es.[15–18] In addition, the importance of oxidatively modified LDL has been demonstrated in this process.[19,20] In fact, plasma Ox-LDL levels have been shown to be elevated in patients with ACS.[9] Effects of Ox-LDL on vascular cells in atherosclerotic progression and plaque rupture appear to be mediated by its receptors.[21] Lectin-like oxidized LDL receptor-1 (LOX-1) is a receptor with an expression that is not constitutive but dynamically inducible by proinflammatory stimuli, angiotensin II, and Ox-LDL, which are risk factors for ACS.[22–28] In human atherosclerotic lesions, LOX-1 is expressed prominently by intimal smooth muscle cells and lipid-laden macrophages in the advanced plaques.[29] Furthermore, LOX-1 plays an important role in Ox-LDL–induced apoptosis of vascular smooth muscle cells[30,31] and production of matrix metalloproteinases,[32] which may directly be linked to plaque rupture. LOX-1 is also expressed on the surface of activated platelets,[33] which may also be involved in thrombus formation after plaque rupture.

LOX-1 expressed on the cell surface can be proteolytically cleaved at its membrane proximal extracellular domain and released as soluble forms (sLOX-1).[34] Therefore, we have established a specific and sensitive assay to measure concentrations of sLOX-1 in human sera. The present report shows that serum sLOX-1 levels are elevated in ACS from its early stage, suggesting its usefulness as an early diagnostic marker of ACS.

Methods

Patient Sample

We enrolled 427 patients who underwent diagnostic coronary angiography (CAG) at the cardiovascular center and 34 patients who visited the emergency department and immediately were hospitalized in the Osaka Red Cross Hospital because of severe noncardiac acute diseases such as infectious diseases, trauma, and asthmatic fit and 60 patients with chronic problems in the outpatient department of internal medicine. All subjects were consecutively identified. All patients in this study gave written informed consent. Consecutive patients undergoing CAG were assigned to 4 groups depending on CAG findings and clinical features. Fifty-two patients whose CAG did not show any apparent atherosclerotic lesions were assigned to the group of patients with intact coronary. One hundred twenty-two patients who had documented coronary atherosclerosis by CAG but had been free of episodes of angina or documented cardiac ischemia for at least 3 months were assigned to the group of patients with controlled coronary heart disease (CHD). One hundred seventy-three patients who had significant coronary stenosis and ischemic symptoms (stable angina) and required elective coronary artery revascularization procedures such as percutaneous coronary intervention (PCI) or CABG were assigned to the group of patients with ischemic CHD. Eighty patients presented with ACS, which was defined as acute onset of prolonged chest pain or discomfort accompanied by ST-segment elevation or depression evolving into pathological Q waves or T-wave inversion and emergency CAG-documented total occlusion or marked delayed filling of a coronary artery. Among ACS patients, those without ST-segment elevation or pathological Q waves were defined as non–Q-wave ACS (NQ-ACS).

In another group of 40 ACS patients, serum sLOX-1 and TnT were serially measured on admission (at 4.4±4.2 hours after onset), immediately after emergency PCI, and at days 1, 3, 5, and 7. Patients with symptomatic peripheral vascular diseases were excluded from this study.

This study, carried out in accordance with the principles of the Declaration of Helsinki, was approved by local ethics committees.

Measurement of sLOX-1 and Other Serum Markers

Serum samples were collected at coronary angiography for patients undergoing CAG or at time of visit for patients with acute illness and chronic illness. In a time-dependent analysis, serum samples were collected serially at the indicated time periods. These samples were stored at −80°C until assays were performed. Serum sLOX-1 levels were determined by a sandwich ELISA using 2 different human LOX-1–specific antibodies. Antibodies were obtained after purification of serum from 2 different rabbits that had been immunized with a recombinant protein corresponding to the extracellular domain of human LOX-1. One of these antibodies was used to coat the plates; the other was fragmented into Fab′ and labeled with horseradish peroxidase for enzymatic detection. Standard curves were obtained by use of a recombinant protein corresponding to the extracellular domain of human LOX-1. Intra-assay and interassay coefficients of variation were 2.0% to 11.8% and 0.0% to 8.1%, respectively. The lower limit of the detection for sLOX-1 was 0.5 ng/mL. All assays were carried out by personnel who had no knowledge of the clinical diagnosis of the patients. Measurement of diluted serum samples by the same ELISA (see the Figure in the online-only Data Supplement) and immunoprecipitation followed by immunoblotting (data not shown) showed comparable results, indicating the accuracy and reliability of this ELISA for sLOX-1. Levels of hs-CRP and TnT were determined on the same serum samples as those for sLOX-1 by commercially available electrochemiluminescent immunoassay kit (F. Hoffmann–La Roche Ltd. and particle-enhanced immunonephelometry (Dade Behringer Ltd), respectively.

Statistical Analysis

We performed statistical analysis using Stat-View, version 5, and SPSS. The 1-way ANOVA was used to compare clinical continuous variables with the Tukey-Kramer test for multiple comparisons and 2-way cross-tabulation with the χ^2 test for binary variables, when appropriate, to compare differences between groups. When sLOX-1 was undetectable by ELISA, the sLOX-1 level was assigned 0. Levels of sLOX-1 did not distribute normally; therefore, the Kruskal-Wallis and Dunn's tests were used for multiple comparisons. Association between sLOX-1 and hs-CRP, LDL cholesterol, HDL cholesterol, triglycerides, or TnT was evaluated by Spearman's rank correlation coefficient. Multivariable logistic regression analysis was performed to assess the correlation between ACS and age, gender, hypertension, diabetes, smoking, LDL cholesterol, HDL cholesterol, triglycerides, hs-CRP, or sLOX-1.[35] Transformed values of hs-CRP in logarithm were used as variables for statistical analyses. Time profiles of serum sLOX-1 and TnT levels were analyzed after conversion of the individual's serial sLOX-1 levels into relative ratios to each individual's maximum value by 1-way repeated-measures ANOVA and multiple comparisons with Bonferroni's test. Receiver-operating characteristic (ROC) analysis was also carried out on the levels of sLOX-1 and hs-CRP for ACS and ACS without apparent ST elevation or pathological Q waves (NQ-ACS) separately. This analysis plots the true-positive fraction (sensitivity) against the false-positive fraction (1−specificity) by changing the cutoff value for the test. Areas under the ROC curves indicate the relative accuracy of diagnostic tests.[36] All probability values are 2 sided. Values of $P<0.05$ were considered statistically significant.

Results

Clinical Characteristics of the Study Samples

Table 1 summarizes age, gender, conventional cardiovascular risk factors, and lipid profiles in each group of patients, as well as the combined non-ACS patients, undergoing CAG. Patient characteristics, including age, gender, and incidence of hypertension, diabetes, and hypercholesterolemia, were comparable between the ACS group and the combined non-ACS CAG, except that the

TABLE 1. Characteristics of Consecutive CAG Patients

Characteristics	Intact Coronary	Controlled CHD	Ischemic CHD	Non-ACS CAG	ACS
Patients, n	52	122	173	Subtotal, 347	80
Age (mean ± SD), y	66 ± 9	66 ± 10	67 ± 9	67 ± 10	64 ± 12
Male sex, n (%)	32 (62)	89 (73)	128 (74)	249 (67)	59 (74)
Risk factors, n (%)					
Hypertension	22 (42)	65 (53)	82 (47)	168 (48)	30 (38)
Diabetes	8 (15)†	43 (35)	63 (36)	114 (33)	26 (33)
Smoking	19 (37)	61 (50)	57 (33)‡	136 (39)*	43 (54)*
Hypercholesterolemia	14 (27)	47 (39)	84 (49)§	144 (41)	27 (34)
Lipid profile (mean ± SD), mg/dL					
LDL cholesterol	122 ± 38	125 ± 35	121 ± 35	121 ± 36	122 ± 35
HDL cholesterol	50 ± 16	45 ± 13	45 ± 14	46 ± 14¶	41 ± 11¶#
Triglycerides	137 ± 103	141 ± 63	132 ± 52	136 ± 68	140 ± 75
hs-CRP (mean ± SD), ng/mL	3.10 ± 0.75	3.09 ± 0.65	3.11 ± 0.87	3.10 ± 0.78¶	3.41 ± 0.87 ¶

Values for hs-CRP were transformed in logarithm of 10. One-way ANOVA was followed up with Tukey-Kramer pairwise comparisons among means.

