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Short communication

Hepatic Lipase C514T Polymorphism and its Relationship with Plasma HDL-C Levels and Coronary Artery Disease in Koreans

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Hepatic lipase is a key enzyme that is involved in HDL-C metabolism. The goal of this study was to find out the frequency of the hepatic lipase C514T polymorphism, and evaluate its relationship with plasma HDL-C levels and coronary artery disease (CAD) in Koreans. Two hundred and twenty four subjects with no previous history of lipid-lowering therapy, 118 patients with significant CAD, and 106 controls were examined with respect to their genotypes, lipid profiles, and other risk factors for CAD. The frequency of the -514T allele was 0.37 in men and 0.35 in women, which were higher than the frequency that was reported in Caucasians, but lower than the frequency that was reported in African-Americans. The -514T allele was associated with significantly higher HDL-C levels in women. After controlling for age, gender, BMI, DM, and smoking, the non-CC genotype was significantly associated with HDL-C levels, and explained 6% of the HDL-C variation in this study. When the genotypes-distribution was compared between the CAD and non-CAD patients, the hepatic lipase C-514T polymorphism was not associated with the presence of CAD. Koreans have a higher frequency of the hepatic lipase gene 514T allele than Caucasians, and the -514T allele is associated with higher plasma HDL-C levels in Korean women, and perhaps non-smoking men. However, our data does not suggest an association between the polymorphism and an increased risk of CAD.

Keywords: Coronary artery disease, Genetic polymorphism, HDL-cholesterol, Hepatic lipase.

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Introduction

Hepatic lipase is a lipolytic enzyme (glycoprotein) that is synthesized and secreted by the liver (Bensadoun and Berryman, 1996). It is bound to the surface of sinusoidal endothelial cells and the external surface of microvilli of parenchymal cells in the space of Disse (Sana *et al.*, 1997), and catalyzes the hydrolysis of triglycerides and phospholipids (Kuusi *et al.*, 1982). Hepatic lipase is involved in high-density-lipoprotein-cholesterol (HDL-C) metabolism and reverse-cholesterol transport (RCT) by hydrolyzing the triglycerides and phospholipids of the intermediate density lipoproteins (IDL) and large triglyceride-rich HDL_2 into the atherogenic small, dense LDL and the small, dense HDL_3 (Tall, 1998; Connely, 1999).

The hepatic lipase gene (LIPC) is located in chromosome 15q21. It is composed of nine exons that span about 35 kb of DNA (Datta *et al.*, 1988). A C-514T polymorphism in the promoter region of LIPC is reportedly associated with low hepatic lipase activity (Tahvanainen *et al*., 1998; Vega *et al.*, 1998; Cohen *et al.*, 1999; Deeb and Peng, 2000), and higher plasma HDL-C levels (Guerra *et al.*, 1997; Cohen *et al.*, 1999; Couture *et al.*, 2000). However, the results have not always been concordant in all populations, and investigations into such associations in Asians, especially Koreans, have been rare (Hong *et al.*, 2000). Also, previous studies did not exclude patients on lipid-lowering therapy or women on hormone replacement therapy, both of which may confuse true associations between the hepatic lipase polymorphism and plasma HDL-C levels.

The association between hepatic lipase and coronary artery disease (CAD) has been controversial. The inverse relationship between hepatic lipase activity and plasma HDL-C (Blades *et al.*, 1993), a well known protective factor against CAD (Gordon and Rifkind, 1989), and the positive association of hepatic lipase with small dense LDL-C (Zambon *et al.*, 1993), a possible risk factor of CAD

(Lamarche *et al.*, 1997), have pointed towards the proatherogenic role of hepatic lipase. However, reports that patients with the hepatic lipase deficiency developed premature CAD (Breckenridge *et al.*, 1982) and animal studies, which showed that the hepatic lipase transgenic mice have less aortic cholesterol deposition (Busch *et al.*, 1994), have pointed towards the antiatherogenic role of hepatic lipase.

The aim of this study was to find out the frequency of the C-514T polymorphism of the hepatic lipase gene in Koreans and its relationship with plasma HDL-C. We also evaluated the frequency differences between the CAD and non-CAD subjects in order to investigate whether hepatic lipase C-514T polymorphism is associated with the presence of CAD.

