Common Variants in the Promoter of the Hepatic Lipase Gene Are Associated With Lower Levels of Hepatic Lipase Activity, Buoyant LDL, and Higher HDL_2 Cholesterol

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Abstract—Increased hepatic lipase (HL) activity is associated with small, dense, low density lipoprotein (LDL) and low high density lipoprotein$_2$ (HDL_2) cholesterol (-C) levels. A polymorphism in the promoter region of the HL gene (*LIPC*) is associated with HDL-C levels. To test whether this association is mediated by differences in HL activity between different *LIPC* promoter genotypes, the *LIPC* promoter polymorphism at position −250 (*G*→*A*), HL activity, LDL buoyancy, and HDL-C levels were studied in white normolipidemic men and men with coronary artery disease (CAD). The less common *A* allele (frequency=0.21 and 0.25 in normal and CAD subjects, respectively) was associated with lower HL activity ($P<0.005$ by ANOVA) and buoyant LDL particles ($P\leq0.01$) in both groups. Normal and CAD subjects heterozygous for the *A* allele had lower HL activity (by 24% and 29%, respectively) and significantly more buoyant LDL particles. Homozygosity for this allele (*AA*) was associated with an even lower HL activity in normal (−26%) and CAD (−46%) subjects. The *A* allele was associated with higher HDL_2-C in CAD patients ($P=0.007$); heterozygotes and homozygotes for the *A* allele had a 92% and a 140% higher HDL_2-C level ($P<0.01$) than did *GG* individuals. In a small number of normolipidemic subjects, the same trend in HDL_2-C was seen. In a univariate analysis, the *LIPC* genotype accounted for 20% to 32% of the variance in HL levels among normal subjects and CAD patients, respectively. After adjustment for HL, the association between *LIPC* genotype and LDL buoyancy was no longer significant, suggesting that the effect of *LIPC* genotype on LDL buoyancy is mediated by its effects on HL activity. The *LIPC A* allele was more frequent in Japanese-Americans and African-Americans than in whites. In summary, these results suggest that variants in the *LIPC* promoter may significantly contribute to the variance in levels of HL activity and consequently, to the prevalence of the atherogenic small, dense, LDL particles and low HDL_2-C levels. **(*Arterioscler Thromb Vasc Biol.* 1998;18:1723-1729.)**

Key Words: LDL ■ HDL ■ triglycerides ■ cardiovascular disease ■ lipoprotein lipase

Hepatic lipase (HL) is a glycoprotein that catalyzes the hydrolysis of triacylglycerols and phospholipids.[1–3] Hepatic lipase is synthesized and secreted by the liver and is bound to the surface of sinusoidal endothelial cells and the external surfaces of microvilli of parenchymal cells in the space of Disse.[4] The human HL gene (*LIPC*), located on chromosome 15q21, comprises 9 exons and 8 introns, spans >30 kb of DNA, and encodes a protein of 449 amino acids, with a signal peptide of 23 amino acids.[5,6]

HL has emerged as a key player in the metabolism of both LDL and HDL. Plasma HDL cholesterol (HDL-C) levels are inversely correlated with HL activity[7]; specifically, HL promotes the conversion of large, buoyant HDL_2 to small, dense HDL_3 by modulating the phospholipid content of these particles.[8,9] Epidemiological studies in humans have indicated that a low level of plasma HDL-C is one of the major risk factors for coronary artery disease (CAD).[10,11] The plasma HDL concentration is modulated by environmental factors such as obesity,[12] cigarette smoking,[13] and a sedentary lifestyle.[14] A strong contribution by genetic factors has also been suggested by family[15,16] and twin[17,18] studies. It has been estimated that between 40% and 60% of the interindividual variation in HDL-C levels is accounted for by genetic variability.[17] These studies have generated intense interest in identifying specific genetic polymorphisms that may influence HDL-C levels. The first evidence for involvement of the *LIPC* locus in influencing HDL-C levels was provided by Cohen et al.[19] Their results suggested that in normolipidemic subjects, allelic variation at the *LIPC* locus accounted for 25% of the interindividual variation in plasma HDL-C levels. These findings were subsequently confirmed by the same group in a much larger population.[20] In addition, they observed 4 polymorphism in the 5′-flanking region of *LIPC*: *G*→*A* at position −250, *C*→*T* at −514, *T*→*C* at −710, and

