rapid communication

The C-514T polymorphism in the human hepatic lipase gene promoter diminishes its activity

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Abstract **Four common polymorphisms in the promoter of the hepatic lipase gene (*LIPC*) that are in almost complete linkage disequilibrium have been described in different ethnic groups. The T and A alleles at positions −514 and −250, respectively, were observed to be associated with decreased hepatic lipase (HL) activity and increased triglyceride content of HDL and LDL particles. We investigated whether these polymorphisms have any effect on transcriptional activity of the proximal promoter (−639 to +29) in transient transfection assays. We found that the promoter with T at position −514 had approximately 30% lower activity than the one with C at the same position ($P < 0.0005$) regardless of the genotype at position −250. In conclusion, these data indicate that the −514T allele may contribute significantly to decreased HL activity and the resultant increase in plasma levels of triglyceride-rich HDL_2 and large buoyant LDL particles. In addition, this promoter variant may explain the finding that its presence attenuates the increase in HL activity with increasing intra-abdominal fat in women.**—Deeb, S. S., and R. Peng. **The C-514T polymorphism in the human hepatic lipase gene promoter diminishes its activity.** ***J. Lipid Res.*** **2000.** 41: **155–158.**

Supplementary key words hepatic lipase • promoter • polymorphisms • activity

Hepatic lipase (HL) is a glycoprotein that catalyzes the hydrolysis of mono-, di-, and triacylglycerols, phosphatidylcholines, and phosphatidylethanolamines (1; reviewed in refs. 2, 3). The majority of HL is synthesized and secreted by the liver and is bound to heparan sulfate proteoglygans on the surfaces of sinosoidal endothelial cells and external surfaces of microvilli of parenchymal cells in the space of Disse (4–6). The human hepatic lipase gene (*LIPC*), located on chromosome 15q21, is comprised of 9 exons, spans over 30 kb of DNA, and encodes a protein of 449 amino acids (7, 8).

HL plays a major role in remodeling of high density lipoprotein (HDL) by promoting the conversion of large triglyceride-rich HDL_2 to small, dense HDL_3 particles (9, 10). High HL and low lipoprotein lipase activity levels are associated with low HDL-C (especially HDL_2) levels (9, 11). In addition, HL catalyzes the hydrolysis of triglycerides and phospholipids of the intermediate density lipoproteins and large buoyant low density lipoprotein (LDL) to form the more atherogenic small, dense LDL particles. The presence of small dense LDL is a common trait in the general population and is associated with an increased risk of coronary artery disease (12–15).

HL participates with surface proteoglycans and the LDL receptor-like protein in promoting hepatic uptake of lipoproteins (2, 16–19).

Recent studies have identified four common polymorphisms (G-250A, C-514T, T-710C, A-763G) in the *LIPC* promoter (20). These polymorphisms have attracted considerable interest because they were initially observed to be associated with total HDL cholesterol levels (20). The four polymorphisms are in almost complete linkage disequilibrium and, therefore, define two haplotypes. The frequency of the less common haplotype was found to range between 0.15 to 0.21 among Caucasians (21), 0.45–0.53 among African Americans (21, 22) and is 0.47 among Japanese Americans (21). The −514T allele was shown to be associated with a statistically significant decrease in post-heparin HL activity (21, 23–27) which in turn conferred a favorable lipoprotein profile comprised of increased HDL_2-cholesterol (but not necessarily total cholesterol) levels and large buoyant LDL particles (21, 28). The change in HL activity upon intensive lipid treatment was shown to be a strong predictor of regression of coronary artery stenosis (29). Moreover, we recently showed that the *LIPC* promoter variant attenuates the increase in HL activity with increasing intra-abdominal fat in women (28). All these observations indicate that the *LIPC* promoter polymorphisms are potentially of clinical significance as genetic markers for the above traits.

The observed associations of the *LIPC* promoter polymorphisms with the above traits do not constitute causal-

Abbreviations: *LIPC*, hepatic lipase gene; LDL, low density lipoprotein; HDL, high density lipoprotein.

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ity. These polymorphisms may alter promoter activity and HL levels or they may be in linkage disequilibrium with other polymorphisms of the gene that may impact these variables. In the present study, we addressed this question by determining the effect of the polymorphisms at positions −250 and −514 on activity of the proximal *LIPC* promoter in transient transfection assays.

METHODS

LIPC promoter constructs

Plasmid constructs in which promoter alleles direct expression of the luciferase gene were prepared by PCR amplification of a promoter segment extending from −639 to +29 using total genomic DNA as template and the following primers:

Forward 5′ tcatgagctcCAGTCCTCTACACAGCTGGAAC 3′
Reverse: 5′ tagtaagcttCGGGGTCCAGGCTTTCTTGG 3′.

