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SHORT COMMUNICATION

Replication studies for the association of *PSMA6* polymorphism with coronary artery disease in East Asian populations

Kunihiko Hinohara[1], Toshiaki Nakajima[1,2], Taishi Sasaoka[3], Motoji Sawabe[4], Bok-Soo Lee[5], Jimin Ban[5], Jeong-Euy Park[5], Toru Izumi[3] and Akinori Kimura[1,2]

Coronary artery disease (CAD) has become a major health problem in many countries because of its increasing prevalence and high mortality. Recently, an association of a functional sequence variation, −8C>G, in the human proteasome subunit α type 6 gene (*PSMA6*) with the susceptibility to CAD was reported. To validate the association, we investigated a total of 1330 cases and 2554 controls from Japanese and Korean populations for *PSMA6* genotypes, and no evidence of the association was obtained in both Japanese (odds ratio (OR)=1.03, 95% confidence interval (CI); 0.90–1.19, *P*=0.66, allele count model) and Korean populations (OR=1.00, 95% CI; 0.86–1.17, *P*=0.95, allele count model). However, when a meta-analysis of data from this study and previously reported six replication studies was done, OR was 1.08 for the G allele (95% CI; 1.02–1.14, *P*=0.0057), suggesting that the contribution of *PSMA6* to CAD was not large enough to be readily replicated. Further studies are required to establish the contribution of this variant in the susceptibility to CAD.
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INTRODUCTION

Coronary artery disease (CAD) is caused by thrombotic occlusion or spasm of coronary artery and becomes a major health problem in many countries. CAD is based on the coronary atherosclerosis and often manifests with sudden chest pain due to reversible (angina pectoris, AP) or irreversible (myocardial infarction, MI) ischemia in the heart caused by decreased blood flow in coronary arteries. Although environmental factors, such as smoking, hypertension, hypercholesterolemia and diabetes mellitus (DM) significantly contribute to the development of CAD,[1] considerable evidence indicates the involvement of genetic factors in the pathogenesis of CAD[2] and several genome-wide association studies have recently identified susceptibility genes and loci for CAD.[3–5] However, not all of the reported associations could be replicated in other studies even if middle to large size samples were investigated in the original report,[6] indicating that the contribution of reported genetic factors was not large enough to be replicated in the other studies. Therefore, validation of the association in other samples is crucial to establish the role of disease-related genes.

Ozaki *et al.*[7] recently reported that a functional sequence variation −8C>G in the 5′ untranslated region of proteasome subunit α type 6 gene (*PSMA6*) was associated with MI in Japanese because G allele frequencies were 0.343 and 0.302 in the patients and controls, respectively, (OR=1.21, 95% CI; 1.11–1.31, $P=4.4\times10^{-6}$) in the initial study group and 0.374 and 0.337, respectively, (OR=1.16, 95% CI; 1.02–1.33, *P*=0.023) in their own replication study. *PSMA6* plays a key role in the vascular inflammatory processes through activation of nuclear factor-kappa B, and the variation −8C>G enhanced the transcription of *PSMA6*.[7] However, there were significant differences in the G allele frequencies among the two case groups (OR=0.88, 95% CI=0.89–0.99, *P*=0.02) as well as among the two control groups (OR=0.85, 95% CI=0.77–0.95, *P*=0.003) reported by Ozaki *et al.*,[7] raising a possibility of sampling biases in their studies and hence the association should be re-evaluated in other populations. In this study, we investigated the association of *PSMA6* −8C>G with CAD (MI and AP) in East Asians, Japanese and Korean populations.

