampling method. *J Hypertens* 2002; **20**: 2191–2197.
19. Kawasaki T, Itoh K, Uezono K, *et al*: A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. *Clin Exp Pharmacol Physiol* 1993; **20**: 7–14.
20. Milne FJ, Gear JSS, Laidley L, *et al*: Spot urinary electrolyte concentrations and 24 hour excretion. *Lancet* 1980; **2**: 1135.
21. Tanaka T, Okamura T, Miura K, *et al*: A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. *J Hum Hypertens* 2002; **16**: 97–103.
22. Luft FC, Aronoff GR, Sloan RS, *et al*: The efficacy of quantitative and qualitative chloride titrators in the estimation of human salt intake. *Klin Wochenschr* 1985; **63**: 62–67.
23. Tabata S, Onishi T, Nakamura H, *et al*: Evaluation of the measurement of the urinary salt concentration using Salt Paper in health screening. *Hokuriku J Public Health* 1987; **14**: 42–46 (in Japanese).
24. Yamasue K, Tochikubo O, Kono E, *et al*: Self-monitoring of home blood pressure with estimation of daily salt intake using a new electrical device. *J Hum Hypertens* 2006; **20**: 593–598.

Journal of Human Hypertension (2007) 21, 212–219

www.nature.com/jhh

ORIGINAL ARTICLE

Reverse white-coat effect as an independent risk for left ventricular concentric hypertrophy in patients with treated essential hypertension

M Tomiyama, T Horio, K Kamide, S Nakamura, F Yoshihara, H Nakata, H Nakahama and Y Kawano

Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, Suita, Japan

Recent studies have shown that the converse phenomenon of white-coat hypertension called 'reverse white-coat hypertension' or 'masked hypertension' is associated with poor cardiovascular prognosis. We assessed the hypothesis that this phenomenon may specifically influence left ventricular (LV) structure in treated hypertensive patients. A total of 272 outpatients (mean age, 65 years) with chronically treated essential hypertension and without remarkable white-coat effect were enrolled. Patients were classified into two groups according to office and daytime ambulatory systolic blood pressure (SBP); that is subjects without (Group 1: office SBP $\geqslant$daytime SBP, $n=149$) and with reverse white-coat effect (Group 2: office SBP $<$ daytime SBP, $n=123$). LV mass index and relative wall thickness were echocardiographically determined. In all subjects, LV mass index and relative wall thickness were positively correlated with daytime and 24-h SBP, but not with office SBP. In addition, these two indices were inversely correlated with office – daytime SBP difference. LV mass index (136 ± 31 and 115 ± 28 g/m^2, mean $\pm$ s.d.) and relative wall thickness (0.49 ± 0.09 and 0.46 ± 0.07) were significantly greater in Group 2 than in Group 1. As for LV geometric patterns, Group 2 had a significantly higher rate of concentric hypertrophy compared with Group 1 (48 and 28%). Multivariate analyses revealed that the presence of reverse white-coat effect was a predictor for LV concentric hypertrophy, independent of age, sex, hypertension duration, antihypertensive treatment and ambulatory blood pressure levels. Our findings demonstrate that reverse white-coat effect is an independent risk factor for LV hypertrophy, especially concentric hypertrophy, in treated hypertensive patients.

Journal of Human Hypertension (2007) **21**, 212–219.
doi:10.1038/sj.jhh.1002127; published online 14 December 2006

Keywords: blood pressure; ambulatory; cardiac hypertrophy; geometry

Introduction

Ambulatory blood pressure (BP) is an important determinant of target organ damage and a significant predictor for cardiovascular morbidity and mortality in hypertensive patients.[1–6] There is often a discrepancy between office and ambulatory BPs, such as white-coat hypertension, a normal ambulatory but elevated office BP. On the other hand, the converse phenomenon of white-coat hypertension called 'reverse white-coat hypertension' or 'masked hypertension', that is, a high ambulatory but normal (or well-controlled) office BP, has received little attention.[7] Whereas, some studies have revealed that the proportion of subjects with reverse white-coat condition reaches 20–40% of the general population and hypertensives.[8,9] In treated hypertensive patients with this phenomenon, particularly, the chance of active and sufficient antihypertensive treatment may be lost by an apparent well-controlled BP in the office. Recent studies suggested that an elevated ambulatory or home BP despite a well-controlled office BP is associated with poor cardiovascular prognosis in treated hypertensive patients.[10,11] However, it remains unclear what mechanism is involved in the association of reverse white-coat phenomenon with cardiovascular prognosis.

Left ventricular hypertrophy (LVH), which is a common cardiac consequence of hypertension, is well known to be an independent risk factor for cardiovascular complications and death.[12,13] In addition, left ventricular (LV) morphologic

Correspondence: Dr T Horio, Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, 5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan.
E-mail: thorio@ri.ncvc.go.jp
Received 4 August 2006; revised 23 October 2006; accepted 30 October 2006; published online 14 December 2006

alteration in hypertensive patients is not uniform, and concentric hypertrophy among various LV geometric patterns is shown to be most closely related to poor cardiovascular prognosis.[13]

Thus, we hypothesized that the presence of reverse white-coat effect may promote LV hypertrophy, especially concentric hypertrophy, in treated hypertension. To assess the hypothesis, the present study investigated the influence of reverse white-coat effect on LV mass and geometry in treated hypertensive patients.

Methods

Subjects

From consecutive patients with essential hypertension who were chronically treated and underwent a 24-h ambulatory BP monitoring at an outpatient clinic of our hospital between May 2000 and December 2003, 272 subjects (142 men and 130 women; mean age, 65 years) in whom satisfactory echocardiographic data were simultaneously obtained were enrolled in the present study. Patients with secondary hypertension, stroke, ischaemic heart disease including myocardial infarction, congestive heart failure, renal failure (serum creatinine $\geqslant 160\,\mu$mol/l) or poorly controlled (haemoglobin A1c $\geqslant 8.0\%$) or insulin-treated diabetes mellitus were excluded from this study. Individuals with a remarkable white-coat effect (described below) were also excluded. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria, such as a fasting plasma glucose of $\geqslant 7.0$ mmol/l and/or a plasma glucose level at 2 h after a 75-g oral glucose load of $\geqslant 11.1$ mmol/l, or when medication was taken for treatment of hyperglycaemia. A diagnosis of hyperlipidemia required a serum total cholesterol level of $\geqslant 5.69$ mmol/l and/or a serum triglyceride level of $\geqslant 1.69$ mmol/l or the use of lipid-lowering drugs, according to the Japan Atherosclerosis Society guidelines.[14]

All patients had taken antihypertensive drugs for at least 1 year (average, 12 years). One hundred and ninety-five patients (72%) were treated with Ca channel blockers, 140 (51%) with renin angiotensin system inhibitors (i.e., angiotensin II receptor blockers and angiotensin converting enzyme inhibitors), 82 (30%) with β-blockers, 53 (19%) with diuretics and 29 (11%) with other classes of agents. All subjects gave their informed consent to participate in the present study. All procedures of the present study were carried out in accordance with institutional and national ethical guidelines for human studies.

Measurement of BP

In each visit, office BP was measured twice by a physician in a hospital outpatient clinic with the patient in a sitting position after over 20 min of rest, using an appropriate-size cuff on the left arm and mercury sphygmomanometer. The first and fifth Korotkoff sounds were used to identify systolic and diastolic values, respectively, and measurements were taken to the nearest 2 mm Hg. Office BP was determined by averaging six measurements taken on three separate occasions during a 3-month period.

In the same study period, all subjects underwent 24-h ambulatory BP monitoring. BP was measured every 30 min during the day and night by the oscillometric method using an automatic monitoring device (TM-2421, A&D Co Ltd, Tokyo, Japan).[15] The accuracy and performance of this device have been demonstrated previously.[16] The patients were instructed to carry on with their normal daily activities during measurements and note their activity and location in a diary. According to the diary, daytime and night time were determined as the waking and sleeping periods of the patient, respectively, and mean values of daytime, night time and 24-h BP (systolic and diastolic) were calculated. Nocturnal BP dipping was determined as $100 \times$ (daytime BP$-$night time BP)/daytime BP.

In the present study, all subjects were classified into two groups by the difference between office and daytime ambulatory systolic BP levels; that is, subjects without reverse white-coat effect (Group 1: office systolic BP$\geqslant$daytime systolic BP, and office systolic BP$-$daytime systolic BP<20 mm Hg) and with reverse white-coat effect (Group 2: office systolic BP$<$daytime systolic BP). Subjects with a remarkable white-coat effect (office systolic BP$-$daytime systolic BP $\geqslant 20$ mm Hg) were excluded from the study.

Echocardiography

A comprehensive 2-dimensional and M-mode echocardiography was performed using a cardiac ultrasound unit (Sonos 5500, Philips Medical Systems, Andover, MA, USA) as described previously.[17] Echocardiographic parameters were measured by the consensus of two experienced investigators who were blinded to the clinical data including office and ambulatory BP of the subjects. Interventricular septal thickness (IVSTd), posterior wall thickness (PWTd), LV diameter at end-diastole (LVDd), and LV diameter at end-systole (LVDs) were measured according to the American Society of Echocardiography recommendations.[18,19] Fractional shortening was calculated as $100 \times$ (LVDd$-$LVDs)/LVDd. Relative wall thickness (RWT) was calculated as (IVSTd + PWTd)/LVDd. LV mass was estimated using the formula validated by Devereux and Reichek[20]: LV mass (g) $= 1.04 \times \{(\text{IVSTd} + \text{PWTd} + \text{LVDd})^3 - \text{LVDd}^3\} - 13.6$. LV mass was normalized for body surface area and expressed as the LV mass index (LVMI). LVH was defined as a LVMI of $\geqslant 125$ g/m^2 in men and 110 g/m^2 in women.[21] The intra-observer and inter-observer coefficients of variation of LVMI were 6.7 and 9.8%, respectively.

The geometry of LV was stratified into four different patterns according to the values of LVMI (< or ⩾125/110 g/m^2, men/women) and RWT (< or ⩾0.44). Patients with increased LVMI and increased RWT were considered to have concentric hypertrophy, and those with increased LVMI and normal RWT were considered to have eccentric hypertrophy. Those with normal LVMI and increased or normal RWT were considered to have concentric remodelling or normal geometry, respectively.

