Original Article

The S447X Polymorphism of Lipoprotein Lipase: Effect on the Incidence of Premature Coronary Disease and on Plasma Lipids

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Summary

Objective: **The objective of this study was to evaluate the effect of polymorphism S447X on plasma lipids of patients with premature coronary artery disease (CAD).**

Methods: **Plasma lipids and genotypes were determined in 2 groups: 313 patients with premature CAD (<55 years of age) and 150 controls without CAD.**

Results: **Frequency of the S447X polymorphism was 18% in patients with CAD and 23% in the control group. The S447X polymorphism of lipoprotein lipase is related to a decrease in plasma triglyceride concentrations in male patients with CAD, but this correlation is not observed in female patients.**

Conclusion: **The presence of the S447X lipoprotein lipase polymorphism was not associated with the incidence of CAD.**

Key words: **Lipoprotein lipase mutations, cholesterol, lipoproteins, triglycerides.**

Introduction

Lipoprotein lipase is the enzyme that catalyzes the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL). This reaction occurs on the endothelial surface of blood capillaries, where the lipase is attached through molecules of proteoglycans. Triglyceride-rich lipoproteins bind with lipase through apolipoprotein (apo) CII present on the lipoprotein surface, which also stimulates enzymatic activity. The products of triglyceride degradation - fatty acids and glycerol - are absorbed by tissues, such as adipose and muscle tissues, where they are esterified and stored[1]. This mechanism enables the organism to store and make use of its most important energy source: fats.

A decline in lipoprotein lipase activity can influence plasma lipid concentrations, causing various degrees of either isolated hypertriglyceridemia or in association with hypercholesterolemia[2]. More than 60 different mutations of the lipoprotein lipase gene have been described to date, and they can lead to a reduction in enzyme synthesis and activity. Additionally, hypertriglyceridemia causes a decrease in HDL[3,4]. This happens by the law of mass action and by the presence of lipid transfer proteins, such as cholesterol ester transfer protein (CETP) that tends to transfer cholesterol esters from HDL to VLDL. Hence, mutations of the lipoprotein lipase gene that affect enzyme activity may contribute to the risk of CAD by the impact on plasma lipid concentrations[5,6].

The lipoprotein lipase gene (LPL) is located in the chromosome 8p22[7]. It comprises 10 exons, interrupted by 9 introns, with a molecular mass of approximately 30 Kb, and it codifies a protein with 475 amino-acids[8,9]. Of the several mutations described in the LPL gene, Asp9Asn, Asn291Ser, and S447X are the most important because of their greater frequency and influence on susceptibility to atherosclerosis[3,10,11].

The S447X polymorphism is one of the most frequent among the various proteins involved in intravascular metabolism of lipids, and shows a 17 to 22% incidence in the Caucasian population[12-14]. It occurs in exon 9 of the LPL gene, where cytosine (C) is replaced by guanine (G) in position 1595[15,16]. This results in the exchange of a serine for a terminal codon and the suppression of the last two amino acids, serine and glycine, in position 447 of the protein[11]. The effect of the polymorphism on lipoprotein lipase activity is controversial. When tested in vitro, this activity has been described both as markedly increased[6] and increased[17] and as slightly decreased[18], but in the majority of studies it was unchanged[19,20].

Some studies indicate that the S447X variant may be favorable to catabolism of VLDL, diminishing fasting concentrations of triglycerides[6,13,14,21,22] with a slight elevation of HDL-cholesterol concentrations[13,14]. Hypertriglyceridemia associated with a lowered HDL concentration represents an important risk factor in CAD development.

Therefore, the S447X polymorphism could play an anti-atherogenic role here. Bearing in mind the high frequency of this variant and the heterogeneous results of the study in other populations, we considered it important to evaluate its impact both on plasma lipids and on the incidence of

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premature CAD in a sample of the Brazilian population. In this population, there is intense racial miscegenation, not observed in other populations where the polymorphism has been described.

Methods

Subjects - One hundred and fifty individuals were analyzed with no clinical history of CAD (no CAD): 60 male and 90 female were employees of the Heart Institute of the Medical School of the Universidade de São Paulo (FMUSP), and 313 were patients with premature CAD, defined as the clinical manifestation of the disease before 55 years of age, with 182 males and 131 females selected in the outpatient clinic of the Heart Institute.

The group with no CAD enrolled individuals with normal clinical, cardiologic, and resting and stress electrocardiographic assessments. The CAD group enrolled patients with arteriographically documented disease, or those with a myocardial infarct episode diagnosed by precordial pain and characteristic electrocardiographic changes.

