Assessment of genetic risk for myocardial infarction

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Summary

Although lifestyle and environmental factors influence the prevalence of myocardial infarction, genetic epidemiological studies have suggested that several genetic variants increase the risk for this condition. We have performed a large-scale association study to identify gene polymorphisms for reliable assessment of the genetic risk of myocardial infarction. The study population comprised 3,483 unrelated Japanese individuals (1,913 men; 1,570 women), including 1,192 subjects with myocardial infarction and 2,291 controls. The genotypes for 164 polymorphisms of 137 candidate genes were determined with an oligonucleotide ligation assay based on analysis of fluorescent microspheres with suspension array technology. Multivariable logistic regression analysis with adjustment for age, sex, body mass index, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia revealed that the 677C→T (Ala222Val) polymorphism of *MTHFR*, the 1595C→G (Ser447Stop) polymorphism of *LPL*, and the –108/3G→4G polymorphism of *IPF1* were significantly associated with the prevalence of myocardial infarction. A stepwise forward selection procedure demonstrated that *IPF1*, *MTHFR*, and *LPL* genotypes significantly affected the prevalence of myocardial infarction. Combined genotype analysis of these polymorphisms yielded a maximum odds ratio of 2.54 for the combined genotype of *TT* for *MTHFR*, *CC* for *LPL*, and *3G3G* for *IPF1*. The genotypes for *MTHFR*, *LPL*, and *IPF1* may prove reliable for assessment of genetic risk for myocardial infarction. Determination of the combined genotype for these genes may contribute to primary, personalized prevention of this condition.

Keywords

Genetics, polymorphism, myocardial infarction, coronary heart disease, atherosclerosis

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Introduction

Completion of the Human Genome Project has the potential to provide substantial benefits to clinical medicine, including the development of panels of genetic markers for the assessment of disease risk (1). One approach to this goal is to evaluate selected polymorphisms of genes that are possibly associated with a disease, either because they are known to encode proteins related to the disease process or because they are located within chromosomal regions identified in linkage studies.

Coronary heart disease (CHD) is the single largest killer of men and women in the United States. The total numbers of individuals affected by CHD or by myocardial infarction (MI) in 2003 were 13.2 million and 7.2 million, respectively. Despite recent advances in therapy for these conditions, nearly 480,000 and 170,000 patients die annually from CHD or MI, respectively (2). In Japan, the total number of individuals with CHD is 0.9 million and nearly 50,000 people die annually from MI (Ministry of Health, Labor, and Welfare of Japan). Disease prevention is an important strategy for reducing the overall burden of CHD and MI, and the identification of markers for disease risk is key both for risk prediction and for potential intervention to reduce the chance of future events.

Several whole-genome linkage analyses of families or sibling-pairs (3–6) and various association studies of unrelated individuals (7–15) have attempted to identify genetic variations that contribute to CHD or MI. The genetic components of these conditions have not been determined definitively, however. We have now performed a large-scale association study for 164 polymor-

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Table 1: Characteristics of the 3,483 study subjects.

Characteristic	Myocardial infarction	Controls
No. of subjects	1192	2291
Age (years)	63.7 ± 10.6*	62.5 ± 11.8
Sex (male/female, %)	77.7/22.3†	43.1/56.9
Body mass index (kg/m^2)	23.7 ± 3.2‡	23.4 ± 3.1
Current or former smoker (%)	22.6†	16.1
Hypertension (%)	73.0†	44.3
Diabetes mellitus (%)	48.7†	20.0
Hypercholesterolemia (%)	56.9†	29.6

Data for age and body mass index are means ± SD. Smoker: smoking of ≥10 cigarettes daily. Hypertension: systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg (or both), or taking antihypertensive medication. Diabetes mellitus: fasting blood glucose of ≥6.93 mM (126 mg/dl) or glycosylated hemoglobin of ≥6.5% (or both), or taking antidiabetes medication. Hypercholesterolemia: serum total cholesterol of ≥5.72 mM (220 mg/dl) or taking lipid-lowering medication. *P < 0.005, †P < 0.001, ‡P < 0.01 versus controls.

phisms of 137 candidate genes and MI in 3,483 Japanese individuals. The purpose of the present study was to identify gene polymorphisms that confer susceptibility to MI and thereby to provide a basis for the primary, personalized prevention of this condition.

