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ORIGINAL ARTICLE

SNPs on chromosome 5p15.3 associated with myocardial infarction in Japanese population

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Myocardial infarction (MI) occurs as the result of complex interactions of multiple genetic and environmental factors. By conducting a genome wide association study in a Japanese population using 210 785 single nucleotide polymorphism (SNP) markers, we identified a novel susceptible locus for MI on chromosome 5p15.3. An SNP (rs11748327) in this locus showed significant association in several independent cohorts (combined $P = 5.3 \times 10^{-13}$, odds ratio=0.80, comparison of allele frequency). Association study using tag SNPs in the same linkage disequilibrium block revealed that two additional SNPs (rs490556 and rs521660) conferred risk of MI. These findings indicate that the SNPs on chromosome 5p15.3 are novel protective genetic factors against MI.

INTRODUCTION

Myocardial infarction (MI) leads to principal cause of death in developed countries. MI is characterized by the rapid development of coronary thrombosis following atherosclerotic plaque instability,[1] which leads to necrosis of myocardium and might result in sudden death. Despite the change in lifestyle and recent development of biomarkers, pharmacological intervention and percutaneous coronary intervention using drug eluting stents, the mortality is still high.

We started genome wide association studies (GWAS) of this disorder using nearly 90 000 gene-based single nucleotide polymorphisms (SNPs) (http://snp.ims.u-tokyo.ac.jp/)[2] by high-throughput multiplex-PCR invader assay system,[3] and identified several genes conferring risk of MI including *LTA*.[4–6] Although the roles of these susceptible genes in MI pathogenesis are under investigation, these findings showed the potent power of GWAS, which is hypothesis free, to identify unexpected anchors to further understand the disease. Through examining the LTA cascade by combination of biological and genetic analyses, we have identified additional MI susceptible genes.[7–9]

Genetic variants that confer susceptibility to MI have been indicated to be present on several chromosomal loci.[10–17] These studies, however, were conducted in individuals from European decent. Therefore, we carried out a systematic GWAS using 210 785 SNPs for MI in Japanese population. We report here identification of SNPs on chromosome 5p15.3 as a novel protective genetic factor against MI. We also examined Taiwanese population to see its universality in another population.

MATERIALS AND METHODS

DNA samples

For the genome wide association study and subsequent second-stage screening, MI case and control subjects (mixed cases with other diseases including asthma, breast cancer, lung cancer, hyperthyroidism, osteoporosis, chronic obstructive pulmonary disease, pollinosis and atopic dermatitis) were obtained from the BioBank Japan project (http://biobankjp.org/). The characteristics of the third, fourth cohorts and the diagnosis of define MI has been described previously.[8,9] For Taiwanese population, subjects were recruited from the Kaohsiung Medical University Hospital, Taiwan.[9] All Taiwanese subjects are of Chinese decent. All

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study subjects provided written informed consent to participation in this study, or if they were under 20 years old, their parents gave consent. Characteristics of the study subjects were summarized in Supplementary Table 1. The protocol was approved by the Ethical Committee at the Center for Genomic Medicine, The institute of physical and chemical research (RIKEN), Yokohama and of each participating institution and by the Internal Review Board of the Kaohsiung Medical University Hospital, Kaohsiung.

SNP genotyping

The genotyping methods for GWAS and second-stage screening were described previously.[18] For third- and fourth-stage screening, we used multiplex-PCR invader assay described previously.[3] In the Taiwanese population, the SNPs were genotyped using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Haplotype block and haplotype frequency were estimated using Haploview v4.0.[19] We used this software to select tag SNPs with a pairwise tagging mode and applied a permutation test for haplotype analysis. We also applied haplotype analysis using the program THESIAS[20] and conditional log-likelihood with Akaike information criterion (AIC): AIC=$-2\times$(the maximized value of the conditional log-likelihood)+$2\times$(the number of parameters). As the number of parameters, we used the number of alleles/haplotypes with frequencies >0.01 that were used for each model. In the logistic regression analysis of an SNP, we first applied a one degree of freedom (1 d.f.) likelihood ratio test to determine whether a 1-d.f. multiplicative allelic effects model of a 2-d.f. full genotype model was more appropriate.[20] As we found no significant difference from the full genotype model ($P>0.05$), we assumed a multiplicative allelic effects mode. Next, we performed a forward logistic regression analysis, where we started by assessing whether the most significant SNP was sufficient to model the association among the SNP set. For this, we used a 1-d.f. likelihood ratio test for adding each of the remaining SNPs to the model by assuming multiplicative allelic effects for the additional SNPs. Relationship between patients' clinical profile and genotype information were assessed by one-way analysis of variance and χ^2-test.

