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

	No. of MetS Components	Population at risk, n	No. of Events	Multivariate-Adjusted HR (95% CI)	*P* for Trend
Cardiovascular disease					
Central obesity (−)	0	509	31	1 (referent)	
	1	563	64	1.32 (0.85–2.04)	
	2	311	32	1.07 (0.64–1.78)	
	3	91	13	1.39 (0.71–2.73)	0.58
Central obesity (+)	0	259	10	1 (referent)	
	1	354	25	1.13 (0.53–2.40)	
	2	261	47	2.47 (1.21–5.04)	
	3	104	24	3.09 (1.40–6.79)	<0.001
Ischemic stroke					
Central obesity (−)	0	509	19	1 (referent)	
	1	563	39	1.37 (0.78–2.40)	
	2	311	16	1.02 (0.51–2.01)	
	3	91	4	0.84 (0.28–2.52)	0.76
Central obesity (+)	0	259	6	1 (referent)	
	1	354	15	1.31 (0.50–3.42)	
	2	261	30	2.95 (1.18–7.34)	
	3	104	16	3.99 (1.47–10.84)	<0.001
Coronary heart disease					
Central obesity (−)	0	509	16	1 (referent)	
	1	563	31	1.13 (0.61–2.09)	
	2	311	16	0.84 (0.41–1.73)	
	3	91	10	1.76 (0.77–4.02)	0.56
Central obesity (+)	0	259	4	1 (referent)	
	1	354	11	1.10 (0.34–3.57)	
	2	261	24	2.85 (0.95–8.56)	
	3	104	13	3.50 (1.07–11.49)	0.001

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

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

In the present study, the risk of CVD occurrence was higher for the modified Japanese MetS criteria than for the IDF criteria despite the identical condition regarding central obesity. One possibility for this is that the definitions of hyperglycemia and dyslipidemia are different between the 2 sets of MetS criteria. In our subjects, the definitions of hyperglycemia and dyslipidemia in Japanese criteria were superior to those in the other criteria for the prediction of the development of CVD (data not shown). These facts may explain why the modified Japanese criteria had a higher HR. Further studies are needed to optimize the cutoff points of fasting plasma glucose and lipid levels for predicting cardiovascular events.

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

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

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

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

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

‡$P<0.01$ versus DM (+)+MetS (−) or HT (+)+MetS (−).

DM indicates diabetes; HT, hypertension.

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

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

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

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

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

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

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

Acknowledgments

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

Sources of Funding

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

Disclosures

None.

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Hypertension Research (2009) 32, 1119–1122

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ORIGINAL ARTICLE

Development and validation of a cardiovascular risk prediction model for Japanese: the Hisayama study

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The objective of this paper is to develop a new risk prediction model of cardiovascular disease and to validate its performance in a general population of Japanese. The Hisayama study is a population-based prospective cohort study. A total of 2634 participants aged 40 years or older were followed up for 14 years for incident cardiovascular disease (stroke and coronary heart disease (myocardial infarction, coronary revascularization and sudden cardiac death)). We used data among a random two-thirds (the derivation cohort, n=1756) to develop a new risk prediction model that was then tested to compare observed and predicted outcomes in the remaining one-third (the validation cohort, n=878). A multivariable cardiovascular risk prediction model was developed that incorporated age, sex, systolic blood pressure, diabetes, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and smoking. We assessed the performance of the model for predicting individual cardiovascular event among the validation cohort. The risk prediction model demonstrated good discrimination (c-statistic=0.81; 95% confidence interval, 0.77 to 0.86) and calibration (Hosmer–Lemeshow χ^2-statistic=6.46; P=0.60). A simple risk score sheet based on the cardiovascular risk prediction model was also presented. We developed and validated a new cardiovascular risk prediction model in a general population of Japanese. The risk prediction model would provide a useful guide to estimate absolute risk of cardiovascular disease and to treat individual risk factors.
Hypertension Research (2009) **32,** 1119–1122; doi:10.1038/hr.2009.161; published online 18 September 2009

Keywords: cardiovascular disease; epidemiology; risk factors; risk prediction model

INTRODUCTION

Cardiovascular disease is estimated to be one of the leading causes of death in Japan, as well as other countries around the world, placing a burden on the community.[1] Although the incidence and mortality of cardiovascular disease in Japan have declined over several decades, the risk of cardiovascular events remains high.[2] Additional protection will require an effective strategy for prevention of cardiovascular disease. Among a number of cardiovascular prevention strategies, high-risk approaches are likely to be one of the most effective strategies for prevention of cardiovascular disease.[3] To identify individuals at high risk of cardiovascular disease, a number of risk prediction tools have been developed.[4–15] However, currently available risk prediction tools of cardiovascular disease are derived mainly from studies carried out in Western populations and few risk prediction tools are developed for general Japanese populations. The objective of this paper is to develop a new cardiovascular risk prediction model and to validate its performance in a general population of Japanese.

METHODS

Study design and participants

Since 1961, we have been conducting a long-term prospective cohort study of cardiovascular disease in the town of Hisayama, a suburb of Fukuoka City in Southern Japan.[2,16,17] In 1988, a screening survey for this study was performed in the town. A total of 2742 residents aged 40 years or older (80.9% of the total population of this age group) consented to participate in the examination.[2,18–21] After the exclusion of 106 subjects with a history of cardiovascular disease and two subjects who died during the examination, the remaining 2634 individuals were enrolled in this study.

The ethics committee of Kyushu University approved this study, participants provided written informed consent, and the procedures followed were in accordance with national guidelines.

Follow-up survey

The subjects were followed up prospectively from December 1988 to November 2002 by repeated health examinations. A detailed description of the study methods has been published previously.[2,18–21] In brief, the health status of any subject who had not undergone a regular examination or who had moved out

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Received 29 June 2009; revised 13 August 2009; accepted 20 August 2009; published online 18 September 2009

of town was checked yearly by mail or telephone. We also established a daily monitoring system among the study team and local physicians or members of the town's Health and Welfare Office. When a subject died, an autopsy was performed at the Departments of Pathology of Kyushu University. During the follow-up period, 577 subjects died, of whom 438 (75.9%) underwent autopsy. Only one participant was lost to follow-up.

Outcomes

The primary outcome of the present analysis was cardiovascular disease. Cardiovascular disease was defined as first-ever development of coronary heart disease or stroke. The criteria for a diagnosis of coronary heart disease included first-ever acute myocardial infarction, silent myocardial infarction, sudden cardiac death within 1 h after the onset of acute illness, or coronary artery disease followed by coronary artery bypass surgery or angioplasty.[2] Acute myocardial infarction was diagnosed when a subject met at least two of the following criteria: (1) typical symptoms, including prolonged severe anterior chest pain; (2) abnormal cardiac enzymes more than twice the upper limit of the normal range; (3) evolving diagnostic electrocardiographic changes; and (4) morphological changes, including local asynergy of cardiac wall motion on echocardiography, persistent perfusion defect on cardiac scintigraphy, or myocardial necrosis or scars >1 cm long accompanied by coronary atherosclerosis at autopsy. Silent myocardial infarction was defined as myocardial scarring without any historical indication of clinical symptoms or abnormal cardiac enzyme changes, and was detected by electrocardiography, echocardiography, cardiac scintigraphy or autopsy. Stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for >24 h. The diagnosis of stroke and the determination of its pathological type were based on the clinical history, neurological examination and all available clinical data, including brain CT/MRI and autopsy findings.[2]

Risk factors

Sitting blood pressure was measured three times at the right upper arm using a sphygmomanometer after 5 min of rest; an average of three measurements was used for the analysis. Plasma glucose levels were determined by the glucose-oxidase method, and diabetes was defined by a 75 g oral glucose tolerance test and by fasting ($\geqslant 7.0\,mmol\,l^{-1}$) or postprandial ($\geqslant 11.1\,mmol\,l^{-1}$) blood glucose levels or by the use of hypoglycemic agents. Total cholesterol, high-density lipoprotein cholesterol and triglyceride levels were determined enzymatically. Low-density lipoprotein (LDL) cholesterol level was estimated using the Friedewald formula.[22] Information on smoking habits was obtained using a standard questionnaire and was classified as either current or not.

