Validation of the Association of Genetic Variants on Chromosome 9p21 and 1q41 With Myocardial Infarction in a Japanese Population

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Background Recent large-scale genome-wide association studies have identified several loci associated with the risk of coronary artery disease (CAD). The aim of the present study was to examine whether the previously reported CAD-associated single-nucleotide polymorphisms (SNPs) confer susceptibility to myocardial infarction (MI) in a study population of 2,475 controls and 589 cases of MI. The effect of the CAD-associated SNPs on cardiovascular risk factors in the control group was also investigated.

Methods and Results Significant associations were observed between 2 SNPs, rs1333049 on chromosome 9p21 and rs17465637 on chromosome 1q41, and MI, with odds ratios adjusted for age, sex, diabetes, hypertension and smoking habit of 1.47 (95% confidence interval (CI), 1.15–1.89; corrected $p=0.006$) and 1.45 (95%CI, 1.15–1.83; corrected $p=0.006$) for rs1333049 and rs17465637, respectively. None of the genotypes was associated with body mass index, plasma lipid profile, blood pressure, glucose, or hemoglobin A_{1c}. The genotypes also had no effect on the marker of inflammation (C-reactive protein) or atherosclerosis (mean and maximum carotid intima-media thickness).

Conclusions Although the underlying mechanisms are not clearly understood, the previously reported association between the 2 SNPs (rs1333049 and rs17465637) and MI was reproduced in this Japanese sample. (*Circ J* 2008; 72: 1213–1217)

Key Words: Genetics; Myocardial infarction; Polymorphism

Based on recent large-scale genome-wide association studies, several loci associated with the risk of coronary artery disease (CAD) have been identified^{1–3} among which the chromosome 9p21 has been the most consistent locus showing a strong association with CAD. A genome-wide scan conducted in Canadians (322 cases, 312 controls) and subsequent validation studies involving more than 2,326 cases and 10,427 controls found the genetic variations in the chromosome 9p21 region (rs10757274 and rs2383206) to be associated with CAD². Similarly, the genome wide association analysis in an Icelandic population demonstrated the association between myocardial infarction (MI) and the single-nucleotide polymorphisms (SNPs) on the chromosome 9p21 locus (rs1333040, rs2383207, and rs10116277)¹. Combined analysis of the initial Icelandic and 4 replication groups from Iceland and the United States revealed rs10757278 on chromosome 9p21 as the most significant SNP, with the population attributable risk for MI being estimated as 21% in general¹. In the analysis of the Wellcome Trust Case Control Consortium (WTCCC) study³

genotyping was carried out in 1,926 CAD cases and 2,938 controls using the GeneChip Human Mapping 500K array (Affymetrix). Nine chromosomal regions were found to be significantly associated with CAD, 3 of which remained significant after testing for the association with MI in the German MI Family Study composed of 875 cases with MI and 1,644 controls⁴, with the chromosome 9p21.3 locus showing the strongest association in both studies. Combined analysis of the WTCCC study and the German MI Family Study revealed an additional 4 loci associated with the increased risk of CAD.

Despite the different SNPs investigated from study to study, associations between the SNPs on chromosome 9p21 and the risk of CAD or MI have been consistently reported in case-control studies from different countries including South Korea⁵, Italy^{6,7}, Germany, Sweden, and the United Kingdom⁶. The underlying mechanisms for the association between these susceptibility loci identified through genome-wide scanning and the risk of CAD/MI remain to be elucidated. There has been no evidence of an association between the risk alleles of SNPs on chromosome 9p and conventional risk factors such as lipid parameters or blood pressure levels^{2,6}. As stressed by the National Cancer Institute-National Human Genome Research Institute (NCI-NHGRI) Working Group on Replication in Association Studies⁸, validation of the postulated gene-disease associations in a population different from that of the initial study is of great value. Thus, the aim of the present study was to examine whether the previously reported CAD-associated SNPs, including those located on chromosome 9p21, confer

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Table 1 Clinical Characteristics of the Control and MI Groups

	Controls	MI cases	<i>p</i> value
<i>n</i> (M/F)	2,475 (1,102/1,373)	589 (510/79)	
Age (years)	65.1±11.0	61.3±10.3	<0.0001
BMI (kg/m ²)	22.8±3.1	23.7±3.0	<0.0001
% current smokers	16.3	59.9	<0.0001
% current drinkers	45.4	40.3	0.026
% subjects with hypertension	36.8	55.1	<0.0001
% subjects with diabetes	9.3	42.0	<0.0001
% subjects with hyperlipidemia	52.0	55.7	0.10

Age and BMI are presented as mean ± standard deviation; other variables are shown as percentage.

