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Research Article

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Association of Variation in Hepatic Lipase Activity with Promoter Variation in the Hepatic Lipase Gene

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Abstract

The associations between six genetic polymorphisms in the hepatic lipase (HL) gene (*LIPC*) and variation in postheparin HL activity and fasting serum lipoproteins were evaluated in 395 male Finnish coronary heart disease patients with HDL cholesterol concentrations ≤ 1.1 mmol/liter. The *LIPC* promoter polymorphism at position -514 was highly significantly associated with variation in HL activity ($P = 0.0000008$), with mean activities of 20.4, 17.5, and 13.2 μmol free fatty acid/ml per hour in subjects having C/C, C/T, and T/T genotypes, respectively. Furthermore, the triglyceride content of low density lipoprotein, intermediate density lipoprotein and HDL, and the cholesterol content of intermediate density lipoprotein were found to be associated with variation at *LIPC* position -514. However, there was no association of this polymorphism with coronary heart disease. These data suggest that the *LIPC* promoter variation is likely to be the basis for variation in HL activity, which underlies the variation in serum lipoprotein phenotypes in this sample. (*J. Clin. Invest.* 1998. 101:956–960.) Key words: polymorphism • hepatic lipase activity • high density lipoproteins • atherosclerosis • *LIPC*

Introduction

Human hepatic lipase (HL;¹ triacylglycerol lipase, EC 3.1.1.3) has monoglyceride, diglyceride, triglyceride hydrolase, and phospholipase A₁ activities (1, 2). HL is synthesized and secreted by the liver, and found extracellularly in liver and in ste-

roidogenic organs, primarily bound to proteoglycans (3–5). The HL gene (*LIPC*) is located in chromosome 15q21 and composed of nine exons spanning about 35 kb of DNA (6, 7). Among dyslipidemic patients genetic variations in *LIPC* that affects HL activity have been detected at positions S267F, T383M (8), L334P (9), and R186H (10). In addition, a splice site mutation in the first intron has been described (11). The most constant effects of HL deficiency on lipoprotein profiles have been triglyceride enrichment of LDL and HDL, presence of circulating β -VLDL, and abnormal catabolism of chylomicrons (9, 11–15). Also *LIPC* polymorphisms, which do not affect HL activity, have been described. Two polymorphisms, V73M and N193S (12), result in an amino acid substitution. In addition, polymorphisms that do not result in amino acid changes have been reported in codons V133V, T202T (8), T457T (16), G175G (17), and T344T (9).

Some of the individuals with HL deficiency have premature atherosclerosis (13, 18), but studies concerning the association of HL with premature atherosclerosis have been impeded by two factors. One is the lack of large patient and control groups in which the HL activity has been measured, mainly because the measurement of HL activity can only be done in postheparin plasma which is usually not available from large trials or from healthy individuals. The other reason is that there have been no reports of common polymorphisms that would correlate with HL activity and thus circumvent the need for postheparin sampling. Recently a C to T substitution with a population frequency of about 0.25, has been described in nucleotide -514, or -480 using a different numbering (6, 7), in the promoter region of *LIPC* by two independent groups (19–21). This polymorphism, thereafter designated -514C/T, has been reported to be associated with variation in HDL cholesterol (HDL-C) levels in normolipidemic individuals and it may have an effect on HL activity (19–21). That polymorphism has been found to be in complete linkage disequilibrium with three other polymorphisms -50G/A, -710T/C, and -763A/G in the promoter region of *LIPC* (21).

The aim of this study was to study the effects of six common *LIPC* polymorphisms on HL activity and on clinical and biochemical phenotypes in 395 Finnish coronary artery disease patients with low HDL-C. The genotype frequencies are compared with those reported for Finnish students (20).

Methods

Study population. In this study, the baseline data from 395 men participating in a secondary prevention trial of coronary artery disease

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1. Abbreviations used in this paper: CAD, coronary artery disease; HL, hepatic lipase; LPL, lipoprotein lipase.