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A→G at −763, with respect to the transcription start site.[20] These 4 polymorphisms were observed to be in complete linkage disequilibrium in the white population studied and in a subsequent African-American population[21] and were defined a single haplotype. Association studies indicated that the presence of the *T* allele at position −514 was associated with elevated plasma HDL-C and apo A-I levels in men but not in women.[20] The authors speculated that the *T* allele at −514 is either directly or indirectly (in linkage disequilibrium with another mutation) associated with lower HL activity.

Low HL activity is also associated with more buoyant, less atherogenic LDL particles.[22–25] HL catalyzes the hydrolysis of phospholipids in the IDL and large LDL to form smaller, denser LDL particles. The prevalence of small, dense LDL is associated with an increased risk of premature CAD[26–30] and is a common trait in the general population.[31] Complex segregation analysis indicated that a major gene influences small, dense LDL, with an allele frequency of 0.19 to 0.32.[32,33] A study by Nishina et al[34] has suggested a positive linkage (log of the odds score >4.0) to a locus at or near the LDL receptor on chromosome 19. A recent report from the same group, however, shows no sequence variants in the LDL receptor gene in these families.[35] One study suggested multigenic control of LDL size, including the cholesteryl ester transfer protein and manganese superoxide dismutase loci, as well as the LDL receptor locus,[36] although the role of these loci in determining LDL size variability remains to be established. Recent evidence has shown that HL activity is an important modulator of LDL density in both normal subjects and patients with CAD.[22]

Since the present study was completed, 2 groups reported an association between *LIPC* and HL activity. The *LIPC* rare allele was shown to be associated with decreased HL activity and modest elevations in HDL-C levels in Dutch patients with CAD.[37] *LIPC* has also been reported to account for the decrease in HL activity[21,37] and the elevation in HDL-C levels seen in African-American males.[21] We confirm these findings in the current study. However, the changes in HDL-C have been demonstrated to be confined to HDL_2-C. Importantly, the rare allele was associated with more buoyant LDL particles.

Methods

Study Subjects

Sixty-eight healthy, unrelated white subjects were studied: 40 women and 28 men aged 33±9 years (mean±SD) with no known lipid disorders or other major diseases affecting lipid metabolism and who were not taking any lipid-altering medication, including β-blockers or estrogens. In addition, 60 unrelated dyslipidemic men with elevated apo B levels, who had originally participated in a clinical intervention trial,[38] aged ≤62 years at entry and with CAD diagnosed by coronary angiography, were also included in the study. Both the normolipidemic[31] and CAD [38] subjects have been characterized previously. For comparison of ethnic groups, 31 healthy Japanese-Americans and 56 healthy African-Americans were evaluated. When studied, patients with CAD were not taking any lipid-lowering medication and were free from diabetes, liver, thyroid, or kidney disease. Five CAD patients were taking β-blockers at the time of the study. All subjects read and signed a consent form approved by the institutional review board.

After an overnight fast, blood specimens were collected in 0.1% EDTA for DNA isolation, lipoprotein density gradient distribution, and plasma lipid measurements. An intravenous heparin bolus of 60 IU/kg was then administered, and after 10 minutes, blood was collected into iced lithium-heparin tubes for measurement of HL activity. Blood samples were immediately processed by centrifugation at 4°C and stored at −70°C for later determination of HL activity.[22]

Lipid and Lipoprotein Determinations

LDL, HDL, HDL_2, and HDL_3 cholesterol and plasma triglycerides were measured at the Northwest Lipid Research Laboratories as previously described.[31,38] HDL-C was measured in the supernatant after precipitation of apo B–containing lipoproteins with dextran sulfate.[39] A second precipitation with high-molecular-weight dextran sulfate was performed on the supernatant containing HDL to separate the HDL_2 and HDL_3 subspecies.[39]