Materials and methods

Study population

The study population comprised 3,483 unrelated Japanese individuals (1,913 men; 1,570 women) who either visited outpatient clinics of or were admitted to one of the six participating hospitals (Gifu Prefectural Gifu, Tajimi, and Gero Hot Spring Hospitals; Hirosaki University Hospital; Reimeikyo Rehabilitation Hospital; and Yokohama General Hospital) between October 2002 and March 2005. The 1,192 subjects with a first MI (926 men; 266 women) all underwent coronary angiography and left ventriculography. The diagnosis of MI was based on typical electrocardiographic changes and increases both in the serum activities of enzymes such as creatinine 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 on 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 control subjects comprised 2,291 individuals (987 men; 1,304 women) who visited the outpatient clinics of participating hospitals for an annual health checkup. They had no history of CHD, peripheral arterial occlusive disease, or other atherosclerotic diseases; of ischemic or hemorrhagic stroke or other cerebral diseases; or of other thrombotic, embolic, or hemorrhagic disorders. 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, and participating hospitals, and written informed consent was obtained from each participant.

Selection of polymorphisms

Our aim was to identify genes associated with MI in the Japanese population in a case-control association study by examining the relation of one to three polymorphisms of each candidate gene to MI. With the use of public databases [including PubMed (NCBI), Online Mendelian Inheritance in Man (NCBI), and GeneCanvas (INSERM, Paris, France; http://ecgene.net/genecanvas/news.php)], we selected 137 candidate genes that have been characterized and were suggested to be associated with MI on the basis of a comprehensive overview of vascular biology (from the viewpoint of atherosclerosis, arterial spasm, or arterial aneurysm); platelet function; leukocyte, lymphocyte, and monocyte-macrophage biology; coagulation and fibrinolysis cascades; neurological factors (from the viewpoint of regulation of the circulation, blood pressure, or endocrine function); as well as lipid and adipose tissue metabolism, insulin and glucose metabolism, peripheral insulin sensitivity, homocysteine metabolism, and other metabolic factors. On the basis of published studies and searches of PubMed, we further selected 164 polymorphisms of these genes – most located in the promoter region, exons, or splice donor or acceptor sites of introns – that might be expected to result in changes in the function or expression of the encoded protein (see Supplementary Table 1 online at www.thrombosis-online.com). 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 mM ethylenediaminetetraacetic acid (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 164 polymorphisms were determined (G&G

Table 2: Characteristics of male and female subjects.

	Men		Women	
	Myocardial infarction	Controls	Myocardial infarction	Controls
No. of subjects	926	987	266	1304
Age (years)	62.1 ± 10.5	62.8 ± 11.7	67.0 ± 10.0*	62.9 ± 11.9
Body mass index (kg/m^2)	23.8 ± 3.1*	23.2 ± 2.8	23.3 ± 3.4	23.4 ± 3.2
Current or former smoker (%)	27.2†	32.6	6.4‡	3.6
Hypertension (%)	71.0*	50.2	80.1*	39.9
Diabetes mellitus (%)	49.0*	23.8	47.7*	17.2
Hypercholesterolemia (%)	53.5*	24.7	68.8*	33.2

Data for age and body mass index are means ± SD. *P < 0.001, †P < 0.01, ‡P < 0.05 versus corresponding controls.

Table 3: Polymorphisms related ($P < 0.05$) to myocardial infarction as revealed by the chi^2-test.

Gene symbol	Polymorphism	*P*	FDR
MTHFR	677C→T (Ala222Val)	0.0003	0.049
LPL	1595C→G (Ser447Stop)	0.0005	0.041
IPF1	–108/3G→4G	0.0007	0.038
CETP	–629C→A	0.0045	0.185
GP1BA	1018C→T (Thr145Met)	0.0052	0.171
APOE	4070C→T (Arg158Cys)	0.0062	0.170
F7	11,496G→A (Arg353Gln)	0.0074	0.173
FABP2	2445G→A (Ala54Thr)	0.0075	0.154
TNF	–863C→A	0.0084	0.153
AGER	268G→A (Gly82Ser)	0.0084	0.138
TNF	–238G→A	0.0106	0.158
AKAP10	2073A→G (Ile646Val)	0.0112	0.153
ACDC	–11,377C→G	0.0197	0.249
PAI1	A→G (Tyr243Cys)	0.0341	0.400
TNFSF4	A→G	0.0379	0.414
APOC3	–482C→T	0.0389	0.399

FDR, false discovery rate.