Statistical analysis

Two-thirds of the study participants (n=1756) were randomly assigned to a risk prediction model derivation cohort and the remaining one-third (n=878) were reserved as an independent validation cohort using random digits generated by the Mersenne Twister method.[23] Among subjects allocated to the derivation cohort, a new risk prediction model was developed using Cox's proportional hazards model. Covariates included in Cox's proportional hazards model were age, sex, systolic blood pressure, diabetes, LDL cholesterol, high-density lipoprotein cholesterol and smoking habits that were traditional risk factors for cardiovascular disease established in the Hisayama study.[16,17,20,21] The performance of the risk prediction model was then tested among subjects allocated to the validation cohort. Ability of the risk prediction model to discriminate persons who experience a cardiovascular disease from those who do not were evaluated using c-statistic,[24] and calibration of the risk prediction model was evaluated using a Hosmer–Lemeshow χ^2-statistic with 8 d.f. The cardiovascular risk prediction model was translated into a risk score sheet using methods developed in the Framingham Heart Study.[25] To facilitate easier understanding of the concept of risk, 'vascular age' was also included in the risk score sheet. An individual's vascular age was calculated as the age of a person with the same predicted risk but with all other risk factor levels in optimal ranges.[10] All analyses were performed using the SAS software package (SAS Institute, Cary, NC, USA).

Table 1 Baseline characteristics in the derivation and the validation cohorts

	Derivation cohort (n=1756)	*Validation cohort (n=878)*
Age, years	59 (12)	59 (12)
Men	43%	40%
Systolic blood pressure, mm Hg	134 (21)	133 (22)
Diastolic blood pressure, mm Hg	78 (12)	77 (11)
Diabetes	11%	13%
LDL cholesterol, mg per 100 ml	131 (43)	133 (41)
HDL cholesterol, mg per 100 ml	50 (12)	50 (12)
Current smoker	24%	27%

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Values are means (s.d.) or frequencies.
SI conversion factors: to convert LDL and HDL cholesterol to millimoles per liter, multiply by 0.0259.

Table 2 Regression coefficients and hazard ratios for the cardiovascular risk prediction model in the derivation cohort

	β	*Hazard ratio*	*95% CI*
Age, years	0.05775	1.059	1.046–1.073
Men	0.55569	1.743	1.264–2.404
Systolic blood pressure, mm Hg	0.01701	1.017	1.011–1.023
Diabetes	0.51977	1.682	1.193–2.370
LDL cholesterol, mg per 100 ml	0.00257	1.003	0.999–1.006
HDL cholesterol, mg per 100 ml	−0.01182	0.988	0.977–1.000
Current smoker	0.35287	1.423	1.024–1.978

Abbreviations: 95% CI, 95% confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
SI conversion factors: to convert LDL and HDL cholesterol to millimoles per liter, multiply by 0.0259.

RESULTS

The baseline characteristics of the subjects allocated to the derivation cohort and those to the validation cohort are shown in Table 1. There were no clear differences in these baseline characteristics between two cohorts.

During 14 years of follow-up, 216 cardiovascular events were observed in the derivation cohort and 125 in the validation cohort. The cardiovascular risk prediction model including covariates of age, sex, systolic blood pressure, diabetes, LDL cholesterol, high-density lipoprotein cholesterol and smoking habits were developed in the derivation cohort. The multivariate-adjusted regression coefficients and hazard ratios for the risk prediction model are shown in Table 2.

The performance of the risk prediction model was then evaluated among the validation cohort. In terms of discrimination, the c-statistic was as high as 0.81 (95% confidence interval, 0.77 to 0.86). Figure 1 demonstrates the calibration plots comparing actual and predicted cardiovascular events by deciles of risk. The calibration χ^2-statistic for the risk prediction model was 6.46 (d.f.=8), indicating excellent goodness of fit (P=0.60). The top 30% of predicted risk identified 70% of subjects who experienced cardiovascular disease during follow-up (sensitivity). Proportion of subjects without cardiovascular events who were not in the top 30% of predicted risk was 79% (specificity).

Tables 3 and 4 provide risk score sheets that can be used for estimation of the multivariable risk of cardiovascular disease at 10

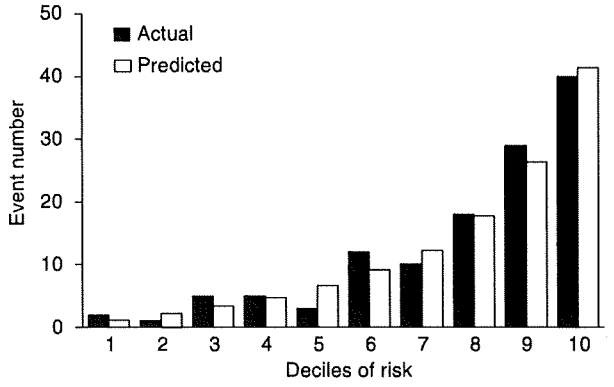

Figure 1 Actual and predicted cardiovascular events by deciles of risk in the validation cohort. Hosmer–Lemeshow χ^2-statistic=6.46, d.f.=8, P=0.60.

Table 3 Cardiovascular risk points

Points	*Age (years)*	*Sex*	*SBP (mm Hg)*	*Diabetic*	*LDL cholesterol (mg per 100 ml)*	*HDL cholesterol (mg per 100 ml)*	*Smoker*
0	40–44	Women	<119	No	<140	⩾40	No
1	45–49		120–139		⩾140	<40	Yes
2	50–54	Men	140–159	Yes			
3	55–59		160–179				
4	60–64		⩾180				
5	65–69						
6	70–74						
7	75–79						
8	⩾80						

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.
SI conversion factors: to convert LDL and HDL cholesterol to millimoles per liter, multiply by 0.0259.

years. Table 4 also provide a different quantification of the same risk in the form of vascular age.

DISCUSSION

In this paper, a new risk prediction model of cardiovascular disease has been developed using data obtained from a prospective cohort study of a general Japanese population. The risk prediction model demonstrated good performance in regard to both discrimination and calibration. A simple risk score sheet based on the cardiovascular risk prediction model was also presented. This simple risk prediction tool of cardiovascular disease for Japanese would provide a useful guide to estimate absolute risk of cardiovascular disease and to treat individual risk factors.

Large-scale cohort studies have developed a number of risk prediction tools of cardiovascular disease.[4–15] However, these risk prediction tools were mainly derived from studies carried out in Western populations and few risk prediction tools are developed among general Japanese populations. The NIPPON DATA 80 derived a cardiovascular risk prediction tool, in which age, sex, systolic blood pressure, glucose levels, total cholesterol and smoking habits were included as risk factors, using data obtained from a 19-year prospective cohort study of general Japanese populations, although the outcome of NIPPON DATA 80 risk charts was death from cardiovascular causes.[5] The Jichi Medical School (JMS) cohort study developed 10-year risk prediction tools for incidence of myocardial infarction[14] and stroke,[15] in which age, sex, systolic blood pressure, diabetes, total cholesterol and smoking habits were included as risk factors, using data obtained from a population-based prospective study of general Japanese populations. The present analysis from the Hisayama study developed a new risk prediction tool for incidence of cardiovascular disease in a general population of Japanese using similar risk factors used in the previous observational studies of Japanese. Cumulative incidence rates of cardiovascular events at 10 years estimated from the present risk prediction tool were almost similar to combined risks of myocardial infarction and stroke obtained from the JMS risk charts[14,15] and this finding supports the validity and the generalizability of the Hisayama risk prediction model.

