Association of six genetic variants with myocardial infarction

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Abstract. Although various genes that confer susceptibility to myocardial infarction (MI) have been identified for Caucasian populations in genome-wide association studies (GWAS), genetic variants related to this condition in Japanese individuals have not been identified definitively. The aim of the present study was to examine an association of MI in Japanese individuals with 29 polymorphisms identified as susceptibility loci for MI or coronary artery disease in Caucasian populations by meta-analyses of GWAS. The study subjects comprised 1,824 subjects with MI and 2,329 controls. Genotypes of the polymorphisms were determined by Luminex bead-based multiplex assay. To compensate for multiple comparisons, we adopted the criterion of a false discovery rate (FDR) of <0.05 for statistical significance for association. Comparisons of allele frequencies by the χ^2 test revealed that rs9369640 of the phosphatase and actin regulator 1 gene (*PHACTR1*, FDR=0.0007), rs4977574 of the CDKN2B antisense RNA 1 gene (*CDKN2B-AS1*, FDR=0.0038), rs264 of the lipoprotein lipase gene (*LPL*, FDR=0.0061), rs599839 of the proline/serine-rich coiled-coil 1 gene (*PSRC1*, FDR=0.0118), rs9319428 of the fms-related tyrosine kinase 1 gene (*FLT1*, FDR=0.0118) and rs12413409 of the cyclin and CBS domain divalent metal cation transport mediator 2 gene (*CNNM2*, FDR=0.0300) were significantly associated with MI. Multivariate logistic regression analysis with adjustment for covariates revealed that rs9369640 (P=0.0005; odds ratio, 0.89), rs4977574 (P=0.0001; odds ratio, 1.50), rs264 (P=0.0405; odds ratio, 0.85), rs599839 (P=0.0003; odds ratio, 0.68), rs9319428 (P=0.0155; odds ratio, 1.20) and rs12413409 (P=0.0076; odds ratio, 0.66) were significantly (P<0.05) associated with MI. *PHACTR1*, *CDKN2B-AS1*, *LPL*, *PSRC1*, *FLT1* and *CNNM2* may thus be susceptibility loci for MI in Japanese individuals.

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Introduction

Myocardial infarction (MI) is a life-threatening disease, the pathogenesis of which is complex and multifactorial. Epidemiological studies have identified several risk factors, including hypertension, diabetes mellitus, dyslipidemia, chronic kidney disease and smoking (1). In addition to these risk factors, recent studies have demonstrated the importance of genetic factors and interactions between multiple genes and environmental factors in the development of MI (2,3). In fact, twin and family studies have indicated that the heritability of coronary heart disease (CHD) is 40-50% (4).

Recent genome-wide association studies (GWAS) have become a powerful approach to identifying genetic variants that are related to common complex diseases (5,6). To date, meta-analyses of GWAS have identified 46 loci associated with CHD or MI at a genome-wide significance level ($P<5x10^{-8}$) (5,6), and an additional 104 genetic variants have been found to be associated with CHD with a false discovery rate (FDR) of <0.05 among individuals of European and south Asian ancestry (5). Although various loci and genes that confer susceptibility to MI or CHD have been identified for Caucasian populations by GWAS (5,6), genetic variants related to these conditions in Japanese individuals have not been identified definitively.

Therefore, the aim of this study was to examine a possible association of MI in Japanese individuals with 29 single nucleotide polymorphisms (SNPs) identified as susceptibility loci for MI or CHD in Caucasian populations by meta-analyses of GWAS.

Materials and methods

Study population. The study population comprised 4,153 Japanese individuals, including 1,824 subjects with MI (1,468 males and 356 females) and 2,329 controls (1,029 males and 1,300 females), who either visited outpatient clinics or

Table I. The 29 single nucleotide polymorphisms (SNPs) examined in the present study.

Chromosomal locus	Gene symbol	dbSNP (NCBI)	Nucleotide substitution	Minor allele[a]
15q24.2	*ADAMTS7*	rs3825807	C→T (Pro214Ser)	C
9p21	*CDKN2B*	rs3217992	G→A	G
9p21.3	*CDKN2B-AS1*	rs4977574	A→G	A
10q24.32	*CNNM2*	rs12413409	G→A	A
13q34	*COL4A1*	rs4773144	G→A	G
13q12	*FLT1*	rs9319428	G→A	A
15q26.1	*FURIN*	rs17514846	A→C	A
4q31.1-q31.2	*GUCY1A3*	rs7692387	G→A	A
14q32	*HHIPL1*	rs2895811	T→C	C
1q21	*IL6R*	rs4845625	C→T	C
6p21	*KCNK5*	rs10947789	T→C	C
6q26	*LPA*	rs3798220	T→C (Ile1891Met)	C
8p22	*LPL*	rs264	G→A	A
20p12.1	*MACROD2*	rs2208454	G→T	T
1q41	*MIA3*	rs17465637	C→A	A
3q22.3	*MRAS*	rs9818870	T→C	T
6p24.1	*PHACTR1*	rs9369640	A→C	C
1p13.3	*PSRC1*	rs599839	G→A	G
6q25.3	*SLC22A3*	rs2048327	A→G	G
5q31.1	*SLC22A4*	rs273909	T→C	C
19p13.2	*SMARCA4*	rs1122608	G→T	T
17p13.3	*SMG6*	rs216172	C→G	G
6q23.2	*TCF21*	rs12190287	C→G	G
19q13	*TOMM40*	rs2075650	G→A	G
17q21.32	*UBE2Z*	rs46522	C→T	C
2p11.2	*VAMP5*	rs1561198	A→G	A
2q33.2	*WDR12*	rs6725887	C→T	C
7q32.2	*ZC3HC1*	rs11556924	C→T (Arg363His)	T
11q23.3	*ZPR1*	rs964184	C→G	G

