SPANDIDOS PUBLICATIONS

Association of genetic variants with myocardial infarction in Japanese individuals with different lipid profiles

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Abstract. Dyslipidemia is an important risk factor for myocardial infarction (MI). We previously showed that gene polymorphisms associated with MI differed among individuals with different lipid profiles. We further examined whether genetic variants that confer susceptibility to MI might differ among individuals with low or high serum concentrations of triglycerides, high density lipoprotein (HDL)-cholesterol, or low density lipoprotein (LDL)-cholesterol. The study population comprised 5270 Japanese individuals, including 1188 subjects with MI and 4082 controls. The 150 polymorphisms examined in the present study were selected by genome-wide association studies of MI and ischemic stroke with the use of the Affymetrix GeneChip Human Mapping 500K Array Set. The initial Chi-square test revealed that the A→G polymorphism (rs12632110) of *SEMA3F* was significantly (false discovery rate <0.05) associated with MI among individuals with high serum HDL-cholesterol or among those with low serum LDL-cholesterol. Subsequent multivariable logistic regression analysis with adjustment for covariates revealed that rs12632110 was significantly ($P<0.01$) associated with MI in individuals with high serum HDL-cholesterol or with low serum LDL-cholesterol. The genetic variants that confer susceptibility to MI differ among individuals with different lipid profiles, and the genetic component for the development of MI is more apparent in individuals at low-risk (high HDL- and low LDL-cholesterol levels) compared to those at high-risk. Stratification of subjects according to lipid profiles may thus be important for personalized prevention of MI based on genetic information.

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Introduction

Myocardial infarction (MI) is a complex disease resulting from an interaction between genetic and environmental factors. Disease prevention is an important strategy for reducing the overall burden of MI, and the identification of markers for disease risk is essential both for risk prediction and for potential intervention to reduce the chance of future events.

Although recent genetic association studies (1-6) have implicated several loci and candidate genes in predisposition to MI, the genes that contribute to genetic susceptibility to MI remain to be identified definitively. We previously showed that genetic variants that confer susceptibility to MI differed between men and women (7,8), between individuals with or without conventional risk factors for atherosclerosis (9), or between individuals with different lipid profiles (10). To further examine whether the association of polymorphisms with MI is influenced by the baseline lipid profiles, we performed an association study of 150 polymorphisms of 144 candidate genes (11) and MI in 5270 Japanese individuals with low or high serum concentrations of triglycerides, high density lipoprotein (HDL)-cholesterol, or low density lipoprotein (LDL)-cholesterol. The purpose of the present study was to identify genetic variants that confer susceptibility to MI in Japanese individuals with different lipid profiles independently and thereby to assess the genetic risk of MI in such individuals separately.

Materials and methods

Study population. The study population comprised 5270 unrelated Japanese individuals (2949 men, 2321 women) who either visited outpatient clinics of or were admitted to one of the participating hospitals (Gifu Prefectural General Medical Center and Gifu Prefectural Tajimi 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 2008 because of various symptoms or for an annual health checkup, or who were recruited to a population-based prospective cohort study of aging and age-related diseases in Gunma Prefecture, Japan. The 1188 subjects with MI (930 men, 258 women) all underwent coronary angiography and left ventriculography. The diagnosis of MI was based on typical electrocardiographic changes and on increases both in the serum activities of enzymes such as creatine kinase, aspartate aminotransferase, and lactate dehydrogenase and in the serum concentration of troponin T. The diagnosis was confirmed by the presence of a wall motion abnormality by left ventriculography and identification of the responsible stenosis in any of the major coronary arteries or in the left main trunk by coronary angiography. The 4082 control individuals (2019 men, 2063 women) were recruited from community-dwelling healthy individuals or patients who visited outpatient clinics regularly for treatment of various common diseases. They had no history of MI or coronary heart disease (CHD), ischemic or hemorrhagic stroke, peripheral arterial occlusive disease, or other atherosclerotic, thrombotic, embolic, or hemorrhagic disorders. The subjects with MI and the controls either had or did not have conventional risk factors for CHD, including hypertension (systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg, or taking antihypertensive medication) and diabetes mellitus [fasting plasma glucose level of ≥6.93 mmol/l or blood glycosylated hemoglobin (hemoglobin A1c) content of ≥6.5%, or taking antidiabetes medication]. Among the total study population, the 3506 and 1764 individuals had low (<1.70 mmol/l) or high (≥1.70 mmol/l) serum concentrations of triglycerides, respectively, and the 948 and 4322 individuals had low (<1.03 mmol/l) or high (≥1.03 mmol/l) serum concentrations of HDL-cholesterol, respectively. The values for LDL-cholesterol were calculated by the Friedewald formula: serum concentration of LDL-cholesterol = (serum concentration of total cholesterol) - (serum concentration of HDL-cholesterol) - [0.2 x (serum concentration of triglycerides)]. Among the total study population, the 4040 and 1230 individuals had low (<3.63 mmol/l) or high (≥3.63 mmol/l) serum concentrations of LDL-cholesterol, respectively.