Density Gradient Ultracentrifugation

Nonequilibrium density gradient ultracentrifugation was used to study LDL density.[40] By layering 2 mL of plasma adjusted to a density of 1.080 g/mL (final volume, 5 mL) underneath 12 mL of a 1.006 g/mL NaCl solution, a discontinuous salt gradient was produced in a Sorvall TV-865B vertical rotor (DuPont). Samples were centrifuged at 65 000 rpm for 90 minutes (total $\omega t^2 = 2.36 \times 10^{11}$) at 5°C; the tubes were then fractionated from the bottom at a flow rate of 1.7 mL/min, and 38 fractions were collected. Total cholesterol was measured in each fraction. The relative flotation rate (Rf), which characterizes LDL peak buoyancy, was obtained by dividing the fraction number containing the LDL-C peak by the total number of fractions collected. The LDL Rf number determined by single vertical-spin density gradient ultracentrifugation was found to be highly reproducible.[22]

Postheparin Plasma Lipase Activity

Total lipolytic activity was measured in postheparin plasma as described previously.[41] Substrate containing tri[1-^{14}C]oleate and lecithin was incubated with aliquots of postheparin plasma for 60 minutes at 37°C, and the liberated free fatty acid radioactivity was extracted and counted. Lipase activity is expressed as nanomoles of fatty acid released per minute per milliliter of plasma nmol · min^{-1} · mL^{-1}. Lipoprotein lipase (LPL) activity was selectively eliminated by incubation with a specific monoclonal antibody (5D2) against LPL. HL activity was defined as the remaining activity detected in the postheparin sample after incubation with the antibody.[42] For each assay, a bovine milk LPL standard and a human postheparin plasma standard were included to adjust for interassay variation.

DNA Analysis

The genotype at position −250 of the *LIPC* promoter was determined by polymerase chain reaction amplification with the use of the following primer pairs: forward, 5′-CCTACCCCGACCTTTGGCAG-3′; reverse, 5′-GGGGTCCAGGCTTTCTTGG-3′. Amplification was carried out in a 10-μL reaction with an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of amplification at 92°C for 15 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at

TABLE 1. *LIPC* Promoter Varients in Unrelated, Random American Populations

Population	n	−250 *A* Allele Frequency
White	68	0.21
Japanese	31	0.47*
African	56	0.45*

*Compared with whites, Japanese-Americans ($\chi^2 = 13.32$, $P < 0.001$) and African-Americans ($\chi^2 = 15.39$, $P < 0.001$) have more frequent occurrences of the −250 A allele.

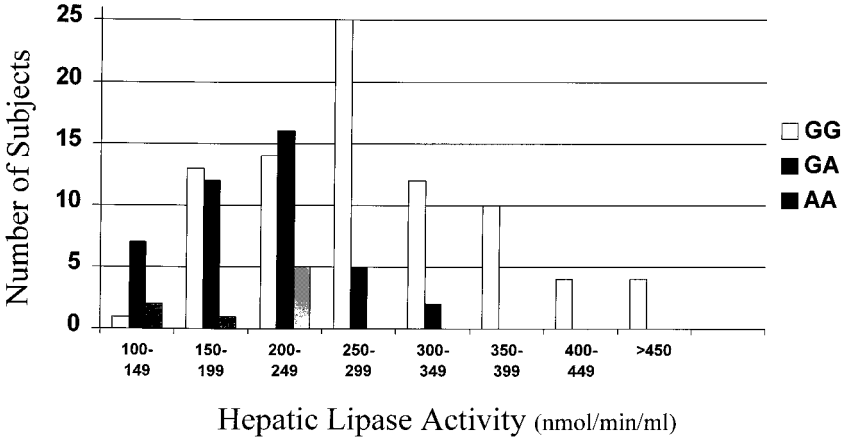

Figure 1. Frequency distribution of *LIPC* −250 polymorphism in normolipidemic and CAD white subjects combined (n=128).