Table 4 Estimated cardiovascular risk at 10 years and vascular age according to risk points

Points	*Risk[a] (%)*	*Vascular age for men[b] (years)*	*Vascular age for women[b] (years)*
0	1.4	—	40–44
1	1.8	—	45–49
2	2.4	40–44	50–54
3	3.2	45–49	55–59
4	4.2	50–54	60–64
5	5.6	55–59	65–69
6	7.4	60–64	70–74
7	9.8	65–69	75–79
8	12.8	70–74	80–84
9	16.7	75–79	85–89
10	21.7	80–84	90–94
11	27.8	85–89	95–99
⩾12	>30	⩾90	⩾100

[a]Estimated cardiovascular risk at 10 years.
[b]Age of a person with the same predicted risk but with all other risk factor levels in optimal ranges.

Several limitations of our study should be discussed. One limitation is a lack of external validation of the risk prediction model. However, split sample validation is an established method for internal validation of a risk prediction model and is widely used in other studies.[9,12] Similarity to the JMS risk chart[14,15] also supports the validity of the Hisayama risk prediction model. Another limitation is that LDL cholesterol, as a continuous variable, did not reach statistical significance in the derivation cohort. However, LDL cholesterol is an established risk factor for cardiovascular disease in the Hisayama study[21] and thus we included LDL cholesterol into the risk prediction model. A third limitation is that our findings are based on a one-time measurement of risk factors (for example, systolic blood pressure, plasma glucose levels, LDL cholesterol levels and high-density lipoprotein cholesterol levels), which may not accurately reflect the status of a study participant. A fourth limitation is that the value of LDL cholesterol was not directly assayed but was calculated by the Friedewald equation,[22] although the equation has been adopted in substantial epidemiologic and clinical studies of LDL cholesterol and cardiovascular disease. These limitations may have resulted in underestimation of the predicted risk among subjects at high risk of cardiovascular disease.

In conclusion, we developed and validated a new cardiovascular risk prediction model in a general population of Japanese. The risk prediction model would provide a useful guide to identify the individuals at high risk of cardiovascular disease in Japan. High-risk

approaches for the prevention of cardiovascular disease using the present risk prediction tool are likely to provide additional protection against the burden of cardiovascular disease in Japan.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported in part by Grants-in-Aid for Scientific Research A (no.18209024) and C (no.19590633) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Original Article

Increased Serum Apolipoprotein B48 Concentration in Patients with Metabolic Syndrome

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Aim: Postprandial hyperlipidemia is characterized by an increase of chylomicron remnants (CM-R), and is a risk factor for atherosclerosis. Apolipoprotein (apo) B48 exists exclusively in chylomicroms and CM-R, and fasting plasma levels of apo B48 may reflect high postprandial levels of chylomicrons and/or CM-R. We hypothesized that fasting apo B48 levels may be increased in metabolic syndrome.
Methods: We investigated 1,349 inhabitants (528 men and 821 women aged 62.4±12.8 y; mean± S.D.) of two towns in rural Hokkaido, who underwent health checks in 2005.
Results: The fasting apo B48 level was significantly higher in males than females (geometric mean 1.92; 95% CI 1.80–2.04 μg/mL, vs. 1.69; 95% CI 1.61–1.76 μg/mL; $p<0.001$). Ln (apo B48) showed a significant positive correlation with total cholesterol and ln (triglycerides), and a negative correlation with HDL-cholesterol. The correlation between ln (apo B48) and ln (triglycerides) was strong. Apo B48 was significantly higher in men and women with than without metabolic syndrome. Regression analysis revealed that ln (apo B48) was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and ln (triglyceride).
Conclusion: Fasting apo B48 levels are raised in individuals with metabolic syndrome.

J Atheroscler Thromb, 2009; 16:517-522.

Key words; Chylomicrons, Hypertriglyceridemia, Apolipoprotein B48, Metabolic syndrome

Introduction

Postprandial hyperlipidemia, which is characterized by increased levels of chylomicron remnants (CM-R), is considered to be a risk factor for atherosclerosis[1, 2]. Chylomicrons are assembled in the small intestine and undergo lipolysis by lipoprotein lipase in the plasma to generate CM-R. Because CM-R are then rapidly taken up by the liver, it has been assumed that fasting plasma levels of these particles are very low[3].

Postprandial hyperlipidemia is related to metabolic syndrome[4-6]. This syndrome is characterized by insulin resistance, hypertension, dyslipidemia, and hyperglycemia, and is an important risk factor for atherosclerosis. In metabolic syndrome, dyslipidemia is characterized by hypertriglyceridemia and a low high density lipoprotein (HDL)-cholesterol level. Recent studies have shown that postprandial hyperlipidemia is a major cause of hypertriglyceridemia associated with metabolic syndrome[6, 7].

Though CM-R are thought to play an important role in dyslipidemia associated with metabolic syndrome, CM-R concentrations in patients with this syndrome have not yet been reported. Apo B48 exists exclusively in chylomicroms and CM-R. Several methods of measuring the apo B48 concentration in plasma or in triglyceride-rich lipoproteins have been reported[8-15]. These methods include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[8-10], SDS-PAGE coupled with Western blotting[11, 12], and competitive enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies[13, 14].

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Received: July 8, 2008
Accepted for publication: June 4, 2009

Sakai *et al.* reported an ELISA for measuring apo B48 in fasting serum that employed a monoclonal antibody against apo B48[15]. We also recently developed an ELISA to measure the serum level of apo B48 using another monoclonal antibody[16].

We hypothesized that the fasting plasma level of apo B48 may be increased in patients with metabolic syndrome; therefore, we measured fasting plasma levels of apo B48 in order to evaluate the relationship between CM-R and metabolic syndrome. We also investigated the factors regulating apo B48 levels in fasting plasma.

Subjects and Methods

The subjects were 1,349 inhabitants (528 men and 821 women) of two towns in a rural area of Hokkaido, Japan, who underwent routine health checks in 2005. Blood samples were collected from all subjects after an overnight fast. Systolic and diastolic blood pressures were measured at rest in the sitting position. The fasting plasma glucose (FPG) level and plasma levels of total cholesterol, triglycerides, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol were measured by enzymatic methods. Immunoreactive insulin (IRI) was measured by an enzyme immunoassay (EIA). Waist circumference was measured at the level of the umbilicus in the standing position. The HOMA-IR (homeostasis model assessment insulin resistance index) was calculated as FPGxIRI/405, after excluding individuals who had an FPG above 126 mg/dL and/or were on treatment for diabetes[17].

Apolipoprotein B48 was measured by EIA[16]. Briefly, a 96-well microtiter plate (Nalge Nunc International, Japan) was coated with an anti-apoB-48 monoclonal antibody (4C8) by overnight incubation at 4℃. After washing the microtiter plate with phosphate-buffered saline, 50-μL aliquots of 100-fold-diluted serum or plasma (diluted with 0.05 mol/L Tris-HCl buffer, pH 7.5, 0.15 mol/L NaCl, and 0.1% Triton X-100) were added in duplicate to the wells and the plate was incubated at room temperature (20–25℃) for 1 hr. Aliquots (50 μL) of the apoB-48 standard (2.5 ng/mL to 160 ng/mL: 7-point calibration curve) were incubated in the same way. After the plate was washed three times, 50 μL biotin-conjugated anti-apoB-48/B-100 (ICN Pharmaceuticals Inc., USA) diluted in 0.01 mol/L phosphate buffer (pH 7.2) with 0.15 mol/L NaCl and 0.1% bovine serum albumin was added to each well and incubated with gentle shaking at room temperature for 1 hr. After the plate was washed, 50 μL horseradish peroxidase-conjugated avidin solution was added followed by incubation at room temperature for 30 min. After the plate was washed, 50 μL chromogenic substrate solution was added to each well and incubated with shaking at room temperature for 20 min until the color developed. Then 50 μL of stop solution was added to each well and plate was read at 450 nm using a Spectra-Fluor-Plus plate reader (Tecan, USA).

