Hepatic Lipase (LIPC) Promoter Polymorphism in Men With Coronary Artery Disease
Allele Frequency and Effects on Hepatic Lipase Activity and Plasma HDL-C Concentrations

Ralph V. Shohet, Gloria L. Vega, Azam Anwar, Joaquin E. Cigarroa, Scott M. Grundy, Jonathan C. Cohen

Abstract—Hepatic lipase is an important determinant of plasma HDL concentration and LDL subclass distribution and may therefore influence susceptibility to coronary artery disease (CAD). To assess the effect of genetic variation in hepatic lipase activity on CAD susceptibility, we determined the frequency of the −514T allele of hepatic lipase in white men with CAD and in controls who did not have CAD. In men with CAD, postheparin plasma hepatic lipase activity was 15% to 20% lower in heterozygotes and 30% lower in homozygotes for the −514T allele. Allele frequencies were similar in cases and controls, however, and were consistent with Hardy-Weinberg expectation in both groups. This finding was confirmed in a second group comprising cases with premature symptomatic CAD and controls who were free of disease. These data indicate that a primary decrease in hepatic lipase activity of as much as 30% does not influence susceptibility to CAD in white men. (Arterioscler Thromb Vasc Biol. 1999;19:1975-1978.)

Key Words: hepatic lipase, risk factor, HDL, atherosclerosis, polymorphism

Epidemiological evidence suggests that high plasma concentrations of LDL cholesterol (LDL-C) and low plasma concentrations of HDL cholesterol (HDL-C) are associated with an increased risk of coronary artery disease (CAD).1 In addition to plasma lipoprotein concentrations, the size distribution of LDL also appears to be associated with CAD susceptibility.2–4 Accordingly, metabolic factors that affect the concentration and subclass distribution of lipoproteins in the circulation may be important determinants of CAD susceptibility.

Hepatic lipase plays a key role in lipoprotein catabolism and is an important determinant of HDL concentration5–7 and LDL subclass distribution.8–10 Accordingly, hepatic lipase activity may influence susceptibility to CAD. Most studies of the relationship between hepatic lipase activity and CAD have been small, case-control comparisons. Some of these studies reported lower hepatic lipase activity in patients with CAD than in healthy controls,11–14 whereas others found that hepatic lipase activity was similar in cases and controls.9 or elevated in men with coronary disease.15 The results of these studies are difficult to interpret, however, because they are subject to confounding by factors such as diabetes,10,16,17 sex steroid hormones,18 and obesity,19,20 which may influence both hepatic lipase activity and susceptibility to atherosclerosis.

Secondary confounding can be avoided by comparing individuals with genetically defined differences in hepatic lipase activity. Mutations that abolish hepatic lipase activity have been associated with CAD,21 but these mutations appear to be extremely rare. More recently, we have identified an allele (designated −514T) of the hepatic lipase gene (LIPC) that was associated with decreased hepatic lipase activity22 and increased plasma HDL-C concentrations in men.23 Jansen et al24 reported that this allele was more common in 782 male patients with angiographically documented CAD than in 316 asymptomatic controls. This finding is intriguing because the −514T allele is associated with increased plasma HDL concentrations, which are considered to be protective against CAD. Analysis of the allele frequencies reported by Jansen et al24 indicates that the distribution of hepatic lipase genotypes among control subjects was consistent with Hardy-Weinberg equilibrium. Among cases, however, only half of the expected number of −514T homozygotes was observed (18 observed versus 36 expected). Given the large sample size, it is highly unlikely that this deviation from Hardy-Weinberg equilibrium is a chance finding; therefore, these data suggest that homozygosity for the −514T allele may in fact be protective against CAD.

To further investigate the relationship between hepatic lipase activity and CAD we compared the prevalence of the
−514T allele in 2 groups of cases and controls. In the first group, cases were men with angiographically documented coronary atherosclerosis, and controls were men who had no detectable CAD by angiography. In the second group, cases were men with symptomatic CAD aged <60 years, and controls were men who did not have CAD symptoms.

Methods

Subjects
This study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center.

The prevalence of the −514T allele in white men with severe CAD was determined in 2 independent case-control study groups. The first group (Group 1) included men who underwent coronary angiography in the Cardiology Division at Parkland Memorial Hospital between 1993 and 1996. Filmed angiograms were used to identify cases (at least 1 vessel with >75% intraluminal obstruction) and controls (<20% intraluminal obstruction).

