Endothelial Lipase Gene Polymorphism is Associated With Acute Myocardial Infarction, Independently of High-Density Lipoprotein-Cholesterol Levels

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Background Endothelial lipase (EL) is a major determinant of high-density lipoprotein-cholesterol (HDL-C) metabolism and promotes monocytes recruitment. The local expression of EL could influence atherogenesis directly, in addition to its systemic role in HDL metabolism. The EL gene has a common 584C/T polymorphism, but it is unclear whether this polymorphism is associated with HDL-C levels or acute myocardial infarction (AMI).

Methods and Results A case–control study of 107 AMI patients and 107 control subjects was conducted. T allele frequency was lower in the AMI group than in controls (0.18 vs 0.26, $p < 0.05$). No significant association was found between the 584C/T polymorphism and HDL-C levels. Multivariate regression analyses showed that the association of the T allele with AMI was statistically significant and independent of other risk factors when age, sex, hypertension, hypercholesterolemia, and diabetes mellitus were included in the analyses (odds ratio (OR), 0.52; 95% confidence interval (95% CI) 0.28–0.98; $p = 0.04$). However, when smoking status was included, the association of the T allele with AMI did not remain statistically significant (OR, 0.61; 95% CI 0.32–1.18; $p = 0.14$).

Conclusions The 584C/T polymorphism of the EL gene was associated with AMI independently of HDL-C levels and thus may be involved in the pathogenesis of AMI. (Circ J 2007; 71: 842–846)

Key Words: Acute myocardial infarction; Endothelial lipase; High-density lipoprotein-cholesterol; Single nucleotide polymorphism; Smoking

The lipoprotein lipase gene family plays a crucial role in lipid metabolism for the regulation of circulating lipoprotein levels and affects the process of atherosclerotic vascular diseases.¹ Recently, the lipoprotein lipase gene family was extended through the characterization of endothelial lipase (protein: EL; gene: *LIPG*; NCBI Locuslink 9388).^{2,3} EL is expressed in endothelial cells and has phospholipase activity, but relatively little triglyceride lipase activity.^{2,4} It has been demonstrated that high-level overexpression of EL in the liver by adenovirus-mediated gene transfer results in a significant decrease in high-density lipoprotein-cholesterol (HDL-C) and apolipoprotein A-I (apoA-I).³ Moreover, we have reported that there was a 57% increase in HDL-C level in male *LIPG* knockout mice as compared with wild-type controls.⁵ Jin et al also reported that inhibition of EL resulted in increased HDL-C levels in mice.⁶ Together, these data strongly indicate that EL is a major determinant of HDL-

C metabolism in mice.⁷ However, the connection to human lipid levels is less robust.

Recently, deLemos et al discovered 6 new, potentially functional variants in the *LIPG* in subjects with high HDL-C levels,⁸ but none showed significant association with plasma HDL-C levels. Among the 17 genetic variants identified in that study, 1 common single nucleotide polymorphism (SNP), 584C/T, in exon 3 deserved greater scrutiny, as it was responsible for a significant amino acid change (threonine at 111 amino acid sequence changes to isoleucine) that could potentially be associated with altered intravascular EL activity. In contrast, Ma et al detected a significant association of the 584C/T SNP in *LIPG* with mean plasma levels of HDL-C.⁹ Patients with the TT allele have a 14% higher mean HDL-C compared with those with the CC allele. Thus, it is controversial whether this SNP is associated with plasma HDL-C levels in humans.

The inverse correlation between serum HDL-C level and atherosclerosis is well known:^{10,11} elevated HDL-C levels are associated with reduced risk of acute myocardial infarction (AMI).¹² In addition, EL activity may have an impact on plaque instability through inflammation. We hypothesized that the 584C/T SNP in the *LIPG* might affect the level of HDL-C and thus might be associated with AMI. To clarify and extend these results, we carried out a more comprehensive assessment of the 584C/T SNP in *LIPG* and its association with HDL-C levels and AMI in a Japanese case–control study.

