

Potentially Protective Effects of the Ser447-Ter Mutation of the Lipoprotein Lipase Gene Against the Development of Coronary Artery Disease in Japanese Subjects Via a Beneficial Lipid Profile

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Several DNA variants at the lipoprotein lipase (LPL) gene locus have been found to be associated with the plasma lipid levels and the prevalence of coronary artery disease (CAD). In particular, the Ser447-termination (Ter) mutation at the exon 9 of the LPL gene has the potential to elevate the plasma high-density lipoprotein (HDL) levels, but it remains unknown in the Japanese population. The present study investigated 93 CAD patients and 96 age- and sex-matched healthy controls. The Ser447-Ter mutation was determined by polymerase chain reaction restriction fragment length polymorphism method. The allelic frequency of the Ser447-Ter mutation was 0.103 in all subjects. The Ser447-Ter (GG and CG) group was associated with significantly higher levels of plasma HDL-cholesterol ($p<0.001$) and lower levels of plasma triglyceride than the CC group ($p<0.02$). The peak particle size of low-density lipoprotein (LDL) was significantly larger in the Ser447-Ter (GG and CG) group than in CC group ($p<0.05$). The frequency of the Ser447-Ter genotype in GG and CG was significantly lower in CAD than in the controls (11.9% vs 26%, odds ratio=0.38; 95% confidence interval, 0.18–0.81; $p<0.02$). These results suggest that the Ser447-Ter mutation of the LPL gene is associated with high plasma HDL-cholesterol levels, low plasma triglyceride levels and a larger LDL particle size. This mutation may have a protective effect against the development of CAD via its favorable lipoprotein profile. (*Jpn Circ J* 2001; 65: 310–314)

Key Words: Coronary artery disease; High-density lipoprotein; Lipoprotein lipase; Ser447-Ter mutation

Lipoprotein lipase (LPL) is an essential enzyme for clearing triglyceride (TG)-rich lipoproteins from the circulation to the liver and peripheral tissues by hydrolyzing TG present in chylomicrons and very low density lipoproteins (VLDL). In addition, LPL modulates plasma high-density lipoprotein (HDL)-cholesterol by the exchange of lipids between TG-rich lipoproteins and HDL.^{1,2} Because LPL plays an important role in lipoprotein metabolism, it will have an influence on the development of coronary artery disease (CAD).

The human LPL gene has been cloned and localized in chromosome 8p22 and spans approximately 30 kb. The first 9 of its 10 exons code for a 475 amino acid protein, including 27 amino acids for a signal peptide that is cleaved post translationally to yield mature LPL with a molecular weight of approximately 60 kd.^{3,4} Several restriction fragment length polymorphisms (RFLPs) have been identified at the LPL locus^{4,5} and have been reported to be associated with plasma lipid levels and the prevalence of CAD. The most closely related association is that between the H2 (presence of restriction site) allele of Hind III RFLPs (intron 8) and increased TG, decreased HDL-cholesterol levels and the prevalence of CAD.^{6–11} Although less consistently, the P2

(presence of restriction site) allele of the Pvu II RFLP (intron 6) has been also found to be associated with high TG and low HDL-cholesterol levels.^{1–13} The first common mutation in a coding sequence was reported to be the Ser447 termination (Ter) caused by a C-G transversion at nucleotide 1595 (exon 9), resulting in a premature termination codon.^{14,15} In some previous studies, this Ser 447 Ter mutation, possessing a strong linkage disequilibrium with H1 (absence of restriction site) and P1 (absence of restriction site) alleles,^{6,9} was not associated with the plasma lipid profile or with CAD.^{6,16} Recently, however, this mutation was reported to have significant effects on HDL-cholesterol levels and might a possible protective role against the development of CAD.^{17,18} Although evidence for the relationship between LPL genotypes and the plasma lipid profile has been accumulated in Caucasians, it is less clearly established in Japanese subjects.

The present study investigated the effects of the Ser447-Ter mutation on the variation of lipid profiles and low-density lipoprotein (LDL) particle size, as well as the differences in genotype frequencies of the Ser447-Ter mutation between patients with angiographically proven CAD and healthy controls in the Japanese population.

Methods

Subjects

We investigated 93 consecutive patients with CAD who underwent coronary arteriography at Juntendo University

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Hospital and 96 healthy subjects who had no past history of hospitalization, resting ECG abnormalities or typical anginal pain as age- and sex-matched controls. We defined the CAD patients as those who had greater than 50% stenosis in at least one of the major branches of the coronary artery tree. Subjects who were taking lipid-lowering drugs were excluded. All subjects gave informed consent.