*$P<0.01$ for comparison with combined all non-ACS and ACS with 2-way cross-tabulation with χ^2 test.

†$P<0.05$ for comparison with controlled CHD, ischemic CHD, and ACS.

‡$P<0.05$ for comparison with controlled CHD and ACS.

§$P<0.05$ for comparison with intact coronary and ACS.

$P<0.05$ for comparison with intact coronary, controlled CHD, and ischemic CHD.

¶$P<0.001$ for comparison between non-ACS CAG and ACS with t test.

#$P<0.05$ for comparison with intact coronary.

ACS group showed higher smoking rate and lower HDL cholesterol levels (Table 1). Table 2 compares the patient characteristics among ACS, non-ACS CAG, and noncardiac acute and chronic illness groups. Patient characteristics were comparable between the ACS and combined non-ACS group, except that HDL cholesterol levels were significantly lower and the incidence of smoking habits was significantly higher in ACS than in the combined all non-ACS group (Table 2), as shown in CAG groups alone (Table 1).

TABLE 2. Characteristics of All Enrolled Patients

Characteristics	Noncardiac Chronic Illness	Noncardiac Acute Illness	Non-ACS CAG	Combined All Non-ACS	ACS
Patients, n	60	34	347	Subtotal, 441	80
Age (mean ± SD, y	67 ± 16	54 ± 18†	67 ± 10	66 ± 13	64 ± 12
Male sex, n (%)	18 (30)	21 (62)	249 (67)	288 (65)	59 (74)
Risk factors, n (%)					
Hypertension	16 (27)	9 (26)	168 (48)§	193 (44)	30 (38)
Diabetes	5 (8)§	2 (6)§	114 (33)	121 (27)	26 (33)
Smoking	9 (15)‖	13 (38)	136 (39)	158 (36)*	43 (54)*
Hypercholesterolemia	25 (42)	6 (18)†	144 (41)	175 (40)	27 (34)
Lipid profile (mean ± SD), mg/dL					
LDL cholesterol	127 ± 30	101 ± 37†	121 ± 36	122 ± 36	122 ± 35
HDL cholesterol	59 ± 18	56 ± 17	46 ± 14	49 ± 16¶	41 ± 11‡¶
Triglycerides	147 ± 106	120 ± 168	136 ± 68	136 ± 86	140 ± 75
hs-CRP (mean ± SD), ng/mL	3.14 ± 0.58	4.17 ± 1.02†	3.10 ± 0.78	3.19 ± 0.83	3.41 ± 0.87#

Values for hs-CRP were transformed in logarithm of 10. One-way ANOVA was followed up with Tukey-Kramer pairwise comparisons among means.

*$P<0.01$ for comparison with combined all non-ACS and ACS with 2-way cross-tabulation with χ^2 test.

†$P<0.05$ for comparison with chronic illness, non-ACS CAG, and ACS.

‡$P<0.05$ for comparison with acute illness, chronic illness, and non-ACS CAG.

§$P<0.05$ for comparison with non-ACS CAG and ACS.

$P<0.05$ for comparison with chronic illness, non-ACS CAG, and ACS.

¶$P<0.001$ for comparison between combined all non-ACS and ACS with t test.

#$P<0.05$ for comparison with non-ACS CAG.

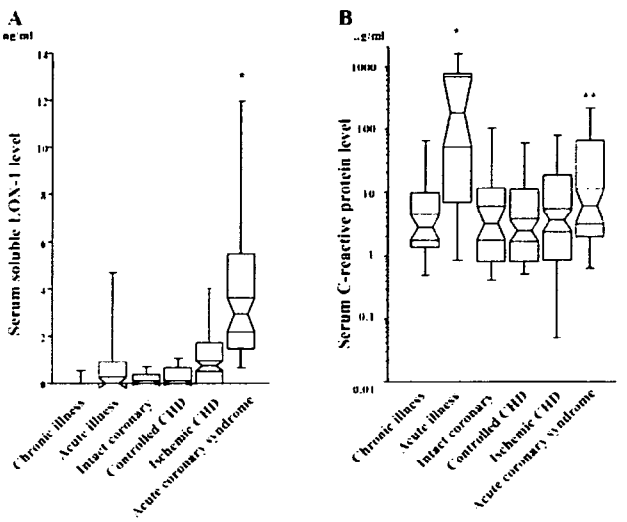

Figure 1. Serum sLOX-1 and hs-CRP levels. In 427 consecutive patients who underwent CAG, consisting of 80 with ACS, 173 with symptomatic CHD (ischemic CHD), 122 with coronary atherosclerosis without ischemia (controlled CHD), and 52 without apparent coronary atherosclerosis (intact coronary) plus 34 with noncardiac acute illness (acute illness) and 60 patients with noncardiac chronic illness (chronic illness), serum LOX-1 (A) and hs-CRP (B) levels were determined and are indicated in box plots. Center horizontal lines indicate median values; inner trapezoidal boxes, 95% CIs for medians; upper and lower edges of outer boxes, 25th and 75th percentiles; and lower and upper bars, 10th and 90th percentiles. *Statistically significant differences among the 6 groups by Kruskal-Wallis test with Dunn's test (A) and 1-way ANOVA with Tukey-Kramer test (B) ($P<0.05$). **Significant differences among 4 CAG groups by 1-way ANOVA with Tukey-Kramer test ($P<0.05$).

Serum sLOX-1 Levels

As shown in Figure 1A, serum sLOX-1 levels were remarkably higher in ACS (median, 2.91 ng/mL; range, <0.5 to 170 ng/mL) when compared among 6 groups including intact coronary (median, <0.5 ng/mL; range, <0.5 to 1.3 ng/mL), controlled CHD (median, <0.5 ng/mL; range, <0.5 to 3.4 ng/mL), ischemic CHD (median, 0.73 ng/mL; range, <0.5 to 14.0 ng/mL), acute noncardiac illness (median, <0.5 ng/mL; range, <0.5 to 6.4 ng/mL), and chronic illness (median, <0.5 ng/mL; range, <0.5 to 3.3 ng/mL). Serum sLOX-1 can discriminate ACS from other CAG groups ($\chi^2=88.2$, $P<0.001$), given a cutoff value of 1.0 ng/mL, with 81% sensitivity and 75% specificity (Table 3).

Lipid Profiles, Conventional Cardiovascular Risk Factors, hs-CRP, and sLOX-1

Serum hs-CRP levels were significantly higher in the ACS than non-ACS groups when compared among 4 CAG groups alone (Table 1 and Figure 1B). Levels of hs-CRP in patients with noncardiac acute illness were significantly higher than in any of other groups because this group contained acute inflammatory diseases (Figure 1B and Table 2). Although levels of hs-CRP in patients with ACS were significantly higher than in any of other groups when compared among CAG patients alone, ACS did not show statistically significant difference in serum hs-CRP levels when compared among all the 6 groups, including noncardiac acute and chronic illness groups (Figure 1B and Table 2).

TABLE 3. Sensitivity and Specificity of sLOX-1 and hs-CRP for ACS Among CAG Patients

	sLOX-1	hs-CRP	TnT
Non-ACS CAG (n=347)			
Positive, n	86	91	...
Specificity	75	74	...
All ACS (n=80)			
Positive, n	65	36	54
χ^2	88.2	12	...
P	<0.001	<0.001	...
Sensitivity	81	45	68
NQ-ACS (n=23)			
Positive, n	21	9	11
χ^2	43.2	1.7	...
P	<0.001	0.22	...
Sensitivity	91	39	48
ACS with TnT negative at the time of visit (n=24)			
Positive, n	20	3	...
χ^2	37.8	3.1	...
P	<0.001	0.2	...
Sensitivity	83	13	...

Cutoff values were 1.0 ng/mL for sLOX-1, 4 μg/mL for hs-CRP, and 0.03 ng/mL for TnT. ACS patients with <0.03 ng/mL TnT determined at the time of visit were defined as cases with TnT negative at the time of visit. χ^2 was determined by the Yates continuity-corrected χ^2 test, and probability values were obtained by comparison with non-ACS patients.

Significant inverse correlation was found between sLOX-1 and HDL cholesterol levels (Spearman's $\rho=-0.17$; $P<0.01$). However, no significant correlation was found between sLOX-1 and either LDL cholesterol (Spearman's $\rho=-0.02$; $P=0.68$) or triglyceride (Spearman's $\rho=-0.01$, $P=0.89$) levels. We also examined the association between sLOX-1 levels and other cardiovascular risk factors such as hypertension, diabetes, and smoking among all enrolled patients. No significant differences were found in sLOX-1 levels between those with and without hypertension, diabetes, or smoking.