Materials and Methods

Study subjects Two hundred and twenty four patients (None had a previous history of lipid lowering therapy and all gave consent to DNA analysis.) were enrolled from February 2001 to July 2001 at the Seoul National University Hospital (SNUH). CAD patients were those that were documented by coronary angiography to have significant coronary artery stenosis, defined as a greater than 50% luminal narrowing in at least one or more coronary arteries. Controls were those who had normal or insignificant coronary angiographic findings, or patients with a normal ECG, who had no chest pain and/or other clinical symptoms/signs of CAD. Lipid profiles (total cholesterol, triglyceride, HDL-C) were obtained before coronary angiography. The presence of traditional coronary risk factors was recorded.

Assay of plasma lipids and lipoproteins Plasma total cholesterol, triglyceride, and HDL-C concentrations were assayed using enzymatic methods. LDL-C was calculated using the Friedewald equation (Friedewald *et al.*, 1972).

Assay of hepatic lipase genotype Genomic DNA was prepared from the leukocytes in blood samples that were taken before the coronary angiography or measurement of lipids. The C to T substitution 514 bp upstream of the transcription initiation site of the hepatic lipase gene was assayed by a polymerase chain reaction (PCR) amplification and restriction digestion with Hsp92II (Promega, Madison, USA). We followed specific protocols that were published in previous studies (Guerra *et al.*, 1997, Zambon *et al.*, 1998). Briefly, PCR was performed using the following primers: 5'-AAG AAG TGT GTT TAC TCT AGG ATC A-3' and 5'-GGT GCC TTC CAC GTG GCT GCC TAA G-3'. Forty pmol of each primer, and 500 ng of genomic DNA were mixed with 0.2 mM dNTPs, 1.4 mM $MgCl_2$, 10 mM Tris pH 8.4, and 0.25 U of Taq polymerase (Promega, Madison, USA). The conditions of amplification were 94°C for 2 min, 35 cycles at 94°C for 15 s, 65°C for 30 s, 72°C for 45 s, and finally 5 min at 72°C. The PCR products were digested with 2 units of restriction endonuclesase Hsp92II at 65°C for 2 h and electrophoresed on a 2% agarose gel.

Data analysis Categorical variables were evaluated using the χ^2 test, and continuous variables by the Students t-test. The observed frequencies of the genotypes were compared with the frequencies that were expected under the Hardy-Weinberg equilibrium by χ^2 tests. Mean plasma lipid and lipoprotein concentrations, age, the presence of diabetes mellitus (DM), hypertension (HTN), and smoking were compared between males and females. The plasma concentrations of lipids and lipoproteins were also compared between the different genotypes using the t-test. An analysis of the covariance was used to test the association between HDL-C and hepatic lipase C-514T polymorphism, controlling for age, gender, DM, body mass index (BMI), and smoking. The genotype distribution was compared between the CAD and non-CAD patients using the χ^2 test. All of the computations were performed with the Statistical Package for Social Sciences (SPSS 10.0 version) for Windows. The statistical significance was defined as $p<0.05$.

Results

Study population Table 1 summarized the baseline characteristics of the patients. One hundred and twenty four men and 100 women were enrolled for the study. Mean age, BMI, the presence of DM, HTN, mean plasma levels of triglycerides, and LDL-C were similar between men and women. There was a significantly higher percentage of smokers in men ($p<0.01$), and the baseline total cholesterol level ($p = 0.04$) and HDL-C level ($p<0.01$) were higher in women.

Allele frequency and genotype distribution of the hepatic lipase C-514T polymorphism and its relationship with CAD The distribution of the CC:CT:TT genotypes in men and women were 38% : 50% : 12% and 36% : 57% : 7%, respectively, giving a -514T allele frequency of 0.37 for men and 0.35 for women. These findings were consistent with the

Table 1. Basic demographics

	Men	Women
Number	124	100
Age	59.8 ± 9.6	60.5 ± 8.7
BMI	24.5 ± 3.0	24.8 ± 3.5
DM (%)	24.20%	31.0%
HTN (%)	43.50%	50.0%
Smoking (%)*	61.30%	6.0%
TotalChol (mg/dL) **	183.6 ± 29.5	193.2 ± 38.8
Triglyceride (mg/dL)	131.5 ± 57.9	140.7 ± 72.0
HDL-C (mg/dL)*	41.5 ± 9.5	46.4 ± 14.4
LDL-C (mg/dL)	115.5 ± 25.3	119.0 ± 32.3

*, $p<0.01$; **, $p = 0.04$; BMI, Body Mass Index; DM, diabetes mellitus; HTN, hypertension; Chol, cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol. Baseline demographics were similar in men and women except for the percentage of smokers, plasma HDL-C levels ($p<0.01$ for both), and total cholesterol levels ($p = 0.04$).