Northern blot analysis

Human multiple-tissue northern blots I, II (Clontech, Palo Alto, CA, USA) or First choice northern blot (Ambion, Austin, TX, USA) 1, 2 were pre-hybridized and hybridized with α-[^{32}P]-dCTP-labeled genomic fragments prepared by PCR using 106 primer pairs as probes (Primer pairs are listed in Supplementary Table 2). Washed membranes were exposed to bioimaging plate for 4–6 h. We detected signal with bioimaging analyzer (FLA7000, FUJIFILM, Tokyo, Japan) according to the manufacturer's instructions.

RESULTS

Genome wide association analysis

We performed staged GWAS that include three screening stages as shown in Figure 1. To avoid false-negative results, we set a very loose threshold in the first-stage screening. We first genotyped 268 068 SNPs with 194 MI cases and 1539 controls (first set of each MI and control) enrolled in BioBank Japan. We successfully obtained genotype information at 210 785 SNP loci. The genomic inflation factor (λ) was 1.03 on the basis of the P-values from the Cochran-Armitage trend test, indicating there is no population stratification. We then selected 8740 SNPs showing P-values <0.02 for the second-stage screening, and genotyped the second set of 1394 MI patients and 1425 control individuals (Supplementary Table 3). Distribution of P-values for these SNPs were summarized in Supplementary Table 4. We also assessed population stratification in second-stage samples by comparing with HapMap samples using principal component analyses,[21] and found that these samples did not show any sign of population stratification (Supplementary Figure 1). After the second-stage screening, we identified two SNPs showing statistical significance after
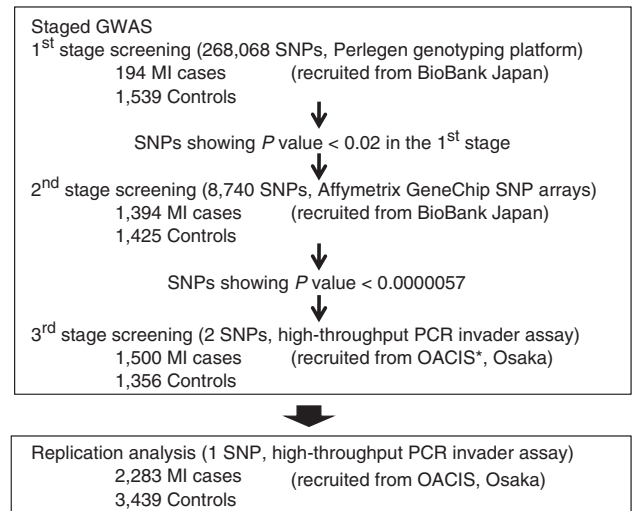

Figure 1 Study design for the GWAS. *Osaka Acute Coronary Insufficiency Study group.

Bonferroni's correction (cutoff P-value<0.0000057). One SNP (rs3782886) was located within *BRAP* on chromosome 12q24 (Supplementary Table 3), previously reported to be associated with susceptibility to MI in two Asian populations.[9] The remaining SNP (rs11748327) on chromosome 5p15.3 showed P-value of 1.8×10^{-6} in second-stage screening, and was verified by genotyping the third-stage samples (1500 cases and 1356 controls). Subsequent joint analyses for the associations of three panels showed the P-value with genome wide significance (combined $P=1.4\times10^{-9}$, odds ratio=0.77; Table 1). This association was further verified by replication panels with 2283 cases and 3439 controls (Table 1). Combined analysis of the four panels using Mantel–Haenszel test showed strong association of the SNP and MI, with a χ^2 value of 56.0 ($P=5.3\times10^{-13}$; comparison of allele frequency) and odds ratio was 0.80 (95% confidence interval: 0.75–0.85; Table 1).