72°C for 7 minutes. Five microliters of the polymerase chain reaction product was digested with the restriction enzyme *Dra*I, followed by electrophoresis on a 1% agarose gel.

Statistical Analysis

Values presented in the tables and figures are expressed as mean±SEM. Comparisons among groups were performed by ANOVA with Tukey's test for all pairwise comparisons between different *LIPC* genotypes. In normolipidemic subjects, although HL activity was lower in women than in men, the relationship between *LIPC* genotype and HL activity was similar between men and women (see Results). Therefore, all quantitative variables (HL activity, LDL-Rf, LDL-C, HDL-C, and triglycerides) were adjusted for sex.[43] Values were all adjusted to male levels for comparison with the CAD subjects, who were all men. Significance level was set at $P<0.05$.

Results

Association of *LIPC* −250 Polymorphism and HL Activity

Subjects in both white populations were divided according to their genotype at position −250 of the *LIPC* promoter. The observed genotypic frequencies were consistent with Hardy-Weinberg equilibrium in both the normolipidemic group (*GG*=44, *GA*=20, and *AA*=4; $\chi^2=0.14$, $P=0.71$) and the CAD group (*GG*=32, *GA*=24, and *AA*=4; $\chi^2=0.03$, $P=0.85$). The frequency of the *A* allele, 0.21 in normolipidemic subjects and 0.25 in CAD subjects, was not different between the 2 groups. The −250 *A* allele was found to occur at a much higher frequency in unselected, unrelated African-Americans (n=56) and Japanese-Americans (n=31) (Table 1). The 4 promoter variants were in strong, but not complete, linkage disequilibrium in these 2 additional populations. In the white population, complete linkage disequilibrium was found.

The distribution of HL activity by genotype was not different between normolipidemic and CAD subjects. Therefore, data from the 2 groups were combined (Figure 1). HL activity in both *GG* and *GA* genotypes was normally distributed. Mean HL activity was higher in subjects with the *GG* than in those with the *GA* or *AA* genotype: 285±9.5, 207±7.0, and 184±10.6 nmol · min^{-1} · mL^{-1} respectively, $P<0.001$ by ANOVA. The same relationship between HL activity and *LIPC* genotype was noted in the normolipidemic women: 202±19, 116±11, and 131±8 nmol · min^{-1} · mL^{-1}, respectively, $P<0.005$ by ANOVA. In the whole group, only 2 subjects with the *GA* genotype had an HL activity value in the 300 to 349 nmol · min^{-1} · mL^{-1} range, and none was found with an HL value ≥350 nmol · min^{-1} · mL^{-1}. The range of HL activity within the *GG* genotype (143 to 538 nmol · min^{-1} · mL^{-1}) was remarkably wider that that of the *GA* genotype (124 to 364 nmol · min^{-1} · mL^{-1}).

Analysis of the association between polymorphism at the *LIPC* promoter region and HL activity in the 2 white

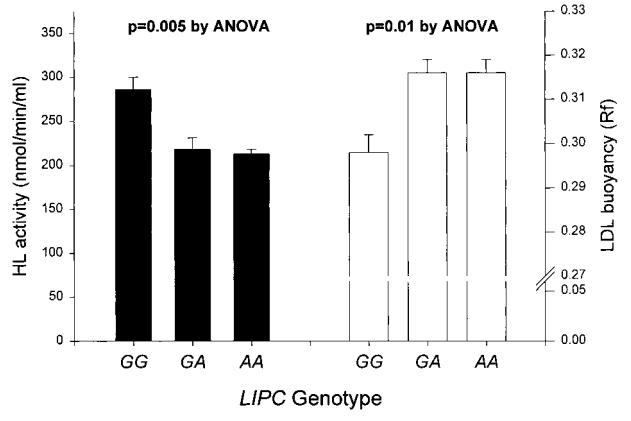

Figure 2. *LIPC* promoter polymorphism at position −250, HL activity (solid bars), and LDL buoyancy (open bars) in normolipidemic white subjects (n=68).

