# Association of lipase lipoprotein polymorphisms with high-density lipoprotein and triglycerides in elderly men

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**ABSTRACT.** Lipoprotein lipase is essential for triglyceride hydrolysis. The polymorphisms S447X in exon 9 and *Hin*dIII in intron 8 have been associated with lower triglyceride levels and lower cardiovascular risk in adult men. We examined the association of these lipoprotein lipase polymorphisms with high-density lipoprotein (HDL) and triglyceride levels in elderly men. Blood samples were obtained from 87 elderly men, 48 of whom had cardiovascular disease and 39 (controls) had no history of cardiovascular events. The lipoprotein lipase polymorphisms were analyzed by PCR-RFLP. Allele frequencies were H- = 27.9% and X = 21.5%. There were no significant differences in allele frequencies or blood lipid levels between cardiovascular disease and control groups. However, the X allele was associated with a lower triglyceride/HDL ratio, 2.30 *vs* 3.02 for X allele absent (P = 0.03); the H-X haplotype was associated with lower triglyceride levels compared to the H+S haplotype (1.22 *vs* 1.58 mM, respectively) and a lower triglyceride/HDL ratio (2.29 *vs* 3.26, respectively). The X allele and H-X haplotype were associated with lower triglyceride/HDL ratios in these elderly men, independent of the history of cardiovascular events.

**Key words:** Lipoprotein lipase; Polymorphism; Triglycerides; HDL cholesterol; Elderly

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## INTRODUCTION

Stroke risk doubles each decade after 55 years of age (Wolf et al., 1992), and the probability of coronary artery disease (CAD) in men is also higher with aging (Barreto et al., 2003). There is an incidence peak of events in elderly people. Part of these events is explained by lipoprotein metabolism and the relationship between HDL cholesterol and triglyceride-rich lipoproteins (Assmann, 2004; Windler et al., 2007). Among older patients with myocardial infarction, HDL cholesterol level is on average 15 mg/dL lower than in controls (Assmann and Gotto Jr., 2004), and the higher ratio of triglycerides to HDL is a strong risk predictor (Lindenstrom et al., 1994; Gaziano et al., 1997).

Lipoprotein lipase is a key enzyme in triglyceride hydrolysis, and its gene is located on chromosome 8p22, with 9 exons and 29.6 kb. Genetic variants can lead to lipid disorders and increase atherosclerosis risk. The *Hin*dIII polymorphism is a base transition of thymine (T) to guanine (G) at position +495 in intron 8, which abolishes the restriction site for the enzyme *Hin*dIII and the H- allele has less affinity for a transcription regulator factor of the LPL gene. The S447X polymorphism is characterized by the substitution of cytosine (C) by guanine (G) at position 1595 in exon 9, resulting in a premature stop codon, which removes the last two amino acids of the protein. The X allele is associated with higher LPL activity (Hata et al., 1990; Kobayashi et al., 1992; Chen et al., 2008).

In the literature, X allele frequency ranges from 10 to 20%, while the frequency of genotype XX is 1%. H- allele frequency ranges from 21 to 30%, while the frequency of genotype HH is 7 to 10%. Previous studies in Brazilians showed the X allele in 12 to 17.8% and H- allele in 28.4 to 34.8% (Almeida et al., 2007; Gigek et al., 2007; Sepetiba et al., 2007).

The exact mechanism of action of these polymorphisms is however controversial, but the X allele and H- allele are associated with protection against myocardial infarction and ischemic stroke. It is probable that benefits are through lipid metabolism, although some research shows an effect independent of blood lipid levels (Morrison et al., 2002).

We studied elderly men to determine if these polymorphisms are associated with HDL and triglyceride levels in this group.

## MATERIAL AND METHODS

### Population

We included a total of 87 men of 65 years of age or more from the Division of Geriatrics and Gerontology of UNIFESP (Federal University of São Paulo); 48 had had acute coronary syndrome or ischemic stroke [cardiovascular (CV) group], and 39 had had no previous cardiovascular event, its equivalents or diabetes (control group) (Sposito et al., 2007). All patients with acute disease, uncontrolled diabetes or systemic disease were excluded. Subjects were considered to be positive for cardiovascular disease when they self-reported previous myocardial infarction or angina pectoris with Intensive Care Unit admission or ischemic stroke and taking specific medication prescribed by physicians. The clinical information obtained pertained to personal and family medical history and lifestyle. Physicians performed a physical examination with weight, height and blood pressure measurements. Blood samples were collected after 12 h fasting for laboratory procedures. The Research Ethics Committee approved this study, and all participants signed informed consent form, according to the Helsinki Declaration.