[a]The minor allele in Japanese individuals was determined by the allele frequency of HapMap-JPT (dbSNP, NCBI).

were admitted to the participating hospitals (Gifu Prefectural General Medical Center, Gifu; Gifu Prefectural Tajimi Hospital, Tajimi; Japanese Red Cross Nagoya First Hospital, Nagoya; Inabe General Hospital, Inabe; Hirosaki University Hospital and Hirosaki Stroke Center, Hirosaki, Japan) between 2002 and 2012, due to various symptoms or for an annual health checkup. The 1,824 subjects with MI all underwent coronary angiography and left ventriculography. The diagnosis of MI was based on typical electrocardiographic changes and on increases both in the serum activity of creatine kinase (MB isoform) and in the serum concentration of troponin T. The diagnosis was confirmed by the presence of stenosis in any of the major coronary arteries or in the left main trunk by coronary angiography. The 2,329 control individuals had no history of CHD or MI, aortic aneurysm or peripheral arterial occlusive disease, or of ischemic or hemorrhagic stroke or any other cerebrovascular diseases, or of any other atherosclerotic, thrombotic, embolic or hemorrhagic disorders. The study protocol complied with the Declaration of Helsinki and was approved by the ethics committees of each participating institution. Written informed consent was obtained from each participant.

Selection and genotyping of polymorphisms. We searched SNPs that were shown to be significantly associated with CHD or MI in Caucasian populations by meta-analyses of GWAS (5,6). We examined these SNPs using the SNP database (dbSNP; National Center for Biotechnology Information, Bethesda, MD, USA) to find SNPs with a minor allele frequency of ≥0.015 in a Japanese population. We finally selected 29 SNPs (Table I) and examined a possible association with MI. The wild-type (ancestral allele) and variant alleles of the SNPs were determined from the original sources.

Venous blood (7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), the peripheral blood leukocytes were isolated and genomic DNA was extracted from these cells using a DNA extraction kit (Genomix; Talent Srl, Trieste, Italy). Genotypes of the 29 SNPs were determined at G&G Science Co., Ltd. (Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes and other conditions for the genotyping of the SNPs examined in this study are shown in Table II. The overall call rate of the

Table II. Primers, probes and other PCR conditions for genotyping.