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(CAD), the LOCAT Study, were used (22). These patients were selected primarily on the basis of a low serum HDL-C concentration, 1.1 mmol/liter, corresponding approximately to the lowest tertile in middle-aged Finnish men. All had undergone coronary bypass surgery previously and were in stable health, with no or only mild angina pectoris. They had no heart failure, and the left ventricular ejection fraction was $\geq 35\%$. Other selection criteria, described elsewhere in detail (22), included LDL cholesterol ≤ 4.5 mmol/liter, serum triglyceride concentration ≤ 4.0 mmol/liter, no manifest diabetes mellitus, no current regular smoking and body mass index < 30 kg/m². Those with uncontrolled hypertension and significant liver, kidney, or thyroid disease were excluded. 81% of the subjects were taking β -blockers. This is difficult to avoid in a group of CAD patients, but other drugs affecting lipoprotein metabolism were not allowed in the study. All participants gave an informed consent and the study was approved by ethical committees in the collaborating hospitals.

To compare the genotypic frequencies in this group of CAD patients with Finnish population frequencies, genotypic data of 194 Finnish university students, aged 18–26 yr, representing both genders, and with no detectable symptoms of CAD, are included. These students were the Finnish participants of the EARS I study (23). Data of these subjects have been previously published (20).

Solid-phase minisequencing. The genomic DNA from white blood cells was isolated using the salting-out procedure (24). The polymorphisms at nucleotide position -514 and at amino acid codons 133, 202, 334, 383, and 457 were determined. The polymorphisms L334F, which was originally described by our group (9), and T383M have been reported to have an effect on HL activity (8, 9). The V133V, T202T, and T457T are the three most common neutral polymorphisms of *LIPC* (8, 16). -514C/T is recently reported to associate with variation in HDL levels (19–21). In the solid-phase minisequencing method used (25) variable nucleotides are identified by a single nucleotide primer extension reaction catalyzed by DNA polymerase from a PCR product on a solid support. Three different primers were used to study each polymorphism; each DNA-fragment, containing a nucleotide to be tested, was first amplified by PCR using a pair of primers and then the product was analyzed by a detection primer required in minisequencing. The used primers have been described (20) except the primers for the polymorphism T383M for which we used 5'GCTGTTACGACTAACTGATTG3' as a forward primer, 5'CTGCCTGGCACAAGTGGGT3' as a reverse primer, and 5'TAAAA-CGTATTCCTTTCTTATCA3' as a detection primer.

Biochemical analyses. Postheparin plasma lipoprotein lipase (LPL) and HL activities were measured 5 and 15 min after injection of heparin (100 IU per kilogram body weight, maximum 10,000 IU) by an immunochemical method (26). For patient selection HDL-C was measured after precipitation of the apoB-containing lipoproteins with phosphotungstic acid and magnesium chloride but for the baseline data reported preparative ultracentrifugation was used. Cholesteryl ester transfer protein activity was measured with a transfer/exchange reaction between lipoproteins (27).

Statistical analysis. Allele frequencies were estimated by gene counting. Possible linkage disequilibrium between loci was tested using a likelihood-ratio test for genotypes, whose empirical distribution is obtained by a permutation procedure (28). In this test the likelihood of a model with linkage disequilibrium is compared with the likelihood of a model assuming linkage equilibrium. Hardy Weinberg equilibrium of genotypes was tested using exact test based on a Markov-chain approach modified from Guo and Thompson (29). The haplotype frequencies were estimated from genotypes using the computer program MYRIAD (30). Standardized linkage disequilibrium coefficients for the less common alleles in each pair of loci (D') were calculated from haplotype data with programs in Arlequin program package (31). The nonrandom distribution of geno- and haplotype frequencies in the CAD patients and in Finnish population was tested with χ^2 test, the P values were corrected for multiple testing by Bonferroni correction. Genotypic effects of each loci on clinical and biochemical characteristics were tested by ANOVA. Pairwise compari-

sons of -514C/T genotypes were done by Scheffe's test. Combined effects of -514C/T polymorphism and the other five polymorphisms on HL activity and on triglyceride content of HDL were analyzed by two-way ANOVA. Triglyceride levels were log-transformed to remove positive skewness. Statistically significant differences ($P < 0.05$) are indicated in tables.