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Table 1 Characteristics of the Study Population

	AMI (n=107)	Control (n=107)	p value
Age (years)	60.1±0.8	58.3±0.5	NS
Sex, M/F (male %)	83/24 (77.7%)	74/33 (69.2%)	NS
Hypertension, n (%)	45 (42.1%)	19 (17.8%)	<0.0005
Hypercholesterolemia, n (%)	37 (34.6%)	20 (18.7%)	<0.05
TC (mg/dl)	188.4±3.5	198.3±3.8	NS
TG (mg/dl)	125.0±5.9	144.9±12.6	NS
HDL-C (mg/dl)	45.0±1.4	50.3±1.6	<0.05
LDL-C (mg/dl)	118.4±3.2	119.1±3.6	NS
Diabetes mellitus, n (%)	37 (34.6%)	10 (9.3%)	<0.0001
Smoking, n (%)	73 (68.2%)	41 (38.3%)	<0.0001

Data are mean ± SEM or number (%) of patients.

For the patients with AMI, the value refers to the age at onset of myocardial infarction, not the current age.

AMI, acute myocardial infarction; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

Table 2 Prevalence of Genotypes and Allele Frequencies in Patients With AMI and Control Subjects

	AMI (n=107)	Control (n=107)	p value
TT+CT	1+36 (34.6%)	3+50 (49.5%)	<0.05
CC	70 (65.4%)	54 (50.5%)	
T allele frequency	0.18	0.26	<0.05
C allele frequency	0.82	0.74	

Data are number (%) of patients. Abbreviations see in Table 1.

Methods

Study Population

The study population comprised 107 patients with AMI and 107 control subjects, all of whom were Japanese. Consecutive patients with AMI aged <80 years were recruited from inpatients admitted to the coronary care unit of Kobe University Hospital (Kobe, Hyogo Prefecture) or Himeji Cardiovascular Center (Himeji, Hyogo Prefecture). The diagnosis of AMI was based on World Health Organization (WHO) criteria and confirmed by coronary angiography and left ventriculography.¹³ All patients had survived a confirmed myocardial infarction (MI).

The controls (n=33) were selected from inpatients of Kobe University Hospital in general medical wards without evidence of MI, angina pectoris (AP), stroke, or peripheral artery disease (PAD). Healthy controls (n=74) were recruited from employees in a heavy-industry factory (Akashi, Hyogo Prefecture) who had an annual medical examination. They completed a series of questionnaires that included details concerning history of AP, other cardiovascular diseases, hypertension (HT), hypercholesterolemia, diabetes mellitus (DM), and smoking habit. None had a documented history of MI, AP, stroke, or PAD. Physical examinations were performed, resting electrocardiograms (ECGs) were recorded, and blood samples were collected for blood chemistry. A cardiologist reviewed all ECGs and confirmed that no patients with MI were included among the control subjects.

Data were obtained for each subject from the official medical record at the time of entry, including current age (for control subjects), age at the time of the first event (for patients with AMI), sex, smoking history, blood pressure, lipid profiles, and DM status. Patients were considered smokers if they had a smoking index (years of smoking ×

amount of smoking [number of cigarettes] per day) >100 and current smoking status. They were considered to have HT if they met the criteria of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7)¹⁴ or already being treated with antihypertensive agents. They were considered to have hypercholesterolemia if they met the criteria of the Adult Treatment Panel III (ATP III)¹⁵ or were already under treatment for hypercholesterolemia. They were defined as having DM if they met the diagnostic criteria of the American Diabetes Association (ADA)¹⁶ or were already under treatment.

This study was approved by the Institutional Committees on Human Research at Kobe University Hospital and Himeji Cardiovascular Center, and all subjects gave written informed consent. The investigation conformed with the principles outlined in the Declaration of Helsinki.