Linkage disequilibrium and haplotype analysis

The marker SNP (rs11748327) was located within $\sim$250 kb linkage disequilibrium (LD) block constructed on the basis of HapMap JPT data (http://www.hapmap.org)[22] using Haploview software[19] (Figure 2). To examine whether other genetic variation(s) in this block is associated with MI, we selected 15 tag SNPs in addition to rs11748327 from SNPs to have minor allele frequency $>5\%$ with pairwise tagging and r^2 threshold of 0.8. We compared allelic frequency of these SNPs in the third panel of each MI and control and found that two additional SNPs (rs490556 and rs521660) were significantly associated with MI after Bonfferoni's correction (Table 2).

The two SNPs, rs490556 and rs521660, were in LD to the marker SNP rs11748327 with r^2 of 0.59 and 0.79, respectively (Table 2). Then, we further genotyped the replication panel of 2283 cases and 3439 controls for the two SNPs and found again significant association between these SNPs and MI (Table 3). Two of the haplotypes based on these SNPs showed significant association with MI (Table 4). Therefore, we further examined the effect of these haplotypes by THESIAS[20] and observed a significant effect on disease susceptibility between the most and second-most frequent haplotypes that could be distinguished by rs490556. Considering the conditional log-likelihoods with AIC, indicating that rs490556 revealed smaller AIC value than the haplotype model, we assumed that rs490556 alone rather than the

Table 1 Association of rs11748327 SNP with MI

		MI							Controls							Comparison of allele frequency			
Stage	Panel	11	%	12	%	22	%	SUM	11	%	12	%	22	%	SUM	χ^2	P-value	OR	95% CI
Staged GWAS	1st	130	67.0	52	26.8	12	6.2	194	866	56.3	575	37.4	97	6.3	1538	5.5	1.9×10^{-2}	0.73	0.56–0.95
	2nd	896	64.4	436	31.3	60	4.3	1392	758	55.3	531	38.7	82	6.0	1371	22.8	1.8×10^{-6}	0.74	0.83–0.65
	3rd	938	62.8	480	32.1	76	5.1	1494	770	57.1	490	36.4	88	6.5	1348	10.2	1.4×10^{-3}	0.82	0.72–0.93
	Combined															40.7	1.4×10^{-9}*	0.77	0.71–0.84
Validation	Replication	1437	62.9	740	32.4	106	4.6	2283	1964	57.5	1254	36.7	197	5.8	3415	16.7	4.4×10^{-5}	0.83	0.76–0.91
	Combined															56.0	5.3×10^{-13}*	0.80	0.75–0.85

Abbreviations: CI, confidence interval; GWAS, genome wide association studies; MI, myocardial infarction; OR, odds ratio; SNP, single nucleotide polymorphism.
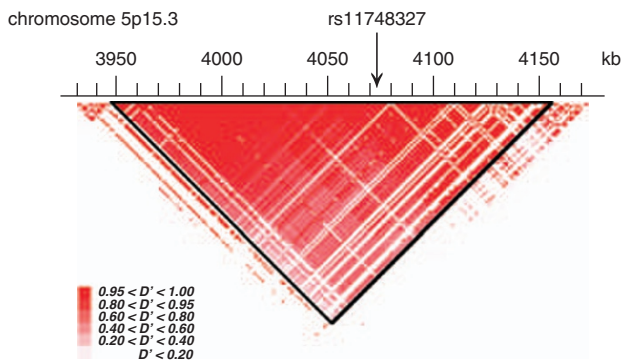*P-value was calculated by Mantel–Haenszel test.

Figure 2 LD (D') block containing the marker SNP (rs11748327) on chromosome 5p15.3.

haplotypes well explained an association with MI. We also applied a logistic regression analysis to search for combinatorial effects of other SNPs to rs490556, but failed to find them. These results indicated that rs490556 itself or other SNPs with high LD to rs490556 are genetically associated with MI.

We also examined the possibility of confounding effect by age, sex and classical risk factors including diabetes, hypertension, smoking, hyperlipidemia within patients group using one-way analysis of variance and χ^2 test, and found no relation between genotype and these factors (data not shown), indicating that the significant SNPs are an independent risk factor of MI.

We further conducted the association between the three tag SNPs and MI with ∼550 cases and 800 controls from a Taiwanese population. However, the results were not consistent with the association for MI in Japanese population (Supplementary Table 5).