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

Selection of polymorphisms. Our aim was to identify genetic variants associated with MI in the Japanese population with different lipid profiles in a case-control association study. A

Table I. Primers, probes, and other conditions for genotyping of single nucleotide polymorphisms (SNPs).

Gene	SNP	Sense primer (5'→3')	Antisense primer (5'→3')	Probe 1 (5'→3')	Probe 2 (5'→3')	Annealing (°C)	Cycles
SEMA3F	A→G (rs12632110)	GGTGCTGCACCGTGGATGTGA	CTCCAGATCACTCCTCTACTACA	TGTGAGTCCTTGCACAGTGGT	CATTTCACCACTGCGCAAGGA	60	50
MCC	A→G (rs6594664)	AAGGGTGTCCCCAAGCCACTC	AGCGGATGCGATCCCTCAGC	CTTGCCAGGTTTATCTTTGCTG	GGCAGCAAAGACAAACCTGG	60	50
USP37	A→G (rs526897)	CCGCGAAGTGGGCGGAGCG	ACAGCCGGACGCGTCCGAC	ACGGCGGCTCATTATTTTC	TGCAGCGGAAAACAATGAGCC	60	50
MEF2D	A→G (rs1109751)	TCTTTTCATCCTGTTAAAACACAGC	TCAGTGAAGACGGTACTCAGAGT	CACCTCATGGTTATTATGACTTA	AAACCTAAGTCACAATAACCATG	60	50
TSPAN9	A→G (rs2011973)	CTTCCTAGCTTCCTCGTGTGTGA	CGTCAGAGAGGGGAGAGCAGA	GGGCCAGACTCACTGCATTC	GGGCCAGACTCGCTGCATT	60	50
MON1B	C→T (rs2232504)	CAACGTGAAGCCTTCCATGCC	TCCCCAAGGGCACGCATGGC	GGCGCCTGGTCGAAGATGG	CATCCCATCTTCAACCAGGC	60	50

Characteristics of subjects with myocardial infarction (MI) and controls among individuals with low or high serum tions of triglycerides.

Characteristic	Low serum triglycerides			High serum triglycerides		
	MI	Controls	P-value	MI	Controls	P-value
No. of subjects	704	2802		484	1280	
Age (years)	67.4±10.3	67.2±10.8	0.6549	64.9±9.8	67.3±9.6	<0.0001
Gender (male/female, %)	76.4/23.6	47.7/52.3	<0.0001	81.0/19.0	53.3/46.7	<0.0001
Body mass index (kg/m^2)	23.3±3.2	23.0±3.3	0.0045	24.4±3.2	24.3±3.2	0.5960
Current or former smoker (%)	24.2	21.3	0.1030	25.8	24.6	0.5981
Hypertension (%)	68.2	50.2	<0.0001	76.2	58.0	<0.0001
Diabetes mellitus (%)	44.0	20.0	<0.0001	50.8	25.6	<0.0001
Serum triglycerides (mmol/l)	1.08±0.36	1.07±0.33	0.2379	2.53±0.71	2.51±0.84	0.1151
Serum HDL-cholesterol (mmol/l)	1.27±0.35	1.50±0.41	<0.0001	1.12±0.31	1.27±0.33	<0.0001
Serum LDL-cholesterol (mmol/l)	3.21±0.87	2.99±0.80	<0.0001	3.17±0.94	3.09±0.94	0.0862
Serum creatinine (μmol/l)	86.7±86.0	71.1±58.4	<0.0001	98.6±113.4	74.9±55.9	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	67.3±21.4	72.4±21.3	<0.0001	64.5±21.2	68.1±18.1	0.0093

Quantitative data are means ± SD. HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate.

Table III. Genotype distributions of single nucleotide polymorphisms (SNPs) related (P-value for allele frequency <0.01) to myocardial infarction (MI) among individuals with low or high serum concentrations of triglycerides as determined by the Chi-square test.

