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Prediction of genetic risk for dyslipidemia

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Abstract

The purpose of the present study was to identify genetic variants that confer susceptibility to dyslipidemia. A total of 5213 individuals from two independent populations were examined: Subject panel A comprised 3794 individuals who visited participating hospitals; subject panel B comprised 1419 community-dwelling elderly individuals. The genotypes for 100 polymorphisms of 65 candidate genes were determined. The χ^2 test and multivariable logistic regression analysis revealed that seven polymorphisms of *APOA5, APOC3, APOA1, ACAT2,* and *LPL* were significantly associated with hypertriglyceridemia, six polymorphisms of *APOA5, LIPC*, and *CYP3A4* with low HDL-cholesterol, and three polymorphisms of *APOE* and *CCR2* with high LDL-cholesterol in subject panel A. For validation of these associations, the same polymorphisms were examined in subject panel B. Six polymorphisms of *APOA5, APOC3, APOA1,* and *LPL* were again significantly associated with hypertriglyceridemia, three polymorphisms of *APOA5* with low HDL-cholesterol, and two polymorphisms of *APOE* with high LDL-cholesterol. Serum triglyceride, HDL-cholesterol, and LDL-cholesterol concentrations differed significantly among genotypes of these corresponding polymorphisms in both subject panels. These results indicate that polymorphisms of *APOA5, APOC3, APOA1,* and *LPL* are determinants of hypertriglyceridemia and that those of *APOA5* and *APOE* are determinants of low HDL-cholesterol and high LDL-cholesterol, respectively, in Japanese individuals.

Keywords: Genetics; Polymorphism; Epidemiology; Hyperlipidemia; Dyslipidemia

Dyslipidemia is a multifactorial disorder caused by an interaction between genetic and environmental factors, the latter including a high-fat and high-calorie diet and physical inactivity [1]. Although genetic linkage analyses [2–4] and candidate gene approaches [5–8] have implicated several loci and candidate genes for predisposition to dyslipidemia, the genes that confer susceptibility to this condition remain to be identified definitively. In addition, given ethnic differences in lifestyle and environmental factors as well as in genetic background, it is important to examine gene polymorphisms related to dyslipidemia in each ethnic group.

We have now performed a large-scale association study for 100 polymorphisms of 65 candidate genes and dyslipidemia in 5213 Japanese individuals. Given that dyslipidemia includes hypertriglyceridemia, a low serum concentration of high-density lipoprotein (HDL) cholesterol, and a high serum concentration of low-density lipoprotein (LDL) cholesterol, each of which is an important risk factor for atherosclerosis, we examined the relations of genetic variants to each of these

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conditions separately. The purpose of the present study was thus to identify gene polymorphisms associated with dyslipidemia and thereby to contribute to the prediction of the genetic risk for this condition.

Results

The characteristics of subject panels A and B are shown in Table 1. Evaluation of genotype distributions by the χ^2 test revealed that seven polymorphisms were significantly (false discovery rate (FDR) <0.05) associated with hypertriglyceridemia, six with low HDL-cholesterol, and three with high LDL-cholesterol in subject panel A (Supplementary Table 1).

The identified polymorphisms were examined further by multivariable logistic regression analysis with adjustment for age, sex, and the prevalence of diabetes mellitus. The −1131T→C, −3A→G, and 553G→T (Gly185Cys) polymorphisms of *APOA5* (all genetic models), the 1100C→T polymorphism of *APOC3* (all models), the 84T→C polymorphism of *APOA1* (dominant and additive 1 and 2 models), the 41A→G (Glu14Gly) polymorphism of *ACAT2* (all models), and the 1595C→G (Ser447Stop) polymorphism of *LPL* (dominant and additive 1 models) were significantly ($p<0.05$) associated with hypertriglyceridemia in subject panel A (Supplementary Table 2). The −1131T→C, 553G→T (Gly185Cys), and −3A→G polymorphisms of *APOA5* (all models), the −250G→A and −514C→T polymorphisms of *LIPC* (all models), and the 13989A→G (Ile118Val) polymorphism of *CYP3A4* (dominant and additive 1 models) were significantly associated with low HDL-cholesterol in subject panel A (Supplementary Table 2). The 4070C→T (Arg158Cys) (dominant and additive 1 models) and 3932T→C (Cys112Arg) (dominant and additive 1 and 2 models) polymorphisms of *APOE* and the 190G→A (Val64Ile) polymorphism of *CCR2* (dominant and additive 1 models) were significantly associated with high LDL-cholesterol in subject panel A (Supplementary Table 2).