The underlined sequences are *Sst*I and *Hind*III cleavage sites for directional cloning of the PCR products. An initial denaturation step at 94°C for 2 min was followed by 25 cycles of 94°C for 15 sec, 66°C for 30 sec, and 72°C for 45 sec, and a final extension step of 4 min at 72°C. The wild-type promoter was amplified from DNA of a Caucasian who was homozygous for C at position −514 and G at position −250 and the double variant promoter was amplified from an African American who was homozygous for T at −514 and A at −250. The amplified segments were then cloned between the *Sst*I and *Hind*III sites of the luciferase vector pXP1 (ATCC #37576)(30) and sequenced.

Variant *LIPC* promoters with C and A or T and G at positions −514 and −250, respectively, were constructed from the wild-type and double mutant clones according to the following strategy. The wild-type and double mutant plasmid vectors were digested with *Hind*III and *Eco47*III which excised a fragment extending from the 3′ multiple cloning site (*Hind* III site) to position −339 (*Eco47*III site between the −250 and −415 polymorphic sites). The digests were mixed and religated to generate promoter constructs having all four possible combinations of alleles at the two sites. The resultant clones were distinguished from each other initially by digestion of the PCR-amplified promoter inserts with *Nla*III (distinguishes between T and C at position −514) and *Dra*I (distinguishes between A and G at position −250). The promoter constructs used in transfection experiments were sequenced to confirm their identity.

Cell culture and transient transfection

AML12 cells were grown in plastic flasks to 70–80% confluence in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum as described (31). Twenty four hours before transfection, 1-ml aliquots of medium containing approximately 10^5 cells were distributed into 24-well plates and incubated at 37°C. The cells were transfected with 0.5 μg plasmid DNA (promoter constructs) and 1.7 μl lipofectamine reagent (Gibco-BRL) for 5 h according to the manufacturer's protocol. The cells were co-transfected with a plasmid (CMV-LacrF) that expresses β-galactosidase under control of the cytomegalovirus promoter in order to correct for transfection efficiency. The transfected cells were harvested 24 h post transfection and cell extracts were assayed for luciferase and β-galactosidase activities and for total protein concentration as described (32). At least two different vector DNA preparations were used in multiple independent transfections, each in quadruplicate.

RESULTS

Activity of the *LIPC* promoter in the murine αML12 cell line

The activity of the human *LIPC* promoter has been investigated by transient transfection of the human hepatocarcinoma cell line HepG2 (33). In general, the promoter exhibited very low activity in these cells. We also have attempted to assess the utility of this cell line to investigate the impact of the *LIPC* promoter variants. The results (data not shown) confirmed earlier reports that the activity in HepG2 cells was too low to reliably detect small differences between alleles. We next investigated the activity of the promoter in the murine hepatocyte cell line AML12. AML12 is a nontransformed hepatocyte cell line isolated in Dr. Nelson Fausto's laboratory (University of Washington) from transgenic mice expressing transforming growth factor alpha, a potent mitogen of hepatocytes (31). This cell line exhibits a high state of hepatocytic differentiation.

Transfection of AML12 cells with the −639/+28 *LIPC* promoter construct driving expression of the luciferase reporter gene resulted in 10- to 20-fold higher activity than transfection of HepG2 cells. This increase was due in part to the 2.5-fold higher transfection efficiency of αML12 cells. The luciferase light units in these assays ranged between 20,000 and 50,000. These results qualify the utility of the AML12 cell line to reliably detect small differences in promoter activity. Therefore, we used these cells to investigate whether the polymorphisms at positions −250 and −514 influenced the activity of the −639 promoter.

LIPC promoter polymorphism at position −514 decreases its activity in AML12 cells

We first performed transfection experiments to compare the activity of the wild-type −639 promoter, with G at −250 and C at −514, to the double variant promoter with A and T at the corresponding positions. The results are shown in **Fig. 1**. The variant promoter, with A and T at positions −250 and −514, respectively, had approximately 70% of the activity of the wild-type promoter ($P = 0.0005$). We next investigated the impact of each promoter polymorphism independently. The promoter with T at position −514 also had 70% of wild-type activity regardless of the genotype at position −250 ($P = 0.0004$). On the other hand, the promoter construct with the G-250A substitution only had diminished activity but was not statistically different from wild-type.