MATERIALS AND METHODS

Subjects

A total of 1330 CAD cases and 2554 control subjects from Japanese and Korean populations were the subjects as reported earlier.[8,9] Briefly, the Japanese panels

[1]Laboratory of Genome Diversity, School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan; [2]Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [3]Department of Internal Medicine and Cardiology, Kitasato University School of Medicine, Sagamihara, Japan; [4]Department of Pathology, Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan and [5]Division of Cardiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
Correspondence: Professor A Kimura, Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan.
E-mail: akitis@mri.tmd.ac.jp
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were composed of CAD cases (n=606) and controls (n=1838). The CAD cases included 554 MI cases and 52 AP cases, whereas the controls included healthy individuals randomly selected from the general population (Jcont-1, n=1382) and consecutive autopsied cases without pathological findings of myocardial infarction (Jcont-2, n=456). The Korean subjects consisted of CAD cases (n=724) including 408 MI cases and 316 AP cases, whereas the controls (n=716) were composed of healthy individuals selected at random from the individuals visited health-check department (Kcont-1, n=182) and cancer patients without ischemic heart diseases (Kcont-2, n=534). The diagnosis of CAD was based on the standard criteria as described earlier.[10] Severity of coronary atherosclerosis was classified according to the number of coronary vessels with significant stenosis (angiographic luminal stenosis >50%) as 0, 1, 2 or 3 vessel disease. Informed consent was given from each participant and the study was approved by the Ethics Review Boards of Medical Research Institute of Tokyo Medical and Dental University, Kitasato University School of Medicine, Tokyo Metropolitan Geriatric Medical Center and Samsung Medical Center.

Genotyping

The *PSMA6* SNP −8C>G (rs1048990) was genotyped using the TaqMan SNP genotyping assay (Applied Biosystems). PCR products were amplified with *PSMA6*–1F (5′-CATGCAAGAGCGGAAGAAAC) and *PSMA6*-1R (5′-ACCTGG TTCACTCACCTACT). Call rate of the assay was over 99% and the samples with uncertain results were genotyped by direct sequencing of the PCR products. Ninety-six samples randomly selected from the Korean and Japanese populations were genotyped by both TaqMan method and direct sequencing method. The results by both methods were completely concordant, indicating the accuracy of TaqMan genotyping method.

Statistical analysis

A statistical analysis for power and sample size computations of case–control design was done by allelic 1 d.f. test using Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html) under the following conditions: high-risk allele frequency; 0.302, controls; unselected (not non-disease control), −8CG genotype odds risk; 1.05, −8GG genotype odds risk; 1.45, type I error rate; 0.05. Frequencies of genotypes and alleles were compared between the cases and controls using a χ^2. Strength of the association was expressed by odds ratio (OR). Meta-analysis was performed using a Mantel–Haenszel method. Significance of the association between the severity of coronary atherosclerosis and rs1048990 was examined by Mann–Whitney U-test. When a P-value was less than 0.05, the association was considered to be significant.

RESULTS AND DISCUSSION

Case–control studies were performed to replicate the association of *PSMA6* −8C>G with CAD. Clinical characteristics of the tested populations are listed in Table 1. Because no significant difference in the G allele frequency was observed among Japanese CAD cases (0.33 in MI cases and 0.32 in AP cases) and Korean CAD cases (0.35 in MI cases and 0.34 in AP cases), data for MI cases and AP cases were combined for Japanese and Korean CAD cases, respectively. In addition, two Japanese controls (0.32 and 0.34 in Jcont-1 and Jcont-2, respectively) and two Korean controls (0.31 and 0.36 in Kcont-1 and Kcont-2, respectively) did not show any significant difference in the G allele frequency, data from the controls were also combined for Japanese and Korean controls, respectively. In addition, there was no statistical difference in the G allele frequencies between Japanese and Korean in both CAD cases and controls. Statistical power analysis of our study design to verify whether it could provide adequate powers in replicating the association reported by Ozaki *et al.*[7] indicated that the power was 80.3% at the type I error rate of 0.05 in assuming that controls were unselected and might include patients, when the Japanese and Korean populations were combined.