Gene Analysis

Genomic DNA was extracted from peripheral whole blood using the Genomix Kit (Tarent, Trieste, Italy). Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism. *LIPG* genotypes were determined using mutagenic oligonucleotide primers with sequences 5'-CAT-GAGCTGAGATTGTTGTCAGTGC-3' and 5'-CAGTCA-ACCACAACCTACATTGGCGTCTTTCTCTCAT-3'. The 254-bp product was typed with restriction enzyme NdeI (New England Biolabs, Beverly, MA, USA). For the C allele, there was no site for NdeI; for the T allele, NdeI digestion produced 217- and 37-bp products.⁹ After restriction enzyme digestion of the amplified DNA, genotypes were identified by electrophoresis on 1.5% agarose gels and visualized with ethidium-bromide staining ultraviolet illumination.

Table 3 Lipids Levels With Respect to the 584C/T Genotype

	HDL-C (mg/dl)	p value	LDL-C (mg/dl)	p value	HDL-C/LDL-C	p value
<i>Control</i>						
TT+CT (n=53)	50.9±2.2	NS	117.6±4.6	NS	0.46±0.03	NS
CC (n=54)	49.7±2.3		120.5±5.6		0.44±0.03	
<i>AMI</i>						
TT+CT (n=37)	44.0±1.9	NS	113.2±5.3	NS	0.43±0.03	NS
CC (n=70)	45.5±1.9		121.2±4.0		0.42±0.03	
<i>Total</i>						
TT+CT (n=90)	47.6±1.5	NS	115.5±3.5	NS	0.45±0.02	NS
CC (n=124)	47.0±1.5		120.9±3.2		0.43±0.02	

Data are mean±SEM (mg/dl). Abbreviations see in Table 1.

Table 4 Univariate Risk Factors of AMI

	OR (95%CI)	p value
TT+CT genotype	0.54 (0.31–0.93)	0.03
HDL-C	0.97 (0.95–0.995)	0.02
Hypertension	3.36 (1.80–6.29)	0.0002
Hypercholesterolemia	2.30 (1.23–4.31)	0.009
Diabetes mellitus	5.13 (2.39–11.0)	<0.0001
Smoking	3.46 (1.97–6.07)	<0.0001

OR, odds ratio; CI, confidence interval. Other abbreviations see in Table 1.

Statistical Analysis

Data are presented as the mean±SEM. The difference between the groups was analyzed by unpaired t-test. The differences in frequencies of sex, smoking, HT, hypercholesterolemia, DM, and genotypes were analyzed by Fisher's exact test. The differences in genotypes were analyzed by the chi-square test, which was also used to test deviations of genotype distribution from the Hardy-Weinberg equilibrium and to determine allele or genotype frequencies between patients and control groups. Univariate analyses were conducted with logistic regression methods and multivariate analyses were conducted with multiple logistic regression methods. The estimation of conditioned relative risk (odds ratio (OR)) and 95% confidence interval (95%CI) were done using StatView 5.0 soft ware (SAS Institute Inc, Cary, NC, USA). In this study, statistical significance was assumed at $p<0.05$.

Results

Characteristics of the Subjects

Table 1 shows the prevalence of age, sex, and the 4 major risk factors for coronary artery disease among the AMI patients and control subjects. There were no significant differences in age or sex. A total of 68.2% of patients and 38.3% of the controls were smokers and there was a statistically significant difference. The frequencies of HT, hypercholes-

terolemia, and DM also differed significantly between the groups. In lipid profiles, there were no significant differences in total cholesterol and low-density lipoprotein-cholesterol (LDL-C) levels, but HDL-C levels of control subjects were significantly higher compared with the AMI patients.

584C/T Genotype Distribution and HDL-C Levels

Genotypic and allelic frequencies of the 584C/T SNP in *LIPG* are summarized in Table 2. The observed genotype frequencies in this study were in Hardy-Weinberg equilibrium and were similar to those reported in other Asian samples,^{17–19} and were in the range reported for Caucasian and African samples.^{9,17–21} Because of the small number of rare allele homozygotes, the TT and CT genotype groups were combined as T allele carriers for statistical analyses. The TT+CT genotype was significantly more frequent in the control group than in AMI patients. The T allele frequency was lower in AMI patients than in controls.