Table 2. Genotype distribution and allele frequency in men and women

Genotype & Allele	Men	Women
CC	38% (47)	36% (36)
CT	50% (62)	57% (57)
TT	12% (15)	7% (7)
C	0.63	0.65
T	0.37	0.35

The numbers in parenthesis are the actual number of patients. The distribution of the genotypes was compatible with the Hardy-Weinberg equilibrium, and similar between males and females. The -514T allele frequency of 0.37 for men and 0.35 for women is comparable to previous reports for Koreans.

Hardy Weinberg expectations (Table 2).

When the genotype distribution was analyzed between the CAD and non-CAD patients, we found no significant associations between the hepatic lipase C-514T polymorphism and CAD. When analyzed separately in men and women, we still found no association between the polymorphism and CAD in both genders (Table 3).

Effect of the hepatic lipase C-514T polymorphism on plasma lipid and lipoprotein concentrations Plasma concentrations of total cholesterol, triglycerides (TG), HDL-C, and LDL-C were compared between the CC homozygotes (CC genotype) and non-CC homozygotes (i.e., the CT and TT genotypes). There were no associations between the genotypes and total cholesterol, TG, and LDL-C. However, HDL-C was significantly higher in the non-CC genotype when compared to the CC genotype (41.4 ± 9.7 vs. 45.0 ± 13.3, $p<0.05$). When men and women were analyzed separately, the association between HDL-C and the genotypes were no longer significant in men (40.7 ± 8.5 vs. 41.9 ± 10.2 in CC and non-CC genotypes, respectively, $p = 0.49$), while the association in women (42.4 ± 11.3 vs. 48.7 ± 15.5 in CC and non-CC genotypes, respectively, $p<0.05$) remained significant (Table 4). Since the only significant difference on the baseline characteristics between men and women was the percentage of smokers, we analyzed only the non-smoking males. We discovered that the mean HDL-C level in the non-CC genotype was slightly higher than the HDL-C level in the CC genotype, although this was not statistically significant (38.6 ± 6.8 vs. 42.7 ± 9.4 in CC and non-CC genotypes, respectively, $p = 0.13$) (Table 5). To exclude the effects of other factors, which might confound the relationship between HDL-C and TaqIB polymorphism, an analysis of the covariance (ANCOVA) that controlled for age, gender, DM, BMI, and smoking showed that the C-514T polymorphism was significantly associated with HDL-C levels ($p<0.05$), and explained the 6% variance in the HDL-C levels ($F = 3.96$, $p = 0.048$).

Table 3. Genotype distribution in regard to coronary artery disease

	Total		Men		Women	
Genotype	CAD group	non-CAD group	CAD group	non-CAD group	CAD group	non-CAD group
CC	37.3% (44)	36.8% (39)	37.3% (28)	38.8% (19)	37.2% (16)	35.1% (20)
non-CC	62.7% (74)	63.2% (67)	62.7% (47)	61.2% (30)	62.8% (27)	64.9% (37)
			p = 0.51		p = 0.49	

CAD, coronary artery disease. When the distribution of the genotypes was evaluated with regard to the presence of CAD, the hepatic lipase C-514T polymorphism was not associated with CAD. This lack of association between the polymorphism and CAD was similar in both men and women.