As shown in Table 2, distributions of *PSMA6* genotypes were not departed from Hardy–Weinberg equilibrium in all the tested populations. We found no significant association between the −8G allele and CAD in both Japanese (OR=1.03, 95% CI; 0.90–1.19, P=NS) and Korean (OR=1.00, 95% CI; 0.86–1.17, P=NS). We could not exclude a possibility that there might be sampling biases in our study. Nevertheless, the allele frequencies of *PSMA6* −8C>G in the independent control samples in this study were nearly identical. It should be noted that the G allele frequency was 0.34 in the Japanese consecutive autopsied cases (Jcont-2, Table 1) in which ischemic changes in the heart such as myocardial infarction were not found in the pathological examination. In addition, the average age of Jcont-2 was 80.5 ± 9.1. Jcont-2 therefore could be a genuine non-MI control, but the G allele frequency in Jcont-2 was similar to or rather higher than that in Jcont-2 (0.32, general population), Japanese MI patients (0.33) or Japanese AP patients (0.32), implying that the G allele of *PSMA6* might not confer a strong selection pressure for survival from CAD-related deaths.

Although we did not find out any significant associations between the *PSMA6* −8C>G and CAD in the Japanese and Korean populations in this study, we could not exclude a possibility that other SNPs of *PSMA6* might be associated with CAD. However, because −8C>G was reported to be a responsible SNP for the association between *PSMA6* and MI because it altered the transcription level of *PSMA6*,[7] it was unlikely that other SNPs not in LD with −8C>G played a major

Table 1 Characteristics of cases and controls

	Japanese				*Korean*			
	CAD patient		*Control*		*CAD patient*		*Control*	
	MI N=*554*	*AP* N=*52*	*Jcont-1* N=*1382*	*Jcont-2* N=*456*	*MI* N=*408*	*AP* N=*316*	*Kcont-1* N=*182*	*Kcont-2* N=*534*
Age (years)	59.1 ± 10.1	65.3 ± 12.2	38.5 ± 10.8	80.5 ± 9.1	59.8 ± 11.6	63.4 ± 10.8	59.5 ± 10.5	57.9 ± 11.4
BMI	23.7 ± 2.9	NA	NA	17.2 ± 3.8	24.6 ± 2.6	25.1 ± 2.9	NA	NA
Gender (% male)	84.0	75.5	55.1	52.9	81.6	70.0	80.0	61.2
Smoking (%)	75.2	NA	NA	NA	72.5	61.4	NA	NA
HT (%)	52.5	50.0	NA	45.7	44.6	50.6	NA	NA
HC (%)	49.3	69.2	NA	17.0	49.5	28.1	NA	NA
DM (%)	30.6	36.5	NA	14.2	26.1	38.2	NA	NA

Abbreviations: 1AP, angina pectoris; BMI, body mass index; DM, diabetes mellitus; HC, Hypercholesterolemia; HT, hypertension; MI, myocardial infarction patients group; NA, data not available; Smoking, history of habitual smoking.
The values are means ± s.d. and percentages where indicated.

Table 2 Association between CAD and *PSMA6* -8C>G (rs1048990)

Genotype	*MI* (N=*554*) N *(%)*	*AP* (N=*52*) N *(%)*	*CAD* (N=*606*) N *(%)*	*Control* (N=*1838*) N *(%)*	*OR (95%CI)*	P-*value*
(a) Japanese						
CC	247 (44.6)	22 (42.3)	269 (44.4)	849 (46.3)		
CG	247 (44.6)	27 (51.9)	274 (45.2)	790 (42.7)		
GG	60 (10.8)	3 (5.8)	63 (10.4)	199 (11.0)	0.96 (0.71–1.29)	0.77
G allele frequency	0.33	0.32	0.33	0.32	1.03 (0.90–1.19)	0.66
HWE (p)	0.99	0.36	0.86	0.75		
Genotype	*MI* (N=*408*) N *(%)*	*AP* (N=*316*) N *(%)*	*CAD* (N=*724*) N *(%)*	*Control* (N=*716*) N *(%)*	*OR (95% CI)*	P-*value*
(b) Korean						
CC	175 (42.9)	137 (43.4)	312 (43.1)	303 (42.3)		
CG	180 (44.1)	142 (44.9)	322 (44.5)	331 (46.2)		
GG	53 (13.0)	37 (11.7)	90 (12.4)	82 (11.5)	1.10 (0.80–1.51)	0.57
G allele frequency	0.35	0.34	0.35	0.35	1.00 (0.86–1.17)	0.95
HWE (p)	0.82	1.00	0.89	0.84		