Results

Genotype and haplotype frequencies. The allele, genotype, and haplotype frequencies for six *LIPC* polymorphisms were studied and the frequencies of five of these loci are compared with the corresponding frequencies in the Finnish population (Table I). The frequencies of the rare alleles were 0.253 (T), 0.438 (G), 0.398 (G), 0.038 (A), 0.014 (T), and 0.299 (A) in the loci -514C/T (C→T), V133V (T→G), T202T (C→G), L334F (A→C), T383M (C→T), and T457T (C→A), respectively. There were no statistically significant deviations from the Hardy Weinberg equilibrium for genotypes. A statistical method was used to calculate haplotype frequencies. The most common haplotype was C-T-G-A-C-C, comprising $> 20\%$ of haplotypes. The distribution of geno- or haplotype frequencies in these study subjects did not differ from Finnish population frequencies. Standardized linkage disequilibrium coefficients between the less common alleles in each loci were determined from haplotype data. The only positive linkage disequilibrium coefficients were noted with polymorphisms -514C/T ($P = 0.002$) and V133V ($P = 0.0001$) with L334F. Because rare

Table I. The Genotype and Allele Frequencies of Six *LIPC* Polymorphisms

<i>LIPC</i> polymorphic site	Genotype	Frequency in:	
		Patients	Students
-C514T	C/C	0.564	0.532
	C/T	0.367	0.403
	T/T	0.069	0.065
N		376	186
V133V	G/G	0.195	0.178
	G/T	0.485	0.533
	T/T	0.289	0.289
N		379	180
T202T	C/C	0.348	0.234
	G/C	0.507	0.609
	G/G	0.145	0.157
N		379	184
L334F	A/A	0.924	0.866
	A/C	0.076	0.134
N		370	186
T383M	C/C	0.973	N.D.
	C/T	0.027	N.D.
N		369	
T457T	A/A	0.071	0.021
	A/C	0.456	0.409
	C/C	0.472	0.570
N		379	186

N.D., not done.

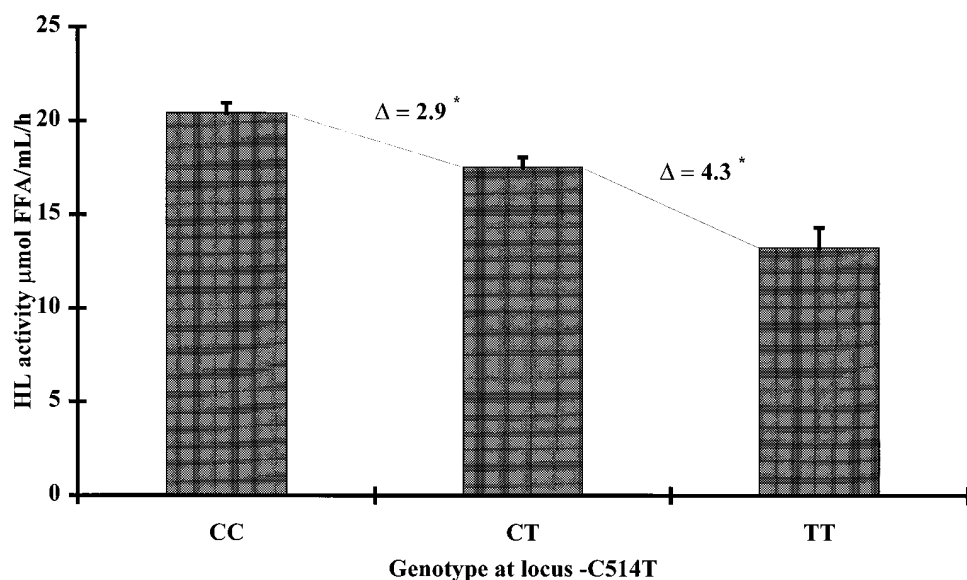


Figure 1. HL activity according to genotype at locus -514C/T. Error bars indicate SEM. Pairwise comparisons between means in C/C and C/T and means in C/T and T/T genotype classes are also shown. * $P < 0.05$ Scheffe's test.

polymorphisms seldom occur together most of the other linkage disequilibrium coefficients were negative or did not reach statistical significance.