Biochemical measurement

Blood samples were obtained in the morning after an overnight fast. Total cholesterol, triglycerides, fasting plasma glucose, haemoglobin A1c and serum creatinine levels were determined by standard laboratory measurements. Creatinine clearance was calculated from the Cockcroft-Gault formula.[22]

Statistical analysis

Statistical analysis was performed using StatView Version 5 Software (Abacus Concepts Inc., Berkeley, CA, USA). Values are expressed as the mean ± s.d. Simple correlations between variables were assessed using univariate linear regression analyses and Pearson's correlation coefficient. An unpaired Student's *t*-test was used for comparison between the two groups. The significance of differences among the three groups was evaluated by an unpaired ANOVA with subsequent Fisher's multiple comparison test. A multiple logistic regression analysis was performed to identify independent determinants of LV mass increase and concentric hypertrophy. A value of $P<0.05$ was accepted as statistically significant.

Results

Simple correlations of office and ambulatory BP levels with two indices of LV structural changes, LVMI and RWT, in all subjects are shown in Table 1. Office systolic or diastolic BP had no correlation with either LVMI or RWT. In contrast, LVMI and RWT were positively correlated with daytime and 24-h systolic BPs, and LVMI was also correlated with night time systolic BP. In addition, these two indices were significantly correlated with the difference between office BP and daytime BP. As shown in Figure 1, LVMI had a close negative correlation with office–daytime systolic BP difference ($r=-0.377$, $P<0.001$). RWT were also inversely correlated with office–daytime systolic BP difference ($r=-0.170$, $P=0.005$). These results suggested that reverse white-coat effect was significantly associated with increases in LVMI and RWT.

Clinical characteristics of the two subject groups classified according to the difference between office and daytime ambulatory systolic BP levels are summarized in Table 2. One hundred and twenty-three (45%) patients were identified as having reverse white-coat effect (Group 2), and the other 149 (55%) patients belonged to Group 1. The proportion of men and the rate of habitual drinkers

Table 1 Correlation of office and ambulatory blood pressure with left ventricular structure in all subjects

	LVMI		*RWT*	
	r	P	r	P
Office systolic BP	0.039	0.526	0.014	0.816
Office diastolic BP	−0.124	0.053	−0.040	0.508
Daytime systolic BP	0.290	<0.001	0.173	0.004
Daytime diastolic BP	0.020	0.742	0.100	0.099
Night time systolic BP	0.318	<0.001	0.113	0.062
Night time diastolic BP	0.099	0.104	0.078	0.198
24-h systolic BP	0.325	<0.001	0.158	0.009
24-h diastolic BP	0.051	0.398	0.096	0.113
(Office − daytime) systolic BP	−0.377	<0.001	−0.170	0.005
(Office − daytime) diastolic BP	−0.211	<0.001	−0.147	0.015

Abbreviations: BP, blood pressure; LVMI, left ventricular mass index; RWT, relative wall thickness.

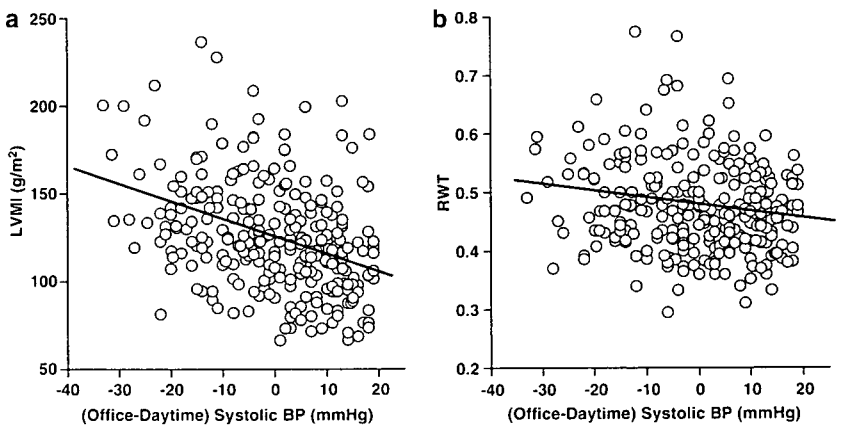


Figure 1 Correlation of the difference between office and daytime systolic BP levels with LVMI (**a**, $r=-0.377$, $P<0.001$) and RWT (**b**, $r=-0.170$, $P=0.005$) in all subjects.

Table 2 Clinical characteristics of two study groups

	Group 1 (n = 149)	*Group 2 (n = 123)*	P
Age (years)	65.8 ± 9.1	65.1 ± 11.0	0.593
Sex (male) (%)	41.6	65.0	<0.001
Body mass index (kg/m^2)	24.2 ± 2.9	24.7 ± 4.0	0.182
Duration of hypertension (years)	17.6 ± 10.8	17.8 ± 11.0	0.850
Diabetes mellitus (%)	19.5	23.6	0.412
Hyperlipidemia (%)	64.9	66.1	0.831
Current smoking (%)	15.6	21.1	0.235
Habitual drinking (%)	50.7	63.6	0.033
Creatinine clearance (ml/min)	81.5 ± 24.8	85.1 ± 32.8	0.297
Fasting plasma glucose (mmol/l)	5.7 ± 1.2	5.8 ± 1.1	0.486
Hemoglobin A1c (%)	5.6 ± 0.8	5.7 ± 0.7	0.257
Total cholesterol (mmol/l)	5.3 ± 0.8	5.2 ± 0.7	0.576
Triglycerides (mmol/l)	1.4 ± 0.7	1.5 ± 0.8	0.126
Antihypertensive treatment			
Period of medication (years)	12.4 ± 9.3	11.7 ± 9.1	0.497
Ca channel blockers (%)	71.8	71.5	0.961
RAS inhibitors (%)	49.7	53.7	0.514
β-Blockers (%)	28.2	32.5	0.440
Diuretics (%)	16.8	22.8	0.216
Others (%)	9.4	12.2	0.458
Total number of classes	1.8 ± 0.9	1.9 ± 0.9	0.141
Office systolic BP (mm Hg)	145.6 ± 12.7	133.8 ± 11.6	<0.001
Office diastolic BP (mm Hg)	83.4 ± 9.9	78.8 ± 10.0	<0.001
Daytime systolic BP (mm Hg)	136.5 ± 12.6	145.1 ± 11.9	<0.001
Daytime diastolic BP (mm Hg)	80.1 ± 9.3	84.8 ± 11.5	<0.001
Night time systolic BP (mm Hg)	126.8 ± 14.9	134.1 ± 15.8	<0.001
Night time diastolic BP (mm Hg)	73.1 ± 9.5	76.9 ± 11.1	0.002
24-h systolic BP (mm Hg)	134.0 ± 12.3	141.6 ± 12.2	<0.001
24-h diastolic BP (mm Hg)	78.2 ± 9.0	82.3 ± 10.5	<0.001
Nocturnal systolic BP dipping (%)	7.1 ± 8.0	7.6 ± 8.0	0.572
Nocturnal diastolic BP dipping (%)	8.5 ± 8.4	9.0 ± 8.5	0.671

Abbreviations: BP, blood pressure; RAS, renin angiotensin system.
RAS inhibitors represent angiotensin II receptor blockers and angiotensin converting enzyme inhibitors. Values are mean ± s.d. or percentage.

were significantly higher in Group 2 than in Group 1. Age, body mass index, hypertension duration, the prevalence of diabetes mellitus and hyperlipidemia, the rate of current smokers, renal function and glucose and lipid parameters did not differ between the two groups. In addition, there were no inter-group differences in the period of medication, the use of any class of antihypertensive agent and the total number of classes of antihypertensive drugs.

Office and ambulatory BP levels had clear differences between the two groups. That is, Group 2 had significantly lower office systolic and diastolic BPs than Group 1, but daytime, night time, and average 24-h ambulatory BPs in Group 2 were significantly elevated compared with those in Group 1. The degree of nocturnal BP dipping, an index of circadian BP variation, did not differ between the two groups.

The comparison of echocardiographic parameters between the two groups is shown in Table 3. Group 2 had a significantly greater LVMI than Group 1, resulting from more increased LV wall thickness and internal dimension. RWT was also significantly increased in Group 2 compared with Group 1. In addition, the prevalence of LVH, defined as an increased LVMI by sex, was significantly higher in Group 2. There was no difference in fractional shortening between the two groups.

Table 3 Comparison of ecchocardiographic parameters between the two groups

	Group 1 (n = 149)	*Group 2 (n = 123)*	P
IVSTd (mm)	10.3 ± 1.5	11.4 ± 1.9	<0.001
PWTd (mm)	10.3 ± 1.4	11.1 ± 1.5	<0.001
LVDd (mm)	44.8 ± 4.5	46.8 ± 4.2	<0.001
LVDs (mm)	26.5 ± 4.9	27.7 ± 4.6	0.037
Fractional shortening (%)	41.1 ± 7.4	41.0 ± 6.8	0.920
LVMI (g/m^2)	115.3 ± 28.3	136.4 ± 30.8	<0.001
RWT	0.46 ± 0.07	0.49 ± 0.09	0.010
Prevalence of LVH (%)	41.6	65.9	<0.001

Abbreviations: IVSTd, interventricular septal thickness at end-diastole; LVDd, left ventricular diameter at end-diastole; LVDs, left ventricular diameter at end-systole; LVMI, left ventricular mass index; LVH, left ventricular hypertrophy; PWTd, posterior wall thickness at end-diastole; RWT, relative wall thickness.
LVH is defined as LVMI of ⩾125 g/m^2 in men and 110 g/m^2 in women.
Values are mean ± s.d. or percentage.

To assess the impact of reverse white-coat effect on LVH, Group 2 was divided into two sub-groups by the extent of its phenomenon. As shown in Figure 2, both LVMI and prevalence of LVH were

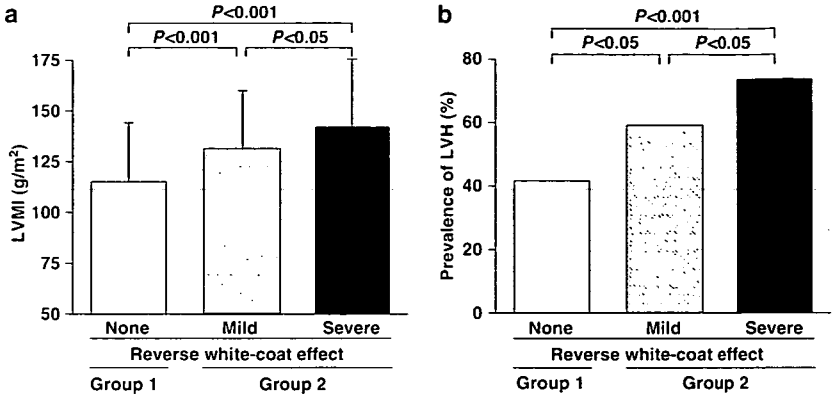


Figure 2 Comparison of LVMI (**a**) and prevalence of LVH (**b**) among the three groups classified by the extent of reverse white-coat effect. None, office systolic BP ≥ daytime systolic BP (i.e., Group 1, $n = 149$); Mild, office systolic BP < daytime systolic BP, but daytime systolic BP−office systolic BP < 10 mm Hg ($n = 63$); Severe, daytime systolic BP−office systolic BP ≥ 10 mm Hg ($n = 60$). LVH is defined as LVMI of ≥125 g/m² in men and 110 g/m² in women. Values are given as the mean ± s.d. (**a**) or percentage (**b**).