Metabolic syndrome was defined according to Japanese criteria[18]. Briefly, a waist circumference of more than 85 cm in men and 90 cm in women combined with more than one of the following factors led to a diagnosis of metabolic syndrome: plasma triglycerides >150 mg/dL and/or HDL cholesterol <40 mg/dL, systolic blood pressure >130 mmHg and/or diastolic blood pressure >85 mmHg, and FPG >110 mg/dL. Some subjects were taking medications, but subjects with or without medications were grouped together for this study.

The mean$\pm$SD or median with interquartile range is shown to summarize the characteristics of the study subjects by sex. Between-group comparisons of the means and median were performed by unpaired t-test and the Wilcoxon rank-sum test, respectively. The relationship of serum lipids and lipoproteins with apo B48 was examined by correlation and multiple regression analysis. Pearson's correlation coefficients were calculated for the correlation. Stepwise multiple regression analysis was used to determine independent predictors of apo B48, with p-to-enter and p-to-retain set at 0.10 each. Statistical significance was declared if the two-sided p value was less than 0.05. Statistical analyses were performed using JMP software (SAS Institute, Cary, NC).

Results

The age of all subjects, men and women was 62.4 ± 12.8 years, 64.2 ± 12.8 years and 61.2 ± 12.7 years (mean$\pm$S.D.), respectively. The mean body mass index (BMI) did not differ significantly between men and women (23.9 ± 3.1 vs. 23.5 ± 3.6, respectively). Plasma levels of total cholesterol, HDL-cholesterol and LDL-cholesterol were significantly lower ($p<0.001$) in men than in women, whereas the values of apo B48, triglycerides, and FPG were significantly higher in men ($p<0.001$) (**Table 1**). **Fig. 1** shows the distribution of apo B48 in men and women. The mean apo B48 level was 1.92 μg/mL in men and 1.69 μg/mL in women.

As shown in **Fig. 1**, the distribution of apo B48 was skewed to the left. Data were therefore normalized by logarithmic transformation for further statistical analysis. The triglyceride and HOMA-IR data were

Table 1. Characteristics of the subjects

	Men (n=524)	Women (n=819)	p-value
Age (years)[a]	64.2±12.8	61.2±12.7	<0.0001
BMI (kg/m^2)[a]	23.9±3.1	23.5±3.6	0.01
Waist (cm)[a]	85.9±8.7	83.1±10.7	0.04
MS with/without	397/125	741/78	<0.0001
T. chol (mg/dL)[a]	192.4±31.1	204.4±30.9	<0.0001
TG (mg/dL)[b]	99 (74-140)	83 (63-117)	<0.0001
HDL-C (mg/dL)[a]	53.9±13.2	62.1±14.3	<0.0001
LDL-C (mg/dL)[a]	106.2±27.6	115.8±27.3	<0.0001
SBP (mmHg)[b]	138 (124-153)	135 (117-151)	<0.001
DBP (mmHg)[b]	78 (70-86)	74.5 (66-83)	<0.0001
FPG (mg/dL)[a]	102.9±23.4	95.0±18.9	<0.0001
IRI (μU/mL)[b]	3.9 (2.6-5.8)	4.1 (2.8-5.8)	0.38
HOMA-IR (mg/dL×μU/mL)[b]	0.924 (0.602-1.387)	0.909 (0.636-1.341)	0.02
ApoB 48 (μg/mL)[b]	1.80 (1.20-3.10)	1.61 (1.14-2.45)	<0.001

[a]Mean±SD
[b]Median (25th and 75th interquartile range)
p values were based on paired t-test and Wilcoxon rank-sum test for mean and median, respectively.

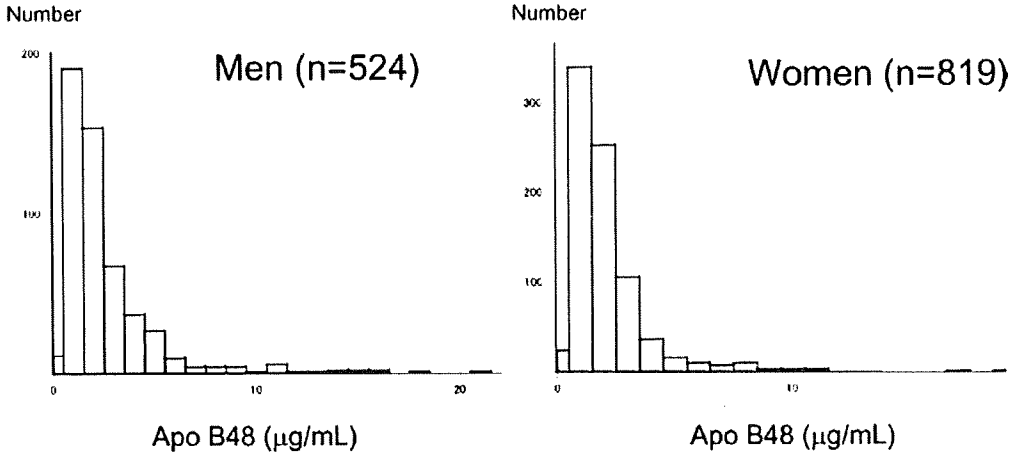

Fig. 1. Distribution of ln (apo B48) in men and women.
Geometric means and 95% central limits are 1.92 μg/mL (1.80-2.04) in men and 1.69 μg/mL (1.61-1.76) in women.

also normalized because of their skewed distribution (data not shown).

The ln (apo B48) showed a weak positive correlation with total cholesterol, and a weak negative correlation with HDL-cholesterol (**Fig. 2**). In addition, ln (apo B48) and ln (triglycerides) showed a strong positive correlation (r=0.53 in men and r=0.48 in women).

Ln (apo B48) also showed a strong positive correlation with ln (HOMA-IR) (**Fig. 2**). The fasting apo B48 level was significantly higher in both men and women with metabolic syndrome than without (**Table 2**).

Ln (apo B48) was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and ln (triglyceride) by multiple regression analysis (**Table 3**).

Discussion

In this study, we measured plasma apo B48 levels with a novel ELISA. According to previous reports, the fasting plasma apo B48 concentration ranges between

