Promoter polymorphisms of hepatic lipase gene influence HDL_2 but not HDL_3 in African American men: CARDIA study

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Abstract Hepatic lipase encoded by the hepatic lipase gene (LIPC) is involved in the metabolism of several lipoproteins. Four promoter polymorphisms in LIPC have been found to be in complete disequilibrium and associated with high density lipoprotein cholesterol (HDL-C) and apolipoprotein (apo)A-I levels in both white and black populations. We investigated the association between the promoter polymorphism and lipid profiles as well as anthropometric phenotypes in African American men in the Coronary Artery Risk Development in Young Adults study. We performed serial cross-sectional analyses and longitudinal analyses of lipids from 578 subjects in five examinations over 10 years of follow-up. Results showed that the allele frequency (0.52) in our black population was consistent with that reported in black subjects but much higher than that reported (~0.2) in white populations. Analysis of covariance tests of the three genotypic means in each examination showed that the *P* values ranged from 0.01 to 0.08 for HDL-C (except $P = 0.54$ in the fourth examination), from 0.006 to 0.01 for HDL_2-C, and from 0.06 to 0.07 for apoA-I. Mean HDL_3-C levels were essentially identical among the three genotypes. Total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides, and apoB, which are mainly involved in the very low density lipoprotein-LDL pathway, were not significantly different according to the promoter polymorphism, except for triglycerides in the third examination ($P = 0.01$). No significant association was found between anthropometric phenotypes and the LIPC polymorphism in any of five examinations. The change of the anthropometric variables was not significantly associated with genotypes. In conclusion, our results indicated that the LIPC promoter polymorphism has exclusive effects on HDL_2-C but not HDL_3-C levels. — Juo, S-H. H., Z. Han, J. D. Smith, L. Colangelo, and K. Liu. **Promoter polymorphisms of hepatic lipase gene influence HDL_2 but not HDL_3 in African American men: CARDIA study. *J. Lipid Res.* 2001.** 42: **258–264.**

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The human hepatic lipase gene (LIPC) is located on chromosome 15q21, and comprises 9 exons and 8 introns (1, 2). The LIPC gene encodes hepatic lipase (HL), which can cleave fatty acids from di- and triglycerides, as well as phospholipids. HL is involved in the metabolism of several lipoproteins, by catalyzing the hydrolysis of their triacylglycerols and phospholipids (3, 4). HL promotes the conversion of buoyant high density lipoprotein 2 (HDL_2) particles to small, dense, high density lipoprotein 3 (HDL_3) particles by remodeling triglycerides and phospholipids (5); and catalyzes the hydrolysis of phospholipids in intermediate density lipoprotein and large low density lipoprotein (LDL) particles to form smaller, denser LDL particles. Low HL activity has been shown to be associated with more buoyant, less atherogenic LDL particles (6–9).

Cohen and coworkers (11) first reported evidence of linkage between the LIPC and a putative locus influencing HDL cholesterol (HDL-C) levels, using sibpair linkage analysis (10); and replicated the result in an independent sample. They identified four novel promoter polymorphisms of the LIPC (−250 G/A, −514 C/T, −710 T/C, and −763 A/G), which were in complete linkage disequilibrium (LD) and significantly influenced HDL-C levels in white men (11). Subsequently, they found that the four polymorphisms were also in complete LD in black men (12). This means that all four polymorphisms are always found together and create two haplotypes. Here we denote haplotype 1 as including alleles A-T-C-G, and haplotype 2 as including alleles G-C-T-A at −763, −710, −514, and −250 polymorphisms.

Jansen et al. (13) independently reported similar results and further indicated that the primary genetic effect

Abbreviations: apoA-I, apolipoprotein A-I; BMI, body mass index; CARDIA Study, Coronary Artery Risk Development in Young Adults study; GEE, generalized estimating equation; HDL-C, high density lipoprotein cholesterol; HL, hepatic lipase; LD, linkage disequilibrium; LIPC, hepatic lipase gene; TC, total cholesterol.

