Association Study of CD14 Polymorphism With Myocardial Infarction in a Japanese Population

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SUMMARY

There have been many studies investigating the association between gene polymorphisms and coronary artery disease (CAD) including myocardial infarction (MI), and some studies have shown that certain gene polymorphisms are associated with CAD/MI. However, the results of the association have sometimes been controversial. The reason may be that the contribution of genetic risk factors to CAD/MI varies depending on the ethnic, environmental, and habitual backgrounds, and differs between males and females. In this study, we analyzed 17 polymorphisms in 12 candidate genes for MI in 136 patients and 200 to 235 controls, and found that there is a significant association of MI with the polymorphisms in the genes for E-selectin and CD14 receptor. To further explore the association, we investigated the C-260 T polymorphism in the promoter region of the CD14 gene in 502 MI patients and 527 control subjects. The genotype distributions of the CD14 polymorphism were as follows; patients; T/T 32.5%, C/T 48.2%, C/C 19.3%, and controls; T/T 25.4%, C/T 52.8%, C/C 21.8%. The frequencies of the T/T homozygotes were significantly higher in the patients (OR = 1.41, $P = 0.013$) than in the control group, confirming the association of CD14 polymorphism with MI in Japanese. Stratification analyses further demonstrated that the association was more prominent in females and in patients with a relatively low body mass index, suggesting that the contribution of the CD14-linked genetic risk to MI differs with respect to gender and habitual background. (Jpn Heart J 2003; 44: 613-622)

Key words: CD14 receptor, Polymorphism, Myocardial infarction, Genetic risk factor

MYOCARDIAL infarction (MI) is usually caused by occlusion of a coronary artery, which is induced by thrombosis and/or rupture of plaque based on atherosclerosis of the coronary arteries.^{1,2)} The disease is multifactorial, where genetic factors as well as environmental factors are suggested to be involved in the patho-

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genesis of coronary atherosclerosis and/or MI. Conventional risk factors for coronary artery disease (CAD) include male gender, smoking, hyperlipidemia, hypertension, diabetes mellitus and obesity. However, not all patients have the conventional risk factors. In addition, most CAD patients do not develop MI, even if exposed to the same environmental factor. Furthermore, not all individuals having the conventional risk factors would suffer from CAD or MI. On the other hand, the incidence of MI in individuals whose first-degree relatives died of MI is 2 to 4 times higher than that in the general population.³⁾ It was also reported that the concordance of MI was higher among monozygotic twins than among dizygotic twins, especially in females.⁴⁾ Based on these observations, it has been suggested that genetic factors may participate in the development of CAD and/or MI, in addition to environmental factors. Because the genetic risk factor(s) contribute to the development of CAD/MI independent of the environmental risk factors, identification of each genetic risk factor may eventually lead to the prevention of CAD/MI.

To identify the genetic risk factors, many studies have examined the association between the polymorphisms in the candidate genes for CAD or MI, and it has been reported that several gene polymorphisms are associated with CAD/MI in certain populations. However, most of the results of these studies were not consistent and some are even controversial. Therefore, the genetic risk factors could not always be identified with certainty. The reason for this controversy may be that the contribution of the genetic risk factors to CAD/MI can vary depending on ethnic, environmental, and habitual backgrounds. It also is possible that the genetic risk factors are different between males and females. Because females are relatively resistant to developing CAD/MI and because the occurrence of conventional risk factors is relatively less frequent in female patients, the genetic risk factors should be accumulated in female MI patients, as is evidenced by the higher odds ratios of the concordance of MI in the twin study.⁴⁾

In order to identify the genetic risk factor or susceptibility gene to MI, and clarify the correlation of each susceptibility gene with other conventional risk factors, a comparison of gene polymorphisms in patients and healthy controls in a large-scale study is indispensable, especially if it is presumed that the contribution of each disease susceptibility gene might be small. Thus, in this research, while ensuring a sufficient sample size required for the analysis to identify the susceptibility gene to MI in a Japanese population, we have investigated the frequencies and distributions of gene polymorphisms which have been reported to be associated with CAD or MI. First, as a screening test, we have tested 17 gene polymorphisms in 12 candidate genes including E-selectin⁵⁾, angiotensin-converting enzyme (ACE)^{5,6)}, endothelial nitric oxide synthase (eNOS)^{7,8)}, β -fibrinogen^{9,10)}, glycoprotein IIIa¹¹⁾, human paraoxonase/arylesterase (HUM-