To validate these associations, we examined in subject panel B the polymorphisms associated with dyslipidemia in subject panel A. Multivariable logistic regression analysis with adjustment for age, sex, and the prevalence of diabetes mellitus revealed that the −1131T→C (all models), −3A→G (all models), and 553G→T (Gly185Cys) (dominant and additive 1 models) polymorphisms of *APOA5,* the 1100C→T polymorphism of *APOC3* (all models), the 84T→C polymorphism of *APOA1* (all models), and the 1595C→G (Ser447Stop) polymorphism of *LPL* (dominant and additive 1 models), but not the 41A→G (Glu14Gly) polymorphism of *ACAT2,* were significantly ($p<0.05$) associated with hypertriglyceridemia in subject panel B (Supplementary Table 3). The −1131T→C (all models), 553G→T (Gly185Cys) (dominant and additive 1 models), and −3A→G (all models) polymorphisms of *APOA5,* but not the −250G→A or −514C→T polymorphisms of *LIPC* or the 13989A→G (Ile118Val) polymorphism of *CYP3A4,* were significantly associated with low HDL-cholesterol in subject panel B (Supplementary Table 3). The 4070C→T (Arg158Cys) and 3932T→C (Cys112Arg) polymorphisms of *APOE* (dominant and additive 1 models), but not the 190G→A (Val64Ile) polymorphism of *CCR2,* were significantly associated with high LDL-cholesterol in subject panel B (Supplementary Table 3).

Genotype distributions in subject panels A or B of the polymorphisms identified by the χ^2 test are shown in Supplementary Table 4. The genotype distributions of these 13 polymorphisms in individuals of subject panels A and B were all in Hardy–Weinberg equilibrium.

We next examined the relations of serum concentrations of triglycerides, HDL-cholesterol, or LDL-cholesterol to genotypes of each polymorphism. Serum triglyceride concentration differed significantly among genotypes for the −1131T→C, −3A→G, and 553G→T (Gly185Cys) polymorphisms of *APOA5,* the 1100C→T polymorphism of *APOC3,* the 84T→C polymorphism of *APOA1,* and the 1595C→G (Ser447Stop) polymorphism of *LPL* in both subject panels A and B (Table 2). Serum HDL-cholesterol concentration differed significantly among genotypes for the −1131T→C, 553G→T (Gly185Cys), and −3A→G polymorphisms of *APOA5* as well as the −250G→A and −514C→T polymorphisms of *LIPC* in both subject panels A and B (Table 2). Serum LDL-cholesterol concentration differed significantly among genotypes for the 4070C→T (Arg158Cys) and 3932T→C (Cys112Arg) polymorphisms of *APOE* in both subject panels A and B (Table 2).

Finally, we examined linkage disequilibrium among polymorphisms of *APOA5, APOA1,* and *APOC3* (located at chromosome 11q23) and of *APOE,* as well as the relations of haplotypes of these polymorphisms to hypertriglyceridemia, low HDL-cholesterol, and high LDL-cholesterol. The −1131T→C, −3A→G, and 553G→T (Gly185Cys) polymorphisms of *APOA5,* the 84T→C polymorphism of *APOA1,* and the

Table 1
Characteristics of the 5213 study subjects

Characteristic	Subject panel A	Subject panel B
No. of subjects	3794	1419
Age (years)	64.8±10.9	72.3±5.6
Sex (male/female, %)	58.5/41.5	47.8/52.2
Body mass index (kg/m^2)	23.6±3.3	23.2±3.0
Current or former smoker (%)	18.4	30.4
Hypertension (%)	64.6	37.1
Diabetes mellitus (%)	35.2	7.0
Serum total cholesterol (mmol/L)	5.25±1.01	5.12±0.86
Serum triglycerides (mmol/L)	1.62±1.39	1.58±9.35
Hypertriglyceridemia (%)	35.1	35.9
Serum HDL-cholesterol (mmol/L)	1.35±0.47	1.51±0.39
Low HDL-cholesterol (%)	20.5	8.2
Serum LDL-cholesterol (mmol/L)	3.15±0.91	2.86±0.73
High LDL-cholesterol (%)	26.3	15.2

Data for age, body mass index, and serum lipid concentrations are means±SD. Smoker, smoking ≥10 cigarettes daily; hypertension, systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg (or both) or taking antihypertensive medication; diabetes mellitus, fasting blood glucose of ≥6.93 mmol/L (126 mg/dl) or glycosylated hemoglobin of ≥6.5% (or both) or taking anti-diabetes medication; hypertriglyceridemia, serum concentration of triglycerides of ≥1.65 mmol/L (150 mg/dl); low HDL-cholesterol, serum concentration of HDL-cholesterol of <1.04 mmol/L (40 mg/dl); high LDL-cholesterol, serum concentration of LDL-cholesterol of ≥3.64 mmol/L (140 mg/dl).