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 2 (see online at www.thrombosis-online.com). Detailed methodology for genotyping was described previously (16).

Statistical analysis

Clinical data were compared between subjects with MI and controls by the unpaired Student's t-test. Qualitative data were compared by the chi^2-test. Allele frequencies were estimated by the gene counting method, and the chi^2-test was used to identify departures from Hardy-Weinberg equilibrium. In the initial screen, the genotype distribution of each autosomal polymorphism was compared between subjects with MI and controls by the chi^2-test (3×2); for polymorphisms on the X chromosome, allele frequencies were compared by the chi^2-test (2×2). The relation of polymorphisms to MI was also examined for men or women separately as well as for individuals aged ≤62 or ≥63 years separately (mean age of total population, 62.9 years). The false discovery rate (FDR) was calculated by the method of Benjamini and Hochberg (17). Calculation of the FDR is an approach to dealing with the problems associated with multiple comparisons and provides a measure of the expected proportion of false positives among data. The FDR threshold is determined from the observed *P* value distribution and is adaptive to the signal level in data. The FDR differs from a *P* value, and much higher FDRs than *P* values can be tolerated. In the present study, the chi^2-test was used as an initial screen, and multivariable logistic regression analysis and a stepwise forward selection procedure were subsequently applied in a more rigorous evaluation of association. The FDR was calculated at each step of the statistical analysis. In the initial screen (the chi^2-test), the FDR was calculated from the distribution of *P* values for the 164 polymorphisms. Polymorphisms with an FDR of <0.05 were further examined by multivariable logistic regression analysis with adjustment for covariates, with MI as a dependent variable and independent variables including age, sex (0 = woman, 1 = man), body mass index (BMI), smoking status (0 = nonsmoker, 1 = smoker), metabolic variables (0 = no history of hypertension, diabetes mellitus, or hypercholesterolemia; 1 = positive history), and genotype of each polymorphism. Metabolic variables were evaluated by measurement of parameters or on the basis of current treatment or clinical history. Each genotype was assessed according to dominant, recessive, and two additive (additive 1 and 2) genetic models, and the *P* value, odds ratio, and 95% con-

Table 4: Polymorphisms related ($P < 0.05$) to myocardial infarction in men or women as revealed by the chi^2-test.

Men				Women			
Gene	Polymorphism	*P*	FDR	Gene	Polymorphism	*P*	FDR
AGER	G→A (Gly82Ser)	0.0005	0.082	*TNF*	–863C→A	0.0023	0.377
LTA	804C→A (Thr26Asn)	0.0042	0.344	*PLAT*	–7351C→T	0.0040	0.328
GNB3	825C→T (splice variant)	0.0069	0.377	*IPF1*	–108/3G→4G	0.0082	0.448
GP1BA	1018C→T (Thr145Met)	0.0101	0.414	*UCP3*	–55C→T	0.0116	0.476
F12	46C→T	0.0111	0.364	*FABP2*	2445G→A (Ala54Thr)	0.0168	0.551
MTHFR	677C→T (Ala222Val)	0.0121	0.331	*CETP*	–629C→A	0.0190	0.519
ACDC	–11,377C→G	0.0148	0.347	*PAX4*	C→T (Arg121Trp)	0.0244	0.572
LPL	1595C→G (Ser447Stop)	0.0168	0.344	*ROS1*	G→A (Asp2213Asn)	0.0274	0.562
F3	–603A→G	0.0185	0.337	*ENG*	C→G (Asp366His)	0.0291	0.530
F7	11,496G→A (Arg353Gln)	0.0284	0.466	*MTHFR*	677C→T (Ala222Val)	0.0307	0.504
TNF	–238G→A	0.0364	0.543	*ITGA2*	1648A→G (Lys505Glu)	0.0319	0.476
TNF	–850C→T	0.0369	0.504	*ABCA1*	1051G→A (Arg219Lys)	0.0393	0.537
APOE	4070C→T (Arg158Cys)	0.0387	0.488				

FDR, false discovery rate.