Gene symbol	SNP	dbSNP	MI	Controls	P-value (allele frequency)	FDR
Low serum triglycerides						
ZNF79	C→T	rs10819291			0.0038	0.2622
	CC		401 (57.9)	1788 (64.2)		
	CT		257 (37.1)	878 (31.5)		
	TT		35 (5.0)	120 (4.3)		
TSPAN9	A→G	rs2011973			0.0091	0.2622
	AA		103 (14.7)	520 (18.8)		
	AG		340 (48.6)	1334 (48.2)		
	GG		257 (36.7)	916 (33.0)		
High serum triglycerides						
SEMA3F	A→G	rs12632110			0.0019	0.2707
	AA		122 (25.3)	255 (20.1)		
	AG		246 (51.0)	633 (49.8)		
	GG		114 (23.7)	383 (30.1)		

Numbers in parentheses are percentages. FDR, false discovery rate.

total of 150 polymorphisms examined in the present study (data not shown) were selected by genome-wide association studies of MI and ischemic stroke (P-value for allele frequency <1.0x10^{-7}) with the use of the GeneChip Human Mapping 500K Array Set (Affymetrix, Santa Clara, CA) (11). The relation of these polymorphisms to MI were not examined in our previous studies (7,8,10,12).

Genotyping of polymorphisms. Venous blood (7 ml) was collected in tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 150 polymorphisms were determined at G&G Science (Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX). Primers, probes, and other conditions for genotyping of polymorphisms related to MI are shown in Table I. Detailed genotyping methodology was described previously (13).

Statistical analysis. Quantitative data were compared between subjects with MI and controls by the unpaired Student's t-test. Categorical data were compared by the Chi-square test. Allele frequencies were estimated by the gene counting method, and the Chi-square test was used to identify departures from Hardy-Weinberg equilibrium. In the initial screen, the allele frequencies of each polymorphism were compared between subjects with MI and controls by the Chi-square test. Given the multiple comparisons of genotypes with MI, the false discovery rate (FDR) was calculated from the distribution of P-values for the allele frequencies of the 150 polymorphisms (14). Polymorphisms with a P-value for allele frequency of <0.01 were further examined by multivariable logistic regression analysis with adjustment for covariates. Multivariable logistic regression analysis was thus performed with MI as a dependent variable and independent variables including age, gender (0, woman; 1, man), body mass index (BMI), smoking status (0, nonsmoker; 1, smoker), serum concentrations of HDL-cholesterol, LDL-cholesterol, triglycerides, or creatinine, history of hypertension or diabetes mellitus (0, no history; 1, positive history), and genotype of each polymorphism; and the P-value, odds ratio, and 95% confidence interval were calculated. Each genotype was assessed according to dominant, recessive, and additive genetic models. Additive models included the additive 1 (heterozygotes versus wild-type homozygotes) and additive 2 (variant homozygotes versus wild-type homozygotes) models, which were analyzed simultaneously with a single statistical model. We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as of other covariates on MI; each genotype was examined according to a dominant or recessive model on the basis of statistical significance in the multivariable logistic regression analysis. The P-levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. With the exception of the initial screen by the Chi-square test (FDR <0.05), P<0.01 was considered statistically significant in comparisons of genotypes with MI. For other clinical background data, P<0.05 was considered statistically significant. Statistical significance was examined by two-sided tests performed with JMP version 6.0 and JMP Genomics version 3.2 software (SAS Institute, Cary, NC).

Results

Genetic variants related to MI in individuals with low or high serum concentrations of triglycerides. The characteristics of the subjects with MI and controls who had low or high serum concentrations of triglycerides are shown in Table II. For individuals with low serum triglycerides, the frequency of male subjects, BMI, serum concentrations of LDL-cholesterol and creatinine, as well as the prevalence of hypertension and diabetes mellitus were greater, whereas the serum concentration of HDL-cholesterol was lower, in subjects with MI than in controls. For individuals with high serum triglycerides, the frequency of male subjects, the prevalence of hypertension and diabetes mellitus, and the serum concentration of creatinine were greater, whereas age and the serum concentration of HDL-cholesterol were lower, in subjects with MI than in controls.

Table IV. Multivariable logistic regression analysis of SNPs related (P-value for allele frequency <0.01) to myocardial infarction by the Chi-square test among individuals with low or high serum concentrations of triglycerides.