Gene	dbSNP	Sense primer	Antisense primer	Probe 1	Probe 2	Tmp (°C)	Cycle (times)
ADAMTS7	rs3825807	CTGTCCACTGGCAGCTAAC	CCGACCGCTGGTGTAGA	TCGAGACTCCAGCTCTGG	CTCCCGTCGAGGCTCCAG	60	50
CDKN2B	rs3217992	CAAACCTCAAACATTATTGGGT	GAAACAGCTAAACCTGTCTGCC	ACTATTTCAATACAACCAGG	ACTATTTTAATACAACCAGGT	60	50
CDKN2B-AS1	rs4977574	CTTGTAACCACATGTCAGTAGCAT	GGTGTTCCAAACAGGACATCTT	TGCATTCTATAGCACAGGATG	TGGAACATCCTGCGCTATAGA	60	50
CNNM2	rs12413409	CCCATATAACTACACGTGAAAAGTG	ATAGACCAGATACAGACTGCAGC	TACAAGCACAACACCATTTACT	GTAGAAGAGTAAATGATGTTGTG	60	50
COL4A1	rs4773144	GTTCCCAGCGTCAATCC	CACTTTCCTGGCCTCTACG	TTTTAAGTTCCCAGTTCCCGTGA	TTTTAAGTTCCCAGCTCCCGTG	60	50
FLT1	rs9319428	TGACTTGATTTTCCCTTGCC	GGACAGCATTTATCTGCATCTATG	GCAACCGAGAACAAGAAC	CAACCAAGAACAAGAACTG	60	50
FURIN	rs17514846	GTTGTTGGATAGAGGGAGGC	GATGGAGCCGCAGCTG	CAGGCGTCAGGCGCAA	GCAGGCTTCAGGCGCA	60	50
GUCY1A3	rs7692387	AGAAATCAAAATCACCTACTTAAGGC	TGGTCAGTTTACAAAGCAAGTA	AGGTCTTTCTTTTTCAAATGTCTC	TCTTTCTTTTTTAAATGTCTCT	60	50
HHIPL1	rs2895811	CACAGCTGCTGGAGATCAC	TGTCTCTCTGCTGACAACAAGC	AGGAGCAGAGTACATGGCTG	ACTCCCAGCCACGTACTCTG	60	50
IL6R	rs4845625	AGAAGTCTGTCCAAGGTGACAT	GATACACTGAAGCAGCTGTTGTC	ATGCAGGTACTACCATGACTG	AGCATTCCAGTCACGGTAGTA	60	50
KCNK5	rs10947789	TCTTTCAGGAGGGGACATTC	GGCTCAGCAGTTCACCC	GTGAAACCTGATTGAAGTAAGAG	CAGCTCACTCTTACCTCAATCA	60	50
LPA	rs3798220	CCAAGAAGTGAACCTCGAATCT	TGCTTAGCTTTAGCAAGGCA	ATGTTCAGGAAATAGAAGTGTC	GACACTTCCATTTCCTGAA	60	50
LPL	rs264	TTCCCTGTGAACTTGCAAC	AGAATCACCCAAGGATATAGTCC	AGGTGIGTGIGCCGCTACCAC	AGGTGIGTGIGCCACTACCAC	60	50
MACROD2	rs2208454	GGCCTGTAGCAATCTCAGTG	ACTCTCCAAAATAAACCAGCAAC	TGATTTTATGTTGGCCTAACT	TGATTTTATTTTGGCCTAACT	60	50
MIA3	rs17465637	TTGATGGAGACACAGAACCA	TGTATCAGCAGCAAAGACATGT	CACTATGACAAAAAATAACTTCTC	TGAGAAGTTCTTTTTTGTCATA	60	50
MRAS	rs9818870	TCAGATCTGTCTCTTGCTGC	GAGTTTTCTGGATGGTGTCCAC	AGTGTATTCGTATCAGAGAG	AGTGTATTCATATCAGAGAG	60	50
PHACTR1	rs9369640	TCAGGAAGTGAGGCAAACC	TGGTACCGGTGTGTAGATACTG	TCTGCTTCTAACAGTGATTTT	CAAAATCACTGTTATAAGCAGA	60	50
PSRC1	rs599839	CAGTTTGTGATAAGCTTACTCTATGAGTC	CCTGGGTGACAGAGCAAGAT	GTATATCTGGAAGTCGATCCT	TGGAAGTTGATCCTGCTC	60	50
SLC22A3	rs2048327	GCTCTCTAGGAGGTCATTGC	GCTAGATGCCTTTCTGAGCAG	TTTGTTGAGCATGAGCAGC	GCTGCTCACGCTCAACAA	60	50
SLC22A4	rs273909	ATGTAGATGCCCTATTGCCTC	GTAAGGAAGTATCTTTGGCTATGATG	TGCAACTCTCACTTCACCT	TGCAACTCCCACTTCACCT	60	50
SMARCA4	rs1122608	TGGGTGGTGCCATGTATAAC	CAGGTGTGGCTCCTGTCTC	GCGAGGCGIGGACTGGCCT	GTGTAAAAGGCCATTCCICA	60	50
SMG6	rs216172	GAATTCCAACTGCTGGGC	AGATCTTTCTCCCTGCAGTCC	TTCTGAGGCAAGGATCAGT	TTCTGAGGGAAGGATCAGT	60	50
TCF21	rs12190287	AGCAATAGTGACCTCATTACCAAG	CCTGACATCTTGAAAAGTCCTC	TGAAGTCACCGAAGTTCTC	ATGAACTCACCGAAGTTCTC	60	50
TOMM40	rs2075650	GTTCTGCTGTGGGTCTCTG	CTTGACACCCTCCATCTGA	GTCACACTCCACTCCAACC	GGGTTGGGGTGGAGTGT	60	50
UBE2Z	rs46522	GGTCACCCTATCCATTGTACTACC	CAGTCAAGCTTGCCCTATTC	TAACGGGATGGGGGAAGA	TAACGAGATGGGGGAAGAG	60	50
VAMP5	rs1561198	CAGAATTGGAAACAACCTGTTG	AATTTCAGAGCATGATTCTTCC	GGTTTGGGACCAGATTGGC	CTGACTTGCCAATCTAGTCCC	60	50
WDR12	rs6725887	TTGGCAGATGCTTTATAGAAAGTG	GGACCAACGTTGTTTCACTACTATAC	ATTTGGTTGAGACATAATATGCT	GTTGAGACACAATATGCTGTT	60	50
ZC3HC1	rs11556924	CCTGGTCCCATTGTCTCTC	GGTGTCTCCTGTTCCCATG	CTCAGGACGGTCAACAGG	GTCCTGTTGACCATCCTGAT	60	50
ZPR1	rs964184	ATCTCTTTCATGGAACTTGAAGTCTAG	CCTGCTTTACATTCCTCCATG	AACAGATCAGGAAAACAGTAC	AACAGATGAGGAAAACAGTAC	60	50

Tmp, annealing temperature.