Patient phenotypes according to genotype. The effects of six polymorphisms, at loci -514C/T, V133V, T202T, L334F, T383M, and T457T in the *LIPC*, on clinical and biochemical phenotypes of 395 men were measured. Characteristics of the major lipoprotein classes in fasting state were analyzed according to genotype. HL activity was associated with genotype only at locus -514T (ANOVA $P = 8 \times 10^{-7}$) so that the lowest activity (mean 13.2 μmol FFA/ml per hour) was measured in patients with genotype T/T and the highest (mean 20.4 μmol FFA/ml per hour) in patients with genotype C/C (Fig. 1). The mean HL activities in these three genotype classes were different ($P < 0.05$) also in pairwise tests (Scheffe). In patients with the T allele in locus -514C/T triglyceride concentrations in IDL ($P = 0.009$), LDL ($P = 0.018$), and HDL ($P = 0.0004$) fractions were elevated, as well as cholesterol in IDL ($P = 0.017$)

(Table II). Association of the T/T genotype in locus -514C/T with triglycerides in HDL was observed in the HDL subfractions HDL₂ ($P = 0.0002$) and HDL₃ ($P = 0.035$). The triglyceride content of HDL₂ in patients with C/C genotype was 0.07 mmol/liter (± 0.0013 SEM) and 0.09 mmol/liter (± 0.0053 SEM) with T/T genotype, and the triglyceride content of HDL₃ was 0.088 mmol/liter (± 0.0017 SEM) and 0.10 mmol/liter (± 0.0044 SEM) in patients with C/C or T/T genotypes, respectively. In a two-way ANOVA test, the combined genetic effects of -514C/T and other polymorphisms on HL activity were consistent with only the locus -514C/T variation having a specific association (data not shown).

There were no statistically significant differences in the age (mean 59 yr), body mass index (mean 26.4), waist-to-hip ratio (mean 0.94), or systolic or diastolic blood pressure (means 135 and 83 mmHg, respectively) in patients with different genotypes. The only exception was that the systolic blood pressure was slightly higher (mean 141 ± 1.0 SEM, $P = 0.027$) in patients

Table II. Biochemical Characteristics: Mean, \pm SEM, and Statistical Significance (ANOVA) According to *LIPC* Genotypes at Locus -514C/T

Genotype	Characteristics	Lipoprotein fraction total	VLDL	IDL	LDL	HDL
				**	*	***
C/C	Triglycerides mmol/liter	1.59 \pm 0.05	1.06 \pm 0.05	0.11 \pm 0.00	0.24 \pm 0.01	0.16 \pm 0.00
C/T		1.59 \pm 0.06	1.03 \pm 0.06	0.12 \pm 0.00	0.26 \pm 0.01	0.16 \pm 0.00
T/T		1.76 \pm 0.12	1.13 \pm 0.10	0.14 \pm 0.01	0.29 \pm 0.02	0.19 \pm 0.01
				**		
C/C	Cholesterol mmol/liter	5.15 \pm 0.05	0.49 \pm 0.02	0.22 \pm 0.01	3.42 \pm 0.04	1.02 \pm 0.01
C/T		5.19 \pm 0.06	0.49 \pm 0.03	0.24 \pm 0.01	3.45 \pm 0.05	1.02 \pm 0.01
T/T		5.27 \pm 0.15	0.57 \pm 0.05	0.30 \pm 0.03	3.38 \pm 0.12	1.03 \pm 0.03
C/C	Protein mg/liter		23.7 \pm 0.73	11.3 \pm 0.23	83.1 \pm 0.95	143 \pm 1.8
C/T			23.2 \pm 0.75	12.5 \pm 0.76	82.6 \pm 1.3	142 \pm 2.5
T/T			24.8 \pm 1.4	13.1 \pm 0.90	81.1 \pm 2.2	148 \pm 5.8