		Dominant		Recessive		Additive 1		Additive 2	
Gene symbol	SNP	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Low serum triglycerides									
ZNF79	C→T	0.0476	1.21 (1.00-1.45)	0.9943		0.0384	1.23 (1.01-1.49)	0.7388	
TSPAN9	A→G	0.0289	1.32 (1.03-1.70)	0.1895		0.0610		0.0249	1.37 (1.04-1.80)
High serum triglycerides									
SEMA3F	A→G	0.0347	0.75 (0.57-0.98)	0.0322	0.75 (0.57-0.97)	0.1390		**0.0091**	0.65 (0.46-0.90)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status, serum concentrations of HDL-cholesterol, LDL-cholesterol, and creatinine, and the prevalence of hypertension and diabetes mellitus. P-values <0.01 are shown in bold.

Characteristics of subjects with myocardial infarction (MI) and controls among individuals with low or high serum tions of HDL-cholesterol.

Characteristic	Low serum HDL-cholesterol			High serum HDL-cholesterol		
	MI	Controls	P-value	MI	Controls	P-value
No. of subjects	372	576		816	3506	
Age (years)	64.7±10.4	68.2±10.3	<0.0001	67.1±10.0	67.1±10.5	0.2764
Gender (male/female, %)	89.3/10.7	70.5/20.5	<0.0001	73.3/26.7	46.0/54.0	<0.0001
Body mass index (kg/m^2)	24.4±3.1	24.0±3.3	0.0858	23.5±3.2	23.3±3.3	0.1292
Current or former smoker (%)	32.3	29.5	0.3706	21.5	21.2	0.8590
Hypertension (%)	67.7	65.6	0.5003	73.2	50.5	<0.0001
Diabetes mellitus (%)	48.4	32.1	<0.0001	46.1	19.8	<0.0001
Serum triglycerides (mmol/l)	1.89±0.94	1.89±0.97	0.4981	1.57±0.85	1.46±0.83	0.0003
Serum HDL-cholesterol (mmol/l)	0.87±0.12	0.88±0.11	0.0264	1.36±0.29	1.52±0.36	<0.0001
Serum LDL-cholesterol (mmol/l)	3.12±0.95	2.96±0.83	0.0244	3.22±0.87	3.03±0.86	<0.0001
Serum creatinine (μmol/l)	94.4±97.4	84.3±94.8	<0.0001	90.2±98.6	70.3±48.7	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	65.9±21.9	69.0±22.8	0.0825	66.2±21.1	71.4±20.0	<0.0001

Quantitative data are means ± SD. eGFR, estimated glomerular filtration rate.

Comparison of allele frequencies with the Chi-square test revealed that two or one polymorphisms were related (P<0.01) to MI in individuals with low or high serum triglycerides, respectively (Table III). Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status, serum concentrations of HDL-cholesterol, LDL-cholesterol, and creatinine, and the prevalence of hypertension and diabetes mellitus revealed that the A→G polymorphism (rs12632110) of *SEMA3F* (additive 2 model) was significantly (P<0.01) associated with MI in individuals with high serum triglycerides (Table IV).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphisms related to MI by the Chi-square test as well as of age, gender, BMI, smoking status, serum concentrations of HDL-cholesterol, LDL-cholesterol, and creatinine, and the prevalence of hypertension and diabetes mellitus on MI (data not shown). For individuals with low serum triglycerides, gender, diabetes mellitus, serum HDL-cholesterol, serum LDL-cholesterol, and smoking, in descending order of statistical significance, were significant (P<0.01) and independent determinants of MI. For individuals with high serum triglycerides, gender, diabetes mellitus, serum HDL-cholesterol, hypertension, age, and the serum concentration of creatinine, in descending order of statistical significance, were significant and independent determinants of MI.

Genetic variants related to MI in individuals with low or high serum concentrations of HDL-cholesterol. The characteristics of the subjects with MI and controls who had low or high serum concentrations of HDL-cholesterol are shown in Table V. For individuals with low serum HDL-cholesterol, the frequency of male subjects, the prevalence of diabetes mellitus, and serum concentrations of LDL-cholesterol and creatinine were greater, whereas age and the serum concentration of HDL-cholesterol were lower, in subjects with MI than in controls. For individuals with high serum HDL-cholesterol, the frequency of male subjects, serum concentrations of triglycerides, LDL-cholesterol, and creatinine, as well as the prevalence of hypertension and diabetes mellitus were greater, whereas the serum concentration of HDL-cholesterol was lower, in subjects with MI than in controls.