Multivariable logistic regression analyses of all patients (Cox and Snell's $R^2=0.263$) showed that sLOX-1 was associated with ACS (odds ratio, 1.51; 95% CI, 1.35 to 1.70; $P<0.001$). Levels of hs-CRP, HDL cholesterol, and smoking habits also were significantly associated with ACS (odds ratio, 1.40, 0.96, and 2.07; 95% CI, 1.00 to 1.94, 0.94 to 0.98, and 1.08 to 3.96; $P<0.05$, $P<0.01$, and $P<0.05$, respectively). However, no significant correlation was found between sLOX-1 and hs-CRP levels among all patients and patients with ACS alone (Spearman's $\rho=0.01$ and -0.06; $P=0.81$ and $P=0.58$, respectively).

sLOX-1 as a Diagnostic Marker of ACS

Figure 2 shows ROC curves for the levels of sLOX-1 and hs-CRP in all 80 ACS patients (Figure 2A) and 24 patients with ACS without ST elevation or abnormal Q waves at the time of visit (NQ-ACS) (Figure 2B) compared with the 347

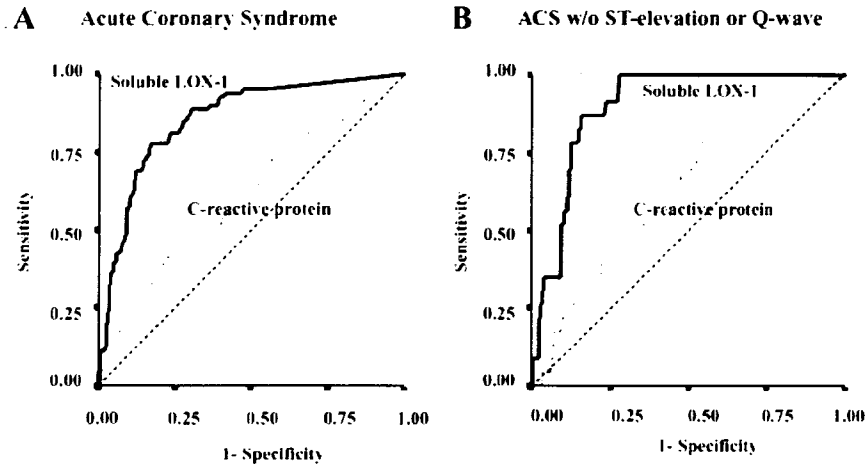

Figure 2. ROC curves of sLOX-1 and hs-CRP for diagnosis of ACS (A) and ACS without ST elevation or abnormal Q-waves (NQ-ACS; B) among consecutive patients undergoing coronary angiography. True-positive fraction (sensitivity as *y* axis) was plotted vs false-positive fraction (1 − specificity as *x* axis) by changing cutoff values for test.

non-ACS CAG patients as a reference group. In all ACS patients, the areas below the curves were 0.86 (95% CI, 0.81 to 0.90) for sLOX-1 and 0.62 (95% CI, 0.55 to 0.69) for hs-CRP. In patients with NQ-ACS, the areas below the curves were 0.90 (95% CI, 0.86 to 0.94) for sLOX-1 and 0.63 (95% CI, 0.52 to 0.74) for hs-CRP. These differences between sLOX-1 and hs-CRP (0.24 and 0.27; 95% CI, 0.20 to 0.28 and 0.21 to 0.33, respectively) are statistically significant ($P<0.05$) in both all ACS and NQ-ACS patients. Given a cutoff value of 1.0 ng/mL for sLOX-1, serum sLOX-1 can significantly discriminate ACS patients from non-ACS patients (non-ACS CAG) among consecutive patients undergoing coronary angiography ($P<0.001$) and showed 81% sensitivity and 75% specificity for the diagnosis of ACS (Table 3). In contrast, an hs-CRP cutoff value of 4 μg/mL, which had comparable specificity (74%), showed lower sensitivity (45%) for the diagnosis of ACS. Values of sLOX-1 at the time of visit efficiently discriminated patients with NQ-ACS ($P<0.001$) from non-ACS CAG with 91% sensitivity; however, sensitivity of TnT (cutoff value, 0.03 ng/mL) for diagnosis of NQ-ACS was 48%. Moreover, sLOX-1 showed 83% sensitivity for diagnosis of ACS even in patients with negative TnT (<0.03 ng/mL) at the time of visit (Table 3).

Time-Dependent Changes in sLOX-1 Concentrations After the Onset of ACS

Serum sLOX-1 and TnT were serially measured in consecutive 40 ACS patients. Figure 3A indicates relative values of serum sLOX-1 and TnT compared with the highest values among serial blood samples obtained from each individual patient. Peak levels of sLOX-1 were observed on admission or after PCI ($P<0.01$). In contrast, the highest TnT values were observed around day 1, which is consistent with previous reports ($P<0.01$).[37,38] In addition, no significant correlation was found between peak levels of sLOX-1 and CPK (Spearman's $\rho=0.28$; $P=0.10$) or TnT (Spearman's $\rho=0.20$; $P=0.20$; Figure 3B).

Discussion

Rupture of atheromatous plaques, followed by thrombus formation, is considered a crucial step in the pathogenesis of ACS. Atherosclerotic plaques with abundant lipid-laden macrophages and activated smooth muscle cells in the intima appear to be prone to rupture.[39] In such vulnerable plaques, LOX-1 is expressed prominently by smooth muscle cells and macrophages and contributes to apoptosis of smooth muscle cells[29–31] and production of matrix metalloproteinases.[32] Under these conditions, enhanced protease activities may cleave

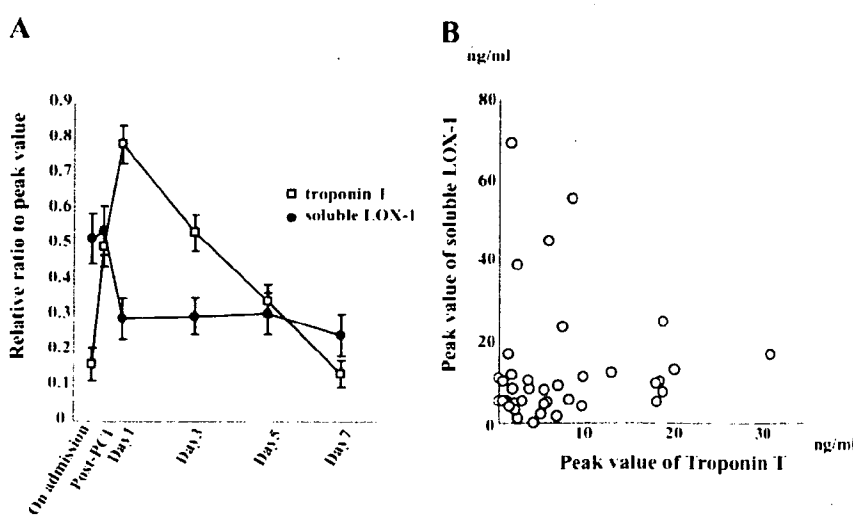

Figure 3. Time-dependent changes in sLOX-1 and TnT levels after onset of ACS (A) and comparison between peak values of sLOX-1 and TnT (B). Blood samples were collected on admission, immediately after PCI (post-PCI), and on days 1, 3, 5, and 7 from 40 ACS patients undergoing emergency PCI. Relative ratios (mean ± SEM) to peak value of each individual patient are indicated (●, sLOX-1; ○, TnT). Statistically significant correlation was not found between peak values of sLOX-1 and TnT during these periods (Spearman's $\rho=0.20$; $P=0.20$).

sLOX-1 from the surface of these vascular cells in which LOX-1 is abundantly expressed, although proteases responsible for LOX-1 cleavage have not been fully identified. Additionally, in the process of thrombus formation after plaque rupture, LOX-1 expression on the surface of platelets may also be abundant by thrombotic activation,[33] as is the case for CD40L.[14] However, LOX-1 can also bind activated platelets[40]; therefore, sLOX-1 might not be liberated from the surface of activated platelets. In fact, we did not observe significant differences in sLOX-1 levels between plasma and serum samples or high levels of circulating sLOX-1 in typical patients with disseminated intravascular coagulation (data not shown). Moreover, LOX-1 expression can be inducible in cardiac myocytes by norepinephrine or endothelin,[41] which may be upregulated by proinflammatory stimuli or ischemia. LOX-1 on the cell surface of cardiac myocytes might possibly be another source of sLOX-1.