## Laboratory exams and DNA extraction

Lipid and lipid fraction measurements were performed using routine enzymatic tests. We calculated risk ratio I = total cholesterol to HDL, desirable, equal to or less than 4.9; risk ratio II = LDL to HDL, desirable, equal to or less than 2.4, and risk ratio III = triglyceride to HDL, desirable, equal to or less than 3.8 (Anonymous, 2001). Blood was collected in tubes containing 0.1% EDTA, and genomic DNA was isolated using a modification of the procedure reported by Lahiri and Nurnberger Jr. (1991).

## Polymorphism detection

Primer sequences, polymerase chain reaction (PCR) conditions and restriction enzyme digestions were as follows (oligonucleotides were synthesized by Promicro, São Paulo, Brazil). For the *Hin*dIII polymorphism, the forward primer was 5'-GATGTCTACCTGGATAAT CAAAG-3, and the reverse primer was 5'-CTTCAGCTAGACATTGCTAGTGT'-3. PCR was carried out in a 50-mL reaction volume containing 100 ng genomic DNA, 0.4 mM of each primer, 0.2 mM dNTPs (Invitrogen, Carlsbad, CA, USA), 2 mM $MgCl_2$ in 10% PCR buffer and 1 U DNA polymerase (DNA directed; Invitrogen, São Paulo, Brazil). PCR involved an initial 5-min denaturation at 96ºC, 40 cycles of denaturation at 94ºC for 1 min, annealing at 57ºC for 1 min and extension at 72ºC for 1 min, with a final extension at 72ºC for 7 min. Aliquots of 8 mL of the PCR products were digested with 2.5 U *Hin*dIII (MBI Fermentas, Vilnius, Lithuania) at 37ºC for 4 h. The common H+ allele has one restriction site and produces two fragments of 210 and 140 bp. For the S447X polymorphism, the forward primer was 5'-TACACTAGCAAT GTCTAGGTGA-3', and the reverse primer was 5'-TCAGCTTTAGCCCAGAATGC-3'. The PCR volume was as described for *Hin*dIII, with initial denaturation for 5 min at 94ºC, 40 cycles of denaturation for 1 min at 94ºC, annealing for 1 min at 60ºC and extension for 30 s at 72ºC, and a final extension for 7 min at 72ºC. Samples were digested with 2.5 U *Mnl*I (MBI Fermentas) for 4 h at 37ºC. The polymorphic X allele has two restriction sites and produces three fragments of 285, 250 and 203 bp. Restriction fragment length polymorphism (RFLP) products were analyzed on 3% agarose gel and stained with ethidium bromide.

## Statistical analysis

SAS 9.1 (Statistical Analysis System, Cary, NC, USA) was used to perform the chi-square test, analysis of variance, logistic regression analysis, and the Student *t*-test. Genotype and allele frequencies were calculated by allele counting (Emery, 1986). Genotype distribution was investigated in relation to Hardy-Weinberg equilibrium using the "Genepop on the web" website (Garnier-Gere and Dillmann, 1992).

# RESULTS

The mean age was 76 years (range: 65-90 years) and 77% were Caucasian. In cardiovascular cases, 52% (25/48) had suffered ischemic stroke, 42% (20/48) acute coronary syndrome and 6% (3/48) both. The prevalence of hypertension, low HDL levels and cardiovascular family cases was higher in the CV group when compared to control subjects (Table 1).

**Table 1.** Baseline characteristics of the controls and cardiovascular (CV) cases.

| Characteristics | Controls (N = 39) | CV cases (N = 48) | P |
|---|---|---|---|
| Age (years) | 75.84 ± 5.83 | 77.33 ± 6.77 | 0.2819 |
| Hypertension | 18 (46.15%) | 34 (70.83%) | 0.0342* |
| SBP (mmHg) | 136.22 ± 16.64 | 144.48 ± 24.07 | 0.0626 |
| DBP (mmHg) | 78.97 ± 9.97 | 84.41 ± 9.74 | 0.0121* |
| BMI (kg/m$^2$) | 26.78 ± 3.84 | 26.12 ± 3.88 | 0.4387 |
| Cholesterol (mM) | 4.70 ± 0.93 | 4.69 ± 0.98 | 0.9361 |
| LDL-C (mM) | 2.72 ± 0.86 | 2.88 ± 0.92 | 0.4116 |
| HDL- C (mM) | 1.29 ± 0.29 | 1.16 ± 0.23 | 0.0196* |
| Triglycerides (mM) | 1.64 ± 0.98 | 1.47 ± 0.82 | 0.4068 |
| Risk ratio I | 3.84 ± 1.22 | 4.18 ± 1.17 | 0.1864 |
| Risk ratio II | 2.20 ± 0.93 | 2.58 ± 0.99 | 0.0759 |
| Risk ratio III | 2.62 (2.12 to 3.26) | 2.69 (2.31 to 3.14) | 0.8480 |
| Family CHD | 5 (12.82%) | 17 (35.42%) | 0.0278* |
| Current smoker | 5 (12.82%) | 6 (12.50%) | 1.00 |
| Previous smoker | 18 (46.15%) | 27 (56.25%) | 0.47 |
| Regular exercise | 22 (56.41%) | 26 (54.17%) | 1.00 |