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of the LIPC polymorphism was on HL activity. However, several groups subsequently reported conflicting results. Jansen et al. (13, 14), Murtomaki et al. (15), and Couture et al. (16) reported significant association between the LIPC polymorphisms and HDL-C levels. Tahvanainen et al. (17), Zambon et al. (7), Hegele et al. (18), and Shohet et al. (19) failed to find a significant genetic effect on HDL-C concentrations. Zambon et al. (7) first reported that the promoter polymorphism significantly influenced HDL_2-C but not HDL-C levels, which is consistent with the previous report that HL activity is primarily associated with HDL_2-C but not with HDL_3-C (20, 21). This result was replicated on the basis of the data from the Framingham Offspring study (16). The allele frequencies between white and black subjects are quite different: the less-common allele of the LIPC polymorphisms in white subjects was the more-common allele in black subjects (7, 12).

To test the hypothesis of the primary genetic effect on HDL_2-C, and to confirm the remarkable difference of the allele frequencies between black and white subjects, we investigated the −250 G/A LIPC polymorphism as a surrogate for the promoter haplotype, using a large black population in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Because HL activity has been suggested to be associated with obesity phenotypes (21), we also tested for the association between the genotype and anthropometric phenotypes [i.e., body mass index (BMI), waist circumference, waist-to-hip ratio, and skinfold] in this sample.

MATERIALS AND METHODS

The study was approved by the Institutional Review Boards at the Rockefeller University (New York, NY) and four CARDIA study participating centers (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA). All participants gave written informed consent.

Subjects

The details of the CARDIA study have been described elsewhere (22). In brief, the CARDIA study is a multicenter, longitudinal study on lifestyle and evolution of cardiovascular disease risk factors in young adults aged 18 to 30 years at initial examination (1985–1986). Participants were randomly recruited from the total community or from selected census tracts in the community for the centers in Birmingham, Chicago, and Minneapolis. For the center in Oakland, participants were randomly recruited from the Kaiser-Permanente health plan membership. Study subjects have received examinations and questionnaires every 2 to 3 years. To test the current hypothesis, we used the data from black men in the CARDIA cohort. Five hundred and eighty-six black men, who have participated in all five examinations over the 10-year follow-up (year 0, 1985–1986; year 2, 1987–1988; year 5, 1990–1991; year 7, 1992–1993; year 10, 1995–1996), have available DNA samples. Various numbers of individuals were further excluded (18 individuals in year 0, 48 in year 2, 54 in year 5, 65 in year 7, and 46 in year 10) in the analyses because they did not fast ≥8 h before the blood draw or because they took antilipid medication.

Measurements of lipoproteins and anthropometric phenotypes

Venous blood was drawn after a 12-h fast. Total HDL-C and HDL_3-C were measured enzymatically after dextran sulfate-magnesium precipitation of apoB-containing particles (23, 24). HDL_2-C was calculated as the difference of these two values. Total cholesterol (TC) and total triglycerides were enzymatically determined (25). LDL-C was estimated using the Friedewald, Levy, and Fredrickson equation (26) (LDL-C = TC − HDL-C − triglycerides/5). Subjects with triglycerides ≥400 mg/dl did not have calculable LDL-C and thus their LDL-C levels were not included in the analyses. Apolipoprotein A-I (apoA-I) (27) and apoB (28) were analyzed by radioimmunoassay. Lipid data in year 2 were systematically elevated because of laboratory drift, and we performed analyses on year 2 data with and without adjustment for this effect. HDL-C, TC, LDL-C, and triglycerides were available in all five examinations, but HDL_2-C, HDL_3-C, apoA-I, and apoB were available only in the first two examinations.

BMI was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured at the minimum abdominal girth with the participant standing. Hip circumference was measured at the maximal protrusion of the hips at the level of the symphysis pubica. The average of the two measurements of each circumference was used to calculate the waist-to-hip ratio (29). Sum of skinfolds was the sum of triceps and subscapular skinfolds as measured by Harpenden calipers (30).