Patients with rheumatic, hepatic, renal, endocrine and neurological diseases, as well as pregnant women, were excluded from this group. All subjects were volunteers who had signed an Informed Consent Form.

Biochemical analyses - Triglyceride, cholesterol, and HDL cholesterol analyses were performed using automated Cobas Integra 700, Roche, by means of enzymatic colorimetric tests, specific of this equipment. LDL-cholesterol levels were calculated utilizing the formula of Friedewald et al[23].

Genotyping - Genomic DNA was isolated from peripheral blood according to the method of Miller et al[24]. Red blood cell lysis was performed by a mixture of ammonium chloride 0.144 M and ammonium bicarbonate 0.01 M in a quantity equal to the blood volume . After centrifugation, cell nuclei were lysed with 3.0 mL buffer of Tris-HCl 10 mM pH 8.0; NaCl 400 mM; Na2EDTA 0.5 M pH 8.0; 200 μL SDS 10%; 500 μL buffer with proteinase K (10 μL of proteinase K; 50 μL of SDS 10%; 2 μL of Na2EDTA 0.5 M pH 8; 0.488 mL of distilled water). Proteins were removed by saline precipitation with 1 mL of NaCl 6 M. The DNA present in the supernatant was isolated, purified by precipitation in absolute ethanol; it then underwent resuspension with 100 μL of buffer (Tris-HCl 10 mM; EDTA 1 mM pH 8) and was maintained at -20oC. In the results, the S447X variant was presented with the nomenclature of the gene, that is, LPL C1595G. The polymorphism was analyzed by amplification of the genomic DNA by means of the polymerase chain reaction (PCR), followed by the restriction fragment length polymorphism (RFLP) technique. Initiators possessing the oligonucleotidic sequences were described by Groenemeijer et al[13].

The product of the PCR reaction (488 pb) was submitted to the action of the restriction enzyme Mnl I, 10 U of the enzyme (MBI Fermentas), at 37°C for 18 hours, and underwent 2.5% agarose gel electrophoresis in order to identify the products of digestion. Presence of the 288 and 200 pb fragments indicate homozygosis of the wild allele (CC), while the presence of the 288, 200, and 50 pb products indicates homozygosis of the mutant allele (GG), and the presence of 288, 238, 200, and 50 pb products indicates heterozygosis (CG genotype). The S447X polymorphism represents the GG and CG genotypes. Documentation of the gels was performed using the Eagle Eye system.

Statistical analysis - Descriptive analysis was used to evaluate continuous variables. For categorical variables, incidence tables were used. Values with asymmetric distributions and great variability underwent logarithmic transformation ($\log_{10}$) for subsequent application of statistical analyses.

The chi-square test was employed to verify Hardy-Weinberg equilibrium (HWE) in the groups studied (no-CAD and CAD). In order to assess the effect of polymorphism in the variation of the biochemical parameters analyzed, Student's t test was used to compare variables with two categories.

Covariance analysis (ANCOVA) was used to examine the influence of age, smoking, diabetes, hypertension, and family history variables on lipid levels of CC versus CG+GG genotypes.

Multivariate logistic regression was used to analyze the influence of risk factors for CAD with the following independent variables: age >40 years, BMI >25 Kg/m^2, smoking, arterial hypertension, diabetes, family history, triglyceride concentrations >200 mg/dL, total cholesterol >238 mg/dL, LDL-c >159 mg/dL, and HDL-c <40 mg/dL. Reference values are those suggested by the Guidelines of the Brazilian Society of Cardiology, 2001[25]. CAD was the dependent variable.

Significance level adopted for the statistical tests was 5% ($p < 0.05$). Statistical analyses were performed using the SAS for Windows (Statistical Analysis System) program, version 6.12. (SAS Institute Inc, 1989-1996, Cary, NC, USA).

Results

Frequency of LPL C1595G polymorphism - Table 1 shows that there was no difference between the no-CAD and CAD groups in incidence of the LPL C1595G variant. As to the distribution of genotypes CC, CG, and GG, the no-CAD was in Hardy-Weinberg equilibrium; the same was not true for the CAD group. Frequency of genotypes CC, CG, and GG was no different on comparison of the no-CAD and the CAD groups.

Table 1 also shows the evaluation of frequency of the polymorphism and genotype according to sex. No differences appeared in these groups between the two sexes, either in the no-CAD or the CAD groups.

LPL C1595G polymorphism and plasma lipids - Table 2 shows that in both the no-CAD and CAD groups there were no differences between individuals with and without the polymorphism as to plasma concentrations of triglycerides, total cholesterol, LDL-c, and HDL-c.