Table 4. Lipid profiles according to genotype

	Total		Men		Women	
	CC	non-CC	CC	non-CC	CC	non-CC
Number	83	141	47	77	36	64
Total-chol	188.5 ± 35.5	187.6 ± 33.6	189.6 ± 32.3	180.0 ± 27.0	187.1 ± 39.6	196.7 ± 38.3
Triglyceride	132.2 ± 55.9	133.4 ± 64.1	131.4 ± 52.9	123.3 ± 48.2	133.3 ± 60.3	144.9 ± 77.9
HDL-C	41.4 ± 9.7*	45.0 ± 13.3*	40.7 ± 8.5	41.9 ± 10.2	42.4 ± 11.3*	48.7 ± 15.5*
LDL-C	119.7 ± 28.5	115.5 ± 28.7	120.2 ± 26.3	112.6 ± 24.5	119.1 ± 31.5	118.9 ± 32.9

*; $p<0.05$, CC versus non-CC genotypes. Lipid levels given as mean ± standard deviation (mg/dl); HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol. HDL-C level was significantly lower in the CC genotype when compared with non-CC genotype, which was primarily due to a significant association between the polymorphism and HDL-C levels in women ($p<0.05$). There were no differences in HDL-C levels in men ($p = 0.49$)

Table 5. Lipid levels in non-smoking men

	CC	non-CC
n	19	29
Total-cholesterol	177.8 ± 29.6	178.4 ± 21.7
Triglyceride	116.3 ± 59.8	111.2 ± 42.3
HDL-C	38.6 ± 6.8	42.7 ± 9.4
LDL-C	114.6 ± 22.8	112.9 ± 20.9

Lipid levels given as mean ± standard deviation (mg/dl); HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol. Although HDL-C was not significantly different between the CC and non-CC genotypes in non-smoking men, the HDL-C level in the non-CC genotype was slightly higher than that in the CC genotype (CC versus non-CC genotype for HDL-C, $p = 0.13$).

Discussion

Our study shows that a common polymorphism of the hepatic lipase gene is associated with significantly higher HDL-C levels in women, and this association in men may be confounded by smoking. Data from this study also shows that the hepatic lipase C-514T polymorphism lacks an association with CAD in Koreans. Our study excluded all patients on, or with a history of, lipid-lowering therapy; thereby, excluding the possibility of lipid level modulation by lipid-lowering drugs. Also, none of the females in our study were on hormone replacement therapy (HRT). This study confirms prior reports of a significant role of the hepatic lipase gene in the genetic modulation of HDL-C levels.

The frequency of the -514T allele of the hepatic lipase gene was more common in Koreans than in Caucasians. In our study, the -514T allele frequency was 0.37 in men and 0.35 in women. This frequency is slightly lower than a previous report on Koreans (Hong *et al.*, 2000). However, the frequency was confirmed in a preliminary data of 99 patients before the onset of this study, which showed a similar -514T allele frequency. Previous studies in different races showed that the frequencies of the allele were 0.15-0.21, 0.45-0.53, and about 0.47 in Caucasians, African Americans, and Japanese Americans, respectively (Zambon *et al.*, 1998). Our data support previous reports on the variability of the hepatic lipase polymorphism in different races (Vega *et al.*, 1998; Zambon *et al.*, 1998; Hong *et al.*, 2000; Tan *et al.*, 2001). Whether the difference in the HDL-C level between different races can be explained by the difference in hepatic lipase gene frequency is a much more complex issue, and extends beyond the scope of this article. However, along with data from previous studies on African-Americans and Japanese, our study shows that a higher frequency of the hepatic lipase C-514T polymorphism may partially contribute to the ethnic difference in the HDL-C level.

Controversy exists on whether hepatic lipase acts in a proatherogenic or antiatherogenic fashion. Likewise, the association of the C-514T allele with coronary artery disease is controversial. Jansen *et al.* reported an increased prevalence of the -514T allele in men with CAD (Jansen *et al.*, 1997), although patients with the -514T allele had higher HDL-C levels than those with the CC genotype. In addition, Dugi *et al.* showed that low hepatic lipase activity is an independent risk of CAD, and the hepatic lipase C-514T polymorphism is significantly associated with a higher CAD extent score (Dugi *et al.*, 2001). Investigators postulated that the increase in the formation of nascent, pre-β HDL particles that mediate cholesterol efflux, and the increase in selective cholesteryl ester uptake form HDL to the liver via the HDL receptor SR-B1, may be the mechanism behind the antiatherogenic effect of hepatic lipase. However, these results have been argued by others who reported that the -514T allele does not significantly affect the risk of CAD (Wilson *et al.*, 1980; Shohet *et al.*, 1999; Hong *et al.*, 2000). Our study showed that the genotype distribution of the hepatic lipase C-514T polymorphism was similar between the CAD and non-CAD patients. This lack of association was universal in both men and women, and even after a multivariate analysis with known risk factors, we found no appreciable increase in the risk of CAD in the non-CC genotype (data not shown). This leads us to believe that the -514T allele is not associated with the development of CAD, at least in this Korean population. CAD is a complex disease that is affected by multiple factors, and one genetic factor may not be enough in determining the development of such a complex disease.