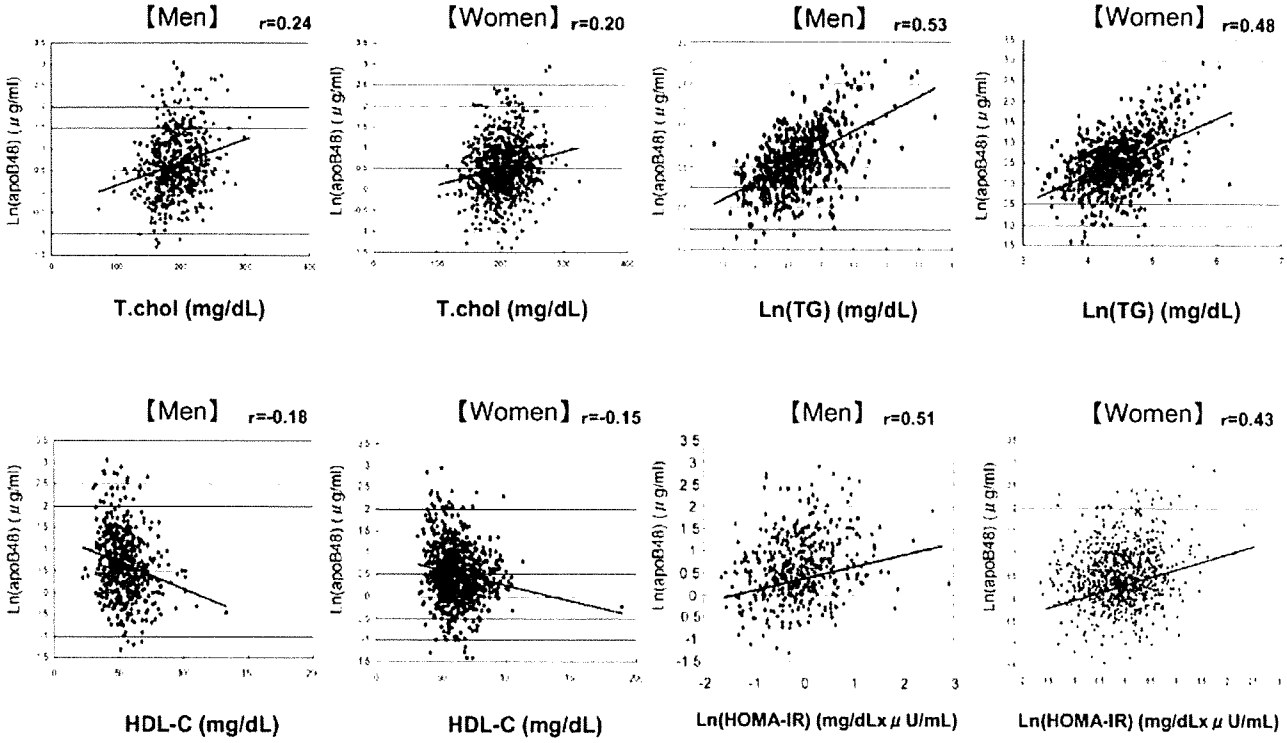

Fig. 2. Correlation of ln (apo B48) with total cholesterol, ln (triglycerides), HDL-cholesterol, and ln (HOMA-IR) in men and women.

Table 2. Apo B48 levels according to metabolic syndrome in men and women

Sex	Metabolic syndrome	N	Geometric mean (95% CI)
Men	(−)	397	1.76 (1.65–1.89)
	(+)	125	2.50 (2.17–2.88)
			$p<0.0001$
Women	(−)	741	1.64 (1.57–1.72)
	(+)	78	2.19 (1.86–2.59)
			$p<0.0001$

CI, confidence interval
p-value was based on unpaired *t*-test.

0.08 μg/mL and 60 μg/mL[8-15]. Our data (men: 1.92 μg/mL; women: 1.69 μg/mL (mean value)) were also in this range, and were similar to the level reported by Sakai *et al.* for normolipidemic subjects (5.2±3.8 mg/mL) using a similar ELISA with another monoclonal antibody against apo B48[15]. Among the methods available to measure apo B48, ELISA systems based on monoclonal antibodies are valuable because they are simple and quantitative methods.

In this study, we measured fasting plasma levels of apo B48. It has been suggested that a high fasting apo B48 level reflects high postprandial concentrations of chylomicrons and/or CM-R[12]; therefore, we assumed that a high fasting plasma level of apo B48 indicated the existence of postprandial hyperlipidemia.

The B48 concentration was higher among men than women (**Fig. 1**). Sakai *et al.* previously found that men also had higher apo B48 levels than women among normolipidemic subjects[15]. These results may indicate that women show more rapid catabolism of chylomicrons and/or CM-R, or less intestinal fat absorption, or both.

Apo B48 showed a significant and strong correlation with triglycerides (**Fig. 2**). Cortner *et al.* reported that delayed catabolism of CM-R leads to hypertriglyceridemia[3]. Since very low density lipoprotein (VLDL) and VLDL remnants are considered the main contributors to plasma triglyceride concentration, the close relationship between apo B48 and triglyceride levels indicates that delayed catabolism of CM-R leads to the accumulation of VLDL or VLDL remnants. It is interesting that the plasma concentration of apo B48, which is far lower than that of apo B100 (0.15–0.2 vs.

Table 3. Stepwise multiple regression analysis of ln (apoB48) in relation to serum lipids, lipoproteins, and glucose-related parameters ($n = 1,089$)

	Regression coefficient	S.E.	t value	p value
Age	−0.0069	0.0013	−5.12	<0.0001
BMI	−0.0213	0.0599	−3.55	0.0004
Total cholesterol	0.0066	0.0021	3.13	0.0018
HDL cholesterol	−0.0068	0.0022	−3.15	0.0017
LDL cholesterol	−0.0047	0.0020	−2.28	0.0227
ln (Triglycerides)	0.5520	0.0715	7.72	<0.0001
ln (HOMA-IR)	0.0583	0.0337	1.73	0.0939

Sex, SBP, DBP, total cholesterol, HDL cholesterol, LDL cholesterol, apo E, ln (triglyceride), and ln (IRI) were also included as explanatory variables in the model, but they did not remain in the final model.

100–120 mg/dL), has such a significant relationship with the VLDL or VLDL remnant level. This may because the triglyceride content of CM-R is very high when compared to VLDL or VLDL remnants.

Apo B48 was also positively correlated with HOMA-IR (**Fig. 2**), which is a marker of insulin resistance, and the apo B48 level was significantly higher in subjects with metabolic syndrome than without (**Table 2**). These results indicate that apo B48 increases in the presence of insulin resistance and/or metabolic syndrome. Since insulin resistance is considered to be involved in the development of metabolic syndrome[19, 20], insulin sensitivity might influence the level of apo B48. It has been reported that insulin resistance shows a negative correlation with lipoprotein lipase mRNA expression and activity in adipose tissue[21]. Thus, defects of lipoprotein lipase may cause the accumulation of apo B48 particles. In fact, it has been reported that insulin resistance might lead to postprandial hyperlipidemia[22, 23].

Because our subjects with metabolic syndrome showed higher fasting plasma concentrations of apo B48, there is a possibility that CM-R may play a role in the increased risk of atherosclerosis related to this syndrome. Vine *et al.* reported that impaired postprandial metabolism of apo B48 led to atherosclerosis in rats with metabolic syndrome[24]. On the other hand, Velero *et al.* reported that the fasting apo B48 level does not predict the risk of coronary heart disease[25]. Thus, whether the fasting apo B48 level influences the risk of atherosclerosis remains to be determined.

Multiple regression analysis revealed that apo B48 was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride. Gender does not affect apo B48 by this method, which may be due to an other factor related to gender (i.e., LDL cholesterol or HDL cholesterol) having a strong association with apo B48.

In conclusion, the fasting plasma level of apo B48 was correlated with the serum triglyceride concentration, and apo B48 levels were higher in rural Japanese subjects with metabolic syndrome than those without; however, further studies of other populations are needed to confirm these results.

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Original Article

A Promoter Polymorphism of Lamin A/C Gene is an Independent Genetic Predisposition to Arterial Stiffness in a Japanese General Population (The Tanno and Sobetsu Study)

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Aim: We examined the hypothesis that there is a positive, independent association between polymorphisms of lamin A/C gene (*LMNA*) and arterial stiffness in Japanese.
Methods: The subjects were 261 men (mean age, 64.4±0.7 years) selected from inhabitants of the towns of Tanno and Sobetsu in a rural area of Japan who underwent medical check-ups. We conducted clinical examinations, including measurement of bilateral brachial-ankle pulse wave velocity (baPWV) as a marker of arterial stiffness, and genetic analysis. Subjects with atrial fibrillation, subjects with ankle-brachial index <0.9, and subjects taking any medication were excluded. We selected two single nucleotide polymorphisms (SNPs) as markers of *LMNA*, 1908C/T in exon 10 and -1030C/T in the promoter region, which we have recently identified. All genotypes were clearly determined by the TaqMan PCR method.
Results: Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT+TT genotype (1,652±22.1 cm/s vs. 1,552±43.0 cm/s, $p=0.039$); however, no significant difference was found for 1908C/T polymorphism. The baPWV was found to be significantly associated with age, body height, systolic blood pressure, and smoking habit; therefore, we next performed multiple regression analysis including these parameters, and found an independent, significant association between baPWV and -1030C/T polymorphism.
Conclusion: Promoter -1030C/T polymorphism of *LMNA* is a possible genetic predisposition to arterial stiffness in the Japanese population.