Abbreviations: AP, angina pectoris; CAD, coronary artery disease; CI, confidence interval; HWE, Hardy–Weinberg Equilibrium; MI, myocardial infarction patients group; OR, odds ratio.
Frequencies of genotypes and alleles were compared between the CAD cases and controls using a χ^2 test.

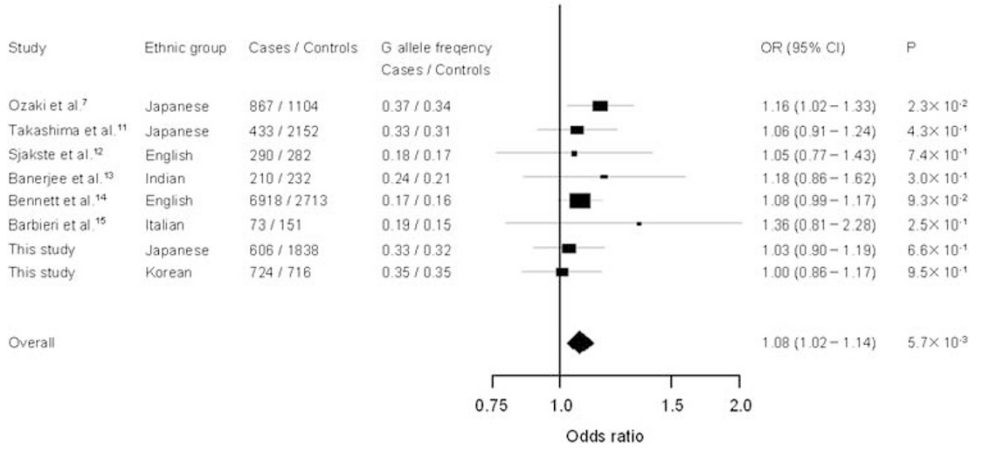

Figure 1 Meta-analysis of data from replication studies for the association between PSMA6 −8C>G and CAD. Odds ratio and 95% confidence interval (95% CI) are schematically indicated for each replication study. Study: authors and reference; Ethnic group: tested populations; Cases/Controls: number of cases and controls; G allele frequency in Cases/Controls: frequencies of G allele in cases and controls; OR (95% CI): odds ratio and 95 confidence interval in parenthesis; *P*: *P*-values.

role in the pathogenesis of CAD. Another possibility for the reason of failure to detect the association was that the power of our study was not enough to detect the modest or weak association. So far there are six other replication studies including that by Ozaki *et al.*,[7] and five of them except for Ozaki's replication study failed to replicate the significant association of *PSMA6* −8C>G with CAD.[11–15] Therefore, we performed a meta-analysis of the data obtained in this study for the Japanese and Korean populations and the data in the previous replication studies. As shown in Figure 1, the association was found to be modest with a statistical significance (OR=1.08, 95% CI; 1.02–1.14, *P*=0.0057). These results implied that the sample sizes of our study and the previous replication studies were not large enough to catch the association. On the other hand, it should be noted here that, when a meta-analysis was confined to the replication studies in the East Asian populations, the association rendered to be weak and did not reach statistical significance (OR=1.07, 95% CI; 1.00–1.15, *P*=0.063) and was further weakened with no statistical significance when the replication study by Ozaki *et al.*[7] was excluded (OR=1.03, 95% CI; 0.95–1.13, *P*=0.462). In addition, a meta-analysis of genotype-specific OR for the risk allele in the East Asians suggested that the contribution of the risk allele to CAD was not large because the combined population attributable risk was 0.059 (Table 3). These observations indicated that further replication studies would be required to establish the role of *PSMA6* −8C>G in CAD (or MI) in the East Asian populations.