In female patients there was no difference between patients with and without the polymorphism in levels of

Table 1 - Distribution of genotypes and relative frequencies of LPL C1595G polymorphism alleles in no-CAD and CAD groups

LPL C1595G	CAD n (%)	CAD n (%)
Total		
CC (wild)	115 (76)	257 (82)
CG (S447X polymorphism)	34 (23)	47 (15)
GG (S447X polymorphism)	1 (1)	9 (3)
Hardy-Weinberg Equilibrium	p>0,05	p<0,05
Relative frequencies of alleles		
Allele C	0.88	0.90
Allele G	0.12	0.10
Female		
CC (wild)	69 (77)	109 (83)
CG (S447X polymorphism)	20 (22)	17 (13)
GG (S447X polymorphism)	1 (1)	5 (4)
Hardy-Weinberg Equilibrium	p>0,05	p<0,05
Relative frequencies of alleles		
Allele C	0.88	0.89
Allele G	0.12	0.11
Male		
CC (wild)	46 (77)	148 (81)
CG (S447X polymorphism)	14 (23)	30 (17)
GG (S447X polymorphism)	0 (0)	4 (2)
Hardy-Weinberg Equilibrium	p>0,05	p>0,05
Relative frequencies of alleles		
Allele C	0.88	0.90
Allele G	0.12	0.10

Due to the low frequency, some tests were performed considering CG and GG together in one group. Fisher's test: females (Control vs CAD (CC vs CG vs GG) p=0,140; males (Control vs CAD) (CC vs CG vs GG) p=0,321; Chi square: females (Control vs CAD) (CC vs CG+GG) p=0,228; males (Control vs CAD) (CC vs CG+GG) p=0,433; control (females vs male) (CC vs CG+GG) p=1,0; CAD (female vs male) (CC vs CG+GG) p=0,667; CAD (females vs male) (CC vs CG vs GG) p=0,509. CAD = coronary artery disease.

triglycerides, total cholesterol, LDL-c, and HDL-c, in both the no-CAD and CAD groups. In male patients, triglyceride levels were lower in the CAD group in the presence of polymorphism compared to the patients without the polymorphism. However, there were no differences as to the levels of total cholesterol and fractions.

Genotypes and plasma lipids: covariance analysis - Bearing in mind that it was only in male patients from the CAD group that the presence of the polymorphism was related to lower levels of triglycerides, we analyzed the possible influence of variables such as age, smoking habits, diabetes, hypertension, and family history on plasma lipid concentrations using covariance analysis (ANCOVA).

Plasma lipid concentrations were similar among the wild and S447X variant groups when classic risk factors for CAD were not considered for the control group (both sexes) and the CAD group (females). The results presented on Table 3, however, confirm that there was a difference in plasma concentrations of triglycerides when the wild genotype was compared to the S447X genotype in male patients of the CAD group. Analyses were corrected considering classic risk factors (age, smoking, diabetes, hypertension, and family history).

S447X polymorphism as a protective factor against CAD: multivariate regression analysis Multivariate logistic regression analysis was performed in order to enhance the influence of risk factors and to determine the relative risk for genotypes in the presence of CAD.

Risk factors included in this analysis were triglycerides, total cholesterol, LDL-c, HDLc, smoking habits, age, and family history. Hypertension and diabetes risk factors were excluded because their low incidence in the control group resulted in an overestimated relative risk. Race and BMI variables were excluded since they did not distinguish CAD cases from the controls.

According to the results shown in Table 4, we see that the variables selected by multivariate logistic regression analysis as possible candidates for prognostic factors were: age (OR=9.05; p<0.001); smoking (OR=9.02; p<0.001); family history (OR=2.27; p<0.001); triglycerides (OR=8.06; p<0.001); total cholesterol (OR=5.38; p=0.003); and HDL-c (OR=2.58; p<0.001). The presence of S447X polymorphism genotypes was not considered a protective factor against CAD.

The results presented in Table 4 indicate that in females the variables selected by multivariate logistic regression analysis as possible candidates for prognostic factors were: age (OR=11.53; p<0.001); smoking (OR=6.86; p<0.001), and triglycerides (OR=11.14; p<0.023). The presence of the S447X variant was not considered a protective factor against CAD in females.

The results shown in Table 4 indicate that in males the variables selected by multivariate logistic regression analysis as possible candidates for prognostic factors were: age (OR=9.21; p<0.001); smoking (OR=13.48; p<0.001); triglycerides (OR=7.43; p=0.003), and HDL-c (OR=3.21; p=0.001). The presence of the S447X polymorphism was not considered a protective factor against CAD in males.