Statistical significance for triglyceride levels, cholesterol level in VLDL and IDL fractions, and protein level in VLDL, IDL and HDL fractions is tested with log-transformed data. ANOVA, $P = * < 0.05$, $** < 0.01$, and $*** < 0.001$.

with the A/A genotype in locus T457T. There were no statistically significant differences in cholesteryl ester transfer protein or LPL activities among patients with different HL genotypes.

Discussion

In this study, six polymorphisms in the HL gene (*LIPC*) were determined in a well-characterized group of CAD patients and associations with HL activity as well as with the distribution and composition of different lipoproteins were assessed. One of the polymorphisms, a C to T transition at position -514 in the promoter region of the *LIPC*, showed a strong association with HL activity and with the triglyceride content of IDL, LDL, and HDL and with the cholesterol content of IDL. These results are in line with previous studies, which have reported that HL has an effect on triglyceride levels in these lipoprotein classes. This study also confirms the result of Jansen et al. (19) that the -514C/T (-480C/T) polymorphism has a marked effect on HL activity.

The HDL-C level is one of the most important metabolic risk factors of premature atherosclerosis (32–35). It has been shown that the plasma HDL-C level is influenced by the activities of HL and LPL; high HL activity and low LPL activity are both associated with low HDL levels (36, 37). Analysis of polymorphic markers close to the *LIPC* on chromosome 15 and of the apolipoprotein AI/CIII/AIV gene complex on chromosome 11 have revealed that the *LIPC* locus accounts for 25% and the apo AI/CIII/AIV gene cluster for 22% of the total interindividual variation in plasma HDL-C level (21, 38). At least one study supports the hypothesis that high HL activity may contribute to the development of atherosclerosis; elderly men with abdominal obesity and asymptomatic myocardial ischemia had higher HL activity and lower HDL-C plasma concentration than control subjects (39). HL activity correlates also with presence of small and dense LDL particles (40). On the other hand, the hypothesis that high HL activity might be atherogenic is challenged by studies in which high HL activity has been shown to be correlated with a low degree of accumulation of aortic cholesterol in transgenic mice (41) and inversely correlated with the development of calcific atherosclerosis in homozygous familial hypercholesterolemia (42). In this study, there were no statistically significant differences in allele frequencies of HL polymorphisms between groups of coronary heart disease patients and Finnish students.

Further studies are needed to reveal the mechanisms of the variation in HL activity that are associated with -514C/T polymorphism. No mutations affecting HL activity have been found in exons of the HL gene in individuals having the T allele (19, 21), supporting the hypothesis that the real defect(s) is in the promoter region. In this study five other polymorphisms that affect HL activity were studied to reveal their possible combined effects on HL activity. One of these polymorphisms, L334F, was in linkage disequilibrium with -514C/T ($D' = 0.45$, $P = 0.002$), but its F allele is rare and can not explain our findings. However, when it is present it further reduces, as does also the M allele of the even rarer polymorphism T383M ($D' = 0.18$, $P = \text{NS}$), the HL activity in individuals with the T allele at locus -514. Possible candidate loci for negative regulatory elements for HL have been reported and some of these match with loci -514C/T and -250G/A (43, 44).

The role of HL in atherogenesis is not clear, but this study emphasizes that HL has an important role in HDL metabo-

lism. The polymorphism -514C/T will be an important tool in future studies assessing the question of whether a low or high HL activity, perhaps in combination with other defects of lipoprotein metabolism, is associated with the risk for atherosclerosis.

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