Our study also investigated the correlation between the -514T allele expression and a high HDL cholesterol level. There have been several previous reports that the 514T allele was associated with higher plasma HDL-C concentrations (Guerra *et al.*, 1997; Couture *et al.*, 2000). However, some investigators reported that this association is gender dependent, and only significant in certain subgroups. For example, Grundy *et al.* reported an association of the -514T allele with increased plasma HDL-C concentrations in only healthy, normotriglyceridemic men who did not smoke or use lipid-lowering drugs (Grundy *et al.*, 1999). Also, Shohet *et al.* showed that the -514T allele was associated with higher HDL-C levels only in healthy men, and not in men with CAD (Shohet *et al.*, 1999). In the only other study on Koreans, the hepatic lipase C-514T polymorphism was not associated with HDL-C in either men or women, and was only associated with apoB levels in women with CAD, and with apoA1 levels in healthy men (Hong *et al.*, 2000). Much of the discrepancy in prior studies may be due to the influences of other factors on hepatic lipase activity and HDL-C. These include gender (Kauma *et al.*, 1996; Tan *et al.*, 2001), obesity (Castro *et al.*, 2001), smoking (Kong *et al.*, 2001), hormone replacement therapy (Yamakawa-Kobayashi *et al.*, 2002), and the presence of DM (Tan *et al.*, 2001), as suggested by previous studies.

In this study, we excluded all patients on, or with a history of, lipid-lowering therapy. Also, none of the females in our study were on hormone replacement therapy (HRT). We found that the non-CC genotypes were associated with

significantly higher plasma HDL-C levels when compared to the CC genotype in women. In men, however, such significant association was not present. Only in non-smoking men could we find a mild trend toward a higher HDL-C level in the non-CC genotype. In addition, after controlling for age, gender, DM, BMI, and smoking (risk factors known to confound the association between hepatic lipase polymorphism and HDL-C levels), the C-514T polymorphism was significantly associated with HDL-C levels, and explained 6% of the variance in HDL-C levels. These results led us to believe that when various factors which affect hepatic lipase activity and HDL-C levels are fully controlled, there is a significant association between the C-514T polymorphism and higher HDL-C levels.

One limitation of this study is that the hepatic lipase activity was not obtained. The C-514T polymorphism has been reported to affect hepatic lipase activity (Tahvanainen *et al*., 1998; Deeb and Peng, 2000). Because we did not obtain the hepatic lipase activity, we fell short of showing that the higher HDL-C level in the non-CC genotypes was in fact due to the actions of a lowered hepatic lipase activity. Another minor limitation was the small number of patients that were enrolled. Although 224 patients is usually not considered to be a small study population, association studies to find out the effect of certain genotypes on disease states and various metabolic states need to be based on a much larger number of patients. We think that the lack of significance in the association of the polymorphism with the HDL-C level in non-smoking men, would have been overcome with a larger study population.

In conclusion, our study shows that Koreans, when compared to Caucasians, have a higher frequency of the hepatic lipase gene C-514T polymorphism, which demonstrates the frequency variation among different ethnic groups. Our data also confirms previous observations that the non-CC genotype of the hepatic lipase gene is associated with a higher plasma HDL-C level in women and perhaps in non-smoking men. However, hepatic lipase C-514T polymorphism does not seem to be associated with the presence of CAD in Koreans. In view of the importance of hepatic lipase in HDL-C metabolism, and in turn the importance of HDL-C in coronary artery disease development, these suggested associations of hepatic lipase polymorphism with HDL-C levels should be studied further in a larger population of patients.

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