DISCUSSION

We have shown, by transient transfection assays of the murine hepatocyte cell line AML12, that the −514 T allele of the *LIPC* promoter has approximately 30% lower activity than the C allele. The G-250A polymorphism does not seem to have any impact on promoter activity in this transient transfection system. The promoter with T at −514 and A at −250 has the same activity as that with T at

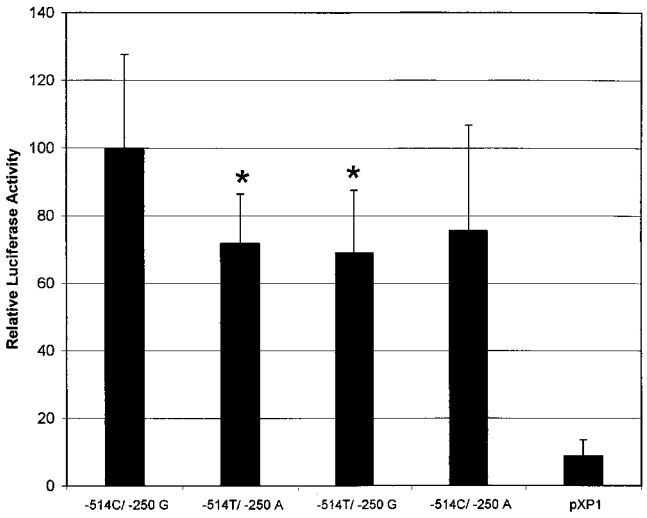

Fig. 1. Activity of the *LIPC* proximal promoter variants in AML12 cells. The data (mean ± standard deviation) were derived from 24 independent transfections, 4 different experiments, and two independent plasmid DNA preparations. The luciferase light units in these assays ranged between 20,000 and 50,000. * Indicates statistically significant difference from activity of the −514C/−250G promoter, $P < 0.001$(*t*-test).

−514 and G at −250. Therefore, there does not appear to be synergy between the two promoter substitutions. The two other polymorphisms (T-710C and A-763G) have not been investigated and could potentially contribute to variation in activity. The *LIPC* promoter variant at position −514 was previously tested for its influence on activity by transient transfection assays in the human hepatocarcinoma cell line HepG2 (26, 34). The activities of the *LIPC* promoter constructs in HepG2 were very low (as was also indicated by our studies) which precluded observing any difference between the C and T alleles. HepG2 cells, not being fully differentiated, may lack critical transcription factors for the *LIPC* promoter.

Association studies suggest but do not prove causality. The promoter polymorphisms could be in linkage disequilibrium with other polymorphisms of the gene that may impact activity levels. The functional consequence of the C-514T substitution reported in this study supports causality in decreasing the level of HL activity and the consequent increases in HDL and LDL particle size and density. In support of this notion is evidence from other laboratories pointing to the absence of mutations in the coding sequence of HL that would account for variance in HL activity (20, 23, 24, 26, 35). Therefore, it is unlikely that the promoter variants are in linkage disequilibrium with coding sequence variants that decrease HL activity. However, it is still possible that the four promoter polymorphisms are in linkage disequilibrium with yet unidentified regulatory mutations in intronic or distal enhancer elements.

The proximal region (from −1,865 to +129) of the *LIPC* promoter has been subjected to in vitro DNase I footprint and electrophoretic mobility shift analyses (EMSA) using rat liver (36) and HepG2 (37) nuclear protein extracts. The results revealed 9 footprints and 6 DNase I hypersensitive sites, none of which includes position −514. However, this does not preclude the possibility that a transcription factor(s) may bind to the −514 region in vivo. A database (http://pdap1.trc.rwcp.or.jp/research/db/TFSEARCH.html) search for potential transcription factor binding sites revealed that the −514 polymorphism lies within a potential binding site (CAC*GGG, the asterisk indicates the C/T polymorphism) for specific upstream stimulatory factors (USF). USF1 and 2 have been implicated in mediating insulin action on fatty acid synthase gene transcription by binding to the insulin response element (38). The consensus sequence for USF is NCACGTGN. The homologous sequence in the *LIPC* promoter lacks the palindrome and may not be as effective a binding site. The C to T substitution may disrupt potential binding to this sequence element. The CACGTG palindrome constitutes the core of a recognition site for N-Myc as well (39).

In conclusion, we have provided evidence that the C-514T substitution in the *LIPC* promoter diminishes transcriptional activity in transient transfection assays and, therefore, may underlie the observed association of this polymorphism with diminished hepatic lipase levels and with HDL and LDL composition and size. In addition, the lower activity of the −514T allele may explain our observation that this variant attenuates the increase in HL activity with increasing intra-abdominal fat in women (28).

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