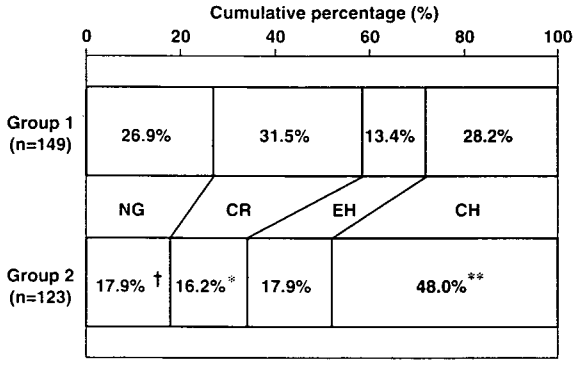


Figure 3 Comparison of LV geometric patterns between the two groups. NG, normal geometry (normal LVMI and RWT); CR, concentric remodelling (normal LVMI and increased RWT); EH, eccentric hypertrophy (increased LVMI and normal RWT); CH, concentric hypertrophy (increased LVMI and RWT). †$P<0.05$, *$P<0.01$, and **$P<0.001$ vs Group 1.

significantly greater in subjects with mild reverse white-coat effect (office systolic BP < daytime systolic BP, but daytime systolic BP−office systolic BP < 10 mm Hg) than in those without reverse white-coat effect (i.e., Group 1), and these values were further increased significantly in the sub-group with severe reverse white-coat effect (daytime systolic BP−office systolic BP ≥ 10 mm Hg).

Figure 3 shows the comparison of LV geometric patterns between the two groups. Group 2 had a significantly higher rate of concentric hypertrophy compared with Group 1 (48 vs 28%, $P<0.001$). In contrast, the rates of patients with normal geometry and concentric remodelling were significantly lower in Group 2 than in Group 1.

To confirm whether the influence of reverse white-coat phenomenon on LV mass increase and specific geometric change was independent of various clinical parameters, we investigated possible predictive factors using a multiple logistic regression analysis in all subjects (Table 4). Although average 24-h systolic BP was the strongest predictor for both LVH and concentric hypertrophy, the presence of reverse white-coat effect (i.e., Group 2) was found to be a significant determinant for these LV structural changes, independent of age, sex, body mass index, hypertension duration, the use of any class of antihypertensive agent and 24-h systolic and diastolic BP levels (for LVH: odds ratio 2.42 vs Group 1, $P=0.005$; for concentric hypertrophy: odds ratio 1.89, $P=0.039$). The significant predictive value of reverse white-coat effect remained even when daytime systolic and diastolic BPs, instead of 24-h BPs, were adopted as independent predictors (data not shown).

Discussion

This study has demonstrated that the presence of reverse white-coat effect is one of the independent predictors for LVH, especially for LV concentric hypertrophy, in patients with treated essential hypertension. The new findings suggest that reverse white-coat phenomenon, independent of average ambulatory blood pressure levels, may have an unfavourable influence on left ventricular geometry in essential hypertension.

The present subjects with reverse white-coat effect (Group 2) had a controlled office BP in spite of elevated ambulatory BP, indicating that the group took on an aspect of masked hypertension. There have been a few studies reporting the possible association between masked hypertension and cardiac and carotid arterial structural changes in the general population. Liu *et al.*[23] found that LV mass and carotid wall thickness in patients with masked hypertension were significantly greater than those in true normotensive subjects and similar to those in patients with sustained hypertension. The data from the PAMELA Study also showed that LVMI was

Table 4 Independent predictors for left ventricular mass increase and concentric hypertrophy by multiple logistic regression analysis

	LVH		*Concentric hypertrophy*	
	OR (95% CI)	P	*OR (95% CI)*	P
Age (10 years)	0.88 (0.58–1.34)	0.544	0.70 (0.46–1.05)	0.087
Sex (male)	0.60 (0.30–1.16)	0.128	0.82 (0.42–1.60)	0.557
Body mass index (1 kg/m^2)	1.10 (1.00–1.23)	0.046	1.10 (1.00–1.22)	0.053
Hypertension duration (1 year)	1.02 (0.99–1.05)	0.123	1.03 (1.00–1.06)	0.028
Diabetes mellitus (yes)	1.04 (0.48–2.22)	0.929	1.02 (0.49–2.13)	0.959
Hyperlipidemia (yes)	1.49 (0.82–2.71)	0.186	1.35 (0.73–2.47)	0.339
Current smoking (yes)	0.99 (0.46–2.13)	0.978	1.25 (0.60–2.58)	0.553
Habitual drinking (yes)	1.09 (0.58–2.06)	0.783	1.22 (0.64–2.32)	0.541
Creatinine clearance (10 ml/min)	0.91 (0.77–1.06)	0.231	0.87 (0.74–1.02)	0.095
Ca channel blocker (yes)	1.36 (0.67–2.77)	0.402	1.09 (0.53–2.24)	0.808
RAS inhibitor (yes)	1.15 (0.60–2.21)	0.678	1.57 (0.82–2.99)	0.170
β-Blocker (yes)	1.89 (0.99–3.59)	0.052	1.36 (0.73–2.55)	0.337
Diuretic (yes)	1.04 (0.49–2.22)	0.921	0.72 (0.34–1.56)	0.407
24-h systolic BP (10 mm Hg)	2.35 (1.66–3.33)	<0.001	1.97 (1.45–2.68)	<0.001
24-h diastolic BP (10 mm Hg)	0.60 (0.40–0.90)	0.014	0.67 (0.45–0.98)	0.041
Reverse white-coat effect				
Absence (Group 1)	1 (reference)		1 (reference)	
Presence (Group 2)	2.42 (1.31–4.48)	0.005	1.89 (1.03–3.44)	0.039

Abbreviations: BP, blood pressure; CI, confidence interval; LVH, left ventricular hypertrophy; OR, odds ratio; RAS, renin angiotensin system. RAS inhibitor represents angiotensin II receptor blocker or angiotensin converting enzyme inhibitor. LVH is defined as LVMI of $\geqslant$125 g/m^2 in men and 110 g/m^2 in women. Concentric hypertrophy is defined as LVH combined with increased RWT ($\geqslant$0.44).

increased in untreated subjects with masked hypertension and sustained hypertension than in those with true normotension.[24] In addition, our recent study showed that masked hypertension was associated with advanced target organ damage in treated hypertensive patients, comparable to that in cases of sustained hypertension.[25] Furthermore, prospective studies have revealed that a high ambulatory or home BP is a powerful predictor for cardiovascular morbidity and mortality in the general population and treated hypertensive patients even when their office BP is normal or well controlled.[10,11,26–28] As for the association between LV geometry and cardiovascular prognosis, it was reported that hypertensive patients with concentric hypertrophy among four LV geometric patterns had the highest incidence of cardiovascular events and death.[13] Taken together, it is likely that advanced target organ changes including LV concentric hypertrophy in patients with masked hypertension or reverse white-coat condition are linked to poor cardiovascular prognosis in such patients.

A higher level of ambulatory BP is a major determinant of target organ damage in hypertensive patients.[1,2] In the present study, however, the presence of reverse white-coat effect was a significant predictor for LVH and concentric hypertrophy, independent of average 24-h ambulatory BP levels. Other factors than a higher ambulatory BP could contribute to target organ damage in reverse white-coat hypertension. Our study has not provided the specific mechanism by which reverse white-coat effect could promote LV concentric hypertrophy in patients with treated hypertension. Therefore, further investigations are required to clarify how reverse white-coat or masked hypertension has a specific unfavourable effect on the hypertensive target organ.

There were some limitations in our study. The present findings were derived from cross-sectional data on the basis of one-time examination of ambulatory BP monitoring and echocardiography. Our subjects were divided into subgroups based on office–daytime difference only in systolic BP, not considering diastolic BP difference. In addition, cardiac magnetic resonance imaging might be more adequate than echocardiography in evaluating LV mass exactly.

All patients in the present study had received antihypertensive medication. As another limitation of this study, therefore, we must consider the possibility that different classes of antihypertensive drugs may have differently affected the development of LVH, partly independently of their BP-lowering effects. Renin angiotensin system inhibitors, particularly, are known to have BP fall-independent protective effects on hypertensive target organ. However, the percentage of patients treated with angiotensin II receptor antagonists or angiotensin converting enzyme inhibitors did not differ between the two study groups. Our multivariate analysis also showed that the association of reverse white-coat effect with LVH and concentric hypertrophy was independent of the use of any class of antihypertensive agent.

In conclusion, the present study indicates that reverse white-coat effect is a significant determinant of LVH, especially concentric hypertrophy, in patients with treated essential hypertension, independent of average ambulatory BP levels and

various other clinical risk factors. Our findings suggest that the presence of this phenomenon may be an independent risk for the adverse LV geometric change in treated hypertensive patients and ambulatory BP monitoring seems to be necessary to unmask this latent risk that is not detectable by routine BP measuring in the office.

What is known about this topic

- Ambulatory blood pressure is an important determinant of target organ damage and a predictor for cardiovascular morbidity and mortality in hypertensive patients.
- The converse phenomenon of white-coat hypertension called 'reverse white-coat hypertension' or 'masked hypertension' is associated with poor cardiovascular prognosis.
- Left ventricular hypertrophy, especially concentric hypertrophy, is a significant risk factor for cardiovascular complications and death.

What this study adds

- Reverse white-coat effect was an independent predictor for left ventricular hypertrophy, especially for concentric hypertrophy, in treated hypertensive patients.
- The presence of reverse white-coat phenomenon, independent of average ambulatory blood pressure levels, may have an unfavourable influence on left ventricular geometry.

Acknowledgements

This study was supported by the Grant for Cardiovascular Disease (11C-5) and the Health and Labor Sciences Research Grants (H14-kouka-021) from the Ministry of Health, Labor and Welfare of Japan, and the Grant from Japan Cardiovascular Research Foundation. We thank Chikako Tokudome, Yoko Oikawa, Yoko Saito, and Miho Nishibata for their secretarial assistance.