Data are reported as means ± SD and number with percent in parentheses, except for Risk ratio III. *P < 0.05. SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; Risk ratio I = total cholesterol/HDL-C; Risk ratio II = LDL-C/HDL-C; Risk ratio III = triglyceride/HDL-C (geometric mean 95%CI); family CHD = family history of premature cardiovascular disease.

In Table 2, we present the genotype frequency in control and CV groups, and there were no differences between them. The S447X and the *Hin*dIII polymorphisms were in linkage disequilibrium. The H- allele and X allele frequencies in the CV group were 25.53 and 22.34% and 30.77 and 20.51% in the control group, respectively. There were no differences in allele frequencies between the groups.

**Table 2.** Comparison of the genotype frequencies of S447X and *Hin*dIII polymorphisms of the LPL gene in controls and cardiovascular (CV) cases.

| Genotype | Controls | CV cases | Total | P |
|---|---|---|---|---|
| H+H+ | 19 (48.72%) | 26 (55.32%) | 45 (52.33%) | 0.762[1] |
| H+H- | 16 (41.03%) | 18 (38.30%) | 34 (39.53%) | |
| H-H- | 4 (10.26%) | 3 (6.38%) | 7 (8.14%) | |
| SS | 23 (58.97%) | 26 (55.32%) | 49 (56.98%) | 0.828[2] |
| SX | 16 (41.03%) | 21 (44.68%) | 37 (43.02%) | |
| XX | 0 | 0 | 0 | |

Data are reported as number with percent in parentheses. [1]Fisher exact test; [2]Pearson chi-square test.

The influence of these polymorphisms on lipid blood levels was determined by comparing the presence and absence of H- and X alleles in all subjects. The presence of X allele was associated with lower triglyceride/HDL ratio (risk ratio III = 2.30, 95%CI = 1.92 to 2.76 *vs* 3.02, 95%CI = 2.53 to 3.57 - for X allele absent, P = 0.03), as shown in Table 3. The *Hin*dIII alleles were not associated with risk ratio and lipid levels. Medications to treat lipid disorders were distributed equally between X and H- allele carriers and non-carriers (data not shown; P = 0.78 and P = 0.53, respectively). In Table 4, the H-X haplotype was associated with lower triglyceride levels compared to the H+S haplotype (1.22 *vs* 1.58 mM, geometric mean 95%CI, respectively) and lower triglyceride/HDL ratio (risk ratio III = 2.29, 95%CI = 1.82 to 2.86 *vs* 3.26, 95%CI = 2.58 to 4.11). There were no differences in the medications used to treat lipid disorders between any polymorphism haplotype carriers (data not shown; P = 0.81).

**Table 3.** Blood lipid levels according to the *Hind*III and the S447X polymorphisms of the LPL gene in all the sample.