J Atheroscler Thromb, 2009; 16:404-409.

Key words; Arterial stiffness, Nuclear lamina, Genetics, Single nucleotide polymorphism (SNP)

Introduction

Hutchinson-Gilford progeria syndrome (HGPS; Online Mendelian Inheritance in Man #176670) is a rare sporadic disorder with premature aging, and patients with this syndrome are likely to have coronary artery disease, stroke, or other cardiovascular diseases[1-3]. HGPS induces severe systemic arterial stiffness, which leads to fatal myocardial infarction or stroke before an average age of 13 years. Approximately 80% of HGPS cases are caused by a single base change of C to T in position 1824 on exon 11 of a gene encoding nuclear lamins A and C[4,5]. Lamins are structural protein components of nuclear lamina, a protein network underlying the inner nuclear membrane that determines nuclear shape and size, and

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Received: December 17, 2008
Accepted for publication: January 29, 2009

constitute a class of intermediate filaments. The gene encoding lamins A and C is named lamin A/C gene (*LMNA*; Gene ID 4000), and it spans approximately 24 kb and contains 12 exons on chromosome 1q21. Alternative splicing within exon 10 of *LMNA* gives rise to 2 different mRNAs that code for prelamin A and lamin C.

HGPS is associated with premature arterial stiffness, and it is therefore thought that *LMNA* is involved in the pathophysiology and genesis of arterial stiffness that occurs concurrently with the accelerated aging process. It has been reported that *LMNA* has several single nucleotide polymorphisms (SNPs). The 1908C/T polymorphism (rs #4641), one of the SNPs on *LMNA*, in exon 10 is associated with a risk for developing metabolic traits, including insulin resistance. A positive association between 1908C/T polymorphism and metabolic abnormalities has been reported in Inuit[6], Japanese[7], Pima Indians and Armish[8].

However, there is no data on the association between *LMNA* and arterial stiffness in a general population. Arterial stiffness is mainly determined by measuring pulse wave velocity (PWV)[9]. PWV reflects systemic arteriosclerosis as well as relating to cardiovascular risk factors[10] and ischemic heart disease in type 2 diabetes mellitus[11]. PWV is also a predictor of cardiovascular mortality in patients with end-stage renal disease[12] or hypertension[13] and in elderly individuals[14], independently of age, blood pressure, and cardiac mass.

The purpose of this study was to examine the relationship between polymorphisms of *LMNA* and arterial stiffness in a cross-sectional epidemiological study of a Japanese general population, the Tanno and Sobetsu study.

Materials and Methods

We recruited 586 male inhabitants of Tanno Town and Sobetsu Town who had undergone medical checkups in 2003. Tanno and Sobetsu are located in Hokkaido, the northernmost island of Japan. The Tanno and Sobetsu study was started in 1977 with a population-based prospective cohort design. Detailed epidemiological findings have already been reported[15-18].

The subjects completed a standard questionnaire regarding their medical history and their smoking and drinking habits. We measured anthropometric parameters, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, plasma glucose, immunoreactive insulin (IRI), highly sensitive C-reactive protein (hs-CRP), and adiponectin in all subjects. Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) were measured using Form® PWV/ABI (Omron Colin Co., Ltd., Tokyo, Japan) and the average of right baPWV and left baPWV was adopted[19]. Insulin sensitivity was determined by homeostasis model assessment of the insulin resistance (HOMA-IR) index, which was calculated as plasma glucose (mg/dL) × immunoreactive insulin (μU/L)/405. Blood samples were collected in the early morning after fasting for 8–11 hours. Blood pressure was measured twice after 5 minutes of rest, with the subjects seated.

Exclusion criteria were atrial fibrillation, suspected arteriosclerosis obliterans (ASO) defined as ABI on any side lower than 0.9, and taking any medication, in order to rule out drug effects. After excluding 219 of the 586 male subjects according to the above criteria, we conducted genetic analysis. Finally, 261 male subjects were successfully genotyped. All subjects gave written informed consent to participate in the genetic analysis and in all other procedures associated with the study. The Institutional Review Board (IRB) of Osaka University and the IRB of Sapporo Medical University both approved the study protocol.

Genomic DNA was extracted from 200 μL buffy coat using a QIAamp DNA Blood Kit (QIAGEN K. K., Tokyo, Japan). C-to-T transversion at nucleotide position 1908 in exon 10 of the lamin A/C gene (*LMNA* 1908C/T; rs #4641) and C-to-T transversion at nucleotide position -1030 in the promoter region of *LMNA* (*LMNA* -1030C/T; no rs#) were determined by the TaqMan-polymerase chain reaction (PCR) method. The *LMNA* 1908C/T polymorphism was detected using the following primers and probes: forward, 5'-CGA GGA TGA GGA TGG AGA TGA C-3'; reverse, 5'-CCT CAG CGG CGG CTA C-3'; cytosine base (C)-specific probe, 5'-VIC-CAC TCA CGT GGT GGT G-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-CAC TCA CAT GGT GGT G-MGB-3'. The *LMNA* -1030C/T polymorphism was detected using the following primers and probes: forward, 5'-CCA CTA CCT TCT TTC TGG CTG AA-3'; reverse, 5'-ACT AGG TCC CAG ATT TCT GTG GTT-3'; cytosine base (C)-specific probe, 5'-VIC-CAG CCA ATG TTG GGT C-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-ACA GCC AAT ATT GGG TC-MGB-3'. PCR was carried out using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: initial denaturation at 95℃ for 10 min, followed by 40 cycles of 92℃ for 15 sec and 60℃ for 60 sec. The fluorescence level of PCR

Table 1. Baseline characteristics of study subjects ($n=261$)

	Male ($n=261$)
Age (years)	64.6 ± 0.7
BMI (kg/m^2)	23.5 ± 0.2
SBP (mmHg)	133 ± 1.3
DBP (mmHg)	75 ± 0.7
Total cholesterol (mg/dL)	193 ± 2.0
Triglyceride (mg/dL)	112 ± 4.6
HDL cholesterol (mg/dL)	52 ± 0.8
Current smoker (%)	33.0
HOMA-IR	1.0 ± 0.07
hsCRP (mg/dL)	0.107 ± 0.008
Adiponectin (ng/mL)	6.1 ± 0.2
baPWV (cm/s)	1,631 ± 19.7

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

products measured using an ABI PRISM 7900HT Sequence Detector (Applied Biosystems) differentiated the three genotypes of these two polymorphisms.

Associations between the polymorphisms and clinical variables were analyzed using one-way analysis of variance (ANOVA). Differences in genotype or allele distribution were examined by χ^2 analysis. Multiple regression analysis was used to assess the contribution of confounding factors. All numerical values are expressed as the means ± SEM. Significance was defined as $p<0.05$. All statistical analyses were conducted using JMP software version 5.1.2J for Windows (SAS Institute Inc., Cary, NC, USA).