Comparison of allele frequencies with the Chi-square test revealed that five or four polymorphisms were related (P<0.01) to MI in individuals with low or high serum HDL-cholesterol concentrations, respectively (Table VI). Among these polymorphisms, the A→G polymorphism (rs12632110) of *SEMA3F* was significantly (FDR<0.05) associated with MI in individuals with high serum HDL-cholesterol. Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status, serum concentrations of triglycerides, LDL-cholesterol, and creatinine, and the prevalence of hypertension, and diabetes mellitus revealed that the A→G polymorphism (rs6594664) of *MCC* (dominant and additive 1 models), and the A→G polymorphism (rs526897) of *USP37* (dominant model) were significantly (P<0.01) associated with MI in individuals with low serum HDL-cholesterol, and that the A→G polymorphism (rs12632110) of *SEMA3F* (dominant and additive 1 and 2 models), the A→G polymorphism (rs1109751) of *MEF2D* (dominant and additive 1 models), and the A→G polymorphism (rs2011973) of *TSPAN9* (dominant and additive 2 models) were significantly associated with MI in individuals with high serum HDL-cholesterol (Table VII).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphisms related to MI by the Chi-square test as well as of age, gender, BMI, smoking status, serum concentrations of triglycerides, LDL-cholesterol, and creatinine, and the prevalence of hypertension and diabetes mellitus on MI (data not shown).

Table VI. Genotype distributions of SNPs related (P-value for allele frequency <0.01) to myocardial infarction (MI) among individuals with low or high serum concentrations of HDL-cholesterol as determined by the Chi-square test.

Gene symbol	SNP	dbSNP	MI	Controls	P-value (allele frequency)	FDR
Low serum HDL-cholesterol						
MCC	A→G	rs6594664			0.0020	0.1139
	AA		305 (84.2)	517 (90.9)		
	AG		55 (15.2)	51 (9.0)		
	GG		2 (0.6)	1 (0.1)		
PIGQ	C→G	rs1045274			0.0023	0.1139
	CC		335 (90.5)	517 (90.9)		
	CG		35 (9.5)	51 (9.0)		
	GG		0 (0.0)	1 (0.1)		
SORCS2	A→G	rs2285780			0.0025	0.1139
	AA		1 (0.3)	2 (0.3)		
	AG		58 (16.0)	51 (9.0)		
	GG		303 (83.7)	517 (90.7)		
LAMA3	A→G	rs12373237			0.0040	0.1419
	AA		8 (2.2)	7 (1.2)		
	AG		90 (24.4)	100 (17.6)		
	GG		271 (73.4)	463 (81.2)		
USP37	A→G	rs526897			0.0096	0.2657
	AA		307 (84.8)	443 (77.7)		
	AG		52 (14.4)	120 (21.1)		
	GG		3 (0.8)	7 (1.2)		
High serum HDL-cholesterol						
SEMA3F	A→G	rs12632110			0.0002	0.0314
	AA		210 (25.9)	713 (20.6)		
	AG		401 (49.4)	1728 (49.8)		
	GG		201 (24.7)	1028 (29.6)		
MEF2D	A→G	rs1109751			0.0038	0.1468
	AA		363 (44.8)	1767 (50.7)		
	AG		366 (45.2)	1417 (40.7)		
	GG		81 (10.0)	300 (8.6)		
TSPAN9	A→G	rs2011973			0.0038	0.1468
	AA		119 (14.7)	648 (18.7)		
	AG		391 (48.1)	1664 (48.0)		
	GG		302 (37.2)	1157 (33.3)		
CLEC16A	C→T	rs9925481			0.0039	0.1468
	CC		651 (80.0)	2603 (75.1)		
	CT		152 (18.7)	801 (23.1)		
	TT		11 (1.3)	62 (1.8)		

Numbers in parentheses are percentages.

For individuals with low serum HDL-cholesterol, gender, diabetes mellitus, age, serum LDL-cholesterol, *MCC* genotype (dominant model), *USP37* genotype (dominant model), and *SORCS2* genotype (recessive model), in descending order of statistical significance, were significant (P<0.01) and independent determinants of MI. For individuals with high serum HDL-cholesterol, diabetes mellitus, gender, hypertension, serum LDL-cholesterol, smoking, *SEMA3F* genotype (dominant model), *MEF2D* genotype (dominant model), serum concentration of creatinine, and *TSPAN9* genotype (dominant model), in descending order of statistical significance, were significant and independent determinants of MI.

Table VII. Multivariable logistic regression analysis of SNPs related (P-value for allele frequency <0.01) to myocardial infarction by the Chi-square test among individuals with or high serum concentrations of HDL-cholesterol.