Table 2
Serum concentrations of triglycerides, HDL-cholesterol, and LDL-cholesterol according to genotypes in subject panels A and B

Gene symbol	Polymorphism	Subject panel A				Subject panel B			
		11	12	22	*P*	11	12	22	*P*
Serum concentration of triglycerides (mmol/L)									
APOA5	−1131T→C	1.39±0.84 (1640)	1.72±1.45 (1687)	2.02±2.24 (459)	4.8×10^{-28}	1.36±0.73 (617)	1.67±0.97 (627)	2.04±1.21 (175)	4.3×10^{-17}
APOA5	−3A→G	1.39±0.84 (1636)	1.73±1.46 (1686)	2.00±2.24 (459)	1.4×10^{-25}	1.36±0.73 (616)	1.67±0.97 (627)	2.04±1.21 (176)	2.7×10^{-17}
APOA5	553G→T (Gly185Cys)	1.55±1.20 (3263)	1.89±1.42 (488)	3.75±6.85 (30)	8.0×10^{-15}	1.53±0.89 (1200)	1.87±1.12 (210)	2.18±0.99 (8)	9.5×10^{-7}
APOC3	1100C→T	1.41±0.89 (632)	1.62±1.42 (1811)	1.72±1.51 (1344)	2.8×10^{-7}	1.34±0.75 (252)	1.57±0.91 (677)	1.72±1.02 (490)	4.0×10^{-7}
APOA1	84T→C	1.67±1.40 (2384)	1.53±1.42 (1224)	1.43±0.76 (178)	0.0002	1.64±0.97 (847)	1.52±0.91 (493)	1.33±0.62 (79)	0.0011
ACAT2	41A→G (Glu14Gly)	1.67±1.58 (2306)	1.64±1.01 (1301)	1.40±0.77 (174)	0.0050	1.56±0.91 (891)	1.64±0.99 (483)	1.40±0.58 (45)	0.4740
LPL	1595C→G (Ser447Stop)	1.64±1.43 (2917)	1.51±1.12 (815)	1.43±1.60 (55)	5.8×10^{-6}	1.63±0.95 (1090)	1.43±0.86 (309)	1.41±0.80 (20)	0.0007
Serum concentration of HDL-cholesterol (mmol/L)									
APOA5	−1131T→C	1.40±0.42 (1652)	1.33±0.49 (1683)	1.27±0.39 (458)	8.6×10^{-15}	1.56±0.39 (617)	1.46±0.36 (627)	1.40±0.39 (175)	5.8×10^{-9}
APOA5	553G→T (Gly185Cys)	1.38±0.47 (3272)	1.27±0.39 (487)	1.07±0.34 (29)	1.8×10^{-10}	1.53±0.39 (1200)	1.40±0.34 (210)	1.46±0.49 (8)	7.4×10^{-5}
APOA5	−3A→G	1.40±0.42 (1648)	1.35±0.49 (1682)	1.30±0.39 (458)	5.4×10^{-12}	1.56±0.39 (616)	1.46±0.36 (627)	1.40±0.39 (176)	5.9×10^{-9}
LIPC	−250G→A	1.30±0.39 (945)	1.35±0.49 (1844)	1.43±0.47 (1005)	2.9×10^{-11}	1.43±0.34 (316)	1.51±0.36 (733)	1.59±0.42 (370)	4.2×10^{-7}
LIPC	−514C→T	1.33±0.39 (933)	1.35±0.49 (1897)	1.43±0.47 (958)	2.6×10^{-9}	1.43±0.34 (325)	1.51±0.39 (733)	1.59±0.42 (361)	3.8×10^{-7}
CYP3A4	13989A→G (Ile118Val)	1.35±0.47 (3747)	1.51±0.26 (40)		0.0007	1.51±0.39 (1419)			
Serum concentration of LDL-cholesterol (mmol/L)									
APOE	4070C→T (Arg158Cys)	3.17±0.91 (3387)	2.76±0.83 (288)	2.31±1.12 (8)	3.6×10^{-17}	2.91±0.73 (1293)	2.39±0.68 (123)	1.82±0.47 (3)	3.6×10^{-14}
APOE	3932T→C (Cys112Arg)	3.09±0.91 (2949)	3.28±0.88 (696)	3.56±0.91 (38)	4.2×10^{-8}	2.83±0.73 (1155)	2.99±0.75 (252)	2.81±0.94 (12)	0.0068
CCR2	190G→A (Val64Ile)	3.09±0.88 (1885)	3.20±0.91 (1487)	3.15±0.94 (311)	0.0013	2.89±0.75 (734)	2.83±0.73 (573)	2.96±0.75 (112)	0.2413