Gene discovery at 5p15.3 locus

National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov) contained only one expressed sequence tag, DA489076.1, in the genomic region within the LD block. Therefore, we examined expression of DA489076.1 in cDNA derived from 13 human tissues including heart, lung, liver, skeletal muscle, placenta, peripheral blood leukocyte, lymph node, adipose, aorta, brain, fetal brain, coronary artery smooth muscle and coronary artery endothelial cells. However, the expression was not detectable in all tissues examined (data not shown). To explore whether other unidentified transcripts are present in this genomic region, we examined mRNA expression in human adult tissues by northern blot analyses using 106 amplicons as probes (Primer pairs are listed in Supplementary Table 2) that cover the entire genomic region of the block except for repetitive sequences. We

could not find obvious signal in all tissues examined (data not shown). Although we also examined micro-RNA (miRNA) and copy number variation databases (http://www.mirbase.org/search.shtml and https://gwas.lifesciencedb.jp/cgi-bin/cnvdb/cnv_top.cgi, respectively), we were not able to obtain any information for the genomic region.

DISCUSSION

Through a GWAS in a Japanese population using 210 785 SNP markers, we identified SNPs on chromosome 5p15.3 as a novel protective genetic factor against MI. The association that we observed in the Japanese population could not be replicated in the Taiwanese population. This might be due to a lack of the power ($1-\beta$; 0.17 in comparison of allele frequencies for these SNPs) or genetic difference between the two populations. We also cannot exclude the possibility that other variants in this genomic region confer risk of MI in the Taiwanese population. The loci on chromosome 5p15.3 and *BRAP* were not detected in the previous GWAS from Europe and the United States; this failure may be due to the difference among ethnicity in allelic frequencies, which affects power of the study and also the effect size. Other reasons might include the ethnic difference in the precise LD pattern, possibility of unidentified hidden SNPs for Caucasian decent, various biases such as publication bias, leaving open the question of association in other populations for these loci.

We could not find replicated previous results for *LTA* and *PSMA6* in the first-stage screening. The estimated powers of the first-stage screening to replicate positive association for *LTA* and *PSMA6* were 0.28 and 0.24, respectively. The significant SNP in *LGALS2* was not on the SNP list of Perlegen genotyping system. Therefore, we think one of the reasons might be lack of the power of this study. Biases including publication bias, sampling bias, cannot be excluded. For chromosome 9p21 locus, the *P*-value in the first-stage screening was 0.0018 (rs1412834). At the second stage, it was 0.0098 and did not pass the threshold. As our aim did not include replication of the previous findings, threshold *P*-value at each screening stage was not appropriate for replication study.

In the genomic region of the LD block on chromosome 5p15.3, we were not able to detect any transcript by our analyses. It is possible we cannot detect unidentified some small non-coding RNAs <100 base pairs, particularly miRNAs. miRNA has important functions in gene regulation in animals and plants by binding to target sites in the 3′ untranslated regions on mRNAs of protein-coding genes to direct their posttranscriptional repression.[23] In fact, recent studies indicated that a single substitution in the mach of the miRNA seed to its target site can abolish gene repression.[24] SNPs in miRNA including pri-miRNAs, pre-miRNAs and mature miRNA could influence the processing and/or target selection of miRNAs and affect