PONA)¹²⁾, methylenetetrahydrofolate reductase (MTHFR)¹³⁾, platelet-activating factor acetylhydrolase (PAF-AH)¹⁴⁾, transforming growth factor β 1 (TGF β 1)¹⁵⁾, aldosterone synthase (CYP11B2)¹⁶⁾, lymphotoxin- α (LTA),¹⁷⁾ and CD14 receptor¹⁸⁾, in 136 MI patients and 200 to 235 controls. This screening test showed that the polymorphisms in the E-selectin gene and that in the CD14 gene were significantly associated with MI in Japanese. Second, we focused on the CD14 polymorphism, which is relatively frequent in the general Japanese population, in 502 MI patients with 527 controls to confirm the association. Finally, stratification analyses were performed to reveal the correlation of CD14 polymorphism with other conventional risk factors of MI in the patients studied. Here, we report that the CD14 promoter polymorphism is associated with MI and its contribution may be relatively large in female patients and in patients with a relatively low body mass index (BMI), ie, the CD14-linked susceptibility gene is a genetic risk factor in Japanese low-risk patients.

METHODS

Subjects: The study group consisted of 502 patients suffering from MI and 527 control subjects without a history of CAD or MI. Patients with MI were recruited from Kitasato University Hospital, Tachikawa General Hospital, and Shimizu Kohsei General Hospital. Patients were genetically unrelated to each other, and the diagnosis of MI was based on clinical manifestation, physical and laboratory examination data, and electrocardiography and echocardiography findings. There were 422 males and 80 females, and the mean age at onset of MI was 58.9 ± 10.3 years. Of the 471 patients examined by coronary angiography, 120 patients had severe coronary stenosis (all three vessels had $> 50\%$ luminal stenosis), 147 patients had significant luminal stenosis ($> 50\%$) in two vessels, and 204 patients had significant stenosis in one vessel. The control subjects were randomly selected from the general population by the criteria of no history of cardiac disease, and residing on Honshu and Kyushu Islands, two of the main islands of Japan. The controls consisted of 359 males and 168 females, with the mean age of 37.3 ± 14.1 years at the time of blood sampling. All patients and controls were Japanese. The Human Subjects Committees of Tokyo Medical and Dental University and Kitasato University Hospital approved this study. Participation of the patients and controls was voluntary. Informed consent for genetic studies was obtained from each subject before blood sampling.

Genotyping: Genomic DNA was extracted from peripheral blood using a standard method.¹⁹⁾ Polymerase chain reaction (PCR) was performed in a final volume of 30 μ L. First, we selected 17 polymorphisms in the 12 candidate genes including E-selectin⁵⁾, ACE^{5,6)}, eNOS^{7,8)}, β -fibrinogen^{9,10)}, glycoprotein IIIa¹¹⁾,

HUMPONA¹²⁾, MTHFR¹³⁾, PAF-AH¹⁴⁾, TGF β 1¹⁵⁾, CYP11B2¹⁶⁾, LTA,¹⁷⁾ and CD14 receptor¹⁸⁾ that have already been reported to be associated with CAD, vasospasm, and MI. We performed the screening study using 136 MI patients and 200 to 235 healthy controls, randomly selected from 502 MI patients and 527 healthy controls, respectively. The characteristics of 136 MI patients have been reported previously.²⁰⁾ From the results of the screening study, we selected the CD14 polymorphism for further analysis. In the second analysis, 502 MI patients and 527 healthy controls were investigated.

Statistical Analysis: The allele frequencies and genotype distributions were compared between the patients and controls using a chisquare test of 2×2 tables as described previously.²⁰⁾ When the *P* value was less than 0.05, the association was considered to be significant. Prespecified subgroup analyses were done with stratification by average age at onset (57.7 years in males, 64.9 years in females), body mass index (23.7 in males, 23.4 in females), habitual smoking, and presence or absence of hypertension, hyperlipidemia, diabetes mellitus, and a familial history of coronary artery disease.

RESULTS

First, we performed an initial screening test for the 17 polymorphisms of the 12 candidate genes in 136 MI patients and 200 to 235 healthy controls (Table I). The results showed that most of them were not associated with MI, because the odds ratio (OR) was usually around 1 or below, except for the two polymorphisms of E-selectin and CD14 receptor genes which had *P* values less than 0.05. The genetic markers showing significant positive association in the screening were E-selectin 128Arg allele positivity (12.6% in the patients vs 6.7% in the controls, OR = 2.0, *P* = 0.039, 95% confidence interval (CI); 1.05-3.85) and CD14 -260T/T genotype (35.3% vs 24.5%, OR = 1.69, *P* = 0.027, 95% CI; 1.06-2.68).