Isolation of DNA

Genomic DNA was isolated from white blood cells by using a DNA Extractor WB Kit (Wako-Junyaku Co, Osaka, Japan). The isolated DNA was redissolved in Tris-EDTA buffer (10mmol/L Tris, 1 mmol/L EDTA, pH7.6) and stored at 4°C until analysis.

Oligonucleotides

Primers for polymerase chain reaction (PCR) were synthesized by Nihon Bio Service Co Saitama, Japan. The primer sequences were derived from published data^{6,17,18}. Primer sets were as follows: Ser447-Ter mutation 5' upstream, 5'-TACACTAGCAATGRTCTAGGTGA-3' and 3' downstream, 5'-TCAGCTTTAGCCCAGAATGC-3'.

Amplification of Genomic DNA

Genomic DNA (0.2 µg) was amplified in a 25-µl reaction mixture containing 10mmol/L Tris-HCL (pH 8.3); 50 mmol/L KCl; 200 µmol/L each of dATP, dCTP, dGTP, and dTTP; 4mmol/L MgCl₂; 0.5 µmol/L of each primer; and 0.5 U Taq DNA polymerase. The mixture was overlaid with mineral oil. All tubes, pipette tips and buffers were autoclaved. The amplification cycle was performed on a Terminal Sequencer TSR 300 (Iwaki Glass Co Ltd) and entailed 300 s of denaturation at 94°C followed by 35 cycles of 60 s at 94°C, 120 s at 52°C, and 180 s at 72°C. This was followed by 300 s of extension at 72°C^{6,17,18}.

Digestion and Electrophoresis

Amplified products were digested overnight at 37°C with Mnl I according to the manufacturer's recommendations (Takara Suzou, Boehringer Mannheim). After separation on 2% agarose gels, the resulting fragments were stained with ethidium bromide and visualized with an UV transilluminator. The PCR product of 488 bp contains 2 Mnl I restriction sites, of which one is a polymorphic site indicating the Ser447-Ter mutation. Digestion of the PCR product with Mnl I resulted in 3 fragments of 290, 250, and 200 bp^{17,18}. The identified genotypes were named according to the presence or absence of the enzyme restriction sites, so Ser447-Ter GG, CG, and CC are homozygote for the presence of the site, heterozygote for the presence and absence of the site, and homozygote for the absence of the site, respectively (Fig 1).

Lipid Analysis

Venous blood samples were obtained by venipuncture with Vacutainer tubes after a 12 h fasting period. Total cholesterol, HDL cholesterol, and TG levels were measured by standard enzymatic methods. LDL-cholesterol was calculated by the Friedewald formula.

Determination of LDL-Peak Particle Diameter

Nondenaturing 2.5–16% polyacrylamide gradient-gel electrophoresis was performed on whole plasma as previously described by Krauss et al¹⁹. Aliquots of 30 ml were applied on ready-made gels (REALGEL PLATE; Biocraft,

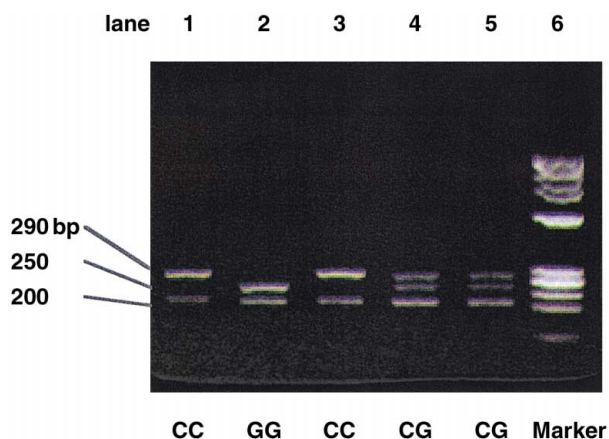


Fig 1. 2% agarose gel electrophoresis cutting with Mnl I. Lane 6, DNA molecular weight marker. Lanes 1–5, PCR product digested with Mnl I. Homozygosity for presence (lane 2), heterozygosity (lane 4, 5), and homozygosity for absence (lane 1, 3) of restriction site are shown.

Tokyo, Japan) in a final concentration of 16% sucrose and 0.2% bromphenol blue. After a 20-min pre-run, electrophoresis was performed at 150 V for 18–20 h with buffer in a final concentration of 0.09 mol/L Tris aminomethane/0.08 mol/L boric acid/0.0025 mol/L EDTA disodium salt at pH 8.3. Each sample and standard LDL (25.5 nm), which was obtained from a healthy young adult male and already evaluated by electric microscope, were stained with 0.04% oil-red O/60% ethanol over a 24 h period, and protein standards of known diameter, such as thyroglobulin, apo-ferritin and catalase, were stained with 0.25% coomassie brilliant blue solution. Judging from the mobility of each sample and standard in each scan on a densitometric image analyzer, the estimated diameter for the major peak was calculated and identified as the LDL-peak particle diameter (LDL-PPD).