References

1 Pickering TG, Harshfield GA, Devereux RB, Laragh JH. What is the role of ambulatory blood pressure monitoring in the management of hypertensive patients? *Hypertension* 1985; **7**: 171–177.
2 Schillaci G, Verdecchia P, Sacchi N, Bruni B, Benemio G, Pede S *et al.* Clinical relevance of office underestimation of usual blood pressure in treated hypertension. *Am J Hypertens* 2000; **13**: 523–528.
3 Cuspidi C, Meani S, Fusi V, Valerio C, Catini E, Magrini F *et al.* Isolated ambulatory hypertension and changes in target organ damage in treated hypertensive patients. *J Hum Hypertens* 2005; **19**: 471–477.
4 Perloff D, Sokolow M, Cowan R. The prognostic value of ambulatory blood pressures. *JAMA* 1983; **249**: 2792–2798.
5 Staessen JA, Thijs L, Fagard R, O'Brien ET, Clement D, de Leeuw PW *et al.* Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. *JAMA* 1999; **282**: 539–546.
6 Verdecchia P, Reboldi G, Porcellati C, Schillaci G, Pede S, Bentivoglio M *et al.* Risk of cardiovascular disease in relation to achieved office and ambulatory blood pressure control in treated hypertensive subjects. *J Am Coll Cardiol* 2002; **39**: 878–885.
7 Pickering TG, Davidson K, Gerin W, Schwartz JE. Masked hypertension. *Hypertension* 2002; **40**: 795–796.
8 Wing LMH, Brown MA, Beilin LJ, Ryan P, Reid CM. 'Reverse white-coat hypertension' in older hypertensives. *J Hypertens* 2002; **20**: 639–644.
9 Bombelli M, Sega R, Facchetti R, Corrao G, Friz HP, Vertemati AM *et al.* Prevalence and clinical significance of a greater ambulatory versus office blood pressure ('reversed white coat' condition) in a general population. *J Hypertens* 2005; **23**: 513–520.
10 Clement DL, De Buyzere ML, De Bacquer DA, de Leeuw PW, Duprez DA, Fagard RH *et al.* Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *N Engl J Med* 2003; **348**: 2407–2415.
11 Bobrie G, Chatellier G, Genes N, Clerson P, Vaur L, Vaisse B *et al.* Cardiovascular prognosis of 'masked hypertension' detected by blood pressure self-measurement in elderly treated hypertensive patients. *JAMA* 2004; **291**: 1342–1349.
12 Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**: 1561–1566.
13 Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann Intern Med* 1991; **114**: 345–352.
14 Hata Y, Mabuchi H, Saito Y, Itakura H, Egusa G, Ito H *et al.* Report of the Japan Atherosclerosis Society (JAS) Guideline for Diagnosis and Treatment of Hyperlipidemia in Japanese adults. *J Atheroscler Thromb* 2002; **9**: 1–27.
15 Kawano Y, Matsuoka H, Takishita S, Omae T. Effects of magnesium supplementation in hypertensive patients: assessment by office, home, and ambulatory blood pressures. *Hypertension* 1998; **32**: 260–265.
16 Imai Y, Sasaki S, Minami N, Munakata M, Hashimito T, Sakuma H *et al.* The accuracy and performance of the A&D TM 2421, a new ambulatory blood pressure monitoring device based on the cuff-oscillometric and the Korotkoff sound technique. *Am J Hypertens* 1992; **5**: 719–726.
17 Horio T, Miyazato J, Kamide K, Takiuchi S, Kawano Y. Influence of low high-density lipoprotein cholesterol on left ventricular hypertrophand diastolic function in essential hypertension. *Am J Hypertens* 2003; **16**: 938–944.
18 Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; **58**: 1072–1083.
19 Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H *et al.* Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989; **2**: 358–367.
20 Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man: anatomic validation of the method. *Circulation* 1977; **55**: 613–618.

21 Cifkova R, Erdine S, Fagard R, Farsang C, Heagerty AM, Kiowski W *et al.* Practice guidelines for primary care physicians: 2003 ESH/ESC hypertension guidelines. *J Hypertens* 2003; **21**: 1779–1786.
22 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31–41.
23 Liu JE, Roman MJ, Pini R, Schwartz JE, Pickering TG, Devereux RB. Cardiac and arterial target organ damage in adults with elevated ambulatory and normal office blood pressure. *Ann Intern Med* 1999; **131**: 564–572.
24 Sega R, Trocino G, Lanzarotti A, Carugo S, Cesana G, Schiavina R *et al.* Alterations of cardiac structure in patients with isolated office, ambulatory, or home hypertension: data from the general population (Pressione Arteriose Monitorate E Loro Associazioni [PAMELA] Study). *Circulation* 2001; **104**: 1385–1392.
25 Tomiyama M, Horio T, Yoshii M, Takiuchi S, Kamide K, Nakamura S *et al.* Masked hypertension and target organ damage in treated hypertensive patients. *Am J Hypertens* 2006; **19**: 880–886.
26 Björklund K, Lind L, Zethelius B, Andrén B, Lithell H. Isolated ambulatory hypertension predicts cardiovascular morbidity in elderly men. *Circulation* 2003; **107**: 1297–1302.
27 Ohkubo T, Kikuya M, Metoki H, Asayama K, Obara T, Hashimoto J *et al.* Prognosis of 'masked' hypertension and 'white-coat' hypertension detected by 24-h ambulatory blood pressure monitoring 10-year follow-up from the Ohasama study. *J Am Coll Cardiol* 2005; **46**: 508–515.
28 Pierdomenico SD, Lapenna D, Bucci A, Di Tommaso R, Di Mascio R, Manente BM *et al.* Cardiovascular outcome in treated hypertensive patients with responder, masked, false resistant, and true resistant hypertension. *Am J Hypertens* 2005; **18**: 1422–1428.

The endothelial nitric oxide synthase gene -786T/C polymorphism is a predictive factor for reattacks of coronary spasm

Tsunenori Nishijima[a], Masafumi Nakayama[a], Michihiro Yoshimura[a], Koji Abe[a], Megumi Yamamuro[a], Satoru Suzuki[a], Makoto Shono[a], Seigo Sugiyama[a], Yoshihiko Saito[c], Yoshihiro Miyamoto[d], Kazuwa Nakao[e], Hirofumi Yasue[b] and Hisao Ogawa[a]

Objective We previously found a -786T/C polymorphism in the 5′-flanking region of the endothelial nitric oxide synthase (eNOS) gene and reported that this polymorphism is strongly associated with coronary spasm. In this study, we examined whether the polymorphism is a prognostic marker in coronary spasm patients.

Methods and results We examined the clinical courses of 201 consecutive patients with coronary spasm who were admitted to our institution: 146 patients with the -786T/T genotype; 50 patients with the -786C/T genotype; and five patients with the -786C/C genotype. The mean follow-up period was 76±60 months. All the patients took calcium channel blockers and/or nitrate during the follow-up period. In this study, no patients died due to a cardiac event. About 25 patients were readmitted owing to cardiovascular disease. Out of these 25 patients, 23 patients were readmitted owing to a reattack of coronary spasm. The -786C allele was significantly associated with readmission due to coronary spasm ($P=0.0072$, odds ratio: 3.37 in the dominant effect). Kaplan–Meier analysis revealed that the occurrence of readmission was significantly higher in the patients with the -786C allele than in the patients without the -786C allele ($P=0.0079$). Further, multiple logistic regression analysis revealed that the -786T/C polymorphism was an independent predictor for readmission due to reattack of coronary spasm ($P=0.006$; relative risk=3.590).

Conclusions The eNOS -786C allele is an independent risk factor for readmission due to a recurrent attack of coronary spasm in patients with coronary spasm, even if the patients have taken calcium channel blockers and/or nitrate. ***Pharmacogenetics and Genomics*** 17:581–587 © 2007 Lippincott Williams & Wilkins.

Pharmacogenetics and Genomics 2007, 17:581–587

Keywords: coronary disease, coronary spasm, genes, nitric oxide synthase, prognosis

[a]The Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, [b]Division of Cardiology, Kumamoto Aging Research Institute, Kumamoto, [c]First Department of Internal Medicine, Nara Medical University, Kashihara, Nara, [d]Division of Atherosclerosis and Diabetes, National Cardiovascular Center, Suita, Osaka and [e]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Sakyou-ku, Kyoto, Japan

Correspondence to Dr Michihiro Yoshimura, MD, Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan
Tel: +81 96 373 5175; fax: +81 96 362 3256;
e-mail: bnp@kumamoto-u.ac.jp

Received 24 May 2006 Accepted 8 August 2006

Introduction

Coronary spasm plays an important role in the pathogenesis of not only variant angina but also ischemic heart diseases in general, including other forms of angina pectoris, acute myocardial infarction, and sudden death [1–3].

We previously reported that the long-term prognosis for patients with coronary spasm is relatively good and that the use of calcium channel blockers (CCBs) improves it [4,5].

We have recommended that patients with coronary spasm take CCB, which dilates the large coronary arteries and thereby prevents the occurrence of coronary spasm; however, there were many patients who were readmitted owing to a recurrent attack of coronary spasm while on the CCB regimen [6]. It is not yet clear what factor(s) predisposes coronary-spasm patients who take CCB to a reattack of coronary spasm.

We previously reported that a -786T/C polymorphism in the 5′-flanking region of the endothelial nitric oxide synthase (eNOS) gene is strongly associated with coronary spasm; it also results in a significant reduction in eNOS gene promoter activity [7]. We further showed that the replication protein A1 binds to the -786C allele and thereby represses eNOS gene transcription [8]. Our clinical research revealed that the polymorphism strongly increases the basal tone of the coronary arteries, and enhances their response to the constrictor effects of acetylcholine (ACh) [9,10]; furthermore, we reported

1744-6872 © 2007 Lippincott Williams & Wilkins

that the polymorphism is significantly associated with myocardial infarction, especially in patients without coronary stenosis [11]. These findings suggest that the -786T/C polymorphism in the eNOS gene compromises endothelial NO synthesis and thereby predisposes the patients to severe coronary spasm.

In this study, we examined the association between the -786T/C polymorphism and the long-term prognosis for coronary-spasm patients.

Methods

Study population

The study population consisted of 201 consecutively admitted patients from July 1984 to July 2000 (100 men; 101 women: mean age in years, 61.9; range 25–81 years) from whom genomic DNA could be obtained. All of them had coronary spasm as defined by an intracoronary injection of ACh. Coronary spasm was defined as total or subtotal occlusion of a coronary artery, which was associated with ischemic electrocardiographic changes and/or chest pain. Patients with significant organic stenosis in the coronary arteries, defined as having more than 50% organic stenosis in at least one coronary artery after nitroglycerine administration, were excluded. Patients who stopped medications were excluded from this study.