It was reported that *PSMA6* had a functional relation with *LTA*,[7] whose sequence variations were associated with the severity of atherosclerosis.[8] In addition, *PSMA6* was reported to be involved in the diabetic atherosclerosis,[16] and the association of −8GG genotype with the thickness of intima-media in carotid artery was recently

Table 3 Genotype-specific OR for the risk allele of rs1048990 (*PMSA* −8G) in East Asians

	Genotype-specific OR[b]			
Study (ethnic group)[a]	*Non-carrier*	*Heterozygous carrier (95% CI)*	*Homozygous carrier (95% CI)*	*PAR*[c]
Ozaki *et al*. (Japanese)[3]	1	1.01 (0.84–1.22)	1.51 (1.14–2.00)	0.076
Takashima *et al*. (Japanese)[11]	1	1.07 (0.86–1.33)	1.13 (0.79–1.61)	0.042
This study (Japanese)	1	1.09 (0.90–1.33)	1.00 (0.73–1.37)	0.041
This study (Korean)	1	0.95 (0.76–1.18)	1.07 (0.76–1.50)	−0.017
Combined (East Asians)	1	1.03 (0.93–1.14)	1.18 (1.01–1.39)	0.059

[a]Same as described in the legend to Figure 1.
[b]Genotype-specific odds ratio for the risk allele. The risk for heterozygous carrier and that for homozygous carrier were compared with the risk for non-carrier.
[c]Population attributable risk.

Table 4 Association between the severity of coronary atherosclerosis and rs1048990

	Japanese CAD		*Korean CAD*	
	G allele (%)	*C allele (%)*	*G allele (%)*	*C allele (%)*
0VD	15 (41.7)	21 (58.3)	10 (45.5)	12 (54.5)
1VD	154 (32.6)	318 (67.4)	158 (33.9)	308 (66.1)
2VD	123 (36.4)	215 (63.6)	114 (37.8)	188 (62.2)
3VD	103 (28.9)	253 (71.1)	67 (31.3)	147 (68.7)
Mann–Whitney *U*	*P*=0.22		*P*=0.65	

Abbreviation: VD, vessel disease with number of significantly affected vessels.

reported.[11] Taken these into account, it was implied that *PSMA6* might be associated with the severity of coronary atherosclerosis and/or DM. As shown in Table 4, there was however no trend of an association between rs1048990 and severity of coronary atherosclerosis in both Japanese and Korean CAD (P=0.22 and P=0.65, respectively). Stratified analyses of rs1048990 with classical risk factors showed a trend of marginal association between the −8GG genotype and DM in Korean (OR=1.75, P=0.044), but it was weak and not significant in Japanese (OR=1.25, P=0.36), implying that the association with DM might be different among the East Asians. It will be interest in the future study to investigate an association between the *PSMA6* SNP with intermediate phenotypes responsible for inflammation in the atherosclerosis, including serum level of inflammatory markers such as a high-sensitive CRP.

In conclusion, although we failed to replicate the association between the *PSMA6* −8C>G polymorphism with CAD, the meta-analysis of data from replication studies including ours showed that the risk was modest OR=1.08. Further analyses are required to establish the contribution of *PSMA6* to the risk for CAD.