| Lipid profile | *Hind*III (N = 86) | | | S447X (N = 86) | | |
|---|---|---|---|---|---|---|
| | H- allele absent (N = 45) | H- allele present (N = 41) | P | X allele present (N = 37) | X allele absent (N = 49) | P |
| Cholesterol (mM) | 4.62 ± 0.94 | 4.76 ± 0.99 | 0.49 | 4.76 ± 0.90 | 4.65 ± 1.01 | 0.61 |
| LDL-C (mM) | 2.72 ± 0.81 | 2.89 ± 0.98 | 0.38 | 2.88 ± 0.88 | 2.76 ± 0.89 | 0.55 |
| HDL-C (mM) | 1.19 ± 0.29 | 1.24 ± 0.24 | 0.49 | 1.27 ± 0.27 | 1.17 ± 0.26 | 0.09 |
| Triglycerides (mM) | 1.69 ± 1.12 | 1.39 ± 0.54 | 0.12 | 1.35 ± 0.56 | 1.71 ± 1.07 | 0.06 |
| Risk ratio I | 4.08 ± 1.29 | 3.98 ± 1.12 | 0.69 | 3.92 ± 1.19 | 4.13 ± 1.21 | 0.43 |
| Risk ratio II | 2.39 ± 0.99 | 2.43 ± 0.98 | 0.85 | 2.39 ± 1.01 | 2.43 ± 0.96 | 0.84 |
| Risk ratio III | 2.90 (2.37 to 3.52) | 2.42 (2.05 to 2.83) | 0.15 | 2.30 (1.92 to 2.76) | 3.02 (2.53 to 3.57) | 0.03* |
| Non-HDL-C (mM) | 3.43 ± 0.95 | 3.52 ± 1.00 | 0.85 | 3.49 ± 0.95 | 3.48 ± 1.00 | 0.95 |

Data are reported as means ± SD, except for Risk ratio III. *P < 0.05. Risk ratio I = total cholesterol/HDL-C; Risk ratio II = LDL-C/HDL-C; Risk ratio III = triglyceride/HDL-C (geometric mean 95%CI); non-HDL-C = total cholesterol-HDL-C.

**Table 4.** Blood lipid levels in the H+S and H-X haplotypes of the LPL gene carriers in all the sample.

| Lipid profile | H+S (CV risk) (N = 32) | H-X (CV protection) (N = 24) | P |
|---|---|---|---|
| Cholesterol (mM) | 4.65 ± 1.06 | 4.84 ± 1.04 | 0.5092 |
| LDL-C** (mM) | 2.82 ± 2.34 to 3.41 | 2.85 ± 2.47 to 3.28 | 0.9429 |
| HDL-C (mM) | 1.14 ± 0.25 | 1.24 ± 0.23 | 0.1299 |
| Triglycerides** (mM) | 1.58 ± 1.32 to 1.90 | 1.22 ± 1.03 to 1.45 | 0.0449* |
| Risk ratio I | 4.27 ± 1.31 | 4.04 ± 1.22 | 0.5354 |
| Risk ratio II | 2.52 ± 12.46 | 2.52 ± 1.08 | 0.9512 |
| Risk ratio III | 3.26 ± 2.58 to 4.11 | 2.29 ± 1.82 to 2.86 | 0.0353* |
| Non-HDL-C (mM) | 3.51 ± 1.06 | 3.60 ± 1.08 | 0.7609 |

Data are repoted as means ± SD. *P < 0.05; **Geometric mean 95%CI. Risk ratio I = total cholesterol/HDL-C; Risk ratio II = LDL-C/HDL-C; Risk ratio III = triglyceride/HDL-C; non-HDL-C = total cholesterol-HDL-C. CV = cardiovascular disease.

There were no differences between haplotype carriers in other lipids and their clinically recommended lipid levels (Table 5). Considering the clinical endpoint, there was a tendency (P = 0.06) towards less family history of premature cardiovascular disease in H-X haplotype carriers (Table 6).

**Table 5.** Proportion of H+S and H-X haplotypes of the LPL gene carriers with clinically recommended blood lipid levels and risk ratios of cardiovascular (CV) disease in all the sample.

| Desirable lipid parameters | H+S (CV risk) (N = 32) | H-X (CV protection) (N = 24) | P |
|---|---|---|---|
| LDL-C less than 2.56 mM | 15 (48.39%) | 10 (41.67%) | 0.6196 |
| HDL-C greater than or equal to 1.03 mM | 21 (61.63%) | 20 (83.33%) | 0.1386 |
| Triglycerides less than 1.69 mM | 19 (59.38%) | 17 (70.83%) | 0.3758 |
| Risk ratio I less than or equal to 4.9 | 24 (75%) | 17 (70.83%) | 0.7275 |
| Risk ratio II less than or equal to 2.4 | 17 (54.84%) | 14 (58.33%) | 0.7950 |
| Risk ratio III less than or equal to 3.8 | 10 (31.25%) | 4 (16.67%) | 0.2123 |
| Non-HDL-C less than 3.33 mM | 16 (50%) | 11 (45.83%) | 0.7575 |

Data are reported as number with percent in parentheses. Risk ratio I = total cholesterol/HDL-C; Risk ratio II = LDL-C/HDL-C; Risk ratio III = triglyceride/HDL-C; non-HDL-C = total cholesterol-HDL-C.