Table 5: Multivariate logistic regression analysis of polymorphisms related to myocardial infarction.

Gene symbol	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P (FDR)	OR (95% CI)	*P* (FDR)	OR (95% CI)	*P* (FDR)	OR (95% CI)	*P* (FDR)	OR (95% CI)
MTHFR	677C→T (Ala222Val)	0.0515 (0.077)		0.0006 (0.002)	1.44 (1.17–1.78)	0.3892 (0.425)		0.0006 (0.002)	1.52 (1.20–1.92)
LPL	1595C→G (Ser447Stop)	0.0132 (0.026)	0.78 (0.64–0.95)	0.1206 (0.145)		0.0289 (0.050)	0.80 (0.65–0.98)	0.0937 (0.125)	
IPF1	–108/3G→4G	0.0004 (0.005)	0.71 (0.59–0.86)	0.4941 (0.494)		0.0004 (0.002)	0.70 (0.57–0.85)	0.0079 (0.019)	0.73 (0.58–0.92)

FDR, false discovery rate; OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia.

fidence 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. For combined genotype analysis, multivariable logistic regression analysis was performed with MI as a dependent variable and independent variables including age, sex, BMI, smoking status, hypertension, diabetes mellitus, hypercholesterolemia, and combined genotypes. Each genotype was assessed according to a dominant or recessive model based on statistical significance, and each combined genotype was compared with a combined genotype that confers the lowest genetic risk for MI. We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as of other covariates on MI. The levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. Given the multiple comparisons of genotypes with MI, we adopted the criterion of FDR < 0.05 for significant association at each step of the statistical analysis. For other clinical background data, we adopted the criterion of $P < 0.05$ for significance. Statistical significance was examined by two-sided tests performed with JMP version 5.1 software (SAS Institute, Cary, NC, USA).

Results

The characteristics of the 3,483 study subjects are shown in Table 1. Age, the frequency of men, BMI, and the prevalence of conventional risk factors for CHD, including smoking, hypertension, diabetes mellitus, and hypercholesterolemia, were all greater in subjects with MI than in controls. The characteristics of subjects with MI and controls separated into men or women are shown in Table 2. For men, BMI and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia were greater, whereas the prevalence of smoking was lower, in subjects with MI than in controls. For women, age and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia were greater in subjects with MI than in controls.

Evaluation of genotype distributions or allele frequencies by the chi^2-test revealed that 16 polymorphisms were related ($P < 0.05$) to the prevalence of MI (Table 3). Among these polymorphisms, the 677C→T (Ala222Val) polymorphism of the 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*), the 1595C→G (Ser447Stop) polymorphism of the lipoprotein lipase gene (*LPL*), and the –108/3G→4G polymorphism of the insulin promoter factor 1 gene (*IPF1*) were significantly (FDR < 0.05) associated with MI. We also examined the relation of polymorphisms to MI for men and women separately (Table 4). Although polymorphisms related to MI appeared to differ between men and women, no polymorphism was significantly associated with MI for men or for women based on the criterion of FDR < 0.05.

Table 6: Genotype distributions of polymorphisms associated with myocardial infarction.

Gene symbol	Polymorphism	Myocardial infarction	Controls
MTHFR	677C→T (Ala222Val)		
	CC	31.5	35.1
	CT	47.8	49.5
	TT	20.7	15.4
LPL	1595C→G (Ser447Stop)		
	CC	79.6	74.2
	CG	19.4	23.9
	GG	1.0	2.0
IPF1	–108/3G→4G		
	3G3G	27.0	21.3
	3G4G	47.1	51.7
	4G4G	25.9	27.0

Table 7: Effects of genotypes and other characteristics on the prevalence of myocardial infarction as determined by a stepwise forward selection procedure.