Table III. Characteristics of the study subjects.

Characteristics	Myocardial infarction	Controls	P-value
Number of subjects	1,824	2,329	
Age	64.6±10.3	62.3±11.8	<0.0001
Gender (male/female, %)	80.5/19.5	44.2/55.8	<0.0001
Body mass index (kg/m^2)	24.0±3.4	23.7±3.2	0.0215
Current or former smoker (%)	36.2	17.5	<0.0001
Hypertension (%)	71.9	43.9	<0.0001
Systolic blood pressure (mmHg)	141±27	141±24	0.6801
Diastolic blood pressure (mmHg)	76±16	79±14	<0.0001
Diabetes mellitus (%)	49.0	20.0	<0.0001
Fasting plasma glucose (mmol/l)	7.56±3.37	6.29±3.34	<0.0001
Dyslipidemia (%)	57.8	29.7	<0.0001
Serum triglycerides (mmol/l)	1.64±1.12	1.46±1.07	<0.0001
Serum HDL-cholesterol (mmol/l)	1.19±0.36	1.47±0.41	<0.0001
Serum LDL-cholesterol (mmol/l)	3.17±0.95	3.03±0.85	0.0004
Serum creatinine (μmol/l)	95.5±104.4	83.1±97.3	<0.0001
eGFR (ml/min/1.73 m^2)	67.2±26.1	70.6±23.2	<0.0001

Quantitative data are shown as the means ± standard deviations. Hypertension was determined by a systolic blood pressure of ≥140 mmHg, a diastolic blood pressure of ≥90 mmHg, or the taking of any antihypertensive medications; diabetes mellitus was determined by fasting plasma glucose levels of ≥6.93 mmol/l or the taking of any antidiabetic medications; dyslipidemia was determined by a serum triglyceride concentration of ≥1.65 mmol/l, a serum high-density lipoprotein (HDL)-cholesterol concentration of <1.04 mmol/l, a serum low-density lipoprotein (LDL)-cholesterol concentration of ≥3.63 mmol/l, or the taking of any antidyslipidemic medications. eGFR, estimated glomerular filtration rate (ml min^{-1} 1.73 m^{-2}) = 194 x [age (years)]$^{-0.287}$ x [serum creatinine (mg/dl)]$^{-1.094}$ x [0.739 if female].

genotypes of the 29 SNPs was 99%. Detailed genotyping methodology was as previously described (7).

Statistical analysis. The χ^2 test was used to compare categorical variables. Quantitative data were compared between 2 groups using the Mann-Whitney U test or among 3 groups using the Kruskal-Wallis test, given that data were not normally distributed [P<0.01 by the Lilliefors (Kolmogorov-Smirnov) test]. The allele frequencies of each SNP were compared between the subjects with MI and the controls using the χ^2 test. To compensate for multiple comparisons of genotypes, a FDR was calculated from the distribution of P-values for the 29 SNPs, and a FDR of <0.05 was considered as statistically significant for association. The statistical power of each SNP was calculated with an Online Sample Size Estimator (http://osse.bii.a-star.edu.sg/calculation2.php). Multivariable logistic regression analysis was performed with MI as a dependent variable and independent variables, including age, gender (0, female; 1, male), body mass index (BMI), smoking status (0, non-smoker; 1, current or former smoker), the prevalence of hypertension, diabetes mellitus and dyslipidemia (0, no history of these conditions; 1, positive history) and the genotype of each SNP; the P-value, odds ratio and 95% confidence interval were calculated. A genotype was assessed according to a dominant (a combined group of heterozygotes and variant homozygotes vs. wild-type homozygotes), recessive (variant homozygotes vs. a combined group of wild-type homozygotes and heterozygotes) and additive model. Additive models included the additive 1 (heterozygotes vs. wild-type homozygotes) and additive 2 (variant homozygotes vs. wild-type homozygotes) models, which were analyzed simultaneously with a single statistical model. Statistical tests were performed using JMP version 11 and JMP Genomics version 6.0 software (SAS Institute Inc., Cary, NC, USA).

Results

Clinical characteristics of the study subjects. The clinical characteristics of the study subjects are presented in Table III. Age, the frequency of the male gender, BMI, the prevalence of smoking, hypertension, diabetes mellitus and dyslipidemia, the fasting plasma glucose levels, the serum concentrations of triglycerides, low-density lipoprotein (LDL)-cholesterol levels and creatinine levels were all higher, whereas diastolic blood pressure, the serum concentrations of high-density lipoprotein (HDL)-cholesterol and the estimated glomerular filtration rate were all lower in the subjects with MI compared to the controls.