Gene symbol	SNP	Dominant		Recessive		Additive 1		Additive 2	
		P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Low serum HDL-cholesterol									
MCC	A→G	**0.0061**	1.83 (1.19-2.83)	0.2712		**0.0095**	1.78 (1.15-2.77)	0.2564	
PIGQ	C→G	0.0123	0.57 (0.37-0.88)	0.7163		0.0344		0.7134	
SORCS2	A→G	0.6990		0.0106	0.58 (0.38-0.88)	0.4406		0.7426	
LAMA3	A→G	0.6255		0.0213	0.68 (0.48-0.94)	0.9551		0.5253	
USP37	A→G	**0.0068**	0.60 (0.41-0.86)	0.3741		0.0101	0.61 (0.41-0.88)	0.3102	
High serum HDL-cholesterol									
SEMA3F	A→G	**0.0010**	0.73 (0.60-0.88)	0.0405	0.82 (0.68-0.99)	**0.0060**	0.75 (0.61-0.92)	**0.0010**	0.68 (0.54-0.85)
MEF2D	A→G	**0.0019**	1.30 (1.10-1.53)	0.2833		**0.0034**	1.30 (1.09-1.54)	0.0644	
TSPAN9	A→G	**0.0059**	1.38 (1.10-1.73)	0.2305		0.0177	1.36 (1.07-1.74)	**0.0088**	1.40 (1.09-1.79)
CLEC16A	C→T	0.0255	0.79 (0.65-0.97)	0.8429		0.0217	0.78 (0.64-0.96)	0.7923	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status, serum concentrations of triglycerides, LDL-cholesterol, and creatinine, and the prevalence of hypertension and diabetes mellitus. P-values <0.01 are shown in bold.

Genetic variants related to MI in individuals with low or high serum concentrations of LDL-cholesterol. The characteristics of the subjects with MI and controls who had low or high serum concentrations of LDL-cholesterol are shown in Table VIII. For individuals with low serum LDL-cholesterol, the frequency of male subjects, BMI, serum concentrations of triglycerides, LDL-cholesterol, and creatinine, as well as the prevalence of hypertension and diabetes mellitus were greater, whereas age and the serum concentration of HDL-cholesterol were lower, in subjects with MI than in controls. For individuals with high serum LDL-cholesterol, the frequency of male subjects, the percentage of smokers, the serum concentration of creatinine, as well as the prevalence of hypertension and diabetes mellitus were greater, whereas the serum concentration of HDL-cholesterol was lower, in subjects with MI than in controls.

Comparison of allele frequencies with the Chi-square test revealed that different sets of three polymorphisms were related (P<0.01) to MI in individuals with low or high serum LDL-cholesterol concentrations, respectively (Table IX). Among these polymorphisms, the A→G polymorphism (rs12632110) of *SEMA3F* was significantly (FDR<0.05) associated with MI in individuals with low serum LDL-cholesterol. Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status, serum concentrations of triglycerides, HDL-cholesterol, and creatinine, and the prevalence of hypertension, and diabetes mellitus revealed that the A→G polymorphism (rs12632110) of *SEMA3F* (dominant and additive 2 models) and the C→T polymorphism (rs2232504) of *MON1B* (dominant and additive 1 models) were significantly (P<0.01) associated with MI in individuals with low serum LDL-cholesterol. No polymorphisms were significantly associated with MI in individuals with high serum LDL-cholesterol (Table X).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphisms related to MI by the Chi-square test as well as of age, gender, BMI, smoking status, serum concentrations of triglycerides, HDL-cholesterol, and creatinine, and the prevalence of hypertension and diabetes mellitus on MI (data not shown). For individuals with low serum LDL-cholesterol, gender, diabetes mellitus, serum HDL-cholesterol, hypertension, smoking, *MON1B* genotype (dominant model), age, and *SEMA3F* genotype (dominant model), in descending order of statistical significance, were significant (P<0.01) and independent determinants of MI. For individuals with high serum LDL-cholesterol, age, diabetes mellitus, serum HDL-cholesterol, and hypertension, in descending order of statistical significance, were significant and independent determinants of MI.

Finally, we examined whether the genotype distributions for the six polymorphisms related to MI were in Hardy-Weinberg equilibrium. The genotype distributions for these polymorphisms were all in Hardy-Weinberg equilibrium in controls (data not shown).