MI, myocardial infarction; BMI, body mass index.

susceptibility to MI in a Japanese population and to investigate the effect of these SNPs on cardiovascular risk factors.

Methods

Study Population

The inclusion criteria and study design of the Suita Study have been described previously^{9–15}. In brief, the sample consisted of 14,200 men and women (30–79 years of age at enrollment), stratified by gender and 10-year age groups (10 groups and 1,420 subjects in each group) who had been randomly selected from the municipal population registry. They were all invited, by letter, to attend regular cycles of follow-up examination (every 2 years). Subjects who were recruited into the Suita Study between April 2002 and February 2004 and were free from CAD served as controls (*n*=2,475). MI cases (*n*=589) were randomly selected in- and outpatients with documented MI who were enrolled in the Division of Cardiology at the National Cardiovascular Center between May 2001 and April 2003. Both controls and MI cases were of the same ethnicity (Japanese) and from the same geographical area. Only those who gave written informed consent were included in the study. The study protocol was approved by the Institutional Ethics Committee and the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center. Subjects with systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg and/or the current use of antihypertensive medication were categorized as having hypertension. Subjects who had total cholesterol (TC) ≥220 mg/dl or triglycerides (TG) ≥150 mg/dl, or were taking antihyperlipidemic medication, were classified as hyperlipidemic. The definition of type 2 diabetes were based on fasting blood glucose levels ≥126 mg/dl, hemoglobin (Hb) A_{1c} ≥6.5%, and/or current treatment for diabetes. Mean and maximum intima-media thicknesses (IMT) of the common carotid artery were determined by high-resolution ultrasonography⁹.

Genotyping Assays

Ten SNPs were selected based on the results of genome-wide association analyses: 7 SNPs (rs2943634, rs6922269, rs1333049, rs599839, rs17465637, rs501120 and rs17228212) from the WTCCC and German MI Family Study⁴; 2 chromosome 9p21 SNPs (rs10757274 and rs2383206) identified by McPherson et al² and rs10757278, the most significant SNP on chromosome 9p21 reported by Helgadottir et al¹. The sequences encompassing the selected SNPs were determined by an ABI 3730 Sequencer (Applied Biosystems, Foster City, CA, USA) in a subgroup of control subjects (*n*=192) for the assessment of minor allele frequency and the

degree of linkage disequilibrium (LD). TaqMan allelic discrimination assays were performed on a 7900 HT (Applied Biosystems) according to the manufacturer's instructions. Genotype frequency distributions for each SNP were tested for agreement with Hardy–Weinberg equilibrium by SNPalyze Ver.5.0.4 Pro (Dynacom, Chiba, Japan). The degree of LD among polymorphisms was evaluated using SNPalyze Ver.5.0.4 Pro (Dynacom).

Statistical Analysis

Data are expressed as mean ± standard deviation. Allele and genotype frequencies between control and MI subjects were compared by chi-square (χ^2) test. Odds ratio (OR) and 95% confidence interval (CI) for the risk allele were estimated by logistic regression analysis with adjustment for age, sex, hypertension, diabetes, and current drinking and smoking habits. Continuous variables were tested for normality of distribution, and logarithmic transformation was applied for those with skewed distributions. Residuals, defined as the observed value minus predicted value, on the basis of confounding factors, were used for the phenotype–genotype association analysis by 1-way analysis of variance tests. Covariates included in the model were derived from multiple logistic regression analysis and used to calculate a residual value for each variable. The level of significance was adjusted for multiple testing by Bonferroni correction. Statistical analysis was performed using the JMP statistical package 7.0 (SAS Institute, Cary, NC, USA).