The patients were divided into two groups: the -786T group consisting of 146 patients with the -786T/T genotype (74 men and 72 women; mean age in years, 61.7; range, 25–81) and the -786C group consisting of 55 patients (28 men and 27 women; mean age in years, 62.2; range, 40–77). The latter group included 50 patients with the -786C/T genotype and five patients with the -786C/C genotype. We examined the following two events during the follow-up period: (i) death from all causes and (ii) readmission due to a coronary arterial event, such as reattack of coronary spastic angina, angina pectoris due to organic stenosis, or acute myocardial infarction.

Cardiac catheterization

All medications taken by the study participants were discontinued at least 48 h before cardiac catheterization. Coronary arteriography was performed in the morning while the patients were in a fasting state. After baseline arteriography of the left and right coronary arteries, an intracoronary injection of ACh was administered as described previously [12–15].

Two consecutive doses (50 and 100 μg) of ACh were administered 4 min apart, injected into the left coronary artery: angiography was performed within 30 s of each injection. Then, 50 μg of ACh was injected into the right coronary artery and angiography was again performed. Finally, both left and right coronary arteriograms were taken after an intracoronary injection of 1 mg of isosorbide dinitrate. We evaluated the degree of organic stenosis after the injection of isosorbide dinitrate.

Screening method for the -786T/C polymorphism in the endothelial nitric oxide synthase gene

An allele-specific oligonucleotide method was used in the screening for the -786T/C polymorphism in the eNOS gene. Hybridization was accomplished with ^{32}P-radiolabeled oligonucleotides corresponding to either the probe for the -786T allele or the probe for the -786C allele. The method has been described earlier. [7]. In brief, the PCR fragments, 236-bp in length, including the -786T/C polymorphism site, were blotted in duplicate onto nylon membranes. Hybridization was accomplished with ^{32}P-radiolabeled oligonucleotides corresponding to either the -786T sequence (5′-GGG TCA GCC AGC CAG GGAA-3′; probe for the -786T sequence) or the -786C sequence (5′-GGG TCA GCCGGC CAG GGAA-3′; probe for the -786C sequence).

Statistical analysis

Continuous variables are expressed as mean ± SD. Mean values were compared using the unpaired Student's *t*-test. The χ^2 test was used for the evaluation of differences between proportions. A probability value < 0.05 was considered to indicate statistical significance.

Multiple logistic regression analysis with forward stepwise selection was performed with SPSS 14.0J for Windows (SPSS Japan Inc). Multiple logistic analysis was used to determine independent predictors of coronary spasms. Independent variables were coded as the following dummy variables: genotype, 0 for the -786T/T genotype and 1 for the -786C/T or the -786C/C genotype; sex, 0 for women and 1 for men; age, 0 for < 55 years and 1 for ≥ 55 years; body mass index, 0 for $< 25\,kg/m^2$ and 1 for $\geq 25\,kg/m^2$; hypercholesterolemia, 0 for < 220 mg/dl and 1 for ≥ 220 mg/dl; Cigarette smoking, 0 for nonsmokers and 1 for ex-smokers (all study participants quit smoking upon admission); hypertension, 0 for normotension and 1 for hypertension; and diabetes mellitus, 0 for an absence and 1 for a presence. A Kaplan–Meier survival curve was used for determining survival and readmission rates in both the -786T group and the -786C group. We compared the survival rates and readmission rates between the -786T group and the -786C group using the log-rank test.

Results

Follow-up periods

The patients in this study were followed up until 1 December 2005. The mean follow-up period was 76 ± 60 months (range 1–252 months) for all study patients, with mean follow-up periods of 74 ± 56 months (range 1–252 months) for the -786T group and 81 ± 68 months (range 1–235 months) for the -786C group.

Clinical characteristics in the study patients

The clinical characteristics of the study patients are shown in Table 1. The incidence of coronary risk factors, including age, sex, hypertension, and cigarette smoking, were compared between the -786T and the -786C groups. No significant differences were seen in these coronary risk factors between the -786T and the -786C groups. No significant differences were seen in the drug regimens, including CCB, nitrates, angiotensin-converting enzyme inhibitors (ACE-I), or antiplatelets between the two groups.

Prognosis of patients and causes of death

In this study population, 192 survived and nine died during the follow-up period. Of the nine patients who died, three died of lung cancer, one of pancreas cancer, one of a brain tumor, one of a ruptured thoracic aortic aneurysm, one of stroke, and two of respiratory failure. Kaplan–Meier analysis revealed that there were no significant differences in the death rates between the -786T group and the -786C group (log-rank test: $P = 0.5945$) (Fig. 1).

Readmission due to coronary arterial disease and the -786T/C polymorphism

Twenty-five patients were readmitted owing to a recurrence of coronary arterial disease. Out of these 25 patients, 23 patients were readmitted owing to a reattack of coronary spasm. In the patients readmitted owing to a reattack of coronary spasm, one patient was readmitted owing to acute myocardial infarction without significant organic stenosis. He had the -786C/C genotype of the eNOS gene. Two patients who were readmitted owing to a progression of coronary stenosis had the -786T/T genotypes.

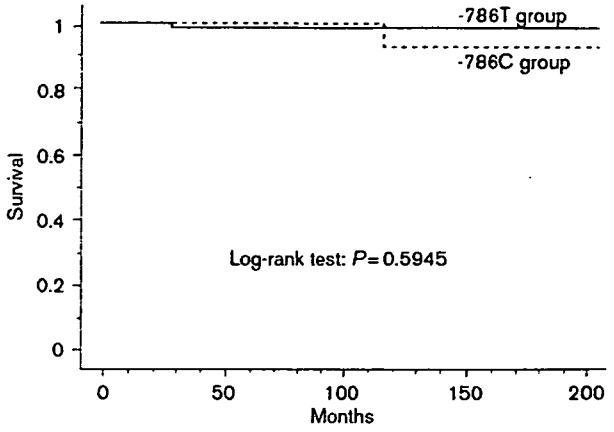
The rate of readmission due to coronary arterial disease was significantly higher in the -786C group than in the -786T group ($P = 0.0134$) [21.8% (12/55) and 8.9% (13/146), respectively, as is shown in Table 2]. The rate of readmission due to a reattack of coronary spasm was significantly higher in the -786C group than in the -786T group ($P = 0.0046$) [21.8% (12/55) and 7.5% (11/146), respectively].

Table 1 **Clinical characteristics of the study patients**

	-786T group ($n = 146$)	-786C group ($n = 55$)	*P* value
Age (years)	62 ± 11	62 ± 10	NS
Men/women	72/74	28/27	NS
Hypertension	44/146 (30%)	15/55 (27%)	NS
Cigarette smoking	78/146 (53%)	33/55 (60%)	NS
Diabetes mellitus	28/146 (19%)	11/55 (20%)	NS
Hypercholesterolemia	41/146 (28%)	13/55 (24%)	NS
BMI (kg/m^2)	23 ± 3	23 ± 3	NS
Pharmacotherapy			
CCB	137/146 (94%)	52/55 (95%)	NS
Nitrates	20/146 (14%)	4/55 (7%)	NS
ACE-I	12/146 (8%)	7/55 (13%)	NS
Antiplatelet	16/146 (11%)	6/55 (11%)	NS
HMG-CoA Reductase inhibitor	18/146 (12%)	2/55 (4%)	NS

Values are numbers of patients or mean ± SD.
ACE-I, angiotensin-converting enzyme inhibitor; BMI, body mass index; CCB, calcium channel blocker; NS, not significant.

Fig. 1



Kaplan–Meier survival curves of cumulative death rates in patients with coronary spasm divided into two groups according to the -786T/C polymorphism.

Table 2 **Readmission rates**

	-786T group ($n = 55$)	-786C group ($n = 146$)	*P* value
Reattack of coronary spasm	11/146 (7.5%)	12/55 (21.8%)	0.0046
Progression of coronary stenosis	2/146 (1.4%)	0/55 (0%)	0.3830
Total	13/146 (8.9%)	12/55 (21.8%)	0.0134

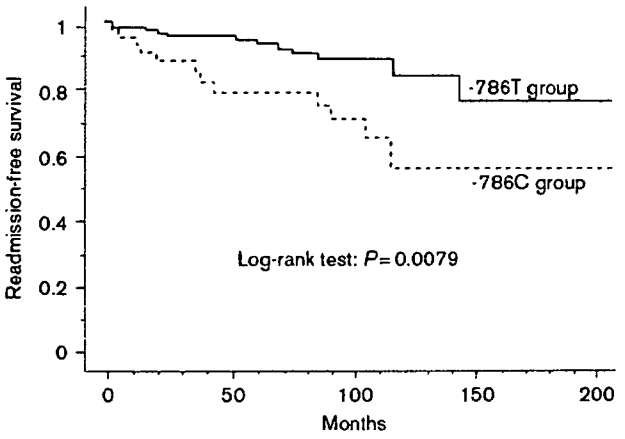
Values are numbers of patients.

Kaplan–Meier analysis revealed that the occurrence of readmission due to coronary arterial disease was significantly lower in the -786T group than in the -786C group ($P = 0.0079$) (Fig. 2). Further, the occurrence of readmission due to coronary spasm was significantly lower in the -786T group than in the -786C group ($P = 0.0032$) (Fig. 3).

Risk factors for readmission due to a reattack of coronary spasm

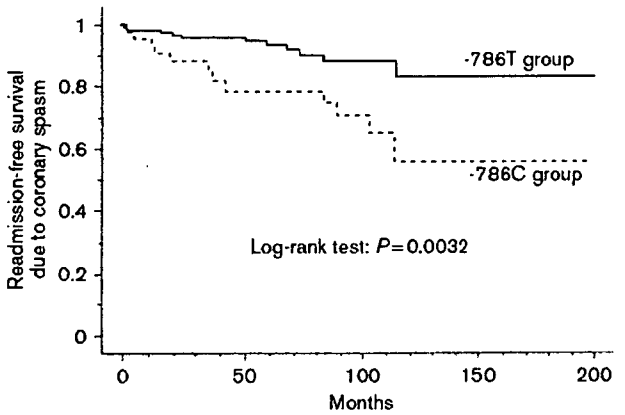
We compared the coronary risk factors, including clinical coronary risk factors and medications, between the readmission and the non-readmission groups as is shown in Table 3. Patients in the group readmission due to a reattack of coronary spasm were significantly younger than those in the non-readmission group ($P = 0.0044$). No significant differences were seen in the other coronary risk factors or in the medications between the readmission and the non-readmission groups.

Fig. 2



Kaplan–Meier survival curves of cumulative readmission rates in patients with coronary spasm divided into two groups according to the -786T/C polymorphism.

Fig. 3



Kaplan–Meier survival curves of cumulative readmission rates due to coronary spasm in patients with coronary spasm divided into two groups according to the -786T/C polymorphism.