HL promoter genotype assay

As there is a discrepancy about the HL transcriptional start site (1, 2), we have adapted the numbering system of Ameis et al. (2). To detect the LIPC promoter haplotype we assayed for the −250 G/A polymorphism, using the restriction endonuclease *Dra*I (cuts at −253 bp) after polymerase chain reaction (PCR) amplification of the HL promoter region from genomic DNA. To amplify a 666-bp product, the sense primer at −603 bp was GGG GGA AGA AGT GTG TTT ACT CTA GGA TCA CC, and the antisense primer at +63 bp was CAC AGG GGA CTT GTG TCC ATT TCT CCG. One microliter of genomic DNA (0.1 μg/μl) was added to 20 μl of a PCR mix containing a PCR buffer, the composition of which has been previously described (31), with 0.15 μl of a mix of the two primers at 50 pmol/ml, and 0.20 μl of *Taq* polymerase. PCR was performed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min. The product was digested with 0.5 μl (10 U) of *Dra*I for 3 h at 37°C. There is an invariant *Dra*I site at position −141, and thus the "G" allele without the *Dra*I site at position −253 yielded bands of 462 and 204 bp, while the "A" allele yielded bands of 350, 204, and 112 bp. The digest was run on a 2% agarose gel and stained with ethidium bromide. The presence of the 462- and/or 350-bp bands was used diagnostically to assign the genotype as GG, GA, or AA, which is correspondent to 11, 12, and 22, respectively, according to the haplotypes defined above.

Measurement of other covariates

Several demographic factors were included in the analysis: age, BMI, education, alcohol consumption (32), smoking history (32), physical activity score (33), calories (34), dietary total saturated fatty acids (SFA) and polyunsaturated fatty acids (PFA) (35–36), and Keys scores (35–37). The details of the measurements of the demographic data have been described elsewhere (32–38). In brief, age, years of education, alcohol intake, and smoking history were ascertained by a self-administered questionnaire. The score of physical activity was based on moderate and intense exercise in the previous year. Dietary intake data were obtained from a detailed diet history questionnaire (38).

Total calories and fat were estimated from the diet history questionnaire. The Keys score is to estimate the combined effect of these dietary factors on serum cholesterol, and it was calculated according to the formula

$$1.35(2S - P) + 1.5Z$$

where S is the percentage of dietary calories from SFA, P is the percentage of dietary calories from PFA, and Z is the square root of dietary cholesterol in milligrams per 1,000 kilocalories.

Statistical methods

Allele/haplotype frequencies were estimated by direct gene counting. Observed numbers of each genotype were compared with those expected for Hardy-Weinberg equilibrium by using the χ^2 test. Analysis of variance was performed to test for any significant difference of baseline demographic characteristics among three genotypes. Analysis of covariance (ANCOVA), which adjusted for age, BMI, alcohol consumption, smoking history, total physical activity scores, and Keys scores, was conducted to test for significant differences in lipid levels among the three genotypes. Similarly, the same covariates, except for BMI, were adjusted for when the genetic effect on anthropometric phenotypes was tested.

Because the data were available in five consecutive examinations, we conducted the above-described statistical tests for each examination to see whether the genetic effect was consistently significant throughout the follow-up period. In addition, the generalized estimating equation (GEE) method (39, 40) was used to examine the longitudinal data in the 10-year follow-up. The method was used to test for different trends in dependent variables among three genotypes over 10 years. A typical model is as follows:

$$\begin{aligned} Y_{it} &= \beta_0 + \beta_1 * 12\ \text{genotype}_i + \beta_2 * 22\ \text{genotype}_i \\ &+ \beta_3 t + \beta_4 * 12 * t + \beta_5 * 22 * t + \beta_6 \text{Baseline age}_i \\ &+ \beta_7 \text{Covariate1}_{it} + \beta_8 \text{Covariate2}_{it} + \ldots + e_{it} \end{aligned}$$