Numbers of subjects are shown in parentheses. 11, wild-type homozygote; 12, heterozygote; 22, variant homozygote.

1100C→T polymorphism of *APOC3* were all in strong linkage disequilibrium (Supplementary Table 5). Haplotype analysis revealed that the haplotypes T (−1131T→C of *APOA5*)–A (−3A→G of *APOA5*)–G (553G→T of *APOA5*)–T (84T→C of *APOA1*)–T (1100C→T of *APOC3*), T–A–G–C–C, and T–A–G–T–C were protective against hypertriglyceridemia, whereas the haplotypes C–G–G–T–T, C–G–T–T–T, C–G–G–C–C, and C–A–G–T–T were risk factors for this condition (Table 3). Similar analysis revealed that the haplotypes T–A–G–T–T and T–A–G–C–C were protective against low HDL-cholesterol, whereas the haplotypes C–G–G–T–T, C–G–T–T–T, C–G–G–T–C, C–A–G–T–T, and T–A–T–T–T were risk factors for this condition (Table 4). The 4070C→T (Arg158Cys) and 3932T→C (Cys112Arg) polymorphisms of *APOE* were in strong linkage disequilibrium ($D'=-0.9866$, $p=1.2\times10^{-8}$). Haplotype analysis revealed that the haplotype C (4070C→T of *APOE*)–C (3932T→C of *APOE*), which results in the production of the Apo E4 isoform, was a risk factor for high LDL-cholesterol, whereas the haplotype T–T, which results in the production of the Apo E2 isoform, was protective against this condition (Table 5).

Discussion

We have examined the relations of 100 polymorphisms in 65 candidate genes to hypertriglyceridemia, low HDL-cholesterol, and high LDL-cholesterol in 5213 Japanese individuals from two independent populations. Our large-scale association study revealed that the −1131T→C, −3A→G, and 553G→T (Gly185Cys) polymorphisms of *APOA5,* the 1100C→T polymorphism of *APOC3,* the 84T→C polymorphism of *APOA1,* and the 1595C→G (Ser447Stop) polymorphism of *LPL* were significantly associated with hypertriglyceridemia; the −1131T→C, −3A→G, and 553G→T (Gly185Cys)

Table 3
Association of hypertriglyceridemia with *APOA5-APOA1-APOC3* haplotypes in combined subject panels A and B

Haplotype	Overall frequency	Frequency		χ^2 test *p* value	Permutation *p* value
		Hypertriglyceridemia (−)	Hypertriglyceridemia (+)		
T–A–G–T–T	0.2740	0.2868	0.2508	8.5×10^{-5}	0.001
C–G–G–T–T	0.2437	0.2253	0.2778	2.7×10^{-9}	0
T–A–G–C–C	0.1925	0.2114	0.1572	2.0×10^{-11}	0
T–A–G–T–C	0.1796	0.1879	0.1647	0.0032	0.001
C–G–T–T–T	0.0622	0.0490	0.0863	5.1×10^{-14}	0
C–G–G–T–C	0.0116	0.0100	0.0149	0.0256	0.084
C–G–G–C–C	0.0106	0.0081	0.0147	0.0014	0.012
C–G–T–C–C	0.0059	0.0048	0.0085	0.0225	0.077
C–A–G–T–T	0.0051	0.0030	0.0084	0.0002	0.001
C–G–T–T–C	0.0042	0.0030	0.0061	0.0171	0.088
T–A–G–C–T	0.0031	0.0029	0.0035	0.5648	0.616
T–G–G–T–T	0.0022	0.0024	0.0018	0.5317	0.561
T–G–G–T–C	0.0013	0.0015	0.0011	0.6490	0.739
T–G–G–C–C	0.0010	0.0006	0.0016	0.0961	0.243
T–G–T–T–C	0.0008	0.0008	0.0006	0.8116	0.882
T–G–T–T–T	0.0004	0.0004	0.0004	0.9763	0.982
C–G–G–C–T	0.0004	0.0006	2.1×10^{-22}	0.1233	0.276
C–A–G–T–C	0.0004	1.1×10^{-7}	0.0007	0.0298	0.088
T–A–T–C–C	0.0003	0.0004	5.0×10^{-8}	0.2275	0.540
T–G–T–C–C	0.0002	0.0006	6.7×10^{-21}	0.1474	0.301
T–A–T–T–C	0.0002	0.0002	0.0004	0.5200	0.886
T–A–T–T–T	0.0002	3.9×10^{-21}	0.0004	0.1034	0.190
C–A–G–C–C	0.0001	0.0005	8.2×10^{-65}	0.1567	0.218
C–A–G–C–T	0.0001	9.2×10^{-34}	0.0002	0.2943	0.352