Association of SNPs with MI. The allele frequencies of each SNP were compared between the subjects with MI and the controls using the χ^2 test, and the SNPs significantly (FDR <0.05) associated with MI are presented in Table IV. Of the 29 SNPs examined in the present study, rs12413409 of the cyclin and CBS domain divalent metal cation transport mediator 2 gene (*CNNM2*), rs9319428 of the fms-like tyrosine kinase-1 gene (*FLT1*), rs264 of the lipoprotein lipase gene (*LPL*), rs599839 of the proline/serine rich coiled coil 1 gene (*PSRC1*), rs4977574 of the CDKN2B antisense RNA 1 gene (*CDKN2B-AS1*) and rs9369640 of the phosphatase and actin regulator 1 gene (*PHACTR1*) were significantly associated with MI. The statistical power of each SNP was calculated with the number of samples and minor allele frequencies of

Table IV. The 6 single nucleotide polymorphisms (SNPs) significantly (allele frequency FDR of <0.05) associated with myocardial infarction (MI).

Gene	dbSNP	Genotype	MI[a]	Controls[a]	P-value (allele)	FDR (allele)	Statistical power (%)
CNNM2	rs12413409				0.0062	0.0300	49.0
		GG	1059 (58.1)	1244 (54.5)			
		GA	664 (36.4)	879 (38.5)			
		AA	99 (5.4)	161 (7.1)			
	MAF		0.2366	0.2629			
	Hardy-Weinberg P		0.7015	0.7363			
FLT1	rs9319428				0.0020	0.0118	58.5
		GG	660 (36.3)	926 (40.8)			
		GA	861 (47.3)	1021 (45.0)			
		AA	299 (16.4)	324 (14.3)			
	MAF		0.4008	0.3675			
	Hardy-Weinberg P		0.5195	0.1172			
LPL	rs264				$6.3x10^{-4}$	0.0061	67.8
		GG	1195 (65.7)	1376 (60.6)			
		GA	557 (30.6)	782 (34.5)			
		AA	68 (3.7)	111 (4.9)			
	MAF		0.1904	0.2212			
	Hardy-Weinberg P		0.7573	0.9938			
PSRC1	rs599839				0.0018	0.0118	60.6
		AA	1583 (87.0)	1875 (83.0)			
		AG	222 (12.2)	372 (16.5)			
		GG	15 (0.8)	13 (0.6)			
	MAF		0.0692	0.0881			
	Hardy-Weinberg P		0.0224	0.2361			
CDKN2BAS1	rs4977574				$2.6x10^{-4}$	0.0038	73.2
		AA	448 (24.6)	651 (28.5)			
		AG	898 (49.3)	1132 (49.6)			
		GG	476 (26.1)	501 (21.9)			
	MAF		0.5077	0.4672			
	Hardy-Weinberg P		0.5491	0.8311			
PHACTR1	rs9369640				$2.3x10^{-5}$	$6.6x10^{-4}$	85.5
		AA	1571 (86.2)	1856 (81.6)			
		AC	242 (13.3)	395 (17.4)			
		CC	9 (0.5)	25 (1.1)			
	MAF		0.0714	0.0978			
	Hardy-Weinberg P		0.9224	0.4401			

[a]Numbers in parentheses are percentages. FDR, false discovery rate; MAF, minor allele frequency.

the cases and the controls, and the significance level (α=0.05) was 49.0-85.5%. The genotype distributions of the 6 SNPs were in Hardy-Weinberg equilibrium (P>0.05) among the control individuals.

Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status and the prevalence of hypertension, diabetes mellitus and dyslipidemia revealed that rs12413409 of *CNNM2* (dominant, recessive and additive 1 and 2 models), rs264 of *LPL* (dominant model), rs599839 of *PSRC*1 (dominant and additive 1 models) and rs9369640 of *PHACTR1* (dominant, recessive and additive 1 and 2 models) were significantly associated with MI, with the minor *A*, *A*, *G* and *C* alleles, respectively, being protective against this condition (Table V). Similar analysis revealed that rs9319428 of *FLT1* (dominant and additive 2 models) and rs4977574 of *CDKN2B-AS1* (dominant, recessive and additive 2 models)

Table V. Multivariable logistic regression analysis of the 6 single nucleotide polymorphisms (SNPs) associated with myocardial infarction.

Gene	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)
CNNM2	rs12413409 (G→A)	**0.0089**	0.82 (0.71-0.95)	**0.0218**	0.70 (0.52-0.94)	**0.0418**	0.85 (0.73-0.99)	**0.0076**	0.66 (0.65-0.85)
FLT1	rs9319428 (G→A)	**0.0155**	1.20 (1.03-1.41)	0.0853		0.0508		**0.0186**	1.30 (1.04-1.62)
LPL	rs264 (G→A)	**0.0405**	0.85 (0.73-0.99)	0.1638		0.0836		0.1030	
PSRC1	rs599839 (A→G)	**0.0007**	0.70 (0.57-0.88)	0.3605		**0.0003**	0.68 (0.56-0.83)	0.4281	
CDKN2BAS1	rs4977574 (A→G)	**0.0063**	1.27 (1.06-1.49)	**0.0004**	1.37 (1.15-1.61)	0.1047		**0.0001**	1.50 (1.22-1.85)
PHACTR1	rs9369640 (A→C)	**0.0005**	0.89 (0.57-0.85)	**0.0132**	0.33 (0.13-0.80)	**0.0021**	0.73 (0.59-0.89)	**0.0096**	0.31 (0.12-0.76)

Multivariable logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status, and the prevalence of hypertension, diabetes mellitus and dyslipidemia. OR, odds ratio; CI, confidence interval. P-values <0.05 are shown in bold.

were also significantly associated with MI, with the *A* and *G* alleles, respectively, representing risk factors for this condition (Table V).