Results

Clinical characteristics of the controls and MI cases included for genotyping are summarized in Table 1. The proportion of male subjects was higher in the MI group than in the control group. Subjects with MI were younger and had a higher prevalence of smoking habit, diabetes and hypertension compared with controls (*p*<0.0001).

Among the 10 SNPs selected for the analysis, 4 that had minor allele frequency of less than 0.15 (rs2943634, rs6922269, rs599839 and rs17228212) were excluded. The 4 SNPs on chromosome 9p21 were highly correlated with each other. For simplicity, we present our results with a particular focus on rs1333049, which was in strong LD with rs10757278 (*r*²=0.98), rs2383206 (*r*²=0.87) and rs10757274 (*r*²=0.83).

Table 2 shows the genotype and risk allele frequencies for the 3 SNPs genotyped in 2,475 controls and 589 MI subjects. OR for MI with the risk allele at rs1333049, rs17465637 and rs501120 estimated by logistic regression analyses with adjustment for age, sex, diabetes, hypertension and smoking habit were 1.47 (95% CI, 1.15–1.89;

Table 2 Logistic Regression Analysis of MI

SNPs	Chr	Risk allele	Risk allele frequency		Genotype frequency						HWE*		OR for risk allele [†] (95% CI)	p value	p value [‡]
			Control (n=2,475)	MI cases (n=589)	Control			MI cases			Control	MI cases			
					OO	OX	XX	OO	OX	XX					
rs1333049	9	C	0.491	0.528	636	1,204	592	137	279	170	0.67	0.32	1.47 (1.15–1.89)	0.002	0.006
rs17465637	1	C	0.538	0.579	526	1,196	711	112	263	204	0.61	0.12	1.45 (1.15–1.83)	0.002	0.006
rs501120	10	T	0.640	0.668	287	1,180	967	62	262	258	0.12	0.78	1.22 (0.98–1.53)	0.079	0.237

Genotype frequencies are given for OO, OX and XX of the risk allele.

*Deviation from HWE was assessed by chi-square test, and p values for controls and MI cases are shown.

[†]All 3 SNPs were included in the logistic regression analyses, with adjustment for age, sex, diabetes, hypertension and smokers. BMI and the presence of hyperlipidemia were not significant predictors for MI and not included in the model.

[‡]Bonferroni correction for multiple testing was applied by multiplying the p value by 3 (the number of SNPs studied for association with MI).

SNP, single nucleotide polymorphism; Chr, chromosome; OO, non-carriers; OX, heterozygous carriers; XX, homozygous carriers; HWE, Hardy-Weinberg Equilibrium; OR, odds ratio; CI, confidence interval. Other abbreviations see in Table 1.

Table 3 Association Analyses of rs1333049 and rs17465637 With Anthropometric and Metabolic Parameters in 2,475 Subjects

n (M/F)	rs1333049			rs17465637			Covariates
	GG	636	(292/344)	CC	711	(317/394)	
	GC	1,204	(553/651)	CA	1,196	(529/667)	
	CC	592	(244/348)	AA	526	(241/285)	
	Genotype	CC v GC + GG	CC + GC v GG	Genotype	CC v CA + AA	CC + CA v AA	
Age (year)	0.81	0.56	0.96	0.81	0.88	0.52	
Res. BMI (kg/m ²)	0.35	0.2	0.28	0.1	0.11	0.41	Sex
Res. mean IMT (mm)	0.29	0.52	0.12	0.77	0.82	0.57	Age, sex, BMI, SBP
Res. maximum IMT (mm)	0.78	0.73	0.65	0.19	0.07	0.52	Age, sex
Res. hs-CRP (mg/dl) ^a	0.79	0.49	0.85	0.07	0.05	0.63	Age, BMI
Res. TC (mg/dl)*	0.17	0.07	0.3	0.58	0.84	0.3	Sex, WHR
Res. TG (mg/dl) ^{a,*}	0.90	0.98	0.65	0.56	0.29	0.59	Sex, BMI, WHR, cigarette consumption
Res. HDL-C (mg/dl)*	0.63	0.36	0.97	0.62	0.93	0.37	Sex, BMI, WHR, cigarette and alcohol consumption
Res. SBP (mmHg)**	0.39	0.19	0.95	0.91	0.99	0.68	Age, BMI, cigarette and alcohol consumption
Res. DBP (mmHg)**	0.37	0.19	0.93	0.31	0.25	0.59	Age ² , BMI, alcohol consumption
Res. glucose (mg/dl)***	0.85	0.93	0.62	0.5	0.8	0.31	Sex, BMI, WHR
Res. HbA _{1c} (%)***	0.92	0.83	0.8	0.83	0.59	0.64	Age, BMI, cigarette consumption

p values by analysis of variance test.