**Table 6.** History of cardiovascular (CV) disease in H+S and H-X haplotypes of the LPL gene carriers in all the sample.

| History of cardiovascular disease | H+S (CV risk) (N = 32) | H-X (CV protection) (N = 24) | P |
|---|---|---|---|
| Personal (case group) | 18 (56.25%) | 13 (54.17%) | 0.8767 |
| Family | 11 (34.38%) | 3 (12.50%) | 0.0614 |

Data are reported as number with percent in parentheses.

## DISCUSSION

In the present study, the X allele was associated with a lower triglyceride/HDL-C ratio, which points to benefits in triglyceride metabolism. The H- allele was not associated with blood lipids. Nevertheless, H-X haplotypes were associated with lower triglyceride levels and lower triglyceride/HDL-C ratio when compared to the H+S haplotype. The frequency of H- allele was 27.9% and of X allele 21.5%, with no differences between CV and control groups.

Medications to treat lipid disorders could reduce triglyceride levels and elevate HDL levels and, as a consequence, could be a factor that reduced our findings of the association between these polymorphism and lipid profile in these subjects. However, we expect that these medications influenced all groups in the same proportion because they were equally distributed between all groups - common and uncommon allele carriers and CV and control groups.

In our sample, 77% were considered to be Caucasian because of parental geographic origin and skin color. In the methods, we decided to ask about parental origin plus personal skin color due to allele frequencies being lower in black people. It seems that there are no differences between Hispanics and white non-Hispanics (Razzaghi et al., 2000). Hence, we understand that this sample is comparable to Caucasians in other studies.

Considering that lipoproteins may be influenced by way of life and genetic polymorphism, the influence of these polymorphisms on lipoprotein levels could be minimized by these elderly men's lifestyle. Current smoking prevalence was 12% and we observed a 70% reduction compared to previous smoking. More than half of the subjects were practicing physical exercise.

There is little information about impact of age on expression of these polymorphisms, and it is possible that there is a difference between elderly and younger adults. The frequency of LPL *Hin*dIII polymorphism was 50% in Russians older than 90 years of age. In elderly Brazilians with age range from 66 to 97 years (mean of 79 years), the H- allele was found in 34.8% and the X allele in 17.6%. Allele H+ was associated with coronary disease in both studies (Malygina et al., 2001; Gigek et al., 2007). In our study, allele H- frequency was slightly lower (27.9%), possibly due to the lower mean age (76 years) or the sample size. It is known that HDL level shows an age-X polymorphism interaction, with association of X allele after 10 years of age and a reduction of effect after 30 years (Chen et al., 2001; Hallman et al., 2001; Thu et al., 2006). Thus, interaction between age, polymorphisms and lipoprotein requires further research. Some studies have also shown that the impact of these polymorphisms on females is not significant (Ahn et al., 1993; Mattu et al., 1994; Humphries et al., 1998; Nicklas et al., 2000; McGladdery et al., 2001; Van Bockxmeer et al., 2001).

Other studies have evaluated these polymorphisms in Brazilian subjects younger than the subjects in our study. A case-control study with 12 normolipidemic subjects with X allele

(mean age: 36 years) and in 13 control subjects without this allele (mean age: 33 years) has shown no differences in chylomicron metabolism evaluated by doubly labeled chylomicron-like emulsion injected intravenously (Almeida et al., 2003). In another case-control study, X allele was associated with lower triglyceride levels and cardiovascular protection in CAD patients of less than 55 years and 150 controls (Almeida et al., 2007). The S447 and *Hin*dIII LPL polymorphisms were assessed in 309 non-diabetic patients with angiographic CAD (mean age: 59.1 years) and in 197 controls (mean age: 55.3 years) in a southern Brazilian population of European descent. H- allele was associated with lower triglycerides and higher HDL-C and the X allele was associated with lower triglyceride levels in males. Carriers of H-S and H-X haplotypes showed lower triglycerides and increased HDL-C levels when compared to the H+S haplotype, and the H- haplotype was associated with a significant protective effect against CAD in the male subjects (Almeida et al., 2003).

In conclusion, our results in elderly men show that carriers of X allele have a better triglyceride/HDL-C ratio and that H-X haplotype carriers have lower triglyceride levels and a better triglyceride/HDL-C ratio. H- allele was not associated with blood lipids or cardiovascular events. Knowledge of polymorphism in this population is a path to a better understanding of genetic influence in triglyceride metabolism and cardiovascular risk in the elderly.

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