Haplotypes consist of the −1131T→C, −3A→G, and 553G→T polymorphisms of *APOA5,* the 84T→C polymorphism of *APOA1,* and the 1100C→T polymorphism of *APOC3.*

polymorphisms of *APOA5* were significantly associated with low HDL-cholesterol; and the 4070C→T (Arg158Cys) and 3932T→C (Cys112Arg) polymorphisms of *APOE* were significantly associated with high LDL-cholesterol.

APOA5, APOA1, and APOC3

The apolipoprotein gene cluster (*APOA5–APOA1–APOC3–APOA4*) on chromosome 11q23 is a region of the genome linked to plasma lipid levels as a quantitative trait. *APOA5* is located ~27 kb upstream of the well-characterized *APOA1–APOC3–APOA4* gene cluster [9] and was found to be strongly associated with plasma triglyceride level [10]. Human *APOA5* consists of four exons and encodes a 369—amino-acid protein [10]. Functional studies in mice indicate that altering the level of *APOA5* expression substantially affects plasma triglyceride level. The plasma triglyceride concentration of transgenic mice that express human *APOA5* was thus found to be one-third that of control mice, whereas that of *APOA5* knockout mice was four times that of controls [10].

The −1131T→C polymorphism in the promoter region of human *APOA5* was found to be independently associated with plasma triglyceride level in populations of various ethnicities, with the variant C allele being a risk factor for increased triglyceride concentrations [7,10–14]. This polymorphism was also associated with HDL-cholesterol level in addition to triglyceride level in both Asian and Caucasian populations, with individuals with the C allele exhibiting reduced HDL-cholesterol concentrations [7,12–14]. The −3A→G [7,15] and 553G→T (Gly185Cys) [16] polymorphisms of *APOA5,* which are in linkage disequilibrium with the −1131T→C polymorphism, have also been associated with variation in triglyceride levels. These observations thus indicate that *APOA5* is important in the regulation of triglyceride and HDL-cholesterol concentrations in humans. We have now shown that the −1131T→C, −3A→G, and 553G→T (Gly185Cys) polymorphisms of *APOA5* were associated with hypertriglyceridemia and low HDL-cholesterol, with the variant C, G, and T alleles, respectively, representing risk factors for these conditions. Our data are thus consistent with the results of previous studies [7,12–16].

The molecular mechanism for the effect of apolipoprotein A–V on triglyceride metabolism remains unclear. Apolipoprotein A–V exhibits a high-affinity, low-elasticity, and slow binding kinetics at hydrophobic interfaces [17], properties that may retard the assembly of triglyceride-rich particles. In addition to the observation that the level of very low density lipoprotein (VLDL) triglycerides was markedly decreased in *APOA5* transgenic mice [10], in vitro studies have shown that recombinant apolipoprotein A–V interacts with and increases the activity of lipoprotein lipase [18]. Apolipoprotein A–V might thus reduce VLDL-triglyceride levels by both down-regulating hepatic VLDL synthesis and increasing VLDL clearance.