Variable	*P*	FDR	*R2*
Sex	<0.0001	<0.001	0.0887
Hypercholesterolemia	<0.0001	<0.001	0.0631
Diabetes mellitus	<0.0001	<0.001	0.0394
Hypertension	<0.0001	<0.001	0.0194
IPF1 (*4G4G* + *3G4G* vs. *3G3G*)	0.0004	<0.001	0.0028
MTHFR (*TT* versus *CC* + *CT*)	0.0006	0.001	0.0033
Age	0.0165	0.024	0.0013
LPL (*GG* + *CG* versus *CC*)	0.0217	0.027	0.0011

FDR, false discovery rate; R^2, contribution rate.

Table 8: Assessment of genetic risk for myocardial infarction with combined genotypes for three polymorphisms.

MTHFR (0 = *CC* = *CT*, 1 = *TT*)	*LPL* (0 = CC, 1 = CG = GG)	*IPF1* (0 = *3G3G*, 1 = *3G4G* = *4G4G*)	No. of subjects with MI/controls	OR (95% CI)	*P*	FDR
1	0	0	56/59	2.54 (1.57–4.11)	0.0001	<0.001
1	1	0	13/15	2.41	0.0611	0.107
0	0	0	208/316	1.73 (1.28–2.34)	0.0004	0.001
1	0	1	145/207	1.70 (1.23–2.35)	0.0015	0.004
1	1	1	33/71	1.34	0.2834	0.331
0	0	1	540/1117	1.21	0.1335	0.187
0	1	0	45/98	1.19	0.4598	0.460
0	1	1	152/408	1.00		

OR, odds ratio; CI, confidence interval; FDR, false discovery rate. Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia.

Polymorphisms that satisfied the condition $P < 0.01$ were evaluated by multivariable logistic regression analysis with adjustment for age, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia for men (see Supplementary Table 3 online at www.thrombosis-online.com) and for women (see Supplementary Table 4 online at www.thrombosis-online.com).

The three polymorphisms associated with MI in the entire study population were examined further by multivariable logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia. The 677C→T polymorphism of *MTHFR* (recessive and additive 2 models), the 1595C→G polymorphism of *LPL* (dominant model), and the –108/3G→4G polymorphism of *IPF1* (dominant and additive 1 and 2 models) were found to be significantly (FDR < 0.05) associated with the prevalence of MI (Table 5). The 677*T* allele of *MTHFR* represented a risk factor for MI, whereas the 1595*G* allele of *LPL* and the –108/*4G* allele of *IPF1* were protective against this condition. The genotype distributions of these polymorphisms both in control subjects and in patients with MI were in Hardy-Weinberg equilibrium (Table 6).

We next performed a stepwise forward selection procedure to examine the effects of genotypes for the 677C→T polymorphism of *MTHFR*, the 1595C→G polymorphism of *LPL*, and the –108/3G→4G polymorphism of *IPF1* as well as of age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia on MI (Table 7). Sex, hypercholesterolemia, diabetes mellitus, hypertension, *IPF1* genotype (dominant model), *MTHFR* genotype (recessive model), age, and *LPL* genotype (dominant model), in descending order of statistical significance, were significant and independent (FDR < 0.05) determinants of the prevalence of MI.

We performed multivariable logistic regression analysis of combined genotypes for assessment of genetic risk for MI. Combined genotype analysis of three polymorphisms (677C→T of *MTHFR*, 1595C→G of *LPL*, and –108/3G→4G of *IPF1*) revealed that the maximal odds ratio of 2.54 was obtained for the combined genotype of *TT* for *MTHFR*, *CC* for *LPL*, and *3G3G* for *IPF1*, whereas the lowest genetic risk was apparent with the combined genotype of *CC* or *CT* for *MTHFR*, *CG* or *GG* for *LPL*, and *3G4G* or *4G4G* for *IPF1* (Table 8).

Finally, we examined the effect of age on the association of the three polymorphisms with MI. Given that the mean age of the total population was 62.9 years, we divided subjects into those aged ≤62 years and those aged ≥63 years. The chi^2-test revealed that the 1595C→G polymorphism of *LPL* and the –108/3G→4G polymorphism of *IPF1*, but not the 677C→T polymorphism of *MTHFR*, were associated (FDR < 0.05) with MI in individuals aged ≤62 years, whereas the 677C→T polymorphism of *MTHFR* and the 1595C→G polymorphism of *LPL*, but not the –108/3G→4G polymorphism of *IPF1*, were associated with MI in individuals aged ≥63 years (Table 9).