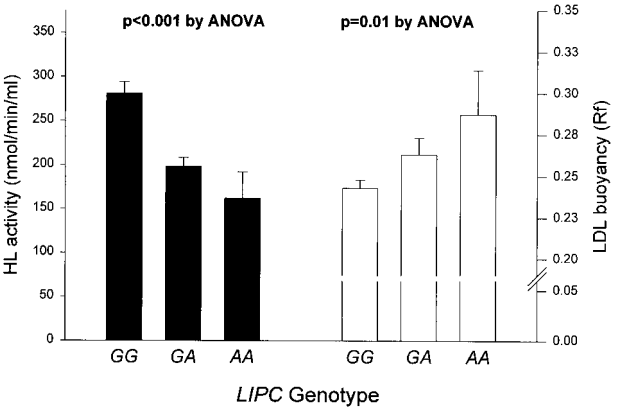

Figure 3. *LIPC* promoter polymorphism at position −250, HL activity (solid bars), and LDL buoyancy (open bars) in white patients with CAD (n=60).

TABLE 2. *LIPC* Promoter Polymorphisms and Plasma Lipid Levels

	Genotype			
Group	*GG*	*GA*	*AA*	*P*
White normolipidemic subjects				
n	44	20	4	
Triglyceride	93±14	85±13	120±40	NS
LDL-C	114±4	116±7	102±8	NS
HDL-C	48±2	53±3	56±6	NS
HDL_2-C	13±6 (9)	24±10 (5)		
HDL_3-C	36±8 (9)	39±3 (5)		*
White CAD subjects				
n	32	24	4	
Triglyceride	229±25	183±29	239±91	NS
LDL-C	184±8	195±13	173±29	NS
HDL-C	35±1	38±2	39±4	NS
HDL_2-C	2.5±0.3	4.8±0.8	6.0±2.1	0.007
HDL_3-C	33±1	33±1	33±2	NS

Values (mean±SEM) are expressed in milligrams per deciliter. Statistical analysis was done by ANOVA.

**GA* and *AA* genotypes were combined owing to small numbers, which appear in parentheses.

populations revealed a significantly lower HL activity in carriers of the *GA* and *AA* genotypes in both normolipidemic ($P=0.005$ by ANOVA, Figure 2) and CAD ($P<0.001$, Figure 3) groups. The *GA* genotype was associated with a 24% and 29% lower HL activity compared with the *GG* genotype ($P<0.05$) in normal and CAD subjects, respectively. In addition, HL activity was even lower in subjects with the *AA* genotype in both groups (by 26% in normal subjects and by 46% in CAD patients). In a univariate analysis, the polymorphism in the promoter region of *LIPC* accounted for 20% and 32% of the variance in HL activity among normolipidemic and CAD subjects, respectively.

Association of *LIPC* −250 Polymorphism and LDL Buoyancy

The polymorphism in the promoter of *LIPC* was associated with LDL buoyancy. LDL particles were significantly more buoyant in subjects with the *GA* and *AA* genotypes in both normolipidemic individuals ($P=0.01$, Figure 2) and patients with CAD ($P=0.01$, Figure 3). In multivariate analysis, after adjustment for HL activity levels, the association between *LIPC* genotype and LDL buoyancy was no longer significant.

Association of *LIPC* −250 Polymorphism and HDL_2-C Levels

No significant association was observed in either group between the *LIPC* promoter polymorphism and plasma triglyceride, LDL-C, or HDL-C levels (Table 2), although there was a trend toward higher HDL-C in *GA* and *AA* subjects compared with *GG* individuals in both groups. It is possible that the lack of a significant association between *LIPC* genotype and HDL-C level is due to the small number of subjects homozygous for the rare allele.

In subjects with CAD, for whom HDL subclasses were analyzed separately, the *LIPC* promoter polymorphism was strongly associated with the HDL_2-C level ($P=0.007$), but not with HDL_3-C (Table 2). Heterozygous and homozygous individuals for the *A* allele had HDL_2-C levels that were 92% and 140% higher, respectively, than those of *GG* individuals. In 14 of 68 normolipidemic whites who had HDL subfractions measured, a similar trend was noted. Within each genotype, HDL_2 was lower in the CAD patients compared with those without CAD. In multivariate analysis, after adjustment for HL activity levels, the association between *LIPC* genotype and HDL_2-C was no longer significant.