Apolipoprotein C-III, a 79-amino-acid glycoprotein, is synthesized by the liver and small intestine and is a major component of triglyceride-rich lipoproteins and HDL. This protein is important in regulation of the serum concentration of

Table 4
Association of low HDL-cholesterol with *APOA5–APOA1–APOC3* haplotypes in combined subject panels A and B

Haplotype	Overall frequency	Frequency		χ^2 test p value	Permutation p value
		Low HDL-cholesterol (−)	Low HDL-cholesterol (+)		
T–A–G–T–T	0.2735	0.2823	0.2304	7.1×10^{-6}	0
C–G–G–T–T	0.2433	0.2379	0.2694	0.0048	0.006
T–A–G–C–C	0.1932	0.1985	0.1658	0.0014	0.003
T–A–G–T–C	0.1802	0.1825	0.1708	0.2421	0.268
C–G–T–T–T	0.062	0.0546	0.0975	7.8×10^{-12}	0
C–G–G–T–C	0.0115	0.0102	0.0175	0.0083	0.039
C–G–G–C–C	0.0105	0.0106	0.0100	0.8209	0.877
C–G–T–C–C	0.0056	0.0062	0.0053	0.6674	0.738
C–A–G–T–T	0.0051	0.0039	0.0109	0.0001	0
C–G–T–T–C	0.0043	0.0032	0.0078	0.0049	0.062
T–A–G–C–T	0.0034	0.0028	0.0059	0.0369	0.076
T–G–G–T–T	0.0022	0.0023	0.0018	0.6616	0.698
T–G–G–T–C	0.0013	0.0012	0.0022	0.2781	0.397
T–G–G–C–C	0.0010	0.0008	0.0011	0.7058	0.796
T–G–T–T–C	0.0007	0.0008	4.6×10^{-11}	0.2202	0.482
T–G–T–T–T	0.0004	0.0006	1.4×10^{-55}	0.3145	0.340
C–G–G–C–T	0.0004	0.0005	2.3×10^{-18}	0.3310	0.267
C–A–G–T–C	0.0004	0.0003	0.0009	0.2314	0.149
T–A–T–C–C	0.0003	0.0002	0.0007	0.2757	0.256
T–G–T–C–C	0.0002	0.0002	0.0006	0.4170	0.486
T–A–T–T–C	0.0002	0.0003	5.5×10^{-19}	0.4985	0.270
T–A–T–T–T	0.0001	5.2×10^{-21}	0.0009	0.0063	0.025
C–A–G–C–C	0.0001	0.0001	0.0002	0.4801	0.344
C–A–G–C–T	4.9×10^{-5}	1.1×10^{-38}	0.0004	0.0677	0.199

Haplotypes consist of the −1131T→C, −3A→G, and 553G→T polymorphisms of *APOA5,* the 84T→C polymorphism of *APOA1,* and the 1100C→T polymorphism of *APOC3*.

triglycerides [19]. It inhibits lipolysis of VLDL, both through inhibition of lipoprotein lipase and hepatic triglyceride lipase activity and by interfering with lipoprotein binding to glycosaminoglycans on cell membranes [20]. Several polymorphisms in or near *APOC3* have been associated with hypertriglyceridemia. A polymorphic *Sst*I site (3238C→G) in the 3′ untranslated region of *APOC3* has been associated with hypertriglyceridemia in various populations [6,9]. The 1100C→T polymorphism of *APOC3* was also shown to be associated with the serum concentration of triglycerides [21]. We have now shown that the 1100C→T polymorphism of *APOC3* was associated with hypertriglyceridemia, with the *T* allele being a risk factor for this condition.

Apolipoprotein A–I is synthesized in the liver and, to a lesser extent, in the small intestine and is the major protein found in HDL [9]. This protein plays an important role in cellular cholesterol homeostasis. It serves as a cofactor for the enzyme lecithin:cholesterol acyltransferase and is therefore a major player in the regulation of reverse cholesterol transport from peripheral tissues to the liver [9]. This pathway is thought to be protective against atherosclerosis. Indeed, transgenic mice that express human *APOA1* have been shown to be resistant to the development of diet-induced atherosclerosis [22]. The 84T→C polymorphism of *APOA1* was previously associated with the serum concentrations of triglycerides and HDL-cholesterol [23]. We have now shown that the 84T→C polymorphism of *APOA1* was associated with hypertriglyceridemia, with the C allele being protective against this condition.

Table 5
Association of high LDL-cholesterol with *APOE* haplotypes in combined subject panels A and B

Haplotype	Overall frequency	Frequency		χ^2 test p value	Permutation p value
		High LDL-cholesterol (−)	High LDL-cholesterol (+)		
C–T	0.8553	0.8572	0.8506	0.4261	0.439
C–C	0.1022	0.0946	0.1262	8.5×10^{-6}	0
T–T	0.0420	0.0478	0.0213	1.6×10^{-8}	0
T–C	0.0005	0.0004	0.0019	0.0261	0.256

Haplotypes consist of the 4070C→T and 3932T→C polymorphisms of *APOE*.