Although LOX-1 expression was prominent in atherosclerotic lesions[29] and remarkably inducible by proinflammatory stimuli,[23,25,26] serum sLOX-1 did not reflect just general inflammation or atherosclerotic lesion sizes but rather instability of atherosclerotic plaques. In fact, sLOX-1 was elevated in the acute phases of ACS, but not in general acute inflammatory diseases in which serum hs-CRP levels were high (Figure 1). In addition, serum sLOX-1 levels were not significantly correlated with those of the inflammatory marker hs-CRP or numbers of affected coronary arteries (data not shown). Although a recent report has shown that CRP can induce LOX-1 expression,[42] LOX-1 can also be induced by a variety of biological stimuli, and regulation of LOX-1 cleavage may not be so correlated with CRP. Circulating Ox-LDL levels, which might be mildly oxidized, have been reported to be elevated in ACS, although its sensitivity or specificity for the diagnosis of ACS was not demonstrated.[9,43] The antibodies used in our ELISA can be bound to sLOX-1 in the presence of Ox-LDL; in fact, the addition of Ox-LDL to sLOX-1 samples did not affect the results of our sLOX-1 ELISA (see the Table in the online-only Data Supplement). Therefore, Ox-LDL in serum does not appear to interfere with the results of our sLOX-1 ELISA.

In addition, sLOX-1 did not show any correlation with TnT (Figure 3B) or CPK, suggesting that sLOX-1 is not a marker for cardiac necrosis or injury. Furthermore, peak time of sLOX-1 in serum was earlier than that of TnT (Figure 3A). This is quite reasonable because plaque instability or rupture precedes cardiac necrosis or ischemic injury and suggests that sLOX-1 appears to be a suitable serum marker for early diagnosis of ACS, especially NQ-ACS without severe cardiac necrosis or damage. In fact, sLOX-1 showed higher sensitivity for early detection of NQ-ACS than TnT or hs-CRP did (Table 3). Moreover, even in ACS patients without significant elevation of TnT levels (<0.03 ng/mL) at the time of visit, 86% of these TnT-negative patients showed sLOX-1 levels >1.0 ng/mL (Table 3), indicating the usefulness of sLOX-1 measurement, in addition to TnT, at the very early stage.

We currently do not know exactly when serum sLOX-1 levels begin to increase before the onset of ACS; however, sLOX-1 levels at the time of visit showed almost the peak values for each patient (Figure 3A), suggesting that serum sLOX-1 levels may begin to rise before the onset of ACS. Further large-scale prospective studies will tell us more about the value of serum sLOX-1 for predicting ACS onset.

Acknowledgments

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Original Article

Polymorphisms in Four Genes Related to Triglyceride and HDL-cholesterol Levels in the General Japanese Population in 2000

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We studied the association of six common polymorphisms of four genes related to lipid metabolism with serum lipid levels. We selected single-nucleotide polymorphisms (SNPs) in the genes for cholesteryl ester transfer protein (*CETP*), lipoprotein lipase (*LPL*), hepatic lipase (*LIPC*), and apolipoprotein CIII (*APOC3*), and studied 2267 individuals randomly selected from the participants of Serum Lipid Survey 2000. There was a significant association of *CETP* polymorphism (D442G, Int14 +1 G → A, and TaqIB), *LPL* polymorphism (S447X), and *LIPC* polymorphism (–514 → CT) with HDL-cholesterol levels. We also found a significant association of *LPL* polymorphism (S447X) and *APOC3* polymorphism (SstI) with triglyceride levels. This is the largest database showing the association of common genetic variants in lipid metabolism with serum lipid levels in the general Japanese population. Further study is necessary to elucidate the role of these gene polymorphisms in cardiovascular events. *J Atheroscler Thromb, 2005; 12: 240–250.*

Key words; Hyperlipidemia, Polymorphism, Cholesterol ester transfer protein, Lipoprotein lipase, Triglyceride lipase, Apolipoprotein CIII

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Introduction

Hyperlipidemia is a major risk factor for coronary artery disease (CAD) (1). In contrast to the sharp decline in both serum cholesterol levels and mortality from CAD in the United States and Western Europe, remarkable increases

in serum cholesterol levels as well as CAD mortality have been anticipated in the Asian-Pacific area due to industrialization and the modernization of lifestyle (2). The importance of lifestyle is also proved by the fact that Japanese who migrated to Hawaii and California, for example, showed higher levels of serum cholesterol and a higher incidence of CAD than people in Japan (3). Thus, dietary habits and other environmental factors affect serum cholesterol levels and CAD mortality in the population. However, genetic traits are also an important determinant of serum lipid levels.

Major mutations have been described coding for the low-density lipoprotein (LDL) receptor, apolipoprotein B, and so forth, affecting mainly serum LDL-cholesterol levels (4, 5). However, plasma triglyceride (TG) and high-density lipoprotein (HDL)-cholesterol levels are also considered established risk factors for CAD (6). Therefore, the association of common variants of candidate genes with changes in TG and HDL-cholesterol levels would be important determinants for CAD risk. Considering the recent prevalence of metabolic syndrome, it would be also intriguing to examine the effect of these genetic polymorphisms on the development of metabolic syndrome. So far in Japan, however, a large-scale analysis has not been performed on common gene variants related to lipid metabolism.

In 2000, we conducted a survey in the general Japanese population, involving 12,839 people from all over the country (7). We tried to examine the frequency of common polymorphisms of four genes related to lipid metabolism and show an association with serum lipid levels. Among the factors involved in lipid metabolism, we chose the following 4 genes because of the association with TG or HDL-cholesterol. Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl ester from HDL to apolipoprotein B-containing lipoproteins (8). CETP is a key protein in reverse cholesterol transport and its deficiency is associated with hyperalphalipoproteinemia (9–11). Among several polymorphisms of the *CETP* gene, a G to A substitution at the 5' splice donor site of intron 14 (Int14 +1 G → A) and a missense mutation in exon 15 (D442G) are common mutations of hyperalphalipoproteinemia in Japanese (12, 13). The Int14 +1 G → A mutation results in a null allele: homozygotes with the mutation have no CETP in plasma and markedly elevated levels of HDL-cholesterol (10). The D442G mutation is near the carboxy terminal region of CETP shown to be essential for its function (14, 15). The TaqIB polymorphism of the *CETP* gene is one of the most studied polymorphisms worldwide. The B2 allele of the TaqIB polymorphism in intron 1 was associated with decreased CETP levels and high HDL-cholesterol levels (16) and with coronary heart disease risk in the Framingham Study (17). Therefore, we selected these three polymorphisms for our analysis.

Lipoprotein lipase (LPL) is one of the key enzymes in the metabolism of TG-rich lipoproteins. Among several polymorphisms of the *LPL* gene we chose S447X, which is common, having an allele frequency of approximately 20% in healthy individuals, and whose mutation is associated with a favorable lipid profile (18–20). Hepatic lipase (LIPC) is also a member of the lipase superfamily and plays an important role in the metabolism and modeling of both pro- and anti-atherogenic lipoproteins (21). Among the several polymorphisms we selected, –514C → T, located in the promoter region of the *LIPC* gene, has been demonstrated to influence LIPC activity levels (22). Apolipoprotein CIII (apoCIII) can inhibit LPL and reduces the uptake of TG-rich remnant particles and the SstI polymorphism of the *APOC3* gene has been shown to be associated with hypertriglyceridemia and CAD in various human populations (23–27). Therefore, we also examined these polymorphisms in the general Japanese population.

The aim of this study was, therefore, to examine the incidence of these gene polymorphisms and their contribution to lipid concentrations in the general Japanese population.

Methods

Designs and data collection

This work is part of the Serum Lipid Survey 2000 from various areas around Japan. The Ethics committee, graduate school and faculty of Medicine, Kyoto University approved the study protocol and all subjects provided written informed consent for the genetic analysis. The DNA samples were handled according to the guidelines from the Ministry of Health, Labor, and Welfare. In the Serum Lipid Survey 2000, a total of 12,839 subjects were recruited at 36 hospitals across the country. The subjects in the present study were participants in the survey at 9 hospitals from whom informed content for genotyping was sought. Of 12,839 subjects, 2267 (17.7%) with no lipid-altering medication were randomly selected for the present study. In some institutes, information on gender was not disclosed.