Discussion

This study demonstrates that the *G*→*A* substitution at position −250 of the *LIPC* promoter is associated with lower HL activity, more buoyant LDL particles, and higher HDL_2-C levels, with no effect on HDL_3-C.

Modest associations between *LIPC* promoter polymorphisms and total HDL-C levels have been detected in a number of population studies.[19,20,36,37,44] Presumably, these changes in HDL-C are a reflection of changes in HDL_2-C. These findings have led to the hypothesis that *LIPC* promoter

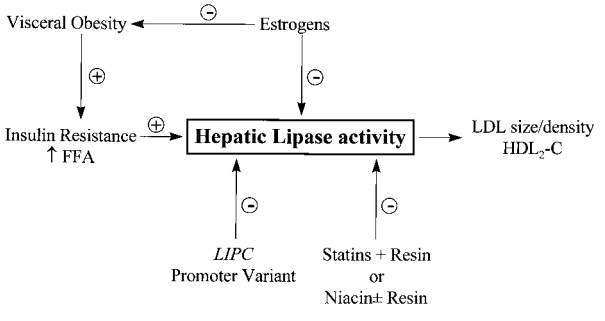

Figure 4. Endogenous and exogenous factors affecting HL activity. − indicates negative (inhibitory) effect; +, positive (stimulatory) effect.

polymorphisms may be associated with lower HL activity values, which are known to be inversely related to HDL-C levels.[7–9] Our study directly addressed this hypothesis and confirmed a strong association between *LIPC* promoter polymorphism and HL activity in normolipidemic subjects as well as in patients with CAD. Normolipidemic and CAD subjects with the *GA* genotype at position −250 had 24% and 29% lower HL activity, respectively, compared with homozygous *GG* individuals. Even lower HL activity (by 26% and 46%, normal subjects and CAD patients) was detected in individuals with the *AA* genotype. Variability in the *LIPC* promoter region accounted for 20% and 30% of the variance in HL activity among normal subjects and CAD patients, respectively. In this study population, HL activity was normally distributed among individuals with either the *GG* or *GA* genotype (Figure 1). However, the range of HL activity among individuals with the *GG* genotype (143 to 538 nmol · min^{-1} · mL^{-1}) was wider then that of *GA* individuals (124 to 364 nmol · min^{-1} · mL^{-1}), indicating a strong negative effect of the *A* allele on HL expression. Since completion of this study, similar findings were reported in Dutch[37] and African-American populations.[21]

In previous studies,[20] no functional mutations were detected in the coding region of *LIPC* that could account for the observed association between polymorphism in the *LIPC* promoter region and plasma HL activity. This suggests that 1 or more of the promoter variants may actually be responsible for diminished transcription of the gene, as has also been described in a recent preliminary report.[45] Our results are consistent with this notion. An alternative explanation is that the promoter polymorphism might be in linkage disequilibrium with other variants located either within *LIPC* itself or within an adjacent gene encoding a protein that regulates *LIPC* expression. The observation[19–21,44,46] that the same *LIPC* haplotype was associated with HDL-C or HL activity in different populations makes this a less likely explanation.