Because CD14 polymorphism is more prevalent than the E-selectin polymorphism in Japanese, we selected the CD14 polymorphism for further testing of the association in a larger scale study. Table II shows the distributions of genotypes and the allele frequencies of CD14 gene polymorphism (C-260T) in the patient and control groups. The c-260T allele was significantly associated with MI (56.6% vs 51.8%, OR = 1.21, *P* = 0.030, 95% CI; 1.02-1.44). In the patient group, 19.3% were C/C, 48.2% were C/T, and 32.5% were T/T. This genotype distribution was significantly different from the distribution in the control group (C/C; 21.8%, C/T; 52.8%, T/T; 25.4%). That is to say, the frequency of the T/T homozygotes was significantly higher in the MI patients than in the control group

Table I. Association of Genetic Markers with Myocardial Infarction in the Screening Test

Gene	Polymorphism	Marker	Freq. in patients ^{#1}	Freq. in controls ^{#2}	OR	P	95% CI
E-selectin	Ser128Arg	128Arg allele positivity	0.126	0.067	2.00	0.039	1.04-3.85
ACE	IVS16 Del vs Ins	DD genotype	0.147	0.124	1.22	NS	0.66-2.25
eNOS	prom -922 A to G	-922 G allele positivity	0.215	0.210	1.03	NS	0.57-1.87
eNOS	IVS4 4a/4b	4a/4a genotype	0.000	0.021	-	NS	-
eNOS	Glu298Asp	298Asp allele positivity	0.169	0.146	1.19	NS	0.63-2.27
β Fibrinogen	prom -453 G to A	-453 GG genotype	0.725	0.707	1.10	NS	0.67-1.80
β Fibrinogen	Arg448Lys	448Lys allele positivity	0.279	0.291	0.94	NS	0.58-1.56
β Fibrinogen	3' region BclI +/-	Bcl I (+) allele positivity	0.256	0.272	0.92	NS	0.55-1.54
GP IIIa	Leu33Pro	33Pro allele positivity	0.000	0.000	-	NS	-
HUMPONA	Arg192Gln	192Gln allele positivity	0.910	0.876	0.92	NS	0.49-1.73
MTHFR	Ala667Val	667Val allele positivity	0.630	0.630	1.00	NS	-
MTHFR	Ala667Val	667Val/Val genotype	0.111	0.148	0.72	NS	0.37-1.40
PAF-AH	Val279Phe	279Phe allele positivity	0.375	0.345	1.14	NS	0.77-1.70
PAF-AH	Val279Phe	279Phe/Phe genotype	0.044	0.032	1.40	NS	0.53-3.71
TGFB1	Leu10Pro	10Leu allele positivity	0.720	0.754	0.84	NS	0.52-1.35
TGFB1	Leu10Pro	10Leu/Leu genotype	0.169	0.193	0.85	NS	0.49-1.48
TGFB1	Leu10Pro	10Leu/Pro genotype	0.552	0.562	0.96	NS	0.63-1.47
TGFB1	Arg25Pro	25Pro allele positivity	0.000	0.000	-	NS	-
CYP11B2	prom -344 T to C	-344 CT genotype	0.507	0.469	1.17	NS	0.77-1.78
CYP11B2	prom -344 T to C	-344 CC genotype	0.096	0.104	0.91	NS	0.45-1.85
LTA	intron1 A252G	GG genotype	0.139	0.112	1.27	NS	0.68-2.38
CD14	prom -260 C to T	-260TT genotype	0.353	0.245	1.69	0.027	1.06-2.68

#1: number of tested patients was 136, #2: numbers of tested controls were from 200 to 235 (depending on loci), NS = Not significant.