Laboratory methods

All serum and blood samples were obtained in the fasting state. All lipid and other analyses were conducted with venous blood samples within one week of collection at BML (Saitama, Japan). Serum cholesterol and TG levels were measured by enzymatic assay. HDL-cholesterol and LDL-cholesterol levels were measured enzymatically with a kit from Daiichi Kagaku Co. Ltd. (Tokyo, Japan). The results of lipid analyses were indirectly standardized according to the criteria of the CDC Lipid Standardization Program (25). DNA was extracted with a QIAamp DNA blood kit (Qiagen, Hilden, Germany).

Detection of gene mutations by Invader® assay

We used the Invader® assay to screen three known mutations of the *CETP* gene, one mutation of the *LIPC* gene, one mutation of the *LPL* gene, and one mutation of the *APOC3* gene, as previously described (26). In brief, the probe/Invader®/$MgCl_2$ mixture was prepared by combining 3 μl of primary probe/Invader® mix and 5 μl of 22.5 mM $MgCl_2$ per reaction. The primary probes/Invader® mixture contained 3.5 μmol/l wild primary probe, 3.5 μmol/l mutant primary probe, 0.35 μmol/l Invader® oligonucleotide, and 10 mmol/l MOPS. Eight microliters of primary probe/Invader®/$MgCl_2$ mixture as well was added into a 96-well plate. Seven microliters of 5 fmol/l synthetic target oligonucleotides, 10 μg/ml yeast tRNA (no target blank), and genomic DNA (15 ng/μl) were added, and denatured by incubation at 95°C for 10 min. After 15 μl of mineral oil (Sigma, St. Louis, MO, USA) was overlayed into each well, the plate was incubated isothermally at 63°C for 4 h in a DNA thermalcycler (PTC-200; MJ Research, Watertown, MA, USA) and then kept at 4°C until fluorescence was measured. The fluorescent intensities were measured using a fluorescence microtiter plate reader (Cytoflour 4000; Applied Biosystems) with excitation at 485 nm/20 nm (Wave length/Band width) and emission, at 530 nm/25 nm for FAM, and excitation at 560 nm/20 nm and emission, at 620 nm/40 nm for RED. The genotyping was based on calculations with the ratios of net counts with wild primary probe to net counts with mutant primary probe. The probes used in this study were designed and synthesized by Third Wave Technologies, Inc (Madison, WI).

Data analyses

Differences in means were evaluated using an analysis of variance. Multiple regression analysis was done to compare age- and sex-adjusted means. The χ^2-test was used to compare the incidence of each genotype. The analysis was performed with the statistical Package for Social Sciences (SPSS Japan Inc. ver. 11.5, Tokyo, Japan).

Results

We investigated the frequency and phenotypic association of the common polymorphisms of *CETP*, *LPL*, *LIPC*, and *APOC3* genes at the population level in 2,267 subjects. Table 1 summarizes the mean serum lipid levels in the participants in this study. The mean age, and total cholesterol, TG, HDL-cholesterol, and LDL-cholesterol levels in this population were similar to the values for all 12,839 participants in Serum Lipid Survey 2000. We also found that the medians of total, LDL-, and HDL-cholesterol levels did not differ appreciably from the means, thereby excluding gross right-hand tailing of the distribution (data not shown). These results indicate that the participants in the gene analysis are representative of the general Japanese population.

Table 1. Lipid profile and age of all the participants.

	All	Men	Women
T-Cho (mmol/l)	5.18 (0.021)	5.23 (0.046)	5.15 (0.046)
TG (mmol/l)	1.31 (0.024)	1.58 (0.050)*	1.11 (0.039)*
HDL-c (mmol/l)	1.53 (0.010)	1.38 (0.020)*	1.65 (0.017)*
LDL-c (mmol/l)	3.00 (0.020)	3.08 (0.044)*	2.93 (0.039)*
Age (years)	47.1 (0.58)	49.5 (0.87)*	45.3 (0.76)*
Men (%)	43		

Data are expressed as the mean (SEM).
* $p < 0.01$, men vs. women.

Table 2 summarizes the association of the gene polymorphisms with serum lipid levels in all the participants. Tables 3 and 4 show the analysis in male and female participants, respectively. Table 5 shows age- and sex-adjusted means with 95% CI. We found that Hardy-Weinberg equilibrium was the case for all the SNPs, supporting the assumptions of random mating in this population except *CETP* Int14 +1 G → A, for which no homozygote was found in this population.

The incidence of heterozygote mutations of D442G and Int14 +1 G → A of the *CETP* gene was 8.1 and 0.6 %, respectively. These mutations were associated with higher HDL-cholesterol levels. The heterozygous mutation of D442G was also associated with lower TG levels only in men. Although the incidence of the homozygous mutation of D442G and heterozygous mutation of Int14 +1 G → A was quite low and the difference was not significant, the TG levels tended to be higher. The incidence of B1B1, B1B2, and B2B2 genotypes of the *CETP* TaqIB polymorphism was 35.8, 48.4, and 15.8%, respectively. The B2 allele of the *CETP* TaqIB polymorphism was associated with higher HDL-cholesterol levels in all the participants, men, and women. Although the difference was not statistically significant, the participants with the B2 allele tended to have lower TG levels, which is different from the results with the homozygous mutation of D442G and heterozygous mutation of Int14 +1 G → A.

We then determined the polymorphisms of *LPL* S447X mutations in this population. The incidence of heterozygous and homozygous mutations in the *LPL* gene was 20.7 and 1.3%, respectively. The mutation of the LPL S447X site was associated with higher HDL-cholesterol and lower TG levels, although the difference in the level of HDL-cholesterol in men or of TG in women was not statistically significant, possibly due to the small sample number.

The incidence of the CC, CT, and TT genotypes of *LIPC* in the Japanese was 24.9, 50.4, and 24.7%, respectively. Overall, the T allele was associated with an increase in HDL-cholesterol levels. However, the difference was not

Table 2. Demographic and lipid profile of all the participants according to genotype.

CETP D442G (rs2303790)

Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	47	91.6	1.53 (0.001)	1.37 (0.025)	3.06 (0.021)
hetero	48.4	8.1	1.75 (0.004)	1.15 (0.061)	2.90 (0.075)
homo	46.5	0.2	1.81 (0.18)	1.60 (0.101)	3.19 (1.580)
			$p = 0.000$	$p = 0.071$	$p = 0.154$

CETP Int14 +1 G → A (rs5742907)

Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	47	99.4	1.54 (0.009)	1.36 (0.024)	3.06 (0.020)
hetero	58.7	0.6	2.12 (0.262)	1.72 (0.362)	3.08 (0.316)
			$p = 0.000$	$p = 0.241$	$p = 0.938$

CETP TaqIB (rs708272)

Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
B1B1	46.8	35.8	1.50 (0.016)	1.36 (0.036)	3.00 (0.033)
B1B2	48.4	48.4	1.54 (0.013)	1.38 (0.038)	3.08 (0.030)
B2B2	48.2	15.8	1.66 (0.024)	1.25 (0.043)	3.08 (0.051)
			$p = 0.000$	$p = 0.160$	$p = 0.362$

LPL S447X (rs328)

Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	47.3	78	1.53 (0.011)	1.37 (0.029)	3.06 (0.023)
hetero	46.2	20.7	1.60 (0.020)	1.24 (0.043)	3.06 (0.046)
homo	48	1.3	1.63 (0.101)	1.08 (0.125)	3.29 (0.189)
			$p = 0.004$	$p = 0.032$	$p = 0.487$

LIPC 514CT (rs1800588)

Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
CC	49.7	24.9	1.49 (0.018)	1.37 (0.046)	3.11 (0.040)
CT	45.6	50.4	1.53 (0.013)	1.33 (0.034)	3.03 (0.029)
TT	47.6	24.7	1.63 (0.020)	1.39 (0.050)	3.06 (0.040)
			$p = 0.000$	$p = 0.520$	$p = 0.255$

APOC3 SstI (rs5128)

Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
S1S1	46.6	42	1.56 (0.015)	1.32 (0.039)	3.06 (0.032)
S1S2	47	45.8	1.54 (0.013)	1.34 (0.033)	3.03 (0.029)
S2S2	48.9	12.2	1.52 (0.025)	1.53 (0.070)	3.11 (0.060)
			$p = 0.413$	$p = 0.021$	$p = 0.434$

Data are expressed as the mean (SEM). Each p-value was based on an analysis of covariance.

significant in men. The TG levels do not seem to be affected by this SNP.