LPL

Lipoprotein lipase (LPL) is the rate-limiting enzyme in lipolysis of triglyceride-rich lipoproteins in the circulation. It is synthesized in parenchymal cells of adipose tissue as well as in skeletal and cardiac muscle, and it is then transferred to heparan sulfate binding sites of the vascular endothelium [24]. The hydrolytic function of LPL is important for the processing of triglyceride-rich chylomicrons and VLDL to remnant particles as well as for the transfer of phospholipids and apolipoproteins to HDL. LPL also plays an important role in the receptor-mediated removal of lipoproteins from the circulation [25]. *LPL* is polymorphic, with amino acid substitutions affecting triglyceride and HDL-cholesterol levels, both of which are implicated in atherosclerosis risk [26]. The 1595C→G

(Ser447Stop) polymorphism of *LPL* results in carboxyl-terminal truncation of LPL by two amino acids. This change is thought to increase the binding affinity of the protein for receptors or to facilitate or otherwise affect its formation of dimers [26]. The *G* allele of the 1595C→G (Ser447Stop) polymorphism has also been shown to be related to decreased plasma triglyceride or increased HDL-cholesterol levels, or both [5,25,26]. Previous observations suggest that the catalytic activity and stability of the truncated variant of LPL may be largely normal, but that it may be present at higher concentrations in the circulation, resulting in a higher level of LPL activity [25,27]. We have now shown that the 1595C→G (Ser447Stop) polymorphism of *LPL* was associated with hypertriglyceridemia, with the G (Stop) allele protecting against this condition, consistent with the previous observations [5,25,26].

APOE

Apolipoprotein E plays an important role in lipid transport and metabolism. Three common alleles (ε2, ε3, and ε4) of *APOE* encode the three major isoforms (E2, E3, and E4), which differ at amino acid positions 112 and 158. Allelic variation of *APOE* accounts for interindividual variability in total cholesterol and LDL-cholesterol concentrations, with studies in human populations demonstrating associations of the ε4 and ε2 alleles with increased and decreased LDL-cholesterol levels, respectively [28–30]. The various apolipoprotein E isoforms interact differently with specific lipoprotein receptors, ultimately affecting circulating levels of cholesterol [31]. Apolipoprotein E from VLDL, chylomicrons, and chylomicron remnants binds to specific receptor cells in the liver. Carriers of the ε2 allele are less efficient at synthesizing VLDL and chylomicrons and at transferring them from plasma to the liver as a result of the binding properties of the Apo E2 isoform. In contrast, these processes are much more efficient in carriers of the ε3 or ε4 alleles. Thus, compared with carriers of the ε3 or ε4 alleles, carriers of the ε2 allele are slower to clear dietary fat from their blood [32]. The difference in uptake of postprandial lipoprotein particles results in differences in regulation of hepatic LDL receptors, which in turn contribute to genotypic differences in total and LDL-cholesterol levels [33–35].

In the present study, we observed that the 4070C→T (Arg158Cys) and 3932T→C (Cys112Arg) polymorphisms of *APOE* were associated with high LDL-cholesterol, with the 4070T allele and the haplotype that encodes the E2 isoform being protective against this condition and with the 3932C allele and the haplotype that encodes the E4 isoform representing risk factors for this condition. Our results are thus consistent with previous observations [8,28–30,33–35].

Conclusion

Our results suggest that *APOA5, APOC3, APOA1,* and *LPL* are determinants of hypertriglyceridemia and that *APOA5* and *APOE* are determinants of low HDL-cholesterol and high LDL-cholesterol, respectively, in the Japanese population. Genotyping of these polymorphisms may prove informative for prediction of the genetic risk for these conditions.

Materials and methods

Study populations

A total of 5213 individuals from two independent populations was examined. Subject panel A comprised 3794 individuals who either visited outpatient clinics of or were admitted to the participating hospitals (Gifu Prefectural General Medical Center, Gifu Prefectural Tajimi Hospital, and Gifu Prefectural Gero Hot Spring Hospital in Gifu Prefecture, Japan, and Hirosaki University Hospital, Reimeikyo Rehabilitation Hospital, and Hirosaki Stroke Center in Aomori Prefecture, Japan) between October 2002 and March 2007 because of various symptoms or for an annual health checkup; subject panel B comprised 1419 community-dwelling elderly individuals recruited to a population-based prospective cohort study of aging and age-related diseases in Gunma Prefecture, Japan.

The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University School of Medicine, Hirosaki University School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology, and the participating hospitals. Written informed consent was also obtained from each participant.