Table II. Frequencies of CD14 Alleles and Genotypes in Japanese Patients with Myocardial Infarction and Healthy Controls

	Patients	Controls	OR*	P	95% CI
Allele frequency (%)	(2n = 1004)	(2n = 1054)			
C allele	436 (43.4%)	508 (48.2%)			
T allele	568 (56.6%)	546 (51.8%)	1.21	0.03	1.02 - 1.44
Genotype frequency (%)	(n = 502)	(n = 527)			
C/C	97 (19.3%)	115 (21.8%)			
C/T	242 (48.2%)	278 (52.8%)			
T/T	163 (32.5%)	134 (25.4%)	1.41	0.013	1.08 - 1.85

*Odds ratio of risk

(OR = 1.41, P = 0.013, 95% CI; 1.08-1.85), suggesting that the T allele may serve as a recessive genetic risk factor for Japanese MI.

We performed stratification analyses in the patients because the contribution of the CD14-linked genetic risk factor to MI may vary depending on the conventional risk factors. As shown in Table III, the number of male patients was much

Table III. Characteristics of MI Patients

Variable	Male patients (<i>n</i> = 422)	Female patients (<i>n</i> = 80)	Male vs. Female <i>P</i> value
Age at onset (years)	57.7 ± 10.0	65.0 ± 9.8	< 0.0001
BMI (kg/m ²)	23.7 ± 2.9	23.4 ± 3.1	NS
Habitual smoking (%)	82.80%	45.00%	< 0.0001
Hypertension (%)	50.20%	53.80%	NS
Diabetes mellitus (%)	30.10%	26.30%	NS
Hyperlipidemia (%)	48.50%	51.30%	NS

NS = not significant.

larger than that of female patients, and a comparison of backgrounds between male and female patients demonstrated that age at onset was significantly younger in the male patients than in the female patients, and that the frequency of habitual smoking was significantly high in male patients. The existence of other conventional risk factors was not significantly different between the male and female patients. This stratification analysis showed that males are more susceptible to MI than females, although habitual smoking was much more prevalent in males, in this study group. Since the T/T genotype of CD14 was associated with MI in the entire patient population, and because there was no significant difference in the CD14 genotype frequencies in age- or gender-grouped controls (data not shown), we have compared the T/T genotype frequencies in various subgroups of MI patients with that in the entire control population (25.4%).

The frequency of T/T genotype in female patients was 37.5% (OR = 1.76, *P* = 0.023, 95% CI; 1.07-2.88) and 31.5% in male patients (OR = 1.35, *P* = 0.038, 95% CI; 1.02-1.79), showing that the association was stronger in females (T/T genotype in the female controls was 25.6% and that in the male controls was 25.3%). When the male and female patients were subgrouped according to the age at onset, the T/T genotype frequencies in the male younger-onset (≤ 57 years) and older-onset (> 58 years) groups were 27.4% and 34.9%, respectively, showing a significant association in the older-onset group (OR = 1.57, *P* = 0.008, 95% CI; 1.12-2.20). On the other hand, the T/T genotype frequency in the female younger-onset (≤ 64 years) group was 42.1% showing significant association (OR = 2.13, *P* = 0.025, 95% CI; 1.09-4.18), while that in the older-onset (> 65 years) group was 33.3%, a higher frequency than in the controls but not statistically significant. The T/T genotype frequency was significantly higher in the patients with a relatively low BMI; 35.9% in patients with a BMI lower than the average (BMI = 23.6, Table III) (OR = 1.64, *P* = 0.003, 95% CI; 1.18-2.28), and was especially high in the females with a BMI ≤ 23.4 (female average) (40.5%. OR = 2.00, *P* = 0.04, 95% CI; 1.01-4.00) and also high in the males with a BMI

≤ 23.7 (male average) (35.0%, OR = 1.58, $P = 0.01$, 95% CI; 1.11-2.24). There were no other significant differences in the T/T genotype frequencies when the male and female patients were subgrouped by the presence or absence of other conventional risk factors, including smoking, hypertension, diabetes mellitus, and hyperlipidemia (data not shown).

DISCUSSION

We examined the association of 17 polymorphisms in the 12 candidate genes for MI in a small scale study testing 136 MI patients and 200 to 235 controls, and found that 128Arg allele positivity for the E-selectin gene and the-260 T/T genotype of the CD14 gene were significantly associated with the disease. We then confirmed in a large scale study that the T/T genotype of the CD14 receptor gene was significantly associated with MI. In addition, stratification analyses revealed that the association was stronger in females and in individuals with a relatively low BMI.