^aLog-transformed values were used for the analysis. *Subjects taking anti-hyperlipidemic medication (n=364) were excluded. **Subjects who were on medication for hypertension (n=609) were excluded. ***Subjects being treated for diabetes (n=113) were excluded.

Covariates included for the calculation of residual values are shown. Alcohol consumption was expressed as ethanol (g) per day. Cigarette consumption was defined as the product of the number of years of smoking and the number of cigarettes consumed per day.

Res, residual; IMT, intima-media thickness; SBP, systolic blood pressure; hs-CRP, high-sensitivity C-reactive protein; TC, total cholesterol; TG, triglycerides; WHR, waist to hip ratio; HDL-C, high-density lipoprotein-cholesterol; DBP, diastolic blood pressure; HbA_{1c}, hemoglobin A_{1c}. Other abbreviation see in Table 1.

nominal p=0.002; corrected p=0.006), 1.45 (95% CI, 1.15–1.83; nominal p=0.002; corrected p=0.006) and 1.22 (95% CI, 0.98–1.53; nominal p=0.079; corrected p=0.237), respectively. The association of rs1333049 and rs17465637 with MI remained significant after adjustment for multiple testing. Inclusion of the 2 positive SNPs (rs1333049 and rs17465637) instead of all 3 SNPs in the logistic regression analysis did not significantly alter the results; ORs adjusted for age, sex, diabetes, hypertension and smoking habit were 1.46 for rs1333049 (95% CI, 1.14–1.87; nominal p=0.0027; corrected p=0.005) and 1.44 for rs17465637 (95% CI, 1.14–1.82; nominal p=0.0024; corrected p=0.005), respectively. The interaction terms (rs1333049*rs17465637, rs1333049*age, rs17465637*age, rs1333049*sex, rs17465637*sex, rs1333049*BMI, rs17465637*BMI) did not reveal any significant association with MI after taking diabetes, hypertension and smoking habit into consideration.

Table 3 shows the association analysis of the 2 positive SNPs (rs1333049 and rs17465637) with anthropometric and metabolic parameters in the 2,475 controls. Confounding factors included in the model to calculate a residual value

for each variable are detailed in Table 3. Irrespective of whether the comparison was made between the genotype groups under the additive genetic model, between homozygous carriers of the risk allele and the combined group of non-carriers and heterozygotes or between those with at least one risk allele and those without it, genetic variants at rs1333049 and rs17465637 were not associated with differences in age, body mass index (BMI), mean and maximal IMT, high-sensitivity C-reactive protein (hs-CRP), TC, TG, high-density lipoprotein-cholesterol (HDL-C), SBP, DBP, fasting glucose, or HbA_{1c} in this Japanese population.

Discussion

The association of the 2 SNPs (rs1333049 and rs17465637) with MI was reproduced in this Japanese sample, yielding an adjusted OR of 1.47 (95% CI, 1.15–1.89) and 1.45 (95% CI, 1.15–1.83) for rs1333049 and rs17465637, respectively. The risk allele frequencies for rs1333049 observed in our study sample (0.53 among cases and 0.49 among controls) were similar to those reported previously in the WTCCC

study (0.55 among cases and 0.47 among controls) and the German MI Family Study (0.54 among cases and 0.48 among controls). During the preparation of this article, a report investigating the association between rs1333049 and CAD in a Japanese population was published and replicated the association.¹⁶ The risk allele frequency reported in that study was similar to ours: 0.49 for controls (n=1,151) and 0.55 for patients with CAD (n=604). Our results, taken together with data from that study, confirm that the previously reported CAD-associated SNP on chromosome 9p21 (rs1333049) confers susceptibility to MI in Japanese.