Table 3 Clinical characteristics in readmission due to coronary spasm and non-readmission groups

	Non-readmission (n=176)	Readmission (n=23)	P value
Age (years)	63±10	56±12	0.0044
Men/women	86/90	12/11	NS
Hypertension	51/176 (29%)	8/23 (35%)	NS
Cigarette smoking	95/176 (54%)	14/23 (61%)	NS
Diabetes mellitus	37/176 (21%)	2/23 (9%)	NS
Hypercholesterolemia	47/176 (27%)	7/23 (30%)	NS
BMI (kg/m^2)	23±3	23±3	NS
Pharmacotherapy			NS
CCB	167/176 (95%)	22/23 (96%)	NS
Nitrates	20/176 (11%)	3/23 (13%)	NS
ACE-I	16/176 (9%)	2/23 (9%)	NS
Antiplatelets	22/176 (13%)	0/23 (0%)	NS
HMG-CoA Reductase inhibitor	16/176 (9%)	2/23 (9%)	NS

Values are numbers of patients or mean ± SD.
ACE-I, angiotensin-converting enzyme inhibitor; BMI, body mass index; CCB, calcium channel blocker; NS, not significant.

Table 4 Genotype frequencies of -786T/C polymorphism in the readmission due to coronary spasm and non-readmission group

	Non-readmission (n=176)	Readmission (n=23)	Odds ratio (95% CI)	P value
-786C/C genotype	4/176 (2%)	1/23 (4%)	-	-
	24%	52%		
-786C/T genotype	39/176 (22%)	11/23 (48%)		
-786T/T genotype	133/176 (76%)	11/23 (48%)	-	-
Additive	-	-	2.56 (1.26–5.21)	0.0097
Dominant	-	-	3.37 (1.39–8.20)	0.0072
Recessive	-	-	1.96 (0.21–18.29)	0.5569

Values are numbers of patients. CI, confidence interval.

The rate of readmission due to a reattack of coronary spasm in the patients with T/T, C/T, and C/C genotypes were 7.6% (11/144), 22.0% (11/50), and 20.0% (1/5), respectively. The incidence of patients with the -786T/C polymorphism was significantly higher in the group of the readmission due to a reattack of coronary spasm than in the non-readmission group (P = 0.0051). When the additive and dominant effect of the -786C allele was analyzed, the -786C allele was significantly associated with readmission due to coronary spasm as is shown in Table 4 (P = 0.0072, odds ratio: 3.37 in the dominant effect).

Subsequently, we performed multiple logistic analysis, with forward stepwise selection for the readmission group, using all the clinical risk factors and the -786T/C polymorphism as shown in Table 5. The analysis revealed that the most predictive independent risk factor for readmission due to a reattack of coronary spasm was the -786T/C polymorphism (P = 0.006, relative risk = 3.590). Other classical coronary risk factors were not significant predictive factors for readmission due to coronary spasm.

CCB and readmission due to coronary spasm

We analyzed compounds and doses of CCBs, which were administered to patients with coronary spasm as shown in Table 6. The incidence of readmission due to coronary spasm was significantly higher in patients who were administered two compounds of CCBs than in patients who were administered one compound of CCBs (P = 0.0016).

We listed nine patients who were administered two compounds of CCBs as shown in Table 7. The incidence of the -786T/C polymorphism in the readmission and non-readmission groups were 4/4(100%) and 1/5(20.0%),

Table 5 Multiple logistic analysis with forward stepwise selection for readmission due to coronary spasm

Variables	β	SE	Relative risk (95% CI)	*P* value
-786T/C Polymorphism	1.278	0.461	3.590 (1.455–8.853)	0.006
Age	−0.931	0.489	0.432 (0.151–1.028)	0.057
Constant	−2.141	0.346	0.117	0.000

CI, confidence interval.

Table 6 Compounds and doses of CCBs in the readmission due to coronary spasm and the non-readmission group

Compounds and doses (dose per day)	Readmission ($n=23$)	Non-readmission ($n=176$)	*P* value
Diltiazem (long acting type)			
200 mg	5/23 (22%)	67/176 (38%)	NS
100 mg	3/23 (13%)	32/176 (18%)	NS
Diltiazem (short acting type)			
240 mg	0/23 (0%)	2/176 (1.2%)	NS
180 mg	1/23 (4%)	3/176 (2%)	NS
150 mg	0/23 (0%)	1/176 (0.6%)	NS
120 mg	2/23 (8%)	8/176 (5%)	NS
90 mg	0/23 (0%)	5/176 (3%)	NS
60 mg	0/23 (0%)	1/176 (0.6%)	NS
Nisoldipine			
20 mg	1/23 (4%)	3/176 (1.7%)	NS
15 mg	1/23 (4%)	0/176 (0%)	NS
10 mg	4/23 (17%)	21/176 (12%)	NS
5 mg	0/23 (0%)	6/176 (3%)	NS
Nifedipine			
80 mg	1/23 (4%)	1/176 (0.6%)	NS
60 mg	0/23 (0%)	1/176 (0.6%)	NS
40 mg	0/23 (0%)	2/176 (1.2%)	NS
20 mg	0/23 (0%)	4/176 (2.2%)	NS
Benidipine			
8 mg	0/23 (0%)	2/176 (1.2%)	NS
Amlodipine			
5 mg	0/23 (0%)	2/176 (1%)	NS
2.5 mg	0/23 (0%)	1/176 (0.6%)	NS
Two CCB compounds	4/23 (17%)	5/176 (3%)	0.0016

Values are numbers of patients.
CCB, calcium channel blocker; NS, not significant.

respectively. In the patients with two compounds of CCBs, the incidence of the -786T/C polymorphism was significantly higher in the readmission group than in the non-readmission group ($P = 0.0164$).

Discussion

Prognosis and readmission in patients with coronary spasm

As with others, we have previously reported that the prognosis for coronary-spasm patients without coronary stenosis was relatively good [4,5]. We have also reported that the intake of CCB, multivessel spasm, and the severity of coronary artery disease are all significant independent predictors of survival for patients without myocardial infarction [4]. In this study population, patients with significant organic stenosis were excluded. All the study patients took CCB and/or nitrate during the follow-up period; there were no cardiac deaths during this period in this study. This result is in accordance with many previous studies. On the other hand, there were 25 study patients (12%) who were readmitted owing to coronary events: 92% of the readmissions were due to a

Table 7 Characteristics of patients who were administered CCB two compounds

Patient no.	Age	Sex	-786T/C genotype	Hypertension	Cigarette smoking	Diabetes mellitus	Hypercholesterolemia
Non-readmission							
1	53	M	T/T	−	+	−	−
2	64	M	T/T	+	+	−	−
3	66	F	T/T	+	+	−	−
4	68	M	T/T	−	+	−	−
5	74	F	C/T	−	−	−	−
Readmission							
6	40	M	C/T	−	+	−	+
7	66	M	C/T	+	−	−	+
8	67	M	C/C	−	+	−	−
9	76	F	C/T	−	−	−	−

F, female; M, male.

reattack of coronary spasm. Sueda *et al.* [6] recently suggested that 42% of the patients with pure coronary spastic angina had a reattack of coronary spastic angina during the administration of CCB. The outcomes in Sueda's coronary-spasm patients are in general agreement with our results.

In this study, the readmitted patients were significantly younger than the non-readmitted patients. We therefore suggest that the disease-activity level of coronary spastic angina is higher in younger patients than in the older ones. Younger patients may be more susceptible to getting coronary spastic angina as a result of coronary spasm. Further study is needed to clarify whether there is a significant difference in the disease activity level of coronary spasm between younger and older patients.

Readmission and the endothelial nitric oxide synthase polymorphism

The incidence of readmission was significantly higher in the -786C group than in the -786T group. The -786T/C polymorphism was significantly associated with readmission due to coronary arterial events. Multiple logistic regression analysis revealed that the -786C allele was the most predictive independent risk factor for readmission in patients with coronary spasm. It is possible that the -786T/C polymorphism reduces eNOS gene expression in the coronary arterial endothelial cells, and thereby predisposes the patients to recurrent coronary spasm even if the patients have taken CCB.

Readmission due to acute myocardial infarction

In this study, there was a patient who had acute myocardial infarction during the follow-up period. Significantly, he had the -786C/C genotype. As this patient had no coronary stenosis, myocardial infarction was most probably caused by coronary spasm. Although this patient had taken CCB, he had an incident of acute myocardial infarction. Thus, it is suggested that the -786T/C polymorphism predisposes patients to have myocardial infarction due to coronary spasm, even while being administered CCB.

Treatment of coronary spasm in patients with the -786C allele

Strict follow-up is necessary in coronary spasm patients with the -786C allele to monitor for reattack and/or acute myocardial infarction. Additional medications like long-acting CCB and/or nitrite and/or other antiangina agents, should be prescribed for coronary-spasm patients with the -786C allele; however, there was no significant difference with the readmission rates between those with long-acting CCB and those with short-acting CCB in this study.

It was revealed that the incidence of readmission due to a reattack of coronary spasm was significantly higher in patients who were administered two CCB compounds than in patients who were administered one CCB compound; moreover, all the readmission patients with two CCB compounds carried the -786C allele. We usually medicate a patient who has severe and/or medicine-resistant coronary spasm, with a combination of two CCB compounds. These results indicate that a combination of two CCBs is effective in patients without the -786T/C polymorphism, but is not effective in the severe coronary-spasm patients with the -786T/C polymorphism. Additional medications such as HMG-CoA reductase inhibitor, ACE-I, or angiotensin II type 1 receptor blocker are possibly needed in the patients with severe coronary spasm with the -786T/C polymorphism.

We recently reported that fluvastatin, an HMG-CoA reductase inhibitor, increases the transcriptional activity of the eNOS gene in the endothelial cells, especially in those with the -786C allele [16]. We therefore suggested that fluvastatin possibly prevents reattacks of coronary spasm, especially in patients with the -786C allele. It was reported that ACE-I or angiotensin II type 1 receptor blocker induces eNOS bioactivity; therefore, those drugs possibly are effective in the patients with coronary spasm [17,18]. A further clinical study is, however, necessary to verify this.

Study limitation

It has been previously suggested that the pathogenesis of coronary-artery spasm is closely related to the process of atherosclerosis [19,20]; however, in this study, there were relatively few patients who were readmitted owing to a progression of coronary stenosis. A longer follow-up period might be necessary to elucidate whether the incidence of patients with coronary stenosis will increase. Also, there may be racial, and/or environmental, and/or lipid-profile differences in the pathogenesis of atherosclerosis. A study on a larger follow-up population will be beneficial to further elucidate this topic.