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1 Wang, Q. Molecular genetics of coronary artery disease. *Curr. Opin. Cardiol.* **20,** 182–188 (2005).
2 Ciruzzi, M., Schargrodsky, H., Rozlosnik, J., Pramparo, P., Delmonte, H., Rudich, V. *et al*. Frequency of family history of acute myocardial infarction in patients with acute myocardial infarction. *Am. J. Cardiol.* **80,** 122–127 (1997).
3 Ozaki, K., Ohnishi, Y., Iida, A., Sekine, A., Yamada, R., Tsunoda, T. *et al*. Functional SNPs in the lymphotoxin-α gene that are associated with susceptibility to myocardial infarction. *Nat. Genet.* **32,** 650–654 (2002).
4 Wellcome Trust Case Control Consortium. Genome-wide association study of 14 000 cases of seven common diseases and 3000 shared controls. *Nature* **447,** 661–678 (2007).
5 Helgadottir, A., Thorleifsson, G., Manolescu, A., Gretarsdottir, S., Blondal, T., Jonasdottir, A. *et al*. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* **316,** 1491–1493 (2007).
6 Morgan, T. M., Krumholz, H. M., Lifton, R. P. & Spertus, J. A. Nonvalidation of reported genetic risk factors for acute coronary syndrome in a large-scale replication study. *JAMA.* **297,** 1551–1561 (2007).
7 Ozaki, K., Sato, H., Iida, A., Mizuno, H., Nakamura, T., Miyamoto, Y. *et al*. A functional SNP in *PSMA6* confers risk of myocardial infarction in the Japanese population. *Nat. Genet.* **38,** 921–925 (2006).
8 Kimura, A., Takahashi, M., Choi, B. Y., Bae, S. W., Hohta, S., Sasaoka, T. *et al*. Lack of association between *LTA* and *LGALS2* polymorphism and myocardial infarction in Japanese and Korean populations. *Tissue Antigens.* **69,** 265–269 (2007).
9 Hinohara, K., Nakajima, T., Takahashi, M., Hohda, S., Sasaoka, T., Nakahara, K. *et al*. Replication of association between a chromosome 9p21 polymorphism with coronary artery disease in Japanese and Korean populations. *J. Hum. Genet.* **53,** 357–359 (2008).
10 Hohda, S., Kimura, A., Sasaoka, T., Hayashi, T., Ueda, K., Yasunami, M. *et al*. Association study of CD14 polymorphism with myocardial infarction in a Japanese population. *Jpn. Heart. J.* **44,** 613–622 (2003).
11 Takashima, N., Shioji, K., Kokubo, Y., Okayama, A., Goto, Y., Nonogi, H. *et al*. Validation of the association between the gene encoding proteasome subunit alpha type 6 and myocardial infarction in a Japanese population. *Circ. J.* **71,** 495–498 (2007).
12 Sjakste, T., Poudziunas, I., Ninio, E., Perret, C., Pirags, V., Nicaud, V. *et al*. SNPs of PSMA6 gene–investigation of possible association with myocardial infarction and type 2 diabetes mellitus. *Genetika* **43,** 553–559 (2007).
13 Banerjee, I., Pandey, U., Hasan, O. M., Parihar, R., Tripathi, V., Ganesh, S. Association between inflammatory gene polymorphisms and coronary artery disease in an Indian population. *J. Thromb. Thrombolysis* **27,** 88–94 (2007).
14 Bennett, D. A., Xu, P., Clarke, R., Zondervan, K., Parish, S., Palmer, A. *et al*. The exon 1–8C/G SNP in the PSMA6 gene contributes only a small amount to the burden of myocardial infarction in 6946 cases and 2720 controls from a United Kingdom population. *Eur. J. Hum. Genet.* **16,** 480–486 (2008).
15 Barbieri, M., Marfella, R., Rizzo, M. R., Boccardi, V., Siniscalchi, M., Schiattarella, C. *et al*. The −8 UTR C/G polymorphism of PSMA6 gene is associated with susceptibility to myocardial infarction in type 2 diabetic patients. *Atherosclerosis* **201,** 117–123 (2008).
16 Marfella, R., D'Amico, M., Esposito, K., Baldi, A., Di Filippo, C., Siniscalchi, M. *et al*. The ubiquitin-proteasome system and inflammatory activity in diabetic atherosclerotic plaques: effects of rosiglitazone treatment. *Diabetes* **55,** 622–632 (2006).