Results

The 261 male subjects had a mean age of 64.6 ± 0.7 years, mean body mass index (BMI) of 23.5 ± 0.2 kg/m^2, and mean brachial-ankle pulse wave velocity (baPWV) of 1,631 ± 19.7 cm/sec. **Table 1** shows the baseline characteristics of all study subjects. The genotype frequencies of the two polymorphisms of *LMNA* examined did not significantly differ from the values predicted by the Hardy-Weinberg equilibrium. The frequencies of CC, CT and TT genotypes of exon 10 1908C/T polymorphism were 60%, 32% and 8%, respectively, and the frequencies of CC, CT and TT genotypes of promoter -1030C/T polymorphism were 79%, 16% and 5%, respectively. Since the number of subjects with TT genotype of these two polymorphisms was small, we adopted a recessive model of the C allele (CC vs. CT+TT) for the two polymorphisms. **Tables 2** and **3** show the clinical parameters of each genotype of the 1908C/T polymorphism and -1030C/T polymorphism of *LMNA*, respectively. Despite previous findings[6-8], there was no significant relationship between the T allele of 1908C/T polymorphism and metabolic traits in our study cohort. BaPWV of subjects with CC genotype of -1030C/T polymorphism was significantly greater than that of

Table 2. Comparison of parameters between CC genotype and CT + TT genotype of 1908C/T polymorphism

	CC ($n=157$)	CT+TT ($n=104$)	p
Age (years)	65.3 ± 0.9	63.5 ± 1.2	0.24
BMI (kg/m^2)	23.5 ± 0.3	23.7 ± 0.3	0.58
SBP (mmHg)	133 ± 1.7	136 ± 2.1	0.30
DBP (mmHg)	75 ± 0.9	77 ± 1.1	0.07
Total cholesterol (mg/dL)	194 ± 2.7	190 ± 3.4	0.09
Triglyceride (mg/dL)	112 ± 5.1	113 ± 10.0	0.91
HDL cholesterol (mg/dL)	52 ± 0.9	52 ± 1.7	0.96
Current smoker (%)	32.4	5.2	0.75
HOMA-IR	1.1 ± 0.1	1.0 ± 0.2	0.56
hsCRP (mg/dL)	0.11 ± 0.008	0.082 ± 0.02	0.10
Adiponectin (ng/mL)	6.1 ± 0.2	6.0 ± 0.4	0.80

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

Table 3. Comparison of parameters between CC genotype and CT + TT genotype of -1030C/T polymorphism

	CC ($n=207$)	CT+TT ($n=54$)	p
Age (years)	64.8 ± 0.8	63.9 ± 1.5	0.61
BMI (kg/m^2)	23.6 ± 0.2	23.2 ± 0.4	0.37
SBP (mmHg)	134 ± 1.4	131 ± 2.8	0.27
DBP (mmHg)	76 ± 0.8	74 ± 1.6	0.24
Total cholesterol (mg/dL)	191 ± 2.2	199 ± 4.4	0.38
Triglyceride (mg/dL)	112 ± 5.9	106 ± 7.3	0.56
HDL cholesterol (mg/dL)	53 ± 1.0	52 ± 1.3	0.88
Current smoker (%)	35.4	28.1	0.26
HOMA-IR	1.1 ± 0.1	1.0 ± 0.1	0.27
hsCRP (mg/dL)	0.11 ± 0.01	0.10 ± 0.01	0.43
Adiponectin (ng/mL)	6.0 ± 0.3	6.2 ± 0.3	0.52

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

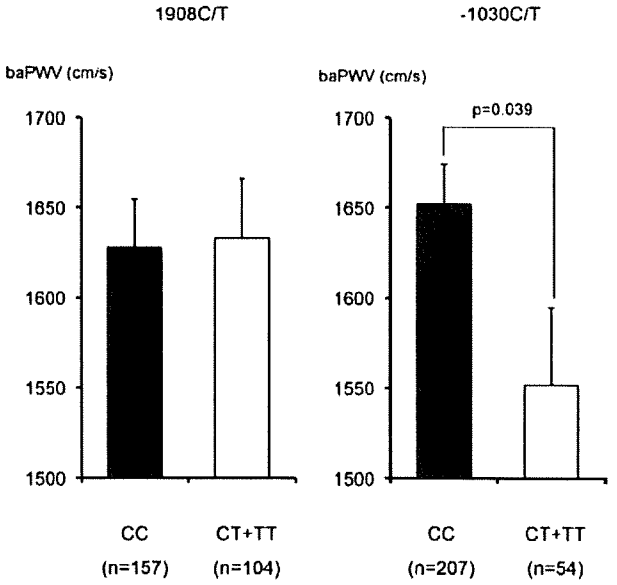

Fig. 1. Comparison of brachial-ankle pulse wave velocity (baPWV) according to genotypes of *LMNA* 1908C/T and -1030C/T polymorphisms.

subjects with CT+TT genotype of -1030C/T polymorphism (1,652±22.1 cm/s vs. 1,552±43.0 cm/s, $p=0.039$), while there was no significant difference in the genotype of 1908C/T polymorphism (**Fig. 1**). BaPWV showed significant positive correlations with age, systolic blood pressure and smoking habit and a significant negative correlation with body height. We therefore selected these factors as covariates of multiple regression analysis for baPWV and *LMNA* -1030C/T polymorphism (**Table 4**). There was an independent relationship between the genotype of -1030C/T polymorphism and baPWV after adjusting covariates. Mean baPWVs of -1030/CC and -1030/CT+TT genotypes were 1,650±17.1 cm/s and 1,571±32.0 cm/s, respectively, after adjusting covariates.

Since there was a strong linear correlation between age and baPWV, we analyzed the genotypic difference of -1030C/T in this correlation. The gradient of the regression line of subjects with CC genotype was significantly high compared to that of subjects with CT+TT genotype (data not shown), indicating that subjects with CC genotype of -1030C/T polymorphism might be susceptible to the progression of arterial stiffness by aging.

Discussion

We examined the hypothesis that there is a positive, independent association between polymorphisms

Table 4. Multiple regression analysis for brachial-ankle pulse wave velocity (baPWV)

Term	Estimate	SE	t	p
Age	12.5	1.58	7.9	<0.0001
Body height	0.684	2.55	0.3	0.79
Systolic blood pressure	7.69	1.15	6.7	<0.0001
Smoking habit	−6.34	15.9	−0.40	0.69
-1030C/T CC vs. CT+TT	−39.4	17.8	−2.2	0.028

$R^2=0.48$ ($n=261$)

of *LMNA* and arterial stiffness in a cross-sectional study of a Japanese population. Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The mean baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT+TT genotype; however, no significant difference was found for 1908C/T polymorphism. On the other hand, age, body height, systolic blood pressure, and smoking habit were significantly associated with baPWV. Multiple regression analysis including covariates revealed that subjects with the -1030T allele had a significantly lower level of baPWV.

The 1908C/T polymorphism of *LMNA* has been reported to be related to metabolic abnormalities or insulin resistance in Japanese[7] and Armish[8], but a relationship between this polymorphism and arterial stiffness was not found in the present study. In addition, no relationship was found between 1908C/T polymorphism and metabolic traits in our population. This may due to the characteristics of our subjects. Subjects taking any medication were excluded from this study, and the number of subjects with type 2 diabetes mellitus or dyslipidemia was therefore small; however, the promoter -1030C/T polymorphism of *LMNA*, which we have recently identified by direct sequencing in the subjects, was independently associated with baPWV as a marker of arterial stiffness. To our knowledge, this is the first report of a relationship between *LMNA* polymorphism and arterial stiffness.

In addition, we investigated the relation between the prevalence of cardiovascular diseases and SNPs in *LMNA* using a cross-sectional method; however, there was no significant relation between cardiovascular diseases and SNPs. Because the subjects of our study were relatively healthy and had a low prevalence of cardiovascular diseases, our investigation might lack statistical power. A prospective study to elucidate this relation is now ongoing. Because of the shortness of the follow-up period, we do not have valuable results

at present, but we will report the obtained results in the future.