Table 3 shows the HDL-C, LDL-C, and their ratio with respect to the 584C/T genotype. We found no significant differences in HDL-C, LDL-C, and their ratio between the TT+CT and CC genotypes in the control group, AMI group, or the overall subjects, respectively. In the present study, the 584C/T SNP was not associated with HDL-C level.

ORs of Risk Factors and 584C/T Genotype

Univariate and multiple logistic regression analyses were conducted to identify variables predictive of AMI; in the univariate analyses these included TT+CT genotype, HDL-C level, HT, DM, hypercholesterolemia, and smoking status (Table 4) and the analyses showed that the TT+CT genotype and HDL-C level were associated with a decreased risk of AMI, with respective OR of 0.54 (95%CI 0.31–0.93) and 0.97 (95%CI 0.95–0.995). Otherwise, HT, DM, hypercholesterolemia, and smoking were found to be significantly associated with AMI.

Multivariate regression analyses (Table 5) showed that the

Table 5 Risk Factors Associated With AMI From Multiple Logistic Regression Analyses

	OR (95%CI)	p value	OR (95%CI)	p value
TT+CT genotype	0.52 (0.28–0.98)	0.04	0.61 (0.32–1.18)	0.14
Age	1.07 (1.02–1.12)	0.009	1.06 (1.01–1.11)	0.02
Sex (male)	3.16 (1.47–6.82)	0.003	1.41 (0.56–3.52)	0.46
Hypertension	3.06 (1.51–6.17)	0.002	3.38 (1.63–7.01)	0.001
Hypercholesterolemia	2.23 (1.10–4.53)	0.03	2.09 (1.01–4.35)	0.047
Diabetes mellitus	5.50 (2.40–12.6)	<0.0001	4.70 (2.00–11.0)	0.0004
Smoking			3.65 (1.68–7.92)	0.001

Abbreviations see in Tables 1, 4.

association of the TT+CT genotype with AMI was statistically significant and independent of other risk factors when age, sex, HT, hypercholesterolemia, and DM were included (OR, 0.52; 95%CI 0.28–0.98; $p=0.04$). However, when smoking status was included in this model, the association of the TT+CT genotype with AMI did not remain statistically significant (OR, 0.61; 95%CI 0.32–1.18; $p=0.14$).

Discussion

We did not detect a significant association between the 584C/T SNP in *LIPG* and the plasma level of HDL-C, which is consistent with the recent study by deLemos et al, who also failed to note an increased prevalence of the T allele in subjects with higher plasma HDL-C levels.⁸ Yamakawa-Kobayashi et al also demonstrated that the 584C/T SNP was not associated with HDL-C levels in healthy school-aged Japanese children.¹⁸ Our observation, however, conflicts with the results of the Lipoprotein and Coronary Atherosclerosis Study (LCAS), which noted significantly higher plasma HDL-C levels in T allele carriers.^{9,22} Discrepancies between the results of our study and those of the LCAS may be attributable to the fact that the latter study included 27% African Americans, whereas the present study sample was homogeneous, comprising all Japanese individuals.

In LCAS there was also a strong association of the 584C/T SNP with the ratios of HDL-C/LDL-C and apoA/apoB, and the mean plasma apoC-III concentration,⁹ but we did not detect a significant association between this SNP and the lipid ratio in the present study. Furthermore, Paradis et al demonstrated that plasma HDL₃-C levels of women homozygous for the mutated allele were significantly higher than in those carrying the wild-type allele.²⁰ We did not examine apolipoproteins and the HDL-C subfractions in the present study, because we could not obtain these data for the healthy subjects, so additional studies are needed to correlate the EL genotype with apolipoproteins and HDL-C subfractions in Japanese subjects.

Our study results demonstrated that the T allele frequency was lower in AMI cases than in the controls, suggesting that the 584C/T SNP is associated with AMI in Japanese. Multivariate regression analyses showed that the association of the T allele with AMI was statistically significant ($p=0.04$) and independent of other risk factors when age, sex, HT, hypercholesterolemia, and DM were included; however, when smoking status was included, the association of the T allele with AMI did not remain statistically significant ($p=0.14$). Mank-Seymour et al reported a trend for an association of the 584C/T SNP with fewer MI, but it was not significant after correction for multiple testing including smoking.¹⁷ Our results, together with theirs, strongly suggest an association may be present.