The -786T/C polymorphism has only a modulatory role in the development and the recurrence of coronary spasms, which possibly also occur in some patients with angiographically detectable stenoses; therefore, further study will be necessary to elucidate other predictive factors for coronary spasm.

Conclusion

Considering the strong association of the C allele of the eNOS gene -786T/C polymorphism with the prognosis for coronary spastic angina patients, we conclude that the -786T/C polymorphism is an independent predictor for readmission in patients with coronary spasm. The -786T/C polymorphism of the eNOS gene is an important factor to consider in determining the clinical course of coronary spastic angina. A strict follow-up is necessary in coronary-spasm patients with the -786C allele. There is no simple test to measure for the -786T/C polymorphism at present; however, if a test is developed in the near future, it will be valuable for treating patients with coronary spasm, especially those with -786T/C polymorphism.

Acknowledgement

No conflicts of interest declared.

References

1 Hillis LD, Braunwald E. Coronary-artery spasm. *N Engl J Med* 1978; 299:695–702.
2 Yasue H, Omote S, Takizawa A, Nagao M. Coronary arterial spasm in ischemic heart disease and its pathogenesis. A review. *Circ Res* 1983; 52 (Suppl I):147–152.
3 Maseri A, Davies G, Hackett D, Kaski JC. Coronary artery spasm and vasoconstriction. The case for a distinction. *Circulation* 1990; 81: 1983–1991.
4 Yasue H, Takizawa A, Nagao M, Nishida S, Horie M, Kubota J, *et al.* Long-term prognosis for patients with variant angina and influential factors. *Circulation.* 1988; 78:1–9.
5 Waters DD, Miller DD, Szlachcic J, Bouchard A, Methe M, Kreeft J, *et al.* Factors influencing the long-term prognosis of treated patients with variant angina. *Circulation* 1983; 68:258–265.
6 Sueda S, Kohno H, Fukuda H, Watanabe K, Ochi N, Kawada H, *et al.* Limitations of medical therapy in patients with pure coronary spastic angina. *Chest* 2003; 123:380–386.
7 Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, *et al.* $T^{-786}\rightarrow C$ mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation* 1999; 99:2864–2870.
8 Miyamoto Y, Saito Y, Nakayama M, Shimasaki Y, Yoshimura T, Yoshimura M, *et al.* Replication protein A1 reduced transcription of the endothelial nitric oxide synthase gene containing a -786T→C mutation associated with coronary spastic angina. *Hum Mol Genet* 2000; 9:2629–2637.
9 Yoshimura M, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Nakamura S, *et al.* A T-786→C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene and coronary arterial vasomotility. *Am J Cardiol* 2000; 85:710–714.
10 Nakayama M, Yoshimura M, Sakamoto T, Shimasaki Y, Nakamura S, Ito T, *et al.* Synergistic interaction of T-786→C polymorphism in the endothelial nitric oxide synthase gene and smoking for an enhanced risk for coronary spasm. *Pharmacogenetics* 2003; 13:683–688.
11 Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Ogawa H, Kugiyama K, *et al.* $T^{-786}\rightarrow C$ mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with myocardial infarction, especially without coronary organic stenosis. *Am J Cardiol* 2000; 86:628–634.
12 Yasue H, Horio Y, Nakamura N, Fujii H, Imoto N, Sonoda R, *et al.* Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. *Circulation* 1986; 74:955–963.

13 Okumura K, Yasue H, Horio Y, Takaoka K, Matsuyama K, Kugiyama K, *et al.* Multivessel coronary spasm in patients with variant angina: a study with intracoronary injection of acetylcholine. *Circulation* 1988; 77:535–542.
14 Horio Y, Yasue H, Okumura K, Takaoka K, Matsuyama K, Goto K, *et al.* Effects of intracoronary injection of acetylcholine on coronary arterial hemodynamics and diameter. *Am J Cardiol* 1988; 62:887–891.
15 Okumura K, Yasue H, Matsuyama K, Goto K, Miyagi H, Ogawa H, *et al.* Sensitivity and specificity of intracoronary injection of acetylcholine for the induction of coronary artery spasm. *J Am Coll Cardiol* 1988; 12:883–888.
16 Abe K, Nakayama M, Yoshimura M, Nakamura S, Ito T, Yamamuro M, *et al.* Increase in the transcriptional activity of the endothelial nitric oxide synthase gene with fluvastatin: a relation with the-786T>C polymorphism. *Pharmacogenet Genom* 2005; 15:329–336.
17 Zhuo JL, Mendelsohn FA, Ohishi M. Perindopril alters vascular angiotensin-converting enzyme, AT (1) receptor, and nitric oxide synthase expression in patients with coronary heart disease. *Hypertension* 2002; 39: 634–638.
18 Landmesser U, Drexler H. Effect of angiotensin II type 1 receptor antagonism on endothelial function: role of bradykinin and nitric oxide. *J Hypertens Suppl* 2006; 24:S39–S43.
19 Ganz P, Alexander RW. New insights into the cellular mechanism of coronary vasospasm. *Am J Cardiol* 1985; 56:11E–15E.
20 Bugiardinini R, Manfrini O, Pizzi C, Fontana F, Morgagni G. Endothelial function predicts future development of coronary artery disease: a study of women with chest pain and normal coronary angiogram. *Circulation* 2004; 109:2518–2523.

Association of Dietary Intake of Soy, Beans, and Isoflavones With Risk of Cerebral and Myocardial Infarctions in Japanese Populations

The Japan Public Health Center–Based (JPHC) Study Cohort I

Yoshihiro Kokubo, MD; Hiroyasu Iso, MD; Junko Ishihara, PhD; Katsutoshi Okada, MD; Manami Inoue, MD; Shoichiro Tsugane, MD; for the JPHC Study Group

Background—Soy and isoflavones have been proposed to reduce the risk of cardiovascular risk factors, but their potential as preventatives for cardiovascular disease remains uncertain. We investigated the association of soy and isoflavone intake with risk of cerebral and myocardial infarctions (CI and MI).

Methods and Results—To examine the association of soy and isoflavone intake with the risk of CI and MI, we studied 40 462 Japanese (40 to 59 years old, without cardiovascular disease or cancer at baseline). They completed a food-frequency questionnaire (1990–1992) and received follow-up to 2002. After 503 998 person-years of follow-up, we documented incidence of CI (n=587) and MI (n=308) and of mortality for CI and MI combined (n=232). For women, the multivariable hazard ratios and 95% confidence limits for soy intake ≥5 times per week versus 0 to 2 times per week were 0.64 (0.43 to 0.95) for risk of CI, 0.55 (0.26 to 1.09) for risk of MI, and 0.31 (0.13 to 0.74) for cardiovascular disease mortality. Similar but weaker inverse associations were observed between intake of miso soup and beans and risk of cardiovascular disease mortality. The multivariable hazard ratios for the highest versus the lowest quintiles of isoflavones in women were 0.35 (0.21 to 0.59) for CI, 0.37 (0.14 to 0.98) for MI, and 0.87 (0.29 to 2.52) for cardiovascular disease mortality. An inverse association between isoflavone intake and risk of CI and MI was observed primarily among postmenopausal women. No significant association of dietary intake of soy, miso soup, and beans and isoflavones with CI or MI was present in men.

Conclusions—High isoflavone intake was associated with reduced risk of CI and MI in Japanese women. The risk reduction was pronounced for postmenopausal women. (***Circulation.* 2007;116:2553-2562.**)

Key Words: soy foods ■ isoflavones ■ cerebral infarction ■ myocardial infarction ■ follow-up studies

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in developed and developing countries.[1] Certain diets exhibit a major ability to modify risk factors for CVD.[2] Evidence is increasing that the consumption of soy protein instead of animal protein lowers blood cholesterol levels and may lower the risk of CVD. A meta-analysis showed that the substitution of soy protein for animal protein significantly lowered total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides without affecting high-density lipoprotein cholesterol.[3] Since then, well-controlled studies of soy protein and soy-derived isoflavones have demonstrated that the impact of soy protein consumption on LDL cholesterol levels was small.[4,5] Dietary soy may be beneficial to cardiovascular health because of its high polyunsaturated fat, fiber, vitamin, and mineral content combined with its low saturated fat content.[6]

Clinical Perspective p 2562

Many studies of soy intake have reported an impact on cardiovascular risk factors, notably on intermediate end points such as hyperlipidemia.[3–5,7,8] However, only a few studies exist on the impact of soy intake on clinical end points such as CVD. A high dietary intake of isoflavones was associated with lower aortic stiffness in postmenopausal

Received December 12, 2006; accepted September 14, 2007.

From the Department of Preventive Cardiology (Y.K.), National Cardiovascular Center, Osaka, Japan; Public Health (H.I.), Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan; Epidemiology and Prevention Division (J.I., M.I., S.T.), Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan; and Academic General Health Center (K.O.), University of Ehime, Ehime, Japan.

The online-only Data Supplement, consisting of tables, is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.106.683755/DC1.

Correspondence to Yoshihiro Kokubo, MD, PhD, Department of Preventive Cardiology, National Cardiovascular Center, 5-7-1, Fujishiro-dai, Suita, Osaka, 565-8565 Japan; (E-mail ykokubo@hsp.ncvc.go.jp). Reprint requests to Shoichiro Tsugane, MD, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; (E-mail stsugane@ncc.go.jp).

Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.106.683755

women.[9] A prospective study of Japanese men and women, in whom average soy intake has been 10 to 70 times higher than in Western people,[10,11] showed that soy intake was weakly and inversely associated with total mortality but not mortality due to CVD.[12] In that study, the inverse association between dietary isoflavones and total mortality did not reach statistical significance after adjustment for cardiovascular risk factors.[12] A prospective study of Dutch women did not support the idea that dietary isoflavones lowered the risk of CVD.[11] The quantity of isoflavones consumed by Dutch women, however, was quite small.[11] Furthermore, no prospective study has examined a potential protective effect of isoflavone intake as an estrogen agonist on risk of CVD among postmenopausal women who have reduced blood estrogen concentrations.[13] We hypothesize that the risk of cerebral infarction (CI) and myocardial infarction (MI) for postmenopausal Japanese women may be reduced through exposure to a large quantity of isoflavones, because estrogen receptors are not occupied with plasma estradiol in postmenopausal women.