Mutations in *LMNA* have been discovered in a staggering variety of inherited diseases called "laminopathies[20]". To date, several laminopathies are more familiar than Hutchinson-Gilford progeria syndrome (HGPS), such as Dunnigan-type familial partial lipodystrophy (FPLD), Emery-Dreifuss muscular dystrophy, Charcot-Marie-Tooth disease, limb-girdle muscular dystrophy, mandibuloacral dysplasia, dilated cardiomyopathy with conduction abnormality and early onset of atrial fibrillation. Laminopathies are caused by a mutation of *LMNA*, and are likely to include cardiovascular diseases[21, 22]. The precise mechanisms of this relationship are unknown, but it is speculated that *LMNA* regulates metabolic traits as well as arterial stiffness and thus results in the aging process. HGPS is a laminopathy that is mainly caused by C-to-T mutation in position 1824 of *LMNA* and results in systemic arteriosclerosis. Recent studies have shown that this mutation causes nuclear blebbing induced by anchoring the mutant lamin A (called "progerin", which lacks 50 amino acids near the carboxy terminus) to the inner nuclear membrane, resulting in dysregulated gene transcription, heterochromatin disorganization[23, 24], and increased vulnerability of the nuclear membrane. Numerous abnormalities present in HGPS are common phenomena that occur in cells not only of HGPS patients but also aged individuals in the general population, such as nuclear blebbing, epigenetic changes and increased levels of DNA damage[25]; therefore, *LMNA* seems to be one of the key genes regulating aging as well as arterial stiffness.

In order to elucidate the function of -1030C/T promoter polymorphism of *LMNA*, we searched for transcription factors likely to bind around -1030C/T, using online databases of TRANSFAC, JASPAR, IMD, and CBIL/GibbsMat (http://www.cbil.upenn.edu/cgi-bin/tess/tess/). Motif analysis revealed one transcription factor, CREB-binding protein/CCAAT recognition factor (CBP/CRF), which has homology with the sequence including the -1030T allele. This may result in a difference in *LMNA* expression. Subjects with the -1030T allele are likely to have a strong expression of lamin A and C matrix and might have a stable nuclear membrane against environmental insult, represented by reactive oxygen species.

Our study has several limitations. First, our study was conducted using a small number of subjects in a Japanese population. Second, this study was is designed as a cross-sectional method. Although arteriosclerosis occurs mostly in aged individuals, the genotype-phenotype relationship showd also be analyzed in a time-considered, longitudinal study. Third, the mechanisms by which transcriptional activities of *LMNA* are regulated by promoter -1030C/T polymorphism are unclear. Further study is required to clarify the function of promoter -1030C/T polymorphism.

In conclusion, promoter -1030C/T polymorphism of *LMNA* might be associated with arterial stiffness in Japanese independent of metabolic traits. The mechanism of the influence of *LMNA* on arterial stiffness may reveal part of the mechanism of a common condition susceptible to arteriosclerosis, aging.

Acknowledgements

We would like to express our gratitude to Ms. Kazuko Iwasa and Ms. Eriko Nagata for their continued support of our investigations. The present study was supported by a Grant-in-Aid for Scientific Research (H17-pharmaco-common-003) from the Japanese Ministry of Health, Labor, and Welfare, by Grants-in-Aid for Scientific Research (18590265, 18590811 and 19650188) from the Ministry of Education, Science, Sports and Culture of Japan, and by research grants from Takeda Science Foundation and the Japan Research Foundation for Clinical Pharmacology.

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Circ J 2009; **73**: 624–625

Uric Acid and Left Ventricular Hypertrophy

Shigeyuki Saitoh, MD

In this issue of the Journal, Mitsuhashi et al report that levels of uric acid (UA) were positively associated with electrocardiographically diagnosed left ventricular hypertrophy (LVH) in healthy Japanese men! The result was independent of body mass index, hypertension, diabetes, hyperlipidemia and age; similar results were obtained in both the normal and high blood pressure (BP) subgroups. Theirs was an epidemiological study, but the reported serum concentrations of UA add important information to the assessment of risk factors and preventing cardiovascular disease, especially heart failure.

Article p667

The relationship between UA and cardiovascular disease has been known since the first half of the 20th century and several studies have identified an association between increased UA and cardiovascular risk in the general population. The positive association between serum UA and hypertension was also observed over a century ago. Although elevated UA levels have been predictive of hypertension in epidemiological studies, the relationship between UA and BP is confounded by numerous factors, including age, diabetes, obesity, alcohol use, and sodium intake or volume status. Recent findings in animal models have helped elucidate possible mechanisms whereby UA may lead to hypertension, and have spurred a renewed interest in discerning a causal role for elevated UA in hypertension.

On the other hand, the presence of hypertensive organ damage signals a condition of increased risk for cardiovascular and renal morbidity and mortality. Thus, the search for LVH, atherosclerosis and microalbuminuria as hypertensive organ damage, which likely reflect both the severity of BP load and other nonhemodynamic risk factors, is currently recommended as part of global risk assessment. Mitsuhashi et al show new findings of a relationship between UA and LVH in Japanese, regardless of the presence of hypertension!

There are already reports of the relation between UA and LVH in Japanese with hypertension. For example, Kurata et al reported that serum UA levels correlated positively with left ventricular (LV) mass and indexed LV mass (LVMI) in male hypertensive patients, but not in female hypertensive patients in a cross-sectional study[2] Iwashima et al also demonstrated that UA is independently associated with LVMI and suggested that the combination of hyperuricemia and LVH is an independent and powerful predictor of cardiovascular disease[3]

With the exception of specific genetic defects in purine metabolism, increased UA is generally associated with important risk factors for atherosclerosis, such as hypertension, abdominal obesity, insulin resistance, metabolic syndrome and heart failure. Many studies have also clearly shown an association between increased UA concentrations and oxidative stress, endothelial dysfunction and inflammation. At the very least, an increased UA level is an independent marker of cardiovascular disease and a risk factor in cardiovascular diseases and hypertension. The question is whether UA is the cause of these risk factors or a morbid vascular change.

Because of being an epidemiological study, the results of Mitsuhashi et al's investigation do not suggest whether an elevation of the serum UA level is the cause or result of LVH! A consideration of the mechanism of UA production and metabolism offers insight into the relationship between UA level and cardiovascular change. Primarily, the association between UA and LVH might relate to an association of UA with other risk factors, especially renal dysfunction, oxidative stress, BP, and obesity. UA is excreted primarily by the kidney, so decreased renal perfusion could lead to increased serum UA and activation of the renin–angiotensin system; angiotensin II is essential for the development of LVH by myocardial remodeling[4] It is well known that angiotensin II induces hypertrophy and hyperplasia of myocytes and vascular smooth muscle cells, as well as influencing the expression of fibrogenic cytokine, and possibly inducing perivascular and interstitial fibrosis[5]

Secondly, UA levels may reflect xanthine oxidase pathway activity, which has the potential to contribute to the progression of LV dysfunction by interfering with myocardial efficiency[6] and myofilament calcium sensitivity[7] UA is a metabolic byproduct of purine metabolism and its serum level may increase because of increased generation, decreased excretion, or a combination of these mechanisms. UA is produced in the terminal step of purine metabolism catalyzed by xanthine oxidase (XO). XO pathway activity also results in the production of superoxide. XO is inhibited by allopurinol, which inhibited progression of cardiac hypertrophy in an animal model of hypertension without changing BP[8]

Furthermore, there are several possible contributors to increased UA production in cardiac disease, especially heart failure, including increased abundance and activity of XO, increased conversion of xanthine dehydrogenase to XO, or increased XO substrate resulting from enhanced ATP breakdown to adenosine and hypoxanthine under such conditions. XO activity participates in both mechano-energetic uncoupling and vascular dysfunction in the failing circulation. Mechano-energetic uncoupling is the process whereby cardiac energy consumption remains the same or increases

The opinions expressed in this article are not necessarily those of the editors or of the Japanese Circulation Society.

(Received February 2, 2009; accepted February 2, 2009)

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