Polymorphisms that satisfied FDR < 0.05 in the chi^2-test were further examined by multivariable logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia. Among individuals aged ≤62 years, the –108/3G→4G polymorphism of *IPF1* (dominant and additive 1 and 2 models) was significantly (FDR < 0.05) associated with MI, whereas the 1595C→G polymorphism of *LPL* was not (Table 10). Among individuals aged ≥63 years, the 677C→T polymorphism of *MTHFR* (recessive and additive 2 models) was significantly associated with MI, whereas the 1595C→G polymorphism of *LPL* was not.

Table 9: Relation of polymorphisms of *MTHFR*, *LPL*, and *IPF1* to myocardial infarction in individuals aged ≤62 or ≥63 years as revealed by the chi^2-test.

		≤62 years (*n* = 530/1106)*		≥63 years (*n* = 662/1185)*	
Gene	Polymorphism	*P*	FDR	*P*	FDR
MTHFR	677C→T (Ala222Val)	0.1418	0.170	0.0017	0.005
LPL	1595C→G (Ser447Stop)	0.0148	0.030	0.0245	0.037
IPF1	–108/3G→4G	0.0004	0.002	0.1775	0.178

FDR, false discovery rate. *Number of subjects with MI/controls.

Discussion

We have examined the relation of 164 polymorphisms in 137 candidate genes to MI. Our large-scale association study with 3,483 subjects revealed that the 677C→T polymorphism of *MTHFR*, the 1595C→G polymorphism of *LPL*, and the –108/3G→4G polymorphism of *IPF1* were significantly associated with the prevalence of MI in a Japanese population. Com-

Table 10: Multivariate logistic regression analysis of polymorphisms related to myocardial infarction in individuals aged ≤62 or ≥63 years.

Gene symbol	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P (FDR)	OR (95% CI)	*P* (FDR)	OR (95% CI)	*P* (FDR)	OR (95% CI)	*P* (FDR)	OR (95% CI)
≤62 years									
LPL	1595C→G (Ser447Stop)	0.0855 (0.195)		0.1125 (0.164)		0.1498 (0.200)		0.0991 (0.198)	
IPF1	−108/3G→4G	0.0002 (0.003)	0.58 (0.43–0.77)	0.2511 (0.309)		0.0005 (0.004)	0.57 (0.42–0.78)	0.0030 (0.010)	0.58 (0.41–0.83)
≥63 years									
MTHFR	677C→T (Ala222Val)	0.0995 (0.177)		0.0009 (0.005)	1.57 (1.20–2.06)	0.5795 (0.580)		0.0015 (0.006)	1.64 (1.21–2.22)
LPL	1595C→G (Ser447Stop)	0.0695 (0.185)		0.3349 (0.357)		0.1040 (0.166)		0.2838 (0.324)	

FDR, false discovery rate; OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index, and the prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia.

bined genotype analysis of these three polymorphisms yielded a maximal odds ratio of 2.54 for predisposition to MI. The association of these three polymorphisms with MI was affected by age: Among individuals aged ≤62 years or ≥63 years, the –108/3G→4G polymorphism of *IPF1* and the 677C→T polymorphism of *MTHFR*, respectively, were associated with MI. Although polymorphisms related to MI appeared to differ between men and women, no polymorphism was significantly associated with this condition in men or in women separately.