Statistical Analysis

Alleles and genotypic frequencies for each of 2 polymorphic loci were estimated by the gene-counting method, and chi-squared analysis was performed to confirm that all groups in the study were in Hardy-Weinberg equilibrium.

Allele and genotype frequencies were analyzed using a chi-squared contingency table (2×2 table). However, when one of the sample sizes was less than 5, the Fischer's exact 2-tail probability was determined.

All results were expressed as mean ± standard deviation (SD). Statistical analysis of serum lipid levels and each parameters was performed by Student's t test.

Results

Clinical Characteristics of Subjects (Table 1)

The body mass index was significantly higher in the CAD group than in the controls (24.1 ± 3 vs 23.1 ± 2; $p < 0.05$). Subjects with CAD had significantly higher levels of TG (169 ± 113 vs 128 ± 89 mg/dl, $p < 0.01$) and lower levels of HDL-cholesterol (40 ± 12 vs 61 ± 16 mg/dl, $p < 0.0001$) compared with the controls. In this study, total cholesterol levels were lower in CAD patients than in the controls (201 ± 36 vs 213 ± 32 mg/dl, $p < 0.05$).

Genetic Characteristics of Subjects

The genotype and allele frequencies of the Ser447-Ter

Table 1 Clinical Characteristics of Patients With Coronary Artery Disease (CAD) and Control Subjects

	CAD (n=93)	Controls (n=96)	p value
Male (%)	82 (88%)	76 (79%)	NS
Age (years)	61±8	59±4	NS
BMI (kg/m ²)	24.1±3	23.1±2	<0.05
TCHO (mg/dl)	201±36	213±32	<0.05
TG (mg/dl)	169±113	128±89	<0.01
HDL-C (mg/dl)	40±12	61±16	<0.0001
FBS (mg/dl)	104±32	96±12	<0.05

Values are mean±SD. BMI, body mass index; TCHO, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; FBS, fasting plasma glucose.

Table 2 Genotypes and Allele Frequencies of Ser 447-Ter Mutation

	n	Genotype			Allele	
		GG	CG	CC	+	-
All	189	3	33	159	0.103	0.897
Control	96	2	23	71	0.142	0.858*
CAD	93	1	10	82	0.065	0.935*

The frequency of Ser447-Ter genotype of GG and CG was significantly lower in CAD patients than in the Controls. Odds ratio: 0.38 (95% confidence interval: 0.18–0.81), $p<0.02$; * $p<0.05$.

Table 3 Association Between the Ser 447-Ter Mutation and Plasma Lipids

	GG+CG	CC	p value
TCHO (mg/dl)	211±30	206±35	NS
TG (mg/dl)	105±46	158±110	<0.05
HDL-C (mg/dl)	59±18	48±16	<0.001
LDL-C (mg/dl)	129±28	127±30	NS
LDL-PPD (nm)	26.23±0.78	25.70±0.94	<0.05

PPD, peak particle diameter.

mutation in the LPL gene are shown in Table 2. In the total group, 36 of 189 subjects carried the Ser447-Ter mutation. The allelic frequency of the total subjects was 0.103, which was similar to previous reports on comparable populations.^{7,17} Moreover, the allelic frequency of Ser447-Ter was significantly lower in CAD patients than in the controls (0.065 vs 0.142, $p<0.05$). The genotype distribution was in accordance with Hardy-Weinberg expectations.

Association of Ser447-Ter Mutation With CAD and the Plasma Lipid Profile

The frequency of the Ser447-Ter genotype of GG and CG was significantly lower in CAD patients than in the controls (11.9% vs 26%, $p<0.02$) (Table 2). The odds ratio (OR) of the Ser (GG and CG) group for CAD was estimated as 0.38 (95% confidence interval (CI), 0.18–0.81; $p<0.02$).

Comparisons of plasma lipid levels based on genotypes at the LPL gene locus are presented in Table 3. In the analysis of the total subjects, the Ser (GG and CG) group was associated with significantly higher levels of HDL-cholesterol (59±18 vs 48±16 mg/dl, $p<0.001$) and lower levels of plasma TG (105±46 vs 158±110 mg/dl, $p=0.014$) compared with the CC group. The LDL-PPD was significantly larger in the Ser (GG and CG) group than in the CC group (26.23±0.78 vs 25.70±0.94 nm, $p=0.024$). The analysis of only the male or control subjects did not change the significant association of this Ser447-Ter mutation with the plasma lipid profile and LDL particle size.