Association of the 6 SNPs to conventional risk factors for CHD. We examined the association of the 6 SNPs associated with MI to conventional risk factors for CHD among genotypes or between 2 groups (dominant or recessive model) (Table VI). Due to the multiple comparisons of genotypes, a P-value of <0.001 (0.05/48) was considered statistically significant. rs599839 of *PSRC1* was significantly associated with the serum concentrations of HDL-cholesterol with the minor *G* allele being related to increased serum HDL-cholesterol. This SNP was also associated (borderline significance) with the serum concentrations of LDL-cholesterol with the *G* allele being related to decreased serum LDL-cholesterol. The rs264 of *LPL* was associated (borderline significance) with the serum concentrations of HDL-cholesterol with the minor *A* allele being associated with increased serum HDL-cholesterol.

Discussion

In the present study, we examined the association of MI in Japanese individuals with 29 SNPs identified as susceptibility loci for CHD or MI in Caucasian populations by meta-analyses of GWAS and observed that 6 SNPs in the *CNNM2*, *FLT1*, *LPL*, *PSRC1*, *CDKN2B-AS1* and *PHACTR1* genes were significantly associated with MI. The association of the SNPs with MI remained significant following adjustment for covariates, suggesting that these genetic variants were independent risk factors for MI.

PHACTR1 is highly expressed in the heart and brain and regulates protein phosphatase 1, an ubiquitous enzyme regulating essential cellular processes (8). The suppression of PHACTR1 in cultured endothelial cells has been shown to result in attenuated tube formation and cell survival (9), as well as in the impaired synthesis of nitric oxide, an important mediator of cardiovascular function (10). In addition, protein phosphatase 1 is considered to be a critical regulator of Ca^{2+} cycling and contractility in smooth muscle cells, and thus, is closely related to arterial elasticity and wave reflection which is recognized as an important factor for cardiovascular disease (11). In the present study, rs9369640 of *PHACTR1* showed the most significant association with MI with the minor *C* allele being protective against this condition, although the role of PHACTR1 in the pathogenesis of MI remains unclear.

CDKN2B-AS1 is located within the CDKN2B-CDKN2A gene cluster at chromosome 9p21. The 9p21.3 locus was first discovered by GWAS to be a risk factor for CHD or MI (12-15). This locus was then shown to be associated with ischemic stroke (16,17) and with intracranial and aortic aneurysms (18). The 9p21.3 locus has also been associated with platelet reactivity and the increased platelet reactivity may explain the association with MI and ischemic stroke (19). The 9p21.3 locus includes *CDKN2B-AS1*, which encodes a functional RNA molecule that interacts with polycomb repressive complex-1 and -2, leading to epigenetic silencing of other genes in this cluster (NCBI Gene) and to the alteration of the expression of several genes related to cellular proliferation (20). Recent studies have also indicated that polymorphisms at 9p21 influence inflamma-

Table VI. Comparisons of coronary risk factors among genotypes or between 2 groups (dominant or recessive model) of the SNPs.