Discussion

Lipoprotein lipase activity is the central event of the catabolic process of lipoproteins in plasma circulation. In catalyzing the hydrolysis of VLDL and chylomicron triglycerides, lipoprotein lipase becomes the primary determinant of the plasma concentration, not only of VLDL and chylomicrons, but also of the remaining lipoproteins and of HDL. In the case of HDL, the accumulation of VLDL because of decreased activity of the enzyme results in a transfer of cholesterol from the HDL to the VLDL by transfer

Table 2 - Distribuition of plasma concentration of lipids in the control and CAD groups per S447X polymorphism

LIPIDS (mg/dL)	Wild Mean ± SD	S447X polymorphism Mean ± DP	Student t test
Control	n=115	n=35	p
Triglycerides	102 ± 44 (37-200)	93 ± 44 (36-200)	0.204
Total cholesterol	186 ± 36 (103-239)	179 ± 31 (119-231)	0.465
LDL-c	121 ± 31 (36-175)	111 ± 26 (56-164)	0.162
HDL-c	48 ± 10 (31-83)	49 ± 12 (30-75)	0.672
CAD	n=257	n=56	
Triglycerides	180 ± 127 (42-997)	153 ± 91 (46-480)	0.114
Total cholesterol	211 ± 54 (76-594)	214 ± 52 (138-358)	0.666
LDL-c	136 ± 45 (35-345)	143 ± 47 (65-284)	0.272
HDL-c	41 ± 11 (14-75)	41 ± 11 (23-70)	0.640

Variables submitted to logarithmic transformation (log10). n=number of individuals; LDL-c = low-density lipoprotein cholesterol; HDL-c = high-density lipoprotein cholesterol; CAD = coronary artery disease.

proteins. This causes the "see saw" in which an increase in triglyceridemia leads to a decrease in HDL cholesterol, and a decrease of triglyceridemia leads to an increase of HDL cholesterol. Therefore, polymorphisms or mutations that affect this enzyme may influence the plasma concentration and the metabolism of all lipoproteins, thus generating risk factors for CAD.

The S447X polymorphism occurs in the C terminal region of the lipoprotein lipase, a region where binding takes place between lipoprotein lipase receptors and lipoproteins. Deletion of the last two amino acids may potentiate this mode of action and increase the removal of triglycerides from chylomicrons and VLDL; this results in a decline of the triglyceride content[26,27]. However, the precise mode of action has not yet been fully clarified.

This study represents the first report on the frequency of the lipoprotein lipase S447X variant in patients with premature CAD in the Brazilian population, characterized by intense racial miscegenation. Our study has the intrinsic limitation of a cross-section design, but prospective studies in premature disease are difficult to carry out because of the necessarily prolonged follow-up period.

In this study, the frequency of the S447X polymorphism was 23%. This incidence was similar to that which had been previously reported in Caucasian populations of Europe, Australia, and North America[4,28,19]. The frequency of this polymorphism in patients with CAD was 18%, similar to what had been described in Caucasian populations with CAD[12,19,28]. On the other hand, male Caucasian patients with hypertriglyceridemia showed a 10% frequency [15,16] and Chinese hypertriglyceridemic patients showed a 9% frequency[11]. The different frequencies found may be related to racial factors and inclusion criteria used to defined the control group.

In this study we also analyzed the effects of the lipoprotein lipase S447X variant on plasma lipids in patients with premature CAD. Epidemiological data showed that the incidence of CAD differs between female and male patients. This fact may be a result of exposure to risk factors involved in the disease and hormonal differences. In women, the presence of the S447X polymorphism had no effect on plasma lipid concentrations, as was seen in the results described in European populations[21,27,29]. In men, the presence of this polymorphism in the CAD group was associated with lower plasma concentrations of triglycerides in comparison with those of patients with CAD without the variant. Similar results were described in the male European Caucasian population with CAD[13,14,21,29]. Conversely, other studies showed that the presence of this variant did not change the plasma concentrations of triglycerides[19,20].

The association between male sex, a reduction in triglyceride concentration, and the S447X polymorphism may be related to the actions of testosterone. Lowered concentrations of testosterone are related to a decrease in activity of lipoprotein lipase[30], and in men with hypogonadism, testosterone replacement leads to an increase in lipoprotein lipase activity[31]. On the other hand, estrogen diminishes the activity of lipoprotein lipase[32] by means of deregulation of gene transcription[33] or because of a possible post-transcriptional modification of the protein[34]. It is interesting to note that a decrease in triglycerides concentration tends to accompany an increase in HDL-c, which did not happen in our study.