Not all studies have detected an association or linkage between HDL-C and markers at the *LIPC* locus. Apparently conflicting results have been presented by Mahaney et al[47] in a Mexican-American population from the San Antonio Family Heart Study. Using model-based linkage analysis, these authors found evidence for a major locus effect on plasma HDL-C levels after adjustment for apo A-I levels but excluded the possibility of linkage between the observed major locus and the *LIPC* locus. A possible explanation for this discrepancy might be that the predominant locus affecting HDL-C levels in Mexican-Americans is different from that in the non-Hispanic white population. An alternative explanation for the apparent discrepancy of these findings may be provided by the results of the current study, showing that only HDL_2-C levels, which represent a fraction of total HDL-C, appear to be strongly associated with *LIPC* polymorphism, whereas the major HDL_3 component is not. It has been known that HL activity is primarily associated with HDL_2-C and not HDL_3-C.[48–50] This suggests that HDL_2-C levels rather than total HDL-C, which in the current report was not significantly associated with *LIPC* promoter polymorphism, may represent a more sensitive parameter for testing the effect of *LIPC* promoter polymorphisms on HDL metabolism. Carriers of the *A* allele with CAD had 92% to 140% higher HDL_2-C levels compared with the *GG* individuals, whereas no differences in HDL_3-C were detected. A similar trend was seen in the normolipidemic subjects, though because of the small number of samples in which HDL_2 was measured, the differences were not statistically significant. It is also possible that the relationship between genotype and HDL_2 is true only for CAD patients. The association between *LIPC* polymorphisms and HDL_2-C levels was mediated by the effects of *LIPC* polymorphisms on HL activity. These findings and perhaps the study size may account for the different results of the current study compared with previous reports in which total HDL-C levels were measured.[19–21,37]

Epidemiological studies have suggested that both low HDL-C[10,11] and the presence of small, dense LDL[26–30] are associated with increased risk of CAD. These lipid abnormalities often coexist in the same subject as part of a multifaceted phenotype referred to as an atherogenic lipoprotein profile.[51] In the current study, the lipid profile characterized by more buoyant LDL and higher HDL_2-C, associated with the presence of the *A* allele and lower HL activity, appears to bear less atherogenic potential than the lipid profile associated with the *GG* genotype (small, dense LDL and lower HDL_2-C). In addition, after adjustment for plasma HL levels, the association between *LIPC* promoter polymorphism and LDL density is no longer significant, suggesting that this association is mediated by the effect of *LIPC* promoter polymorphism on plasma HL activity. This observation is in agreement with previous studies demonstrating an important role of HL activity as a major player in determining LDL size and density,[21–24] and it provides further evidence for genetic regulation of LDL subclass distribution. A number of other factors have also been shown to affect HL activity in association with changes in LDL size and density, HDL_2-C levels, and CAD risk. These include sex,[52] intra-abdominal fat, and insulin resistance.[53,54] In addition, intensive pharmacological intervention with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or niacin plus colestipol, resulted in a significantly lower HL activity, more buoyant LDL, higher HDL_2-C, and regression of CAD.[55] Therefore, plasma HL activity levels are modulated by a number of endogenous (estrogen levels, central adiposity) and exogenous (drugs) factors, in addition to the *LIPC* promoter polymorphisms (Figure 4).

Because the less-common allele frequency of the HL promoter polymorphisms is much higher in African-Americans[21] and Japanese-Americans compared with white Americans, the former 2 populations would offer the possibility of obtaining a large number of homozygotes for association studies. The presence of a common haplotype in all 3 of these populations indicates that these polymorphisms are derived from a common ancient origin.

In summary, the current study demonstrates that the *LIPC* promoter polymorphisms and plasma HL activity levels are strongly associated. This association is potentially clinically important, because the frequency of the *A* allele among whites is ≈0.2. In addition, the clinical relevance of this observation results from the association between *LIPC* promoter polymorphism and both LDL density and HDL_2-C

levels, mediated by the potential effect of these polymorphisms on *LIPC* transcription. Because the polymorphism at −250 is in nearly complete linkage disequilibrium with the 3 other promoter polymorphisms, it is not known which of these potentially affects promoter activity. These findings further highlight the contribution of genetic factors to the regulation of both HDL and LDL subclass distribution. Finally, the strong association of *LIPC* genotype with large HDL_2 but not with smaller HDL_3 particles suggests that HDL_2 concentration may represent a better lipid measure to study the effect of *LIPC* on HDL metabolism. Functional analysis of the wild-type and of the different *LIPC* promoter variants is needed to investigate whether the effect on HL activity is accounted for by 1 or more of these *LIPC* promoter polymorphisms or is due to linkage disequilibrium between these polymorphisms and other as-yet-unidentified variants located within *LIPC* itself or within a gene encoding a protein regulating *LIPC* expression.

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