Measurement of serum lipid profile

Venous blood was collected in the early morning after the subjects had fasted overnight and before they had begun lipid-lowering treatment as appropriate. Blood samples were centrifuged at 1600*g* for 15 min at 4°C, and serum was separated and stored at −30°C until analysis. The serum concentrations of total cholesterol [36], triglycerides [37], and HDL-cholesterol [38] were measured as described previously. The serum concentration of LDL-cholesterol was also measured as previously described [39] or was calculated by the Friedewald formula [serum concentration of LDL-cholesterol = serum concentration of total cholesterol − serum concentration of HDL-cholesterol − (0.2 × serum concentration of triglycerides)] when the serum triglyceride concentration was ≤4.4 mmol/L [40,41].

Selection of polymorphisms

Our aim was to identify genes associated with dyslipidemia in the Japanese population in a case–control association study by examining the relation of one to five polymorphisms of each candidate gene to this condition. With the use of public databases, including PubMed (NCBI) and Online Mendelian Inheritance in Man (NCBI), we selected 65 candidate genes that have been characterized and suggested to be associated with hypertriglyceridemia or low HDL-cholesterol or high LDL-cholesterol concentrations. On the basis of published studies and searches of PubMed, we further selected 100 polymorphisms of these genes—most located in the promoter region or exons—that might be expected to result in changes in the function or expression of the encoded protein (Supplementary Table 6). Wild-type and variant alleles of the polymorphisms were determined from the original sources.

Genotyping of polymorphisms

Venous blood (7 ml) was collected into tubes containing 50 mmol/L EDTA (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 100 polymorphisms were determined at G&G Science (Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with analysis by suspension array technology (Luminex 100 flow cytometer; Luminex, Austin, TX, USA). Primers, probes, and other conditions for genotyping are shown in Supplementary Table 7. Detailed methodology for genotyping was described previously [42].

Statistical analysis

Allele frequencies were estimated by the gene counting method, and the χ^2 test was used to identify departure from Hardy–Weinberg equilibrium. In the initial screen, the genotype distribution of each polymorphism was compared between subjects with hypertriglyceridemia (1=serum triglyceride concentration ≥1.65 mmol/L, 0=<1.65 mmol/L), low HDL-cholesterol (1=serum HDL-cholesterol concentration <1.04 mmol/L, 0=≥1.04 mmol/L), or high LDL-cholesterol (1=serum LDL-cholesterol concentration ≥3.64 mmol/L, 0=<3.64 mmol/L) and individuals without each of these conditions by the χ^2 test (2×2) in subject panel A. Given the multiple comparisons of genotypes with dyslipidemia, we calculated the FDR [43] from the distribution of p values for the 100 polymorphisms. Polymorphisms with an FDR of <0.05 were further examined in a more rigorous evaluation of association by multivariable logistic regression analysis with adjustment for covariates, with hypertriglyceridemia, low HDL-cholesterol, or high LDL-cholesterol as a dependent variable and independent variables including age, sex (0=woman, 1=man), the prevalence of diabetes mellitus (0=no history of diabetes mellitus, 1=positive history), and genotype of each polymorphism. Each polymorphism was assessed according to dominant, recessive, and two additive (additive 1 and 2) genetic models, and the p value, odds ratio, and 95% confidence interval were calculated. Each genetic model comprised two groups: the combined group of variant homozygotes and heterozygotes versus wild-type homozygotes for the dominant model, variant homozygotes versus the combined group of wild-type homozygotes and heterozygotes for the recessive model, heterozygotes versus wild-type homozygotes for the additive 1 model, and variant homozygotes versus wild-type homozygotes for the additive 2 model. Serum concentrations of triglycerides, HDL-cholesterol, or LDL-cholesterol were compared among the three genotype groups for selected polymorphisms by the nonparametric Kruskal–Wallis test or between two groups by the Wilcoxon rank sum test, given that these data were not distributed normally ($p<0.01$ by the Kolmogorov–Smirnov Lilliefors test). We examined linkage disequilibrium among polymorphisms of the same gene or locus as well as the associations of haplotypes with hypertriglyceridemia, low HDL-cholesterol, or high LDL-cholesterol. For statistical analyses other than the initial screen of polymorphisms, a p value of <0.05 was considered significant. Statistical significance was examined using two-tailed tests performed with JMP version 5.1 software (SAS Institute, Cary, NC, USA). Linkage disequilibrium and haplotype analyses were performed with SNPAlyze version 6 software (Dynacom, Yokohama, Japan).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2007.08.001.

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