	Genotype			P-value		
	AA	*AB*	*BB*	Genotypes[a]	Dominant[b]	Recessive[b]
CNNM2 (rs12413409)						
Body mass index (kg/m^2)	23.8±3.3	23.9±3.3	23.7±3.0	0.6506	0.3652	0.9460
Systolic blood pressure (mmHg)	144±27	143±27	140±24	**0.0465**	**0.0219**	0.1243
Diastolic blood pressure (mmHg)	78±15	78±16	77±16	0.6507	0.3587	0.8981
Serum triglycerides (mmol/l)	1.60±1.14	1.60±1.15	1.45±0.87	0.0966	0.6625	**0.0323**
Serum HDL-cholesterol (mmol/l)	1.32±0.40	1.33±0.39	1.33±0.40	0.1015	0.0344	0.7764
Serum LDL-cholesterol (mmol/l)	3.11±0.92	3.13±0.92	3.22±0.91	0.1130	0.0977	0.0926
Fasting plasma glucose (mmol/l)	7.00±3.40	7.02±3.43	6.64±2.79	0.3987	0.4757	0.1913
Blood glycosylated hemoglobin (%)	6.86±1.78	6.86±1.85	6.61±1.41	0.3576	0.4400	0.1694
FLT1 (rs9319428)						
Body mass index (kg/m^2)	23.8±3.2	23.8±3.3	23.8±3.3	0.5774	0.3007	0.6131
Systolic blood pressure (mmHg)	144±26	143±27	143±28	0.1760	0.0694	0.3121
Diastolic blood pressure (mmHg)	78±15	77±15	77±16	**0.0132**	**0.0044**	0.0985
Serum triglycerides (mmol/l)	1.56±1.09	1.60±1.15	1.60±1.14	0.7222	0.4204	0.8155
Serum HDL-cholesterol (mmol/l)	1.33±0.41	1.32±0.40	1.30±0.38	0.3880	0.2471	0.2752
Serum LDL-cholesterol (mmol/l)	3.11±0.91	3.15±0.92	3.09±0.94	0.1223	0.4008	0.1402
Fasting plasma glucose (mmol/l)	6.98±3.53	7.06±3.53	6.81±2.97	0.5706	0.4960	0.5928
Blood glycosylated hemoglobin (%)	6.80±1.74	6.92±1.84	6.79±1.75	0.3054	0.3068	0.4747
LPL (rs264)						
Body mass index (kg/m^2)	23.8±3.2	23.8±3.2	24.0±3.8	0.8921	0.7600	0.6612
Systolic blood pressure (mmHg)	144±27	144±26	142±26	0.8924	0.9272	0.6714
Diastolic blood pressure (mmHg)	78±16	78±15	76±16	0.1158	0.7469	0.0601
Serum triglycerides (mmol/l)	1.61±1.14	1.56±1.13	1.46±0.95	**0.0166**	**0.0230**	**0.0209**
Serum HDL-cholesterol (mmol/l)	1.31±0.40	1.35±0.41	1.37±0.39	**0.0028**	**0.0012**	**0.0473**
Serum LDL-cholesterol (mmol/l)	3.12±0.91	3.13±0.94	3.17±0.90	0.8230	0.8355	0.6126
Fasting plasma glucose (mmol/l)	7.08±3.44	6.80±3.30	7.14±3.06	**0.0124**	**0.0179**	0.2940
Blood glycosylated hemoglobin (%)	6.88±1.79	6.75±1.74	7.19±2.09	**0.0365**	0.1717	0.0854
PSRC1 (rs599839)						
Body mass index (kg/m^2)	23.8±3.2	23.8±3.4	23.3±3.1	0.6409	0.3631	0.6698
Systolic blood pressure (mmHg)	143±26	144±28	147±24	0.6169	0.4197	0.7066
Diastolic blood pressure (mmHg)	78±15	78±16	76±11	0.8203	0.5310	0.8471
Serum triglycerides (mmol/l)	1.59±1.13	1.61±1.12	1.55±1.02	0.9641	0.7988	0.8838
Serum HDL-cholesterol (mmol/l)	1.31±0.40	1.38±0.42	1.43±0.48	**3.9x10^{-4}**	0.2138	**8.0x10^{-5}**
Serum LDL-cholesterol (mmol/l)	3.15±0.93	3.02±0.87	2.93±0.86	**0.0042**	0.3405	**0.0010**
Fasting plasma glucose (mmol/l)	6.98±3.37	7.11±3.48	6.22±2.54	0.3285	0.2202	0.5633
Blood glycosylated hemoglobin (%)	6.84±1.78	6.95±1.85	6.17±1.31	0.1458	0.0850	0.5744
CDKN2BAS1 (rs4977574)						
Body mass index (kg/m^2)	23.8±3.1	23.8±3.3	23.8± 3.3	0.4787	0.5823	0.4071
Systolic blood pressure (mmHg)	144±26	144± 27	142±27	0.3707	0.3150	0.2043
Diastolic blood pressure (mmHg)	78±16	78±16	77±15	**0.0387**	0.8099	**0.0135**
Serum triglycerides (mmol/l)	1.58±1.03	1.60±1.12	1.57±1.23	0.4110	0.5686	0.1844
Serum HDL-cholesterol (mmol/l)	1.34±0.42	1.32±0.39	1.32±0.39	0.7624	0.4879	0.6937
Serum LDL-cholesterol (mmol/l)	3.15±0.93	3.10±0.91	3.14±0.95	0.1639	0.1494	0.4950
Fasting plasma glucose (mmol/l)	7.07±3.57	6.92±3.28	7.05±3.35	0.4114	0.9365	0.2006
Blood glycosylated hemoglobin (%)	6.88±1.80	6.85±1.81	6.82±1.73	0.7465	0.6853	0.6347

Table VI. Continued.