The incidence of the S1S1, S1S2, and S2S2 genotypes of the *APOC3* SstI polymorphism was 42.0, 45.8, and 12.2%, respectively. Although the HDL and LDL-cholesterol levels were similar for all the genotypes, the S2 allele was associated with higher TG levels in all the participants and in men, but not in women. Among the SNPs studied, no polymorphism was found to affect LDL-cholesterol levels. We also determined sex- and age-adjusted means in Table 5 by multiple regression analysis. Due to the limited sample number and large variability of data, a

Table 3. Demographic and lipid profile of male participants according to genotype.

CETP D442G (rs2303790)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	351	1.36 (0.020)	1.60 (0.052)	3.11 (0.045)
hetero	26	1.60 (0.105)	1.19 (0.176)	2.98 (0.194)
		$p = 0.003$	$p = 0.035$	$p = 0.453$

CETP TaqIB (rs708272)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
B1B1	121	1.33 (0.034)	1.64 (0.087)	3.06 (0.073)
B1B2	203	1.36 (0.026)	1.55 (0.068)	3.11 (0.064)
B2B2	53	1.56 (0.063)	1.53 (0.147)	3.13 (0.107)
		$p = 0.001$	$p = 0.664$	$p = 0.758$

LPL S447X (rs328)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	292	1.36 (0.022)	1.65 (0.060)	3.08 (0.047)
hetero	81	1.43 (0.048)	1.36 (0.082)	3.16 (0.112)
homo	4	1.51 (0.386)	0.95 (0.295)	2.80 (0.513)
		$p = 0.278$	$p = 0.029$	$p = 0.617$

LIPC 514CT (rs1800588)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
CC	99	1.32 (0.032)	1.66 (0.094)	3.08 (0.072)
CT	188	1.40 (0.032)	1.51 (0.075)	3.08 (0.069)
TT	90	1.40 (0.041)	1.60 (0.095)	3.08 (0.085)
		$p = 0.266$	$p = 0.499$	$p = 0.996$

APOC3 SstI (rs5128)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
S1S1	165	1.37 (0.031)	1.50 (0.073)	3.16 (0.072)
S1S2	173	1.40 (0.031)	1.58 (0.076)	3.00 (0.060)
S2S2	39	1.31 (0.054)	1.92 (0.162)	3.13 (0.138)
		$p = 0.473$	$p = 0.041$	$p = 0.196$

Data are expressed as the mean (SEM). Each *p*-value was based on an analysis of covariance.

Table 4. Demographic and lipid profile of female participants according to genotype.

CETP D442G (rs2303790)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	440	1.58 (0.018)	1.128 (0.0412)	2.93 (0.041)
hetero	34	1.67 (0.074)	1.15 (0.092)	2.98 (0.140)
		$p = 0.002$	$p = 0.590$	$p = 0.306$

CETP TaqIB (rs708272)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
B1B1	183	1.58 (0.028)	1.13 (0.057)	2.93 (0.062)
B1B2	220	1.67 (0.026)	1.15 (0.066)	2.98 (0.059)
B2B2	72	1.75 (0.043)	0.92 (0.057)	2.85 (0.105)
		$p = 0.004$	$p = 0.127$	$p = 0.461$

LPL S447X (rs328)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	369	1.62 (0.020)	1.14 (0.046)	2.95 (0.046)
hetero	102	1.73 (0.038)	0.99 (0.065)	2.85 (0.081)
homo	4	1.97 (0.164)	0.72 (0.177)	3.89 (0.321)
		$p = 0.010$	$p = 0.185$	$p = 0.054$

LIPC 514CT (rs1800588)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
CC	102	1.59 (0.041)	1.15 (0.089)	2.93 (0.086)
CT	249	1.63 (0.022)	1.04 (0.046)	2.90 (0.050)
TT	124	1.73 (0.037)	1.20 (0.091)	3.03 (0.090)
		$p = 0.014$	$p = 0.210$	$p = 0.406$

APOC3 SstI (rs5128)

Genotype	*n*	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
S1S1	207	1.65 (0.028)	1.05 (0.054)	2.90 (0.062)
S1S2	208	1.62 (0.026)	1.18 (0.067)	2.93 (0.059)
S2S2	60	1.75 (0.045)	1.08 (0.079)	3.03 (0.106)
		$p = 0.078$	$p = 0.272$	$p = 0.608$

Data are expressed as the mean (SEM). Each *p*-value was based on an analysis of covariance.

significant difference was not found in TG levels in *LPL* or *APOC3* polymorphisms.

To determine the contribution of *CETP* and *LPL* gene polymorphisms to hyperalphacholesterolemia (2.58 mmol/l or over) and hypoalphacholesterolemia (1 mmol/l or under), we divided all the participants into 3 groups according to HDL-cholesterol levels; 1 mmol/l or under, 1 to 2.58 mmol/l, and 2.58 mmol/l or over. We then assessed the incidence of each genotype. The incidence of hyper- and hypoalphacholesterolemia was 1.8 and 8.3%, respectively. Among the genes studied, we found 3 gene polymorphisms to be associated with the incidence of high HDL-cholesterol (2.58 mmol/l or over) (Table 6). Participants with the B2B2 genotype of *CETP* TaqIB had a higher incidence of high HDL-cholesterol levels than the others. Heterozygotes of the *CETP* D442G polymorphism had a higher incidence of higher HDL-cholesterol levels than individuals with the wild type. Homozygotes of the *LPL* S447X polymorphism had a higher incidence of higher HDL-cholesterol levels than the others.

Discussion

In this study we have demonstrated the frequency of six common polymorphisms of four genes related to lipid

Table 5. Age- and sex-adjusted means of all the participants according to genotype.

CETP D442G (rs2303790)

Genotype	HDL-c (mmol/l)			TG (mmol/l)			LDL-c (mmo/l)		
	mean	low	upper	mean	low	upper	mean	low	upper
wt	1.53	1.49	1.56	1.37	1.29	1.45	3.05	2.98	3.11
hetero	1.72	1.62	1.83	1.18	0.90	1.46	2.90	2.68	3.12
homo	1.91	1.70	2.13	1.00	0.42	1.55	2.75	2.30	3.20
	$p = 0.0005$			$p = 0.200$			$p = 0.210$		

CETP Int14 +1 G → A (rs5742907)

Genotype	HDL-c (mmol/l)			TG (mmol/l)			LDL-c (mmo/l)		
	mean	low	upper	mean	low	upper	mean	low	upper
wt	1.54	1.51	1.57	1.35	1.27	1.43	3.04	2.97	3.10
hetero	2.13	1.72	2.54	1.70	0.63	2.79	2.97	2.11	3.83
	$p = 0.0048$			$p = 0.514$			$p = 0.877$		

CETP TaqIB (rs708272)

Genotype	HDL-c (mmol/l)			TG (mmol/l)			LDL-c (mmo/l)		
	mean	low	upper	mean	low	upper	mean	low	upper
B1B1	1.47	1.42	1.51	1.41	1.30	1.54	3.03	2.93	3.13
B1B2	1.56	1.53	1.59	1.34	1.25	1.42	3.04	2.97	3.10
B2B2	1.65	1.59	1.71	1.26	1.09	1.41	3.05	3.00	3.12
	$p = 0.0001$			$p = 0.154$			$p = 0.873$		

LPL S447X (rs328)

Genotype	HDL-c (mmol/l)			TG (mmol/l)			LDL-c (mmo/l)		
	mean	low	upper	mean	low	upper	mean	low	upper
wt	1.53	1.49	1.56	1.38	1.30	1.47	3.03	2.96	3.10
hetero	1.60	1.54	1.65	1.24	1.09	1.39	3.07	2.95	3.19
homo	1.66	1.55	1.78	1.11	0.80	1.40	3.11	2.87	3.35
	$p = 0.033$			$p = 0.090$			$p = 0.546$		

LIPC 514CT (rs1800588)

Genotype	HDL-c (mmol/l)			TG (mmol/l)			LDL-c (mmo/l)		
	mean	low	upper	mean	low	upper	mean	low	upper
CC	1.48	1.46	1.51	1.33	1.26	1.40	3.05	3.00	3.10
CT	1.54	1.52	1.56	1.35	1.31	1.39	3.04	3.01	3.07
TT	1.59	1.57	1.62	1.37	1.30	1.44	3.02	2.97	3.07
	$p = 0.0076$			$p = 0.770$			$p = 0.530$		

APOC3 SstI (rs5128)

Genotype	HDL-c (mmol/l)			TG (mmol/l)			LDL-c (mmo/l)		
	mean	low	upper	mean	low	upper	mean	low	upper
S1S1	1.55	1.51	1.60	1.30	1.18	1.41	3.04	2.95	3.13
S1S2	1.54	1.50	1.57	1.38	1.29	1.46	3.03	2.96	3.10
S2S2	1.52	1.45	1.58	1.45	1.29	1.63	3.02	2.89	3.16
	$p = 0.4223$			$p = 0.180$			$p = 0.816$		

Data are expressed as the mean (95% confidence interval). Each *p*-value was based on an analysis of covariance.

metabolism and its incidence and association with serum lipid levels in the general Japanese population. Because this is the largest Japanese population ever analyzed, these data would be useful for future analyses on the general Japanese population.