For the second group (Group 2), cases were white men who had developed symptomatic CAD (a history of coronary artery bypass grafting or coronary angioplasty or angiographic evidence of more than 75% stenosis of at least 1 major coronary artery) before the age of 60. These men were recruited through a large interventional cardiology program in Dallas, Texas and through 6 cardiac rehabilitation programs in the Dallas/Fort Worth area. A control group of white men who were free of symptomatic CAD was recruited from participants in a study of normal families. In addition, hepatic lipase activity was measured in 47 men with CAD (a history of coronary artery bypass grafting or coronary angioplasty or angiographic evidence of >75% stenosis of at least 1 major coronary artery) recruited at the Veterans Affairs Medical Center in Dallas.

Assay of Postheparin Plasma Hepatic Lipase Activity
Postheparin plasma hepatic lipase activity was assayed using a radiolabeled lipid substrate as described previously in detail.6

Assay of Plasma Lpid and Lipoproteins
Plasma cholesterol, triglyceride and HDL-C concentrations were assayed using enzymatic methods.23

Assay of Hepatic Lipase Genotype
The C to T substitution 514 bp upstream of the transcription initiation site was assayed by polymerase chain reaction amplification and restriction digestion.23

Statistical Analysis
The frequency of −514T homozygotes was compared in cases and controls using Fishers exact test. The observed frequencies of the −514C and −514T alleles were compared with the frequencies expected under Hardy-Weinberg equilibrium by χ² tests. Median plasma lipid and HDL-C concentrations were compared with hepatic lipase activities using Wilcoxon rank test.

Results

Frequency of the −514T Allele in Men With CAD

Group 1
Of 596 white men who underwent coronary angiography in the Cardiology Department at Parkland Memorial Hospital between March 1992 and June 1997, 406 had at least 1 vessel with >75% intraluminal obstruction. DNA samples were available from 317 of these patients. Polymerase chain reaction amplification and restriction digestion revealed that the frequency of the −514T allele was 0.20 (see Table 1). The number of −514T homozygotes observed was consistent with Hardy-Weinberg expectation. DNA samples were avail-

Table 1. Frequency of the −514T Allele of LIPC in White Men With CAD and in Healthy Controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Group 1*</th>
<th>Group 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>Obs</td>
<td>Exp</td>
</tr>
<tr>
<td>CC</td>
<td>201</td>
<td>203</td>
</tr>
<tr>
<td>CT</td>
<td>105</td>
<td>102</td>
</tr>
<tr>
<td>TT</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

Data are numbers of individuals.

*Cases in Group 1 had more than 75% luminal stenosis of at least one major coronary artery by angiography. Controls had no detectable luminal stenosis by angiography.

†Cases in Group 2 had premature, symptomatic CAD. Controls were age matched men with no symptoms or history of CAD.

P value for Group 1 cases vs controls=0.50 (χ² test).

P value for Group 2 cases vs controls=0.43 (χ² test).

able from 74 of the 86 men who were studied in this facility and found to have minimal CAD. The frequency of the −514T allele in this group (0.16) was consistent with Hardy-Weinberg expectation and was not significantly different from that found in cases.

Group 2
A second study was then performed in 179 men who developed symptomatic CAD before the age of 60 years. Several of these men used lipid lowering drugs (n=87) and/or β-blockers (n=65) and 17 were diabetic. The frequency of the −514T allele in these men (0.18) was essentially identical to that observed in the angiography patients and was not significantly different from the frequency observed in 220 healthy men (Table 1). The number of cases who were homozygous for the −514T allele was consistent with Hardy-Weinberg expectation.

Effect of the −514T Allele on Postheparin Plasma Hepatic Lipase Activity
In men with CAD, postheparin plasma hepatic lipase activity was significantly lower in 18 men who were heterozygotes for the −514T allele than in 24 men who were homozygotes for the −514C allele (Table 2). Mean hepatic lipase activity was lowest in 5 men who were homozygotes for the −514T allele.

Effect of the −514T Allele on Plasma Lipid and Lipoprotein Concentrations
Plasma cholesterol, triglyceride, and HDL-C concentrations were obtained from cases and controls in Group 2. LIPC genotype was not associated with statistically significant differences in plasma total cholesterol or triglyceride concent-

Table 2. Postheparin Plasma Hepatic Lipase Activity in Men With CAD

<table>
<thead>
<tr>
<th>LIPC Genotype</th>
<th>n</th>
<th>Hepatic Lipase activity (mmol · hr⁻¹ · L⁻¹)</th>
<th>HDL-C (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>24</td>
<td>48±21*</td>
<td>30±7</td>
</tr>
<tr>
<td>CT</td>
<td>18</td>
<td>41±22</td>
<td>29±7</td>
</tr>
<tr>
<td>TT</td>
<td>5</td>
<td>35±28</td>
<td>34±9</td>
</tr>
</tbody>
</table>

*P<0.05 for CC vs CT.
trations in either cases or in controls (Table 3). In CAD cases, plasma HDL-C concentrations were similar in the 3 genotypic groups (CC, CT, and TT). In controls, plasma HDL-C concentrations showed an increasing trend with increasing proportion of the T allele.