Methods

Study Cohort

Cohort I of the Japan Public Health Center–Based (JPHC) Study was a population-based sample of 27 063 men and 27 435 women born between 1930 and 1949 (between 40 and 59 years old); it was registered in 14 administrative districts supervised by 4 public health center (PHC) areas on January 1, 1990. The present study included 12 291 subjects from Ninohe city and Karumai town in the Ninohe PHC area of Iwate prefecture; 15 782 subjects from Yokote city and Omonogawa town in the Yokote PHC area of Akita; 12 219 subjects from 8 districts of Minami-Saku county in the Saku PHC area of Nagano; and 14 206 subjects from Gushikawa city and Onna village in the Ishikawa PHC area of Okinawa.[14] We excluded subjects who reported MIs, angina pectoris, strokes, or cancer before the study, which left a total of 40 462 men and women for the present analysis. The study was approved by the Human Ethics Review Committees of the National Cancer Center and the University of Tsukuba. Informed consent was obtained from each participant implicitly when they completed the baseline questionnaire in which the study purpose and follow-up were described.

Baseline Survey

A self-administered questionnaire was distributed to all registered noninstitutional residents in 1990, asking them to report on their demographic characteristics, medical history, smoking habits, drinking habits, and diet. Of these, 40 462 men and women (74.2%) returned their questionnaires between January 1990 and May 1992, primarily between February and October 1990.

The 1990 food-frequency questionnaire included 44 foods with 3 questions to assess soy, bean, and miso consumption. The 1995 follow-up questionnaire covered 147 foods with 8 questions on soy products.[15] Each participant was asked how often he or she had consumed soy, miso soup, and beans on average during the previous month. Portion size for each food was specified in the 1995 questionnaire but not in the 1990 questionnaire. Responses for each food item ranged from "rarely" to "1 to 2 days per week," "3 to 4 days per week," and "almost every day" in the 1990 questionnaire. For the 1995 questionnaire, possible responses were "rarely," "1 to 3 times per month," "1 to 2 times per week," "3 to 4 times per week," "5 to 6 times per week," "once per day," "2 to 3 times per day," "4 to 6 times per day," and "7 or more times per day." Study participants who answered "almost daily or more frequently" were asked an additional question about the average number of bowls of miso soup consumed per day.

Isoflavone intake was calculated from these 2 questions with a portion size of 100 mL for miso soup (10 g of miso paste, 2.9 mg of genistein, and 2.1 mg of daidzein) and 60 g for soy foods (18.4 mg of genistein and 10.9 mg of daidzein).[16] Total isoflavone intake was defined for the present study as the sum of genistein and daidzein intake. Portion size and isoflavone contents were validated through a study in which 247 subjects provided 28-day dietary records accompanied by blood and urine samples.[16,17] The correlation coefficients between these 2 questionnaires and participants' dietary records were 0.59 for genistein and 0.60 for daidzein, and the correlation coefficients between repeated measurements were >0.7.[16] Estimated mean intakes were ≈30% higher as assessed by the questionnaires (mean±SD 18.3±13.1 mg for daidzein and 31.4±24 mg for genistein) than by the dietary records (mean±SD 14.5±6.6 mg for daidzein and 23.4±10.5 mg for genistein). The associations for miso soup consumption frequency, soy food consumption frequency, and estimated isoflavone intake between the 2 questionnaires administered 5 years apart were 0.70, 0.53, and 0.61, respectively.[18] Information on the consumption of isoflavone supplements was not requested in the 1990 questionnaire. At that time, isoflavone supplements were assumed to be used infrequently. Menopausal status was questioned in the baseline questionnaire in the form of alternatives: premenopause, postmenopause, or surgical menopause.

Stroke and MI: Confirmation and Classification of Stroke Subtypes

A total of 30 hospitals in the 4 PHC areas were capable of performing computed tomography scans and/or magnetic resonance imaging. By region, that included 10 hospitals in the Ninohe PHC area, 4 in the Yokote PHC area, 3 in the Saku PHC area, and 14 in the Ishikawa PHC area. All were major hospitals at which acute stroke and MI cases would be admitted. Medical records were reviewed by registered hospital workers, PHC physicians, or research physicians who did not have access to lifestyle data. Stroke and MI events were registered if they occurred after the date on which the baseline questionnaire was received and before January 1, 2003. To complete the surveillance for fatal stroke and MI, we also conducted a systematic search for death certificates. For all fatal strokes and MIs listed on the death certificates but that had not been registered, medical records in registered hospitals were reviewed by hospital workers, PHC physicians, or research physicians. We regarded these fatal CIs and MIs as probable CIs and MIs, respectively. Details on the surveillance procedures have been described elsewhere.[19,20]

Strokes were confirmed according to National Survey of Stroke criteria. These criteria require the rapid onset of a constellation of neurological deficits that lasts at least 24 hours (or until death). For each stroke subtype (ie, ischemic stroke [thrombotic or embolic stroke], intracerebral hemorrhage, and subarachnoid hemorrhage), a definite diagnosis was established on the basis of the examination of computed tomography scans, magnetic resonance imaging results, or autopsies.[21] MI was confirmed in the medical records according to the criteria of the MONICA project (MONitoring of trends and determinants In CArdiovascular disease), which requires evidence from ECGs, cardiac enzymes, and/or autopsy.[22] Information on the underlying cause of death was obtained by checking against death certificate files with permission to confirm mortality due to CI and MI (ischemic CVD) according to the International Classification of Death, 10th Revision codes I21–I23 and I46 for MI and I60–I61, I63, and I693 for CI.

Changes in residential status were identified through the residential registry in each area. Subjects who moved from their original residence (2% of total participants) were censored at that time.

Statistical Analysis

ANOVAs and χ^2 tests were used to compare mean values and frequencies according to the frequency of dietary soy intake by sex. Time-dependent Cox proportional hazards regression models were used for the association of soy, miso soup, beans, and isoflavones with CI and MI separately for men and women during the 13-year follow-up (from 1990 until the end of 2002). For each subject, person-months of follow-up were calculated from January 1, 1990, to

Table 1. Sex-Specific Baseline Distributions of CVD Risk Factors and Selected Dietary Variables in a Cohort of 40 462 Men and Women According to the Frequency of Dietary Soy Intake at the Baseline Surveillance

	Frequency of Soy Intake, Days per Week							
	Men				Women			
	0–2	3–4	≥5	P*	0–2	3–4	≥5	P*
No. of subjects	5186	7367	6913	...	3981	7432	9571	...
Age at baseline, y	48.4	49.2	50.0	<0.001	48.8	49.2	50.0	<0.001
Mean body mass index, kg/m²	23.6	23.6	23.4	<0.001	23.6	23.5	23.5	0.847
Current smoker, %	77.1	79.9	74.6	0.006	10.8	7.6	6.0	<0.001
Current drinker, %	77.4	80.6	80.4	<0.001	23.7	24.3	22.9	0.085
College or higher education, %	15.0	14.6	12.9	0.001	11.5	12.7	11.1	0.004
Sports at leisure time ≥1 time per week, %	17.7	17.9	16.9	0.256	12.5	14.7	14.6	0.002
History of hypertension, %	11.6	14.7	16.8	<0.001	12.1	12.3	14.5	<0.001
History of diabetes mellitus, %	4.2	4.7	6.3	<0.001	2.4	2.1	2.3	0.623
Menopausal status, %								
Natural menopause	...	...	...	...	40.7	42.7	48.2	...
Surgical menopause	...	...	...	...	7.4	8.0	9.4	<0.001
Mean daily total energy, kcal	1910	2132	2344	<0.001	1227	1363	1491	<0.001
Mean daily intake								
Rice, g/d	293.8	316.3	336.0	<0.001	164.0	171.4	181.9	<0.001
Vegetables, g/d	184.3	229.2	281.0	<0.001	201.5	244.2	287.4	<0.001
Fruits, g/d	76.4	91.4	111.4	<0.001	105.0	127.1	143.2	<0.001
Fish, g/d	38.2	49.9	65.8	<0.001	31.1	40.9	54.2	<0.001
Potassium, mg/d	1800	2121	2501	<0.001	1665	1974	2277	<0.001
Calcium, mg/d	380.3	473.8	596.4	<0.001	373.9	468.7	564.9	<0.001
Carbohydrate, g/d	307.5	333.4	358.2	<0.001	202.0	216.9	231.2	<0.001
Polyunsaturated fatty acid, g/d	5.2	6.6	8.4	<0.001	4.5	5.8	7.3	<0.001
Saturated fatty acid, g/d	10.3	11.9	13.6	<0.001	9.4	11.0	12.1	<0.001
Fiber, g/d	7.7	9.2	11.0	<0.001	7.2	8.7	10.3	<0.001
Isoflavones, mg/d	13.8	24.5	41.5	<0.001	12.1	21.8	37.6	<0.001
Frequencies								
Beans 5–7 times per week, %	5.1	10.0	12.5	<0.001	6.7	11.1	14.4	<0.001
Miso soup 3 cups per day, %	26.1	32.4	46.2	<0.001	17.8	21.5	33.0	<0.001

*ANOVA or χ^2 tests were performed.

whichever came first: the first end point, death, emigration, or December 31, 2002.

The time-dependent Cox proportional hazards regression was fitted on the grouped or categorized consumption (reference group was the lowest consumption level) after adjustment for age and other potential confounding factors, which were the baseline age values (in 5-year categories); sex; smoking status (never, ex-smoker, and current smoker of 1 to 19 or ≥20 cigarettes per day); alcohol intake (nondrinkers [<1 day per month], occasional drinkers [1 to 3 days per month], or weekly ethanol intake of 1 to 149 g, 150 to 299 g, 300 to 449 g, and ≥450 g); body mass index (in quintiles); history of hypertension or diabetes mellitus (yes or no); medication use for hypercholesterolemia (yes or no); quintiles of energy-adjusted dietary intake of fruits, vegetables, and salt; education levels (junior high school, high school, and college or higher); leisure time spent engaged in sports (<1 day per month, 1 to 3 days per month, and ≥1 day per week); and nearest PHC. We updated the quintiles for dietary intake and the confounding factors (except for age, sex, education level, and PHC) using the 5-year follow-up questionnaire, to which 80% of the baseline participants responded.[20] Trend tests were conducted by assignment for frequencies of soy, bean, and miso soup intake and estimated values for isoflavones consumption to test the significance of these variables. We excluded women with surgical menopause from the stratified analysis by menopausal status because the presence or absence of estrogen exposure was uncertain. In addition, isoflavone and menopause (or sex) interactions were tested with use of a cross-product term of isoflavone intake with sex or menopausal status. All statistical analyses were conducted with the SAS statistical package (release version 8.2, SAS Institute Inc, Cary, NC).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

During a follow-up period that averaged 12.5 years, 1230 strokes were documented, of which 1137 were confirmed through imaging or autopsy. These results for strokes comprised 587 CIs, 437 intracerebral hemorrhages, and 206 subarachnoid hemorrhages. In addition, 308 MIs were documented, of which 232 were definite MI on the basis of clinical or autopsy evidence, and 23 were cases of sudden cardiac death.