EL on the endothelial cell surface can promote monocyte adhesion to the vascular endothelium through the interaction with heparin sulfate proteoglycans.²³ Expression of EL by endothelial cells is upregulated by proinflammatory cytokines and is associated with increased EL-specific triglyceride and phospholipase activity in vitro.²⁴ Moreover, it has been reported that EL mediates the uptake and binding of HDL-C and regulates the selective uptake of HDL-C-associated cholesterol esters.²⁵ Another study reported that EL mediates the binding and uptake of LDL-C, as well as that of HDL-C.²⁶ Increased EL expression and activity at the site of chronic inflammation may also increase the uptake

of lipoprotein-associated cholesteryl ester into vascular cells. Taken together, EL may play an important role in lipid metabolism and energy homeostasis in states of acute and chronic inflammation.²⁴ Thus, the findings imply that EL may modulate the progression of vascular diseases, and may have indirect atherogenic actions in vivo through its effect on circulating HDL-C level and direct atherogenic actions through vascular wall processes such as monocyte recruitment and cholesterol uptake.²⁷

The 584C/T SNP results in an amino acid change (from a polar to nonpolar amino acid) that occurs in a relatively poorly conserved area of the EL sequence, far from important sites encoding for the catalytic activity of defining the tertiary structure of the enzyme.²⁸ The high prevalence of the minor allele also suggests that it may not have a large effect on plasma HDL-C levels in the general population.²⁰ We have no experimental evidence to support the notion that the 584C/T SNP influences the expression of *LIPG* and/or the activity of EL. Further in vivo and/or in vitro studies are required to determine the functional change in EL attributable to such a common nucleotide substitution in *LIPG*. The present study was observational, thus we do not show the cause–effect relationship of the 584C/T SNP with AMI. Additional studies are also warranted for investigating an association between *LIPG* genotype and cardiovascular disease.

Environmental factors, including smoking, are known to influence serum lipid levels, especially HDL-C levels.²⁹ We analyzed the relationship between the 584C/T SNP and serum HDL-C levels in nonsmokers and could not find any associations (data not shown). The discovery of EL was made as a result of performing a differential display of THP-1 cells after exposure to oxidized low-density lipoprotein (LDL).³ Cigarette smoke is a source of oxidative stress and induces oxidation of LDL. As regards the effect of smoking, some studies suggest that smoking is a stronger risk factor in women than in men,³⁰ but others have failed to corroborate this.³¹ Recently, Sasaki et al reported that smoking was related to a greater increase in the risk of coronary events in women than in men.³² In our multivariate analyses (Table 5), the association of sex (male) with AMI did not remain statistically significant ($p=0.46$) when smoking was included, suggesting that smoking is related to a greater increase in the risk of AMI in women than in men. On the other hand, the incidence of coronary events was 60% lower in women than in men among the J-LIT participants.³² We have repeated all of our analyses with subjects segregated by gender, however the sample of women was too small, the 584C/T SNP was associated with AMI in the same manner (data not shown).

Study Limitations

We acknowledge that the major limitation of this study is the small number of patients. An additional potential limitation is that we enrolled only survivors of AMI and control subjects by clinical criteria, without any imaging or exercise testing. This may create a potential bias in our results. There is a need to conduct a more extensive and prospective study, especially in nonsmokers, addressing the significance of this SNP and to determine whether the magnitude of this association is comparable in other ethnic groups. It is also important to know whether this genotype influences short- and long-term outcomes in patients with AMI.

In conclusion, the 584C/T SNP of *LIPG* was associated with AMI independently of HDL-C levels in a Japanese

population, suggesting that this SNP may be involved in the pathogenesis of AMI. Modulation of EL and/or *LIPG* may have a potentially beneficial effect on cardiovascular disease in humans.

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