Discussion

The present study demonstrated that the Ser447-Ter mutation in the LPL gene modulated lipoprotein metabolism, resulting in increased levels of HDL-cholesterol, decreased levels of plasma TG and low prevalence of small, dense LDL in a population of Japanese patients with CAD and normal control subjects. Moreover, the OR=0.38 (95% CI, 0.18–0.81) of the Ser447-Ter mutation for CAD indicates this mutation might have a protective role against the development of CAD.

Kozaki et al originally found an approximately 2-fold increase in the enzymatic activity of LPL resulting from the Ser447-Ter mutation at the carboxy terminal of LPL transfected and expressed in vitro.²⁰ However, the functional significance of the carboxy terminal of LPL remains unclear. In other studies of LPL expression in vitro, conflicting results that demonstrated that Ser447-Ter mutation might have either no or an inverse effect on LPL function may have been caused by different methodology. In contrast, most of the previous clinical studies have demonstrated that the Ser447-Ter mutation is associated with increased levels of HDL-cholesterol and decreased levels of plasma TG, which is consistent with our results, in normolipidemic subjects and hypercholesterolemic subjects.^{7,18,21} Although LPL activity in postheparin plasma was not measured in our study, this plasma lipid profile is considered to be induced by the increased enzymatic activity of LPL in the carriers of the Ser447-Ter mutation, based on the initial observation

in vitro.¹⁷ In fact, one of the large scale clinical studies was the substudy of the Regression Growth Evaluation Statin Study (REGRESS) presenting increased postheparin LPL activity by the Ser447-Ter mutation followed by increased HDL-cholesterol and decreased plasma TG levels in CAD patients.¹⁷ In other studies, however, the Ser447-Ter mutation had no significant association with the plasma lipid profile. One reason for these discrepancies may be the different categories of subjects, such as hypertriglyceremia²² and familial combined hyperlipidemia.²³ In origin, the Ser447-Ter mutation would be less prevalent in the hypertriglyceremic subjects, therefore other metabolic factors might be involved.

The present study also demonstrated that the peak particle size of LDL in carriers with the Ser447-Ter mutation was significantly larger than that in non-carriers, which indicates that the potential increased LPL activity by the Ser447-Ter mutation might prevent production of small, dense, atherogenic LDL. Many factors have been reported to be responsible for determining the peak particle size of LDL, including LPL,²⁴ hepatic lipase^{25,26} and various metabolic states, such as diabetes mellitus,²⁷ insulin resistance syndrome^{28–30} and obesity.²⁹ Among these factors, the enzymatic activity of LPL might be one of the strongest for determining the peak particle size of LDL. It is not clear, however, whether LPL activity affects the LDL particle size directly or indirectly by influencing metabolism of plasma TG and HDL-cholesterol³¹ and further investigation of this is necessary.

In our analysis of the association of the Ser447-Ter mutation with CAD, its frequency was significantly lower in the CAD patients than in the controls (11.9% vs 26%, $p < 0.02$) and the OR of this mutation for CAD was 0.38, representing a significantly low risk of CAD. The present results are the first demonstration that the Ser447-Ter mutation may have a protective effect against the development of CAD by its favorable plasma lipid profile; that is, decreased atherogenic effects of both the TG-rich remnant lipoproteins and small, dense LDL and an enhanced anti-atherogenic effect of HDL-cholesterol. Recently, decreased LPL activity has been considered to contribute to the plasma lipid profile and atherogenicity in insulin-resistant metabolism.^{32–34} Furthermore, increased LPL activity is reported to modify lipoprotein metabolism and prevent the development of atherosclerosis in transgenic mice overexpressing the human LPL transgene by cross-breeding with LDL receptor knockout atherogenic mice.³⁵ These results suggest that LPL activity may be one of the important determinant factors for lipoprotein metabolism and atherogenesis. Therefore Ser447-Ter mutation may relate to increased LPL activity, resulting in an anti-atherogenic plasma lipid profile. The present study further supports the hypothesis of the anti-atherogenic role of the Ser447-Ter mutation.

In conclusion, this study demonstrated that the Ser447-Ter mutation at the LPL locus modulates lipoprotein metabolism, resulting in increased levels of HDL-cholesterol, decreased levels of plasma TG and a low prevalence of small, dense LDL in Japanese subjects. The Ser447-Ter mutation may have a protective role against the development of CAD.

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