Coronary atherosclerosis is considered to be an inflammatory disease.²¹⁾ Ongoing inflammation in the vessel wall accelerates progression of atherosclerosis and destabilizes the plaque, the rupture of which causes atherothrombosis.²⁾ It has been established that, in addition to well-known risk factors such as hyperlipidemia, hypertension, diabetes mellitus, obesity, and smoking, infection and inflammation play a role in the development of atherosclerosis and consequently MI. Although the precise involvement of infectious agents as risk factors of coronary atherosclerosis remains unclear, inflammatory cells such as neutrophils, lymphocytes, and monocyte-derived macrophages, which accumulate in atheroma, play a key role in the process of atherosclerosis.^{21,22)} The monocyte receptor for bacterial lipopolysaccharide (LPS, endotoxin), CD14, is a particularly important mediator of the inflammatory response in the process of coronary atherosclerosis. LPS, an outer membrane component of gram-negative bacteria that is produced by infectious agents, is a potent inducer of inflammation²³⁾ and stimulates the synthesis of tumor necrosis factor- α , interleukins, and growth factors in monocytes and endothelial cells.²⁴⁾ When LPS interacts with LPS-binding protein and CD14, the expression of inflammatory genes and adhesion molecule genes is activated through induction of nuclear factor κ B (NF κ B) and mitogen-activated protein-kinase signaling.²⁵⁾ This mechanism is believed to participate in the initiation and development of atherosclerosis.

The CD14 receptor gene is localized on chromosome 5. It consists of 3900 bp organized in 2 exons and encodes a protein of 375 amino acids.²⁶⁾ C-260 T polymorphism in the promoter of the CD14 gene is located near a Sp1 transcription factor binding site and may alter the activity of CD14 gene promoter and

affect the level of CD14 gene expression.^{18,27)} In our study, the T/T genotype of the CD14 gene was significantly associated with MI in a Japanese population.

Although several studies have reported an association between CD14 promoter polymorphism and CAD or MI, several other reports have presented controversial results. In the initial report, Hubacek, *et al* found that CD14 polymorphism was associated with an increased risk of MI.¹⁸⁾ Shimada, *et al* showed that CD14 polymorphism was associated with MI rather than with coronary atherosclerosis and might be one genetic risk factor for MI in Japanese men.²⁸⁾ In addition, Unkelbach, *et al* reported that the T/T genotype was associated with MI in a small subgroup of low-risk, nonsmoker, normotensive patients, although there was no significant correlation between the CD14 polymorphism and patients who survived from MI.²⁹⁾ On the other hand, Zee, *et al* reported that there was no significant association between CD14 polymorphism and the risk of MI,³⁰⁾ Which appears to be in conflict with the above-mentioned observations and our findings in this study. However, Zee, *et al* examined only male patients. Since we revealed that the association of MI with CD14 polymorphism was stronger in female patients than in male patients, this association may not be readily found in an analysis of male patients. It should be noted here that Yamada, *et al* have reported a large scale association study of Japanese MI with candidate gene polymorphisms.³¹⁾ They demonstrated that CD14 polymorphism was significantly ($P < 0.05$) associated with MI in females, but not in males, findings that are consistent in part with our findings.

We also found that the association of CD14 polymorphism with MI was more evident in the patients with a relatively low BMI, both in males and females, suggesting that the CD14-linked genetic risk may contribute to CAD/MI in the low-risk group. This was not consistent with a report of Zee, *et al*, which showed the association of CD14 polymorphism with CAD was found preferentially in male patients with a BMI greater than 25.³⁰⁾ The reason for the discrepancy is not clear, but might reflect an ethnic difference in the contribution of genetic factor (s).

The TT genotype of the CD14 gene was reported to confer the risk of alcoholic liver disease, and the association was strong in females; a similar situation as in Japanese MI found in this study.³²⁾ It also was reported that the CD14 molecule is involved in alcoholic liver damage, being produced in larger quantities in females in a rat model.³³⁾ These observations suggest that the inflammatory reaction through CD14 can be modulated by sex hormones. This may be related to the fact that the contribution of CD14 polymorphism to MI was stronger in the female patients, especially the younger-onset group, and in the older-onset male patients.

In conclusion, we have confirmed that the T/T genotype of the CD14 gene was significantly associated with MI in a Japanese population. A large-scale analysis has enabled us to investigate the correlation of CD14-linked genetic risk factors with conventional risk factors. It was demonstrated that the association was preferential in female patients and in non-obese patients. We have shown clearly for the first time that the contribution to MI of the CD14 polymorphism is dependent on gender and serves as a genetic risk for MI in a low-risk group without obesity.

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