The risk allele frequency of SNP rs17465637 was slightly lower in our study sample (0.54 among controls and 0.58 among cases) than in populations of European ancestry; 0.71–0.74 in controls and 0.75–0.76 in CAD/MI cases.⁴ This observation was comparable to the difference found in the C allele frequency between the HapMap Japanese (JPT, 0.556) and CEU (CEPH European ancestry, 0.750) samples.¹⁷

SNP rs501120 at the 10q11 locus (nearby gene, *LOC728347*) did not show any association with MI in this Japanese sample. The risk allele frequency in the controls (0.640) was almost identical to that from the HapMap JPT data (0.636), but lower than that reported in controls of white European origin (0.84–0.87).⁴

We attempted to investigate the effect of the 2 positive SNPs (rs1333049 and rs17465637) on anthropometric and metabolic parameters in Japanese subjects without MI. None of the genotypes of rs1333049 and rs17465637 was associated with conventional risk factors such as BMI, plasma lipid profile, blood pressures, glucose, or HbA_{1c}. Homozygosity for the risk allele of rs1333049 and rs17465637 also had no effect on circulating levels of hs-CRP, an inflammatory marker that has been shown to be associated with cardiovascular events.^{18–20} Carotid IMT has been used as a non-invasive measure of atherosclerosis.^{21–23} In our present analysis, IMT of the common carotid artery assessed by high-resolution ultrasonography was not associated with the risk allele of rs1333049 or rs17465637.

The lack of an association between the genotypes of rs1333049 and the anthropometric and metabolic parameters observed in our study is in accordance with previous studies showing no effect of the chromosome 9p21 susceptibility locus on cardiovascular risk factors.^{2,6} Results from the Atherosclerosis Risk in Communities Study (ARIC) and Copenhagen City Heart Study (CCHS) demonstrated no significant associations between the genotypes of CAD-associated SNPs (rs10757274 and rs2383206) and the risk factors including BMI, glucose, TC, TG, low-density lipoprotein-cholesterol, HDL-C, SBP, DBP and hs-CRP.² Although the chromosome 9p21 region has emerged as a susceptibility locus for CAD, showing consistent associations across different populations, the increased risk associated with genetic variability at this locus appears not to be accounted for by the established risk factors. In the aforementioned replication study conducted in Japanese,¹⁶ there was no association between the severity of coronary atherosclerosis and the prevalence of the risk allele of rs1333049.

It remains to be determined whether nearby genes, such as *CDKN2A*, *CDKN2B* or *MTAP*, are responsible for the pathogenesis of CAD or if unknown mechanisms are involved. Although a large anti-sense non-coding RNA has been suggested as a candidate susceptibility gene for this locus,⁶ only limited data are available to date.

The association between rs17465637 and MI was reproduced in the present Japanese sample. Although many

studies have focused on the CAD-associated SNPs on chromosome 9p21, an intronic SNP of rs17465637 in the melanoma inhibitory activity family, member 3 (*MIA3*) gene on chromosome 1q41 has received little attention. *MIA3*, also referred to as *ARNT* or *TANGO*, belongs to the melanoma inhibitory activity gene family and may have a role in tumor suppression.^{24,25} As with the case of rs1333049, the classic risk factors appear not to be related to the increased MI risks associated with rs17465637.

In the present study, significant differences in age, sex, BMI, smoking habit, hypertension and diabetes were noted between the MI and control groups, which often occurs in case–control studies. We attempted to minimize the potential effects of background differences with the multiple logistic regression analysis in which several confounding factors were included. However, there is the possibility that an inability to adjust for unknown or undetermined confounders could lead to a spurious or false-negative association. Moreover, in a cross-sectional case–control study design, only survivors from MI would be included in the case group. Thus, a large-scale prospective cohort study will be necessary to confirm that the genetic variants on chromosome 9p21 and/or 1q41 predispose to susceptibility to MI. However, with the accumulating results from many validation studies with positive associations, it is highly likely that the observed associations in this Japanese sample are valid.

Given the lack of associations between the CAD-associated SNPs and classic risk factors, further investigations focusing on the underlying mechanisms will provide new insight into the pathophysiology of cardiovascular disease, leading to improved prevention and treatment strategies.

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