This is possibly due to the fact that the range of triglyceride levels observed in the study groups and the percentage of triglyceridemia reduction determined by the polymorphism

Table 3 - Covariance analysis of genotype and plasma lipids corrected for classic risk factors

Group		Lipids*	p
Control n=150	Wild vs. S447X polymorphism	Triglycerides	0.748
		Total cholesterol	0.423
		LDL-c	0.994
		HDL-c	0.319
Control females n=90	Wild vs. S447X polymorphism	Triglycerides	0.661
		Total cholesterol	0.340
		LDL-c	0.452
		HDL-c	0.328
Control males n=60	Wild vs. S447X polymorphism	Triglycerides	0.300
		Total cholesterol	0.864
		LDL-c	0.801
		HDL-c	0.654
CAD n=313	Wild vs. S447X polymorphism	Triglycerides	0.204
		Total cholesterol	0.326
		LDL-c	0.125
		HDL-c	0.563
CAD females n=131	Wild vs. S447X polymorphism	Triglycerides	0.634
		Total cholesterol	0.463
		LDL-c	0.840
		HDL-c	0.362
CAD males n=182	Wild vs. S447X polymorphism	Triglycerides	0.030
		Total cholesterol	0.606
		LDL-c	0.113
		HDL-c	0.755

Variables which underwent logarithmic transformation (log10). n=number of individuals; LDL-c = low-density lipoprotein cholesterol; HDL-c = high-density lipoprotein cholesterol; CAD = coronary artery disease.

were not sufficient to cause an increase in HDL.

By multivariate regression analysis, our results showed that the presence of the S447X polymorphism was not an independent protection factor against CAD in females. In fact, in agreement with our data, this polymorphism was not considered a protective factor in European women[21,27]. In our study, the presence of the S447X variant in males also did not appear as an independent protection factor against CAD. This is a controversial aspect in literature, as there are some studies that indicate that the polymorphism could confer anti-CAD protection[14,21,27,35], while others do not confirm these findings[35,36]. The protective or anti-atherogenic effects can be partial and independent from the effects on plasma lipids[16,21].

In conclusion, in our study, the lipoprotein lipase S447X polymorphism was related to a decrease in triglyceride concentrations in male patients with CAD, but this association did not exist for the female patients. The presence of the lipoprotein lipase S447X polymorphism was not associated with the incidence of CAD.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Table 4 - Relative risk for CAD due to influence of classic risk factors and S447X polymorphism, calculated by multivariate logistic regression analysis

		Total		Females		Males	
Variable		**OR**	**IC 95% OR**	**OR**	**IC 95% OR**	**OR**	**IC 95% OR**
Age	≤30 years	1.00	---	1.00	---	1.00	---
	31-39 years	4.64***	2.24-9.62	5.94**	1.77-19.85	4.39**	1.67-11.55
	≥40 years	9.05***	4.65-17.61	11.53***	3.74-35.49	9.21***	3.82-22.20
Smoking	No	1.00	---	1.00	---	1.00	---
	Yes	9.02***	4.26-19.06	6.86***	2.29-20.53	13.48**	2.77-65.61
	Former Smoker	5.29***	3.32-8.42	3.29**	1.42-7.59	4.34***	1.96-9.63
Family history	No	1.00	---	1.00	---	1.00	---
	Yes	2.27***	1.45-3.55	1.65	0.91-3.01	1.65	0.91-3.01
Genotypes	CG+GG	1.00	---	1.00	---	1.00	---
	CC	1.58	0.91-2.73	1.80	0.84-3.88	1.44	0.64-3.27
Triglycerides	<200 mg/dL	1.00	---	1.00	---	1.00	---
	≥200 mg/dL	8.06***	2.76-23.56	11.14*	1.39-89.22	7.43**	2.01-27.54
Total cholesterol	<238 mg/dL	1.00	---	1.00	---	1.00	---
	≥238 mg/dL	5.38**	1.18-24.61	6.69	0.60-74.79	4.63	0.60-35.66
LDL-c	<159 mg/dL	1.00	---	1.00	---	1.00	---
	≥ 159 mg/dL	3.38	0.73-15.76	4.75	0.40-56.10	2.79	0.36-21.52
HDL-c	>40 mg/dL	1.00	---	1.00	---	1.00	---
	≤40 mg/dL	2.58***	1.59-4.18			3.21**	1.60-6.43

*OR = odds ratio for CAD (n=150 Controls and n=313 CAD); 95% CI OR= 95% confidence interval for odds ratio LDL-c=low density lipoprotein cholesterol; HDL-c = high-density lipoprotein cholesterol; CAD = coronary artery disease * p<0,05; ** p<0,01; *** p<0,001.*

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