where t = 0, 2, 5, 7, and 10, Y_{it} is the phenotype for the ith individual at year t. Genotype 11 serves as the reference group and the other genotypes are compared with this reference group. Genotypes 12 and 22, as well as baseline age, are time-independent covariates. BMI, smoking, alcohol intake, and physical activity are time-dependent covariates, and e_{it} is the error term. The coefficient β_1 is the covariate-adjusted mean difference of the dependent variable between genotypes 12 and 11 in year 0, and similarly β_2 is between 22 and 11. The coefficient β_3 is the annual change of the dependent variable in the 11 genotype adjusting for the covariates. The coefficient β_4 measures the difference in the annual changes between the 12 and 11 genotypes; similarly, β_5 measures the difference between 22 and 11. The coefficient β_6 measures the association between the dependent variable (averaged over time) and baseline age, adjusting for other covariates. The coefficients β_7, β_8 . . . are the coefficients of the correspondent covariates. Our preliminary results indicated that coefficients of t, 12*t, and 22*t were not significant at all, and thus all the results reported here are from the model not including these covariates. In this reduced model, β_1 and β_2 should be interpreted as the overall differences in the dependent variables between genotypes in 10 years.

To reduce skewness and kurtosis, triglycerides and anthropometric phenotypes were log-transformed in the statistical tests, but untransformed means are presented in the tables. SAS software (SAS Institute, Cary, NC) was used for all statistical analyses. Two-tailed P values < 0.05 were considered significant, and $0.05 \leq P < 0.09$ were considered borderline significant.

RESULTS

Comparison of baseline characteristics

The baseline demographic characteristics among three genotypes are provided in **Table 1**. Except for Keys scores ($P = 0.06$) and dietary total SFA ($P = 0.05$) with borderline significance, there were no significant differences in mean age, BMI, education, alcohol consumption, physical activity scores, proportion of current smokers in each genotype, number of cigarettes per day for current smokers, or dietary PFA among three genotypes in the year 0 examination.

Allele frequency

DNA samples were available from 586 individuals, and 578 individuals were successfully genotyped. There were 137, 283, and 158 individuals in the GG, GA, and AA genotypes, respectively, and the frequency of allele A was 0.52. Thus the frequencies of haplotypes 1 and 2 are 0.48 and 0.52, respectively. The distribution of genotypes was not significantly different from the expectation under the Hardy-Weinberg equilibrium ($P = 0.94$). The A allele frequency in this African American population is similar to the previous reports from other African American populations (7, 12), while it is the less common allele with a frequency of approximately 0.2 in the reported white populations (7, 11–17, 19, 41).

TABLE 1. Demographic characteristics among three LIPC promoter genotypes in year 0

	11 (n = 137)[a]	12 (n = 283)[a]	22 (n = 158)[a]	Analysis of Variance (P)
Mean age (years)	23.9 (3.3)	24.6 (3.0)	24.2 (3.7)	0.16
Years of education	13.0 (2.0)	13.2 (1.9)	13.0 (2.0)	0.75
BMI (kg/m^2)	24.2 (4.0)	24.7 (4.5)	25.1 (4.2)	0.25
Physical activity score	514.9 (340.5)	521.0 (372.5)	540.2 (339.5)	0.81
Current smoker	37%	32%	34%	0.54
Daily smoking (cigarette)[b]	11.7 (7.5)	10.3 (6.9)	10.5 (7.0)	0.50
Daily drinking (ml)	16.7 (25.8)	17.8 (35.7)	18.5 (25.8)	0.88
Keys score	51.7 (11.2)	49.1 (9.7)	49.6 (10.9)	0.06
PFA (% kcal)[c]	6.3 (2.0)	6.6 (1.9)	6.7 (2.0)	0.20
SFA (% kcal)[c]	14.9 (3.1)	14.1 (2.7)	14.3 (2.9)	0.05

[a] Values represent means ± SD.
[b] Data were based on current smokers (50, 88, and 53 in genotypes 11, 12, and 33, respectively).
[c] PFA and SFA indicate dietary polyunsaturated and saturated fatty acid, respectively.