Discussion

We examined the possible relations of 150 polymorphisms of 144 candidate genes to the prevalence of MI in individuals

Table VIII. Characteristics of subjects with myocardial infarction (MI) and controls among individuals with low or high serum concentrations of LDL-cholesterol.

Characteristic	Low serum LDL-cholesterol			High serum LDL-cholesterol		
	MI	Controls	P-value	MI	Controls	P-value
No. of subjects	841	3199		347	883	
Age (years)	66.7±10.2	67.5±10.5	0.0021	65.5±10.0	66.3±10.3	0.1785
Gender (male/female, %)	81.7/18.3	51.6/48.4	<0.0001	70.0/30.0	41.7/58.3	<0.0001
Body mass index (kg/m^2)	23.8±3.2	23.3±3.3	0.0009	23.9±3.2	23.7±3.3	0.3466
Current or former smoker (%)	25.3	23.9	0.3945	23.6	16.7	0.0046
Hypertension (%)	70.9	51.9	<0.0001	72.9	55.4	<0.0001
Diabetes mellitus (%)	46.7	20.8	<0.0001	47.0	24.1	<0.0001
Serum triglycerides (mmol/l)	1.69±0.93	1.51±0.90	<0.0001	1.62±0.79	1.57±0.72	0.3994
Serum HDL-cholesterol (mmol/l)	1.20±0.35	1.43±0.41	<0.0001	1.22±0.31	1.41±0.38	<0.0001
Serum LDL-cholesterol (mmol/l)	2.76±0.57	2.70±0.57	0.0025	4.25±0.63	4.20±0.61	0.2082
Serum creatinine (μmol/l)	92.5±104.0	72.0±52.3	<0.0001	89.2±82.8	73.3±73.9	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	65.9±20.9	71.3±20.0	<0.0001	66.7±22.5	70.2±21.9	0.0143

Quantitative data are means ± SD. eGFR, estimated glomerular filtration rate.

Table IX. Genotype distributions of SNPs related (P-value for allele frequency <0.01) to myocardial infarction (MI) among individuals with low or high serum concentrations of LDL-cholesterol as determined by the Chi-square test.

Gene symbol	SNP	dbSNP	MI	Controls	P-value (allele frequency)	FDR
Low serum LDL-cholesterol						
SEMA3F	A→G	rs12632110			7.7x10^{-5}	0.0115
	AA		221 (26.4)	659 (20.8)		
	AG		416 (49.7)	1585 (50.1)		
	GG		200 (23.9)	923 (29.1)		
MON1B	C→T	rs2232504			0.0016	0.1196
	CC		563 (67.7)	2354 (74.0)		
	CT		254 (30.6)	766 (24.1)		
	TT		14 (1.7)	59 (1.9)		
LAMA3	A→G	rs12373237			0.0095	0.4725
	AA		16 (1.9)	42 (1.3)		
	AG		194 (23.2)	635 (19.8)		
	GG		628 (74.9)	2499 (78.9)		
High serum LDL-cholesterol						
GAS7	A→C	rs16958993			0.0062	0.4270
	AA		0 (0.0)	0 (0.0)		
	AC		36 (10.5)	52 (5.9)		
	CC		305 (89.5)	823 (94.1)		
MYO7B	C→T	rs13015157			0.0064	0.4270
	CC		252 (73.9)	706 (80.7)		
	CT		83 (24.3)	162 (18.5)		
	TT		6 (1.8)	7 (0.8)		
CAMTA1	C→G	r rs845206			0.0093	0.4270
	CC		328 (96.2)	861 (98.4)		
	CG		12 (3.5)	14 (1.6)		
	GG		1 (0.3)	0 (0.0)		

Numbers in parentheses are percentages.

Table X. Multivariable logistic regression analysis of SNPs related (P-value for allele frequency <0.01) to myocardial infarction by the Chi-square test among individuals with low or high serum concentrations of LDL-cholesterol as determined by the Chi-square test.