**Discussion**

High hepatic lipase activity is associated with an atherogenic lipoprotein profile, but the relationship between hepatic lipase activity and CAD has not been fully defined. In the present study, the relationship between hepatic lipase activity and CAD was examined using a polymorphism in the 5′ flanking region of *LIPC* that is associated with decreased *LIPC* expression. Assays of postheparin plasma indicated that hepatic lipase activity was 15% to 20% lower in CAD patients who were heterozygotes and 30% lower in patients who were homozygotes for the less common allele (−514T) of this polymorphism. Because the reduction in hepatic lipase activity associated with this allele is genetically determined, the polymorphism provides a means to examine the effects of primary reduction in hepatic lipase activity that is independent of secondary factors such as drugs, hormone use, or obesity.

The association between the −514T allele and CAD was assessed in 2 independent case-control comparisons. First, we examined a large cohort of men who had undergone coronary angiography. To maximize the difference between cases and controls, filmed angiograms were used to identify men with severe disease (>75% luminal narrowing) and men with minimal disease (<20% luminal narrowing). Individuals with intermediate disease were excluded from the study. The frequency of the −514T allele was not significantly different in cases and in controls. Furthermore, the number of men who were homozygotes for the −514T allele was consistent with expectation under Hardy-Weinberg equilibrium. To confirm this result, the frequency of the −514T allele was determined in a group of men with premature symptomatic CAD and in healthy controls. The results from this analysis were almost identical. Among cases studied by Jansen et al., the prevalence of the −514T allele was 0.213. This compares with an allele frequency of 0.209 among angiographically defined cases and 0.196 among premature CAD patients in the present study. In control subjects, Jansen et al.24 reported an allele frequency of 0.189. The corresponding frequencies in the present study were 0.162 among angiographically defined controls and 0.180 among asymptomatic controls. Therefore despite differences in the outcome of the statistical analyses, the primary data of the 2 studies are essentially identical. Because the prevalence of the −514T allele was slightly higher in CAD patients than in controls in each of the 3 case control comparisons performed in these 2 studies, it might be argued that the data support an association between the −514T allele and CAD. Even under the most extreme case-control comparison (severe CAD versus disease free by angiography), the frequency of the −514T allele in CAD patients and controls is very similar. Accordingly, the magnitude of CAD risk attributable to the −514T allele, if any, is very small. It should be emphasized that the −514T allele analyzed in this study is associated with decreased hepatic lipase activity. Therefore, the present results do not exclude the possibility that low HDL-C resulting from increased hepatic lipase activity may be atherogenic.

Previously we reported that the −514T allele was associated with increased plasma HDL-C concentrations in healthy, normotriglyceridemic men who did not smoke or use lipid lowering drugs. Jansen et al.24 and Murtomaki et al.25 also reported that the −514T allele was associated with higher plasma HDL-C concentrations. In the present study, the −514T allele was associated with increased plasma HDL-C concentrations in healthy men but not in men with CAD. The finding that decreased hepatic lipase activity is associated with increased HDL-C in some individuals but not in others is consistent with observations in hepatic lipase deficiency. It also suggests that a genetic reduction in hepatic lipase activity predisposes an individual to high plasma HDL-C concentrations but is not sufficient to confer high plasma HDL-C concentrations in the presence of HDL lowering factors, such as high plasma triglyceride concentrations or β-blockers. In this study, plasma HDL-C concentrations were measured in 6 men who were homozygotes for the −514T allele. Four of these men were current smokers, 1 was very obese, 1 was diabetic, and 3 were on β-blockers. Thus, many CAD patients may have other genetic and/or metabolic factors that confer low plasma HDL-C concentrations even in the presence of low hepatic lipase activity. The identification of these factors could help to elucidate the association between low HDL-C and CAD.
Acknowledgments
We thank Liangcai Nie, Sijing Niu, Jinping Wang, Hanh Nguyen, Zakir Siddiquee, and Han Tran for excellent technical assistance, and Dick Verstraete for subject recruitment. This work was supported by NIH grant HL-53917, the Southwestern Medical Foundation, and the Moss Heart Foundation, Dallas, TX.

References
Hepatic Lipase (LIPC) Promoter Polymorphism in Men With Coronary Artery Disease: Allele Frequency and Effects on Hepatic Lipase Activity and Plasma HDL-C Concentrations

Ralph V. Shohet, Gloria L. Vega, Azam Anwar, Joaquin E. Cigarroa, Scott M. Grundy and Jonathan C. Cohen

doi: 10.1161/01.ATV.19.8.1975

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/8/1975

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/