TABLE 2. Serial cross-sectional and longitudianl analyses (GEE) of HDL-C, HDL subfractions, and apoA-I levels

	11[a]	12[a]	22[a]	Analysis of Covariance (*P*)
		mg/dl		
HDL-C				
Year 0	51.4 (1.1)	53.2 (0.8)	54.9 (1.0)	0.08
Year 2	52.0 (1.3)	54.8 (0.9)	56.3 (1.2)	0.05
Year 5	48.3 (1.3)	52.6 (0.9)	52.6 (1.2)	0.01
Year 7	49.6 (1.2)	51.0 (0.8)	51.2 (1.1)	0.54
Year 10	45.9 (1.1)	48.4 (0.8)	50.0 (1.1)	0.03
Mean difference in HDL-C by GEE	Reference group	2.78 (1.12); $P = 0.013$	3.85 (1.37); $P = 0.005$	
HDL_2-C				
Year 0	13.6 (0.8)	15.3 (0.6)	17.2 (0.8)	0.006
Year 2	12.1 (0.9)	14.0 (0.6)	15.8 (0.8)	0.01
HDL_3-C				
Year 0	37.8 (0.5)	37.9 (0.4)	37.8 (0.5)	0.97
Year 2	39.9 (0.6)	40.8 (0.5)	40.5 (0.6)	0.52
ApoA-I				
Year 0	138.5 (1.9)	139.9 (1.3)	144.2 (1.8)	0.06
Year 2	142.6 (2.0)	148.0 (1.4)	147.7 (1.8)	0.07

BMI, age, daily cigarette smoking, alcohol intake, and physical activity were adjusted for. HDL_2-C, and apoA-I were available only in years 0 and 2.
[a] Values represent means ± SE.

Effects of the LIPC genotypes on lipid profiles and anthropometric phenotypes

Mean HDL-C, HDL_2-C, and apoA-I were significantly (or borderline significantly) different among three genotypes in each examination (**Table 2**) except for HDL-C in year 7 ($P = 0.54$). Mean HDL_3-C levels were essentially identical among the three genotypes, which suggests that the significant difference in HDL-C levels among the three genotypes was virtually due to the cholesterol in the HDL_2 subfraction. Haplotype 2 appears to have an additive effect on elevating lipid levels. Genotypes 12 and 22 had approximately 13% and 27% higher mean HDL_2-C levels, respectively, compared with genotype 11. From the GEE analysis of longitudinal data on HDL-C, genotypes 12 and 22 were 2.8 and 3.9 mg/dl, respectively, significantly higher than genotype 11. However, adjusted annual change in HDL-C was not significantly different among the three genotypes (data not shown), which suggested no interaction between genotypes and time. In addition, we also tested for interaction between any covariates (i.e., BMI, age, alcohol intake, smoking, and physical activities) and genotypes, and none was significant (data not shown).

On the other hand, mean TC, LDL-C, triglycerides, and apoB levels, which are primarily involved in the very low density lipoprotein-LDL metabolic pathway, were not significantly different among the three genotypes in any examination (**Table 3**), except for triglycerides in year 5 ($P = 0.01$). On the basis of biologic mechanism and serial data, the significant finding of triglycerides in year 5 is more likely to be a type I error. Results from the GEE analysis did not suggest any significant difference in genotypic means or different trends among the three genotypes in the 10-year follow-up (data not shown).

None of the anthropometric phenotypes yielded significant association with the genotypes (data not shown). *P* values ranged from 0.09 to 0.50 for BMI; from 0.16 to 0.76 for waist circumference; from 0.06 to 0.99 for waist-to-hip ratio, and from 0.08 to 0.43 for skinfold. The

TABLE 3. Cross-sectional (year 0) and longitudinal analyses (GEE) of TC, LDL-C, triglycerides, and apoB