Gene symbol	SNP	Dominant		Recessive		Additive 1		Additive 2	
		P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Low serum LDL-cholesterol									
SEMA3F	A→G	**0.0036**	0.75 (0.62-0.91)	0.0425	0.82 (0.68-0.99)	0.0187	0.78 (0.64-0.96)	**0.0024**	0.69 (0.55-0.88)
MON1B	C→T	**0.0001**	1.43 (1.19-1.71)	0.8122		**0.0001**	1.46 (1.21-1.76)	0.9307	
LAMA3	A→G	0.4486		0.0384	0.81 (0.67-0.99)	0.7681		0.3751	
High serum LDL-cholesterol									
GAS7	A→C			0.0279	0.58 (0.36-0.95)				
MYO7B	C→T	0.0310	1.42 (1.03-1.96)	0.3172		0.0459	1.40 (1.00-1.93)	0.2652	
CAMTA1	C→G	0.0207	2.67 (1.15-6.16)	0.7743		0.0326	2.52 (1.06-5.90)	0.7734	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status, serum concentrations of triglycerides, HDL-cholesterol, and creatinine, and the prevalence of hypertension and diabetes mellitus. P-values <0.01 are shown in bold.

with low or high serum concentrations of triglycerides, HDL-cholesterol, or LDL-cholesterol. Our association study with three steps of analysis (Chi-square test, multivariable logistic regression analysis with adjustment for covariates, and stepwise forward selection procedure) revealed that the A→G polymorphism (rs12632110) of *SEMA3F* was significantly associated with MI in individuals with high serum HDL-cholesterol and in those with low serum LDL-cholesterol.

Sema domain, immunoglobulin domain, short basic domain, secreted, 3F (SEMA3F) is a secreted member of the semaphorin III family. All the family members have a secretion signal, a 500-amino acid sema domain, and 16 conserved cysteine residues (15). During the developmental process, SEMA3F plays a pivotal role in axon guidance in the peripheral and central nervous system by interacting with its receptor, neuropilin 2 (NRP2) (16). The SEMA3F-NRP2 signaling pathway guides axonal extension by means of a chemotactic repulsing effect on the axons (17). *SEMA3F* was isolated from a region of 3p21.3 involved in homozygous deletions in small-cell lung cancer cell lines and was recognized as a candidate tumor suppressor gene, given that p53 inhibits tumor vessel formation and cell growth through the SEMA3F-NRP2 pathway (18). Genetic variants of *SEMA3F* have not been associated with MI or CHD. We have now shown that the A→G polymorphism (rs12632110) of *SEMA3F* was significantly associated with the prevalence of MI in individuals with high serum HDL-cholesterol and in those with low serum LDL-cholesterol, with the *G* allele protecting against MI, although the underlying mechanism remains unclear.

Our results suggested that another five polymorphisms were also candidate susceptibility loci for MI: the A→G polymorphism (rs6594664) of *MCC* and the A→G polymorphism (rs526897) of *USP37* in individuals with low serum HDL-cholesterol; the A→G polymorphism (rs1109751) of *MEF2D* and the A→G polymorphism (rs2011973) of *TSPAN9* in individuals with high serum HDL-cholesterol; and the C→T polymorphism (rs2232504) of *MON1B* in individuals with low serum LDL-cholesterol. None of these polymorphisms have previously been shown to be associated with MI. The variants of MCC have been reported as a candidate for the putative colorectal tumor suppressor gene located at 5q21 (19). *USP37* is a member of the ubiquitin-specific protease gene family, which affect the fate and degradation of intracellular proteins and are essential for maintenance of cell-free ubiquitin pools (20). *MEF2D*, expressed in limited areas of the central nervous system, is a member of the myocyte-specific enhancer binding factor 2 (MEF2) gene family (21). *MEF2D* may thus be involved in either the differentiation process or the function of the neurons. The protein encoded by *TSPAN9* is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. Most of these members are cell-surface proteins that are characterized by the presence of four hydrophobic domains. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth, and motility (22). The *MON1B* protein consisted of 547 amino acids and had a significant homology in six eukaryotic species. On the basis of its structure, it was identified as a member of the SAND protein family (23). Given that the functional relevance

remains unclear, further studies will be required to identify the underlying mechanisms of the relations between these genes and the pathogenesis of MI.

Our study has several limitations. i) It is possible that one or more of the polymorphisms associated with MI in the present study are in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of this condition. ii) The functional relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study. iii) Although we adopted the criterion of FDR <0.05 for association to compensate for the multiple comparisons of genotypes with MI, it is not possible to exclude completely potential statistical errors such as false positives. (iv) Given that the results of the present study were not replicated, validation of our findings will require their replication with independent subject panels.

In conclusion, genetic variants that confer susceptibility to MI differ among individuals with different lipid profiles, and that genetic component for the development of MI is more apparent in individuals at low-risk (high HDL- and low LDL-cholesterol levels) compared to those at high-risk. Stratification of subjects according to lipid profiles may thus be important for personalized prevention of MI based on genetic information. Given that our present study may be considered as hypothesis generating, validation of our findings will require their replication with independent subject panels.

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