The prevalence of the D442G and Int14 +1 G → A mutations is very high in the general Japanese population, with heterozygote frequencies of 7 and 1%, respectively

Table 6. Incidence of CETP TaqIB, D442G, and LPL S447X genetypes according to HDL levels.

Genotype	HDL-c (mmol/l)			
CETP TaqIB				
	1.0> (8.3%)	1.0 ≤, 2.58 > (89.9%)	2.58 ≤ (1.8%)	†
B1B1	72 (9.9%)	644 (88.8%)	9 (1.2%)	$p = 0.009$
B1B2	79 (8.2%)	870 (90.2%)	16 (1.7%)	
B2B2	15 (4.8%)	284 (91.6%)	11 (3.5%)	
CETP D442G				
WT	161 (8.7%)	1671 (89.8%)	29 (1.6%)	$p = 0.011$
Hetero	5 (3.6%)	125 (91.2%)	7 (5.1%)	
Homo	0 (0%)	2 (100%)	0 (0%)	
LPL S447X				
WT	134 (8.9%)	1354 (89.4%)	26 (1.7%)	$p = 0.002$
Hetero	21 (5.0%)	390 (93.3%)	7 (1.7%)	
Homo	2 (8.0%)	21 (84.0%)	2 (8.0%)	

Column percentage is shown on top. Each box shows the number of participants in each category and its percentage in each genotype.
† The χ^2-test was used.

(10, 11, 27, 28). Our large-scaled study showed similar frequencies of these mutations, with 8.1 and 0.6%, respectively, indicating that our study population represents the general Japanese population and confirmed that the frequency of these mutations is quite high in Japanese. Because these mutations are associated with lower levels of CETP activity (27), the plasma level of HDL-cholesterol is higher in heterozygotes and homozygotes. We have also confirmed that the incidence of the mutation D442G is higher in people with hyperalphalipoproteinemia (2.58 mmol/l or over).

A genetic *CETP* deficiency is the most important and common cause of hyperalphalipoproteinemia in Japanese and contributes to 60% of hyperalphacholesterolemia (29). However, the role of CETP in atherogenesis is still under debate. A study in the Japanese Omagari area has shown a relatively increased incidence of coronary atherosclerosis in patients with CETP deficiency (30). In the Copenhagen City Heart Study, increased HDL-cholesterol levels caused by mutations in *CETP* were associated with an increased risk of CAD in caucasian females (31). In contrast, the B2 allele of the TaqIB polymorphism is associated with a low CETP mass, higher HDL-cholesterol levels, and a decreased risk of coronary artery disease (17). The reason for this discrepancy is unknown. Dose effects of CETP mass or another genetic abnormality may explain the difference in risk for CAD. Hirano *et al.* showed that people with weak LIPC activity had a higher incidence of CAD (32). Therefore, it is possible that LIPC activity is involved in these differences. More studies are needed to determine the role of CETP in CAD in various populations with different genetic backgrounds.

Our study is consistent with others in terms of the allele frequency of the S447X polymorphism of the *LPL* gene (19, 20, 33). Recent studies showed that the X447 mutation is associated with a favorable lipid profile, and lower TG and higher HDL-cholesterol levels, and that it may confer protection against coronary artery disease (19, 20, 33). We also found a similar tendency in men and women. However, a significant change in HDL-cholesterol levels was found in the total population and women, but not in men. Because the X447 mutation is associated with stronger LPL activity, the TG levels were lower in heterozygotes and homozygotes as expected, although the difference was not significant in women. Homozygotes seem to have lower TG levels than heterozygotes, which reflects the gene dosage effect. Because carriers of S447X have a favorable lipid profile in terms of HDL-cholesterol and TG, and a decreased risk of CAD (35, 36), we should examine whether carriers of S447X have fewer coronary artery events.

In terms of *LIPC* gene polymorphisms, our data clearly indicate that the frequency of the TT genotype is significantly higher in Japanese than in Caucasians (37, 38). However, a higher frequency of the TT genotype is also reported in Koreans and Japanese (39–41). Therefore, this difference might partly explain the higher HDL-cholesterol levels in Asians.

Our results on the allele frequency of the Sst1 polymor-

phism of the *APOC3* gene were almost comparable to the data on Asian Indians (42), but not on Caucasians (43). Caucasians seem to have a lower allele frequency of S2. Although a association of higher TG levels with the S2 allele has been reported in studies carried out in Caucasians (44–46) and Asians (47–49), our data show that such an association was found in the total population and in men, but not in women. Few other studies, however, have found any significant association between the Sst1 polymorphism and hypertriglyceridemia (50–52). The linkage disequilibrium between this polymorphism and the causative mutation might be weakened or absent in some populations (44).

Our data clearly showed that the heterozygotes of the D442G mutation, homozygotes of the LPL S447X mutation, and people with the TaqIB2B2 genotype had a higher incidence of hyperalphalipoproteinemia with HDL-cholesterol levels of 2.58 mmol/l or over. Alcohol consumption and smoking can also affect the levels of HDL-cholesterol. Corbex et al showed that the HDL levels of people with certain polymorphisms of the *CETP* gene are modulated by alcohol consumption (53). Therefore, it might be necessary to take into account environmental factors for the effect of gene polymorphisms on HDL-cholesterol levels as well as on the risk of cardiovascular events.

In summary, we have provided the largest ever database of gene polymorphisms related to lipid metabolism in the general Japanese population. A prospective study is now under way to determine the contribution of these gene polymorphisms to cardiovascular risk in Japanese.

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Appendix

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Original Article

Prevalence of Metabolic Syndrome in the General Japanese Population in 2000

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To determine the prevalence of metabolic syndrome in the Japanese general population, we analyzed data from a nationwide survey conducted in 2000. According to the Japanese new diagnostic criteria for metabolic syndrome in 2005, we analyzed 3,264 people aged from 20 to 79 (men, 1,917; women, 1,347) from the total participants. The incidence of metabolic syndrome was 7.8%. Men had a higher incidence (12.1%) than women (1.7%). Most of the women satisfying the criteria were 50 years old or over, while the incidence in men started to rise from their 30s. When we applied the criteria of Adult Treatment Panel III, the incidence was about 3-fold higher. In this population visceral obesity was associated with metabolic abnormalities, such as higher LDL-cholesterol, triglyceride, glucose, and blood pressure and lower HDL-cholesterol. Thus we determined the incidence of metabolic syndrome and each metabolic abnormality in the Japanese general population in 2000 and found an association of visceral obesity with metabolic abnormalities. Intervention to reduce the incidence of metabolic syndrome in Japan is necessary to reduce the risk of cardiovascular disease.

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Introduction

Metabolic syndrome is a constellation of multiple risk factors, such as dyslipidemia, elevated glucose, and elevated blood pressure. This syndrome has received increased attention due to its association with increased risk for cardiovascular disease and type 2 diabetes[1]. Although the pathogenesis of metabolic syn-

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drome has not been fully understood, the predominant underlying risk factor is considered to be visceral obesity due to an atherogenic diet and physical inactivity in the presence of some unknown genetic background[2-4]. In women the incidence of metabolic syndrome increases after menopause; therefore, hormonal imbalance and aging are also associated with the development of metabolic syndrome[5].