	11[a]	12[a]	22[a]	Analysis of Covariance (*P*)
		mg/dl		
TC (year 0)	175.7 (2.9)	174.1 (2.0)	179.4 (2.7)	0.28
Mean difference in TC by GEE	Reference group	−1.22 (3.49); $P = 0.73$	3.82 (3.94); $P = 0.33$	
LDL-C (year 0)	110.8 (2.7)	106.9 (1.9)	110.4 (2.5)	0.38
Mean difference in LDL-C by GEE	Reference group	−3.53 (3.30); $P = 0.29$	−0.05 (3.73); $P = 0.99$	
Triglycerides* (year 0)	67.7 (2.9)	69.5 (2.1)	70.3 (2.7)	0.65
Mean difference in triglycerides by GEE	Reference group	−2.71 (4.32); $P = 0.64$	2.00 (4.79); $P = 0.30$	
ApoB				
Year 0	89.2 (2.0)	88.3 (1.4)	94.0 (1.9)	0.05
Year 2	90.3 (2.4)	91.8 (1.7)	93.8 (2.2)	0.57

BMI, age, daily cigarette smoking alcohol intake, and physicall activity were adjusted for. *P* values in other examinations were not significant, except for triglycerides in year 5 and year 10 (0.01 and 0.07, respectively). * For triglycerides the genotype means for years 5 and 10 are 81.9, 78.9, 91.6, and 103.9, 91.4, 100.6, respectively. ApoB was available only in years 0 and 2.
[a] Values represent means ± SE.

changes in these variables over time were not significantly different among LIPC genotypes (data not shown).

Keys scores were available only for years 0 and 7, and the results remained similar when Keys scores were included for adjustment (data not shown). The results did not change when we adjusted for the systematic error in year 2 lipid data, and therefore all values in year 2 presented in this article do not account for this error.

DISCUSSION

The present study showed that the LIPC promoter haplotype has significant (or borderline significant) influence on HDL-C, HDL_2-C, and apoA-I levels in young African American men in each examination during the follow-up period, except for HDL-C in year 7. We present the current results on HDL-C, HDL_2-C, and apoA-I levels in the context of the previous reports in white populations conducted by several independent groups (7, 11–19) (**Table 4**). The current study is the first to report an association of the LIPC promoter polymorphism with HDL-C levels in black subjects. Furthermore, the present study found that the genetic effect on HDL-C levels is due to an effect on HDL_2-C but not on HDL_3-C, which has been suggested by two studies (7, 16). In fact, studies have shown that HL activity is specifically correlated with the plasma levels of HDL_2 (13, 20, 21). On the basis of our detailed metabolic ward study (41), we have hypothesized that the LIPC genotypes control HL activity, and in turn HL activity affects HDL particle size, which is highly correlated with HDL-C levels. This indirect effect of the LIPC promoter haplotype on HDL-C can explain why significant associations are not consistently observed among previous studies. Our metabolic ward study (41) found that 38% of the variation of the HL activity was associated with the LIPC promoter haplotypes, but only 25% of variation in HDL-C is associated with HL activity. Thus, with many other factors affecting HDL-C levels, one would not expect to observe significant associations between HDL-C and the LIPC promoter haplotypes in all studies.

Cohen and colleagues (42, 43) performed pharmacogenetic studies with stanozolol, an anabolic steroid, which also offered evidence indicating that the LIPC promoter haplotype affects only HDL_2-C levels. They found that stanozolol, which increases HL activity, markedly reduced the plasma concentration of HDL_2-C but not HDL_3-C. The effect of stanozolol on HL activity was observed in all three LIPC promoter genotypes. This means that the testosterone response element is not altered by the promoter genotypes. Moreover, Deeb and Peng (44) reported that the promoter haplotype 2 has 30% decreased transcriptional activity compared with haplotype 1. In addition to association studies, a linkage study also found linkage be-

TABLE 4. Studies investigating the association between the LIPC promoter haplotype and lipids