	Genotype			P-value		
	AA	*AB*	*BB*	Genotypes[a]	Dominant[b]	Recessive[b]
PHACTR1 (rs9369640)						
Body mass index (kg/m^2)	23.8±3.3	23.9±3.3	23.6±2.5	0.4887	0.2882	0.7502
Systolic blood pressure (mmHg)	143±27	144±26	144±30	0.8251	0.6873	0.7002
Diastolic blood pressure (mmHg)	78±16	77±14	76±12	0.5141	0.2726	0.5745
Serum triglycerides (mmol/l)	1.59±1.11	1.53±0.98	2.14±3.21	0.3316	0.1590	0.8641
Serum HDL-cholesterol (mmol/l)	1.32±0.40	1.36±0.41	1.35±0.37	**0.0313**	**0.0085**	0.6184
Serum LDL-cholesterol (mmol/l)	3.14±0.92	3.07±0.92	3.07±0.77	0.1811	0.0654	0.8264
Fasting plasma glucose (mmol/l)	7.03±3.42	6.77±3.12	7.09±3.36	**0.0431**	**0.0156**	0.8902
Blood glycosylated hemoglobin (%)	6.86±1.80	6.80±1.75	6.92±1.24	0.3906	0.5751	0.2740

[a]Data for each parameter were compared among genotypes using the Kruskal-Wallis test or [b]were compared between 2 groups [dominant (*AB* + *BB* vs. *AA*) or recessive (*BB* vs. *AA* + *AB*) model] using the Mann-Whitney U test. *AA*, wild-type homozygote; *AB*, heterozygote; *BB*, variant homozygote. P-values <0.05 are shown in bold.

tory signaling (21) and vascular cell proliferation (22). In this study, we demonstrated that rs4977574 of *CDKN2B-AS1* was significantly associated with MI in Japanese individuals with the *G* allele representing a risk factor for this condition. These previous data and the data from the present study indicate that *CDKN2B-AS1* at 9p21.3 may be a susceptibility locus for MI, although the underlying molecular mechanisms have not been determined definitively.

The lipoprotein lipase encoded by *LPL* hydrolyzes triglycerides in circulating lipoprotein into 2 free fatty acids and 1 monoacylglycerol molecules. It also binds simultaneously to both lipoproteins and cell surface receptors and promotes the cellular uptake of lipoproteins and free fatty acids. Genetic variants causing decreased activity of LPL have been shown to result in disorders of lipoprotein metabolism, such as hypertriglyceridemia and hypo-HDL-cholesterolemia (23). In addition, genetic variants of *LPL* have been implicated in insulin resistance and type 2 diabetes mellitus (24). In the present study, rs264 of *LPL* was found to be associated with the serum concentrations of HDL-cholesterol with the *A* allele being related to increased HDL-cholesterol levels. We observed that rs264 was significantly associated with MI with the *A* allele being protective against MI. This association may be attributable, at least in part, to the effect of rs264 on the metabolism of HDL-cholesterol.

The rs599839 of *PSRC1* is located in the cadherin, EGF LAG seven-pass G-type receptor 2 (*CELSR2*)-*PSRC1*-sortilin 1 (*SORT1*) gene cluster on chromosome 1p13.3. The SORT1 protein, the higher expression of which is associated with the minor *G* allele of rs599839, is a multiligand transmembrane receptor protein that binds to a variety of ligands, including the LDL-receptor associated protein (25), lipoprotein lipase (26) and apolipoprotein A-V (27), and enhances the endocytosis and intracellular degradation of LDL-cholesterol. In the present study, the minor *G* allele of rs599839 was found to be associated with an increase in serum HDL-cholesterol levels and a decrease in serum LDL-cholesterol levels. We demonstrated that rs599839 of *PSRC1* was associated with MI in Japanese individuals with the *G* allele being protective against MI. The effects of rs599839 on the metabolism of HDL- and LDL-cholesterol may account for its association with MI.

rs12413409 is located in *CNNM2* at 10q24.32. *CNNM2* was identified as a gene that plays an important role in magnesium homeostasis by mediating the epithelial transport and renal reabsorption of Mg^{2+} (28,29). We observed the association of rs12413409 of *CNNM2* with MI in a Japanese population with the minor *A* allele being protective, although the role of *CNNM2* in the pathogenesis of CHD or MI remains unclear.

FLT1 encodes vascular endothelial growth factor (VEGF) receptor 1 in humans and plays an important role in the pathological mechanisms of atherosclerosis. In addition to VEGF, placental growth factor selectively binds to VEGF receptor 1 and accelerates atherosclerotic processes through the enhancement of intramural angiogenesis and monocyte recruitment (30-32). Recruited monocytes, differentiating into macrophages, produce pro-inflammatory cytokines, such as monocyte chemoattractant protein-1 which cause the augmentation of the inflammatory response and plaque vulnerability (33). In this study, we demonstrated that rs9319428 of *FLT1* was significantly associated with MI with the *A* allele representing a risk factor for MI. The effects of rs9319428 on the development of atherosclerosis may account for its association with MI.

In conclusion, the results from the present study suggest that rs12413409 of *CNNM2*, rs264 of *LPL*, rs9369640 of *PHACTR1*, rs9319428 of *FLT1*, rs599839 of *PSRC1* and rs4977574 of *CDKN2B-AS1* are susceptibility loci for MI in Japanese individuals. The determination of genotypes for these SNPs may prove informative for the assessment of the genetic risk for MI. Given that multiple variants, each having a small effect, will ultimately be found to be responsible for a large fraction of the genetic component of MI, further identification of MI susceptibility genes will allow for a more accurate assessment of the genetic component of this condition.

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