Table 2 Association analysis of the 15 tag SNPs with MI

	MI							Controls								
dbSNP ID	11	%	12	%	22	%	SUM	11	%	12	%	22	%	SUM	P-value[a]	r² with rs11748327
rs511664 C>T	1232	86.0	193	13.5	8	0.6	1433	1150	88.3	143	11.0	9	0.7	1302	1.6×10^{-1}	0.03
rs505800 A>G	763	51.4	599	40.3	123	8.3	1485	666	49.6	551	41.1	125	9.3	1342	4.0×10^{-1}	0.3
rs2008927 C>T	700	47.3	614	41.5	166	11.2	1480	650	49.2	524	39.6	148	11.2	1322	7.2×10^{-1}	0.18
rs631942 G>A	795	53.9	558	37.8	122	8.3	1475	660	48.9	569	42.1	121	9.0	1350	2.9×10^{-1}	0.4
rs10060583 C>T	566	38.1	686	46.2	233	15.7	1485	547	40.9	611	45.6	181	13.5	1339	9.1×10^{-1}	0.12
rs490556 T>C	863	58.1	521	35.1	102	6.9	1486	681	50.7	549	40.9	113	8.4	1343	2.4×10^{-3}	0.59
rs521660 G>A	762	55.3	515	37.3	102	7.4	1379	627	49.1	530	41.5	120	9.4	1277	1.5×10^{-2}	0.79
rs1187466 T>A	630	44.3	630	44.3	162	11.4	1422	579	46.9	540	43.8	115	9.3	1234	1.1×10^{-1}	0.11
rs1187463 C>A	743	50.4	600	40.7	130	8.8	1473	607	46.0	563	42.7	150	11.4	1320	7.5×10^{-2}	0.65
rs903083 C>T	1334	89.4	156	10.5	2	0.1	1492	1180	88.0	155	11.6	6	0.4	1341	2.6×10^{-1}	0.02
rs10512709 G>A	607	41.3	682	46.5	179	12.2	1468	559	42.3	611	46.3	151	11.4	1321	8.0×10^{-1}	0.17
rs1187477 C>T	1169	77.9	308	20.5	23	1.5	1500	1025	75.6	311	22.9	20	1.5	1356	3.0×10^{-1}	0.04
rs1209069 C>T	816	58.6	496	35.6	81	5.8	1393	779	61.2	435	34.2	58	4.6	1272	1.4×10^{-1}	0.04
rs1493470 G>A	827	55.9	566	38.3	86	5.8	1479	729	54.5	504	37.7	104	7.8	1337	2.4×10^{-1}	0.78
rs1187483 T>C	578	39.6	663	45.4	220	15.1	1461	478	36.0	635	47.8	215	16.2	1328	1.2×10^{-1}	0.41

Abbreviations: MI, myocardial infarction; SNP, single nucleotide polymorphism.
[a]Comparison of allelic frequency and adjusted for Bonferroni's correction.

Table 3 Association of the rs490556 and rs521660 with MI

		Cases							Controls							Comparison of allele frequency			
dbSNP ID	Samples	11	%	12	%	22	%	Total	11	%	12	%	22	%	Total	χ²	P-value	OR	95% CI
rs490556 T>C	3rd	863	58.1	521	35.1	102	6.9	1486	681	50.7	549	40.879	113	8.4	1343	14.4	1.5×10^{-4}	0.79	0.70–0.89
	Replication	1324	58.4	802	35.4	141	6.2	2267	1786	51.9	1399	40.68	254	7.4	3439	20.6	5.7×10^{-6}	0.82	0.75–0.89
	Combined															33.8	4.0×10^{-9}*	0.81	0.76–0.87
rs521660 G>A	3rd	762	55.3	515	37.3	102	7.4	1379	627	49.1	530	41.504	120	9.4	1277	10.9	9.4×10^{-4}	0.82	0.72–0.92
	Replication	1197	55.9	781	36.5	162	7.6	2140	1597	49.3	1342	41.445	299	9.2	3238	21.8	3.1×10^{-6}	0.81	0.75–0.89
	Combined															32.7	1.2×10^{-8}*	0.81	0.76–0.88

Abbreviations: CI, confidence interval; MI, myocardial infarction; OR, odds ratio; SNP, single nucleotide polymorphism.
*P-values were calculated by Mantel–Haenszel test.

Table 4 Haplotype analysis

	SNP IDs Risk allele			Haplotype frequency		Comparison of haplotype frequency	
	rs490556	rs11748327	rs521660	Case	Control	χ²	P-value
Haplotype	T	C	G				
Haplotype 1	T	C	G	0.727	0.688	30.7	3.0×10^{-8}
Haplotype 2	C	T	A	0.176	0.213	37.1	1.1×10^{-9}
Haplotype 3	C	C	A	0.053	0.058	1.4	2.3×10^{-1}
Haplotype 4	T	T	A	0.031	0.030	0.3	6.0×10^{-1}

Abbreviation: SNP, single nucleotide polymorphism.

miRNA-mediated translational suppression.[25] Therefore, the SNPs on chromosome 5p15.3 might be located within the region encoding unidentified miRNAs, affect their functions, and contribute to the development and/or progression of CAD. Although it is very difficult to reveal function of the SNP with the present knowledge, we think the increasing attention to function and/or higher-order structure of genome and subsequent progress will help to solve this problem.

We believe that knowledge of genetic factors contributing to its pathogenesis provides a useful clue for the development of diagnostic methods, treatments and preventive measures for this common but serious disorder.

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