Homocysteine is a sulfur-containing amino acid that plays a pivotal role in methionine metabolism. 5,10-Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, a reaction that provides a substrate for the methylation of homocysteine to methionine catalyzed by methionine synthase. Individuals with the 677C→T (Ala222Val) substitution of *MTHFR* manifest reduced MTHFR activity and higher homocysteine levels compared with those without it (18–20). Association of the 677C→T polymorphism of *MTHFR* with CHD or MI has been described (21–24). Other studies, however, did not support such an association (20, 25, 26). These apparently contradictory results are attributable, at least in part, to differences in intake of folate and other B vitamins (27). A meta-analysis of the association of the 677C→T polymorphism of *MTHFR* with the risk of CHD in 11,162 cases and 12,758 controls from 40 studies revealed that individuals with the *TT* genotype had an odds ratio of 1.16 for CHD compared with those with the *CC* genotype (28). These observations suggest that impaired folate metabolism, resulting in high homocysteine concentrations, is an important determinant of CHD. Another meta-analysis of the association of the 677C→T polymorphism of *MTHFR* with CHD in 26,000 cases and 31,183 controls from 80 studies yielded an overall odds ratio of 1.14 for the *TT* genotype versus the *CC* genotype; odds ratios for Europe, Australia, and North America were about 1.0, whereas those for the Middle East and Asia were 2.61 and 1.23, respectively (29). These results indicate that the 677C→T polymorphism of *MTHFR* is associated with CHD in the Middle East and Asia, but not in Europe, North America, or Australia, with this geographic variability possibly reflecting higher folate intake in the latter regions (29). These previous observations support our present results showing that the 677C→T polymorphism of *MTHFR* was significantly associated with the prevalence of MI in Japanese, with the *TT* genotype being a risk factor for this condition.

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 (30). The hydrolytic function of LPL is important for the processing of triglyceride-rich chylomicrons and very low density lipoproteins to remnant particles as well as for the transfer of phospholipids and apolipoproteins to high density lipoproteins. LPL also plays an important role in the receptor-mediated removal of lipoproteins from the circulation (31). *LPL* is polymorphic, with amino acid substitutions affecting triglyceride and HDL-cholesterol levels, which are implicated in atherosclerosis risk (32). 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 (32). 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 (31–37). In addition, the *G* (Stop) allele of this polymorphism was found to be associated with a reduced risk of CHD or MI (32, 38). The 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 (31, 39–41). Our present results indicate that the 1595C→G (Ser447Stop) polymorphism of *LPL* is associated with the prevalence of MI, with the *G* (Stop) allele protecting against this condition, consistent with the previous observations (32, 38).

Insulin promoter factor 1 (IPF1) is a homeodomain-containing protein that is a key regulator of the insulin gene in pancreatic β cells (42, 43) and plays an important role in development of the pancreas (44, 45). IPF1-deficient mice thus selectively lack the pancreas at birth (44), and a patient with pancreatic agenesis and insulin-deficient diabetes was found to have a single nucleotide deletion in codon 63 of *IPF1* that caused a frameshift in the transactivation domain (45). A 3G→4G polymorphism of *IPF1* was identified 108 bp upstream of the translation start site in the

Japanese population but was found not to be related to the prevalence of type 2 diabetes mellitus (46, 47). Our results indicate that the 3G→4G polymorphism of *IPF1* was significantly associated with MI, with the *4G* allele protecting against this condition. This is the first demonstration of an association of this polymorphism in *IPF1* with MI, although the underlying molecular mechanism remains to be elucidated.

Given the multiple comparisons of genotypes with MI in the present study, we adopted the criterion of FDR < 0.05 for significant association in each step of the statistical analysis. It is not possible, however, to exclude completely potential statistical errors such as false positives. Validation of our findings will require their replication with independent subject panels. It is also possible that one or more of the polymorphisms associated with MI in our study are in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of this condition. Furthermore, the relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study.

In conclusion, our present results suggest that *MTHFR*, *LPL*, and *IPF1* are susceptibility loci for MI in the Japanese population. Determination of combined genotypes for these polymorphisms may prove informative for assessment of the genetic risk for MI and may contribute to the primary, personalized prevention of this condition.

Appendix

In addition to the authors, the following investigators participated in the study: Y. Matsuno and M. Tomita (Gifu Prefectural Gifu Hospital, Gifu); T. Kameyama and M. Oguri (Gifu Prefectural Tajimi Hospital, Tajimi); S. Tanihata (Gifu Prefectural Gero Hot Spring Hospital, Gero); M. Hiramoto (Yokohama General Hospital, Yokohama); and nursing and laboratory staff at the participating hospitals.

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