Along with the westernization of lifestyle, the incidence of metabolic disorders, such as dyslipidemia, hypertension, and diabetes is increasing in Japan. In spite of the availability of many drugs, such as statins, angiotensin-converting enzyme inhibitors, and aspirin, the incidence of cardiovascular disease is not decreasing in Japan, probably due to these metabolic abnormalities, especially dyslipidemia and diabetes along with obesity according to the national survey by the Ministry of Health, Labour and Welfare (http://www.mhlw.go.jp/toukei/saikin/hw/kenkou/jyunkan/jyunkan00/gaiyo.html). In 2000, we conducted a lipid survey in various districts in Japan[6]. What we found in this survey was that the level of triglyceride increased in middle-aged men along with increased body mass index (BMI) compared with the data in 1990[7]. This increase in BMI also suggests an increase in the incidence of visceral obesity and metabolic syndrome; therefore, knowing the incidence of metabolic syndrome is very important from the standpoint of preventive medicine.

In the last few years, several expert groups have attempted to set forth simple diagnostic criteria to be used in clinical practice to identify patients with metabolic syndrome. The committee of International Diabetes Federation (IDF) adopted waist circumference as the surrogate marker for visceral obesity as an essential component of this syndrome (http://www.idf.org/webdata/docs/IDF_Metasyndrome_definition.pdf). In Japan the committee established diagnostic criteria under the same principle as that used in the IDF criteria, except that the cutoff point for high glucose is 110 mg/dL instead of 100 mg/dL[8]. The cutoff of waist circumference for visceral obesity was adopted as ≧85 cm in men and ≧90 cm in women. Meanwhile, the National Cholesterol Education Program (NCEP) Adult Treatment Panel Ⅲ (ATP Ⅲ) criteria required no single factor for diagnosis, but instead required the presence of at least 3 out of 5 components for diagnosis[9]; thus, complete agreement on the definition and diagnostic criteria has not been achieved so far.

The purpose of this study is to examine the incidence of metabolic syndrome in the Japanese general population and the relationship with the risk factors included in the diagnostic criteria. We also compared the incidence of metabolic syndrome by using the NCEP-ATP Ⅲ new diagnostic criteria.

Methods

Design and Data Collection

The Research Group on Serum Lipid Level Survey 2000 in Japan organized the members of 36 institutes from various areas around Japan. The project was designed to produce representative data about serum lipid levels in the civilian Japanese population. The subjects were people receiving annual health examinations in the general community, companies, and schools, and not patient-visiting hospitals. Among the 12,839 participants we measured the waist circumference of 3,264 people aged 20 to 79 (men 1,917; women, 1,357) and examined the incidence of metabolic syndrome.

Laboratory Methods

All serum and plasma samples were obtained in the fasting state. All lipid and other analyses were conducted on venous blood samples within one week of collection at BML (Saitama, Japan). Serum cholesterol and triglyceride levels were measured by enzymatic assay. HDL-cholesterol and LDL-cholesterol were measured enzymatically using a kit from Daiichi Kagaku Co. Ltd. (Tokyo, Japan). The results of lipid analyses in the four surveys were indirectly standardized according to the criteria of the CDC Lipid Standardization Program[10]. Thus, the cholesterol levels in these five surveys appear comparable. Plasma glucose was determined enzymatically and HbA1c was determined using a kit from Kyowa Medex Co.Ltd (Tokyo, Japan). Serum insulin was determined by immunoradiometric assay (Abbott Diagnostics Division, Abbot Park, IL). Waist circumference at the umbilical level was measured in the late exhalation phase in a standing position.

Definition of Metabolic Syndrome

According to the new definition released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome in April 2005, we defined metabolic syndrome as the presence of 2 or more abnormalities in addition to visceral obesity (waist circumference: 85 cm or more in men, 90 cm or more in women). These three abnormalities are as follows: 1, triglycerides ≧150 mg/dL and/or HDL-cholesterol <40 mg/dL or under treatment for this type of dyslipidemia, 2, systolic blood pressure ≧130 and/or diastolic blood pressure ≧85, or under treatment for hypertension, 3, fasting glucose ≧110 mg/dL or under treatment for diabetes. People treated for dyslipid-

Table 1. Clinical characteristics of the study population

	men (n=1,917)	women (n=1,347)
age	46.3 ± 0.30	45.7 ± 0.46
BMI	23.4 ± 0.07	22.4 ± 0.07*
waist circumference (cm)	84.1 ± 0.20	73.2 ± 0.29*
systolic blood pressure (mmHg)	125 ± 0.40	120 ± 0.49*
diastolic blood pressure (mmHg)	76.3 ± 0.27	72.3 ± 0.31*
T-cho (mg/dL)	201 ± 0.78	200 ± 0.97
TG (mg/dL)	145 ± 2.97	92.1 ± 1.64*
HDLc (mg/dL)	54.8 ± 0.33	64.6 ± 0.39*
LDLc (mg/dL)	118.0 ± 0.99	113.5 ± 1.22**
HbA1c (%)	4.86 ± 0.02	4.82 ± 0.14
fasting glucose (mg/dL)	97.8 ± 0.43	91.1 ± 0.36*
insulin (IU/mL)	6.28 ± 0.11	7.16 ± 0.21*

Data are expressed as the means ± SEM. T-cho; total cholesterol, TG; triglyceride, HDLc; HDL-cholesterol, LDLc; LDL-cholesterol. *$p<0.001$, **$p<0.01$

emia were excluded, because we could not obtain data as to whether they were treated for hypercholesterolemia or hypertriglyceridemia. We also analyzed the incidence of metabolic syndrome by ATP III criteria published in 2005[9]. We modified the criteria by using the Japanese cutoff of waist circumference. Other differences are fasting glucose ≧100 mg/dL and HDL-cholesterol <50 mg/dL in women. Metabolic syndrome in ATP III criteria was defined as the presence of at least 3 abnormalities among visceral obesity, hypertriglyceridemia, low HDL-cholesterolemia, hypertension, and glucose intolerance.

Data Analysis

The results are expressed as the mean value ± standard deviation, and categorical data by the incidence and relation between visceral obesity and various factors were expressed by the odds ratio and 95% confidence interval. Differences in the means were evaluated by analysis of variance (ANOVA) or analysis of covariance (ANCOVA). The relation between visceral obesity and various factors was examined using multiple, logistic regression analysis for multivariate analysis. Analysis was performed using the statistical Package for Social Sciences (SPSS Japan Inc. ver. 11.5, Tokyo, Japan). A p value of 0.05 or less was considered to indicate significant difference.

Results

Table 1 shows the characteristics of the study population. The means of total cholesterol, triglyceride, HDL-cholesterol, and fasting glucose were 200 mg/dL, 123 mg/dL, 59 mg/dL, and 95 mg/dL. These data are almost the same as the means of the total participants (201, 115, 59, 95, respectively)[6]. The means of both genders were also equivalent to the means of the total participants, indicating that this population represents all participants in this Japanese lipid survey in 2000. Although we found no difference in the mean age, total cholesterol, and HbA1c between men and women, the means of BMI, waist circumference, blood pressure, triglyceride, LDL-cholesterol, and fasting glucose were higher in men than in women, while those of HDL-cholesterol and insulin were lower in men than in women.

Table 2. Incidence of metabolic syndrome and metabolic abnormalities by Japanese diagnostic criteria

	men (%)	women (%)	all (%)
metabolic syndrome	12.1	1.7	7.8
visceral obesity	48.2	9.7	32.3
hypertriglyceridemia	31.3	11.2	23.0
low HDL-cholesterolemia	12.4	2.2	8.2
dyslipidemia	35.2	12.1	25.6
hypertension	25.4	19.5	22.9
elevated fasting glucose	14.4	7.0	11.3

Dyslipidemia is defined as hypertriglyceridemia and/or low HDL-cholesterolemia

Using the Japanese diagnostic criteria for metabolic syndrome we determined the incidence of metabolic syndrome (**Table 2**). The incidence of metabolic syndrome in all participants was 7.8%. The incidence in men and women was 12.1, 1.7%, respectively. The incidence was about 7-fold higher in men than in women, reflecting the difference in visceral obesity defined by waist circumference, 48.2% in men and 9.7% in women. The incidence of dyslipidemia, hypertension, and glucose intolerance was also higher in men than in women in this population, indicating a higher prevalence of metabolic abnormalities in men.

It is important for us to intervene from the period of visceral obesity to prevent cardiovascular disease due to these metabolic abnormalities. Therefore, we compared the incidence of visceral obesity, visceral obesity plus one metabolic abnormality, and metabolic syndrome. **Fig. 1** shows the incidence of visceral obesity, visceral obesity plus one metabolic abnormality, and metabolic syndrome. The incidence of visceral obesity plus one metabolic abnormality was about twice the incidence of metabolic syndrome both in men and women.

To compare the incidence of metabolic syndrome