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®/MgCl₂ mixture was prepared by combining 3 µl of primary probe/Invader® mix and 5 µl of 22.5 mM MgCl₂ 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®/MgCl₂ 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 overlaid into each well, the plate was incubated isothermally at 63°C for 4 h in a DNA thermocycler (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 (Cytofluor 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

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

	All	Men	Women
T-Chol (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.

the participants in the gene analysis are representative of the general Japanese population.

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.

<i>CETP</i> D442G (rs2303790)					
Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
			$p = 0.000$	$p = 0.071$	$p = 0.154$
<i>CETP</i> Int14 +1 G → A (rs5742907)					
Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
			$p = 0.000$	$p = 0.241$	$p = 0.938$
<i>CETP</i> TaqIB (rs708272)					
Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
			$p = 0.000$	$p = 0.160$	$p = 0.362$
<i>LPL</i> S447X (rs328)					
Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
			$p = 0.004$	$p = 0.032$	$p = 0.487$
<i>LIPC</i> 514CT (rs1800588)					
Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
			$p = 0.000$	$p = 0.520$	$p = 0.255$
<i>APOC3</i> SstI (rs5128)					
Genotype	Age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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 al-

lele 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.

<i>CETP</i> D442G (rs2303790)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.003	<i>p</i> = 0.035	<i>p</i> = 0.453
<i>CETP</i> TaqIB (rs708272)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.001	<i>p</i> = 0.664	<i>p</i> = 0.758
<i>LPL</i> S447X (rs328)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.278	<i>p</i> = 0.029	<i>p</i> = 0.617
<i>LIPC</i> 514CT (rs1800588)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.266	<i>p</i> = 0.499	<i>p</i> = 0.996
<i>APOC3</i> SstI (rs5128)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.473	<i>p</i> = 0.041	<i>p</i> = 0.196

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

<i>CETP</i> D442G (rs2303790)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.002	<i>p</i> = 0.590	<i>p</i> = 0.306
<i>CETP</i> TaqIB (rs708272)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.004	<i>p</i> = 0.127	<i>p</i> = 0.461
<i>LPL</i> S447X (rs328)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.010	<i>p</i> = 0.185	<i>p</i> = 0.054
<i>LIPC</i> 514CT (rs1800588)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.014	<i>p</i> = 0.210	<i>p</i> = 0.406
<i>APOC3</i> SstI (rs5128)				
Genotype	<i>n</i>	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmol/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)
		<i>p</i> = 0.078	<i>p</i> = 0.272	<i>p</i> = 0.608

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

(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 (mmol/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 (mmol/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 (mmol/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 (mmol/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 (mmol/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 (mmol/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 genotypes according to HDL levels.

CETP TaqIB				
Genotype	HDL-c (mmol/l)			
	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 differ-

ences. 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

Research Group on Serum Lipid Survey 2000 in Japan

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