Study[a]	Participants	Frequency of Haplotype 2	ΔHDL[b]	ΔHDL_2[b]	ΔApoA-I[b]	HL[b]
				%		
Guerra et al., 1997 (11)	133 white M	0.15	14.9[c]	N/A	8.9[c]	N/A
	139 white F		−2.1	N/A	1.7	
Jansen et al., 1997 (13)	612 white M	0.21	2.8	N/A	N/A	−14.5[c]
Murtomaki et al., 1997 (15)	270 white (M + F)	0.26	8.1[c]	N/A	4.7[c]	N/A
Tahvanainen et al., 1998 (17)	395 white CAD M	0.26	0.2	N/A	N/A	−17.5[c]
Vega et al., 1998 (12)	42 black M	Black: 0.52	N/A	N/A	N/A	Black: −21.1[c]
	41 white M	White: 0.17	N/A	N/A	N/A	White: −27.8[c]
Zambon et al., 1998 (7)	60 CAD white M	0.27	9.0	92[c]	N/A	−28.5[c]
	68 normal (M + F)		11.5	N/A	N/A	−41.3[c]
De Oliveira e Silva et al., 1999 (41)	36 F (31 white + 5 nonwhite)	0.21	10.6	N/A	−0.5	−45.7[c]
Jansen et al., 1999 (14)	790 white M	0.23	5.2[c]	N/A	4.8[c]	N/A
Hegele et al., 1999 (18)	657 AH[d] (M + F)	0.042	−1.2 M; −2.4 F	N/A	0.2 M; −0.9 F	N/A
	328 O-C[d] (M + F)	0.274	−4.0 M; 7.1 F	N/A	−2.0 M; 4.3 F	N/A
	210 KI[d] (M + F)	0.167	6.7 M: −3.8F	N/A	N/A	N/A
Shohet et al., 1999 (19)	179 CAD M	0.20	0.6	N/A	N/A	−17.3[c]
	220 healthy M	0.18	8.1	N/A	N/A	N/A
Couture et al., 2000 (16)	1,314 M[e]	0.20	2.3	8.0[c]	1.8[c]	N/A
	1,353 F[e]	0.20	3.4[c]	8.9	2.4[c]	N/A
Juo at al., (present article)	578 black M	0.52	3.0–8.9[c]	17.5–21.0[c]	3.7–6.8[c]	N/A

CAD, Coronary artery disease; N/A, not available; TG, triglycerides; M, male; F, female; M + F, original analysis was performed with combined data from males and females.

[a] Numbers in parentheses indicate reference numbers.

[b] Calculated by [(weighted average of 12 and 22) − 11]/11.

[c] Significant difference between haplotype 2 carriers and noncarriers.

[d] AH, O-C, and KI: Alberta Hutterites, Ontario Oji-Cree, and Keewatin Inuit, respectively.

[e] Most participants are white.

tween the LIPC gene and a putative locus influencing unesterified HDL_{2a}-C levels (45). On the basis of association, linkage, gene expression, and the metabolic studies mentioned above, it is clear that the LIPC promoter haplotypes only determine cholesterol levels in large buoyant HDL_2 particles by influencing HL activity. Another interesting finding is that haplotype 2 appears to have a dose effect on elevating lipid levels. The 12 and 22 genotypes had approximately 13% (12-11/11) and 28% (22-11/11) higher mean HDL_2-C levels, respectively, compared with the 11 genotype. While the present study found that one copy of haplotype 2 increased HDL_2-C levels by approximately 13% in black subjects, in the other two studies in which this was measured, Zambon et al. (7) and Couture et al. (16) reported approximately 90% and 8%, respectively, in white subjects. The higher genetic effect reported by Zambon et al. compared with Couture et al., and by us, can be primarily caused by random variation due to the small sample size in the Zambon et al. study (n = 60, 2,667, and 578 in Zambon et al., Couture et al., and the current study, respectively). In addition, all the subjects in the Zambon et al. study had heart disease, but most participants in the Couture et al. study and all participants in our study did not have heart disease. Table 4 also displays the consistent association of the LIPC haplotype 2 with decreased HL activity in all studies. These population studies of HL activity are in concert with the gene expression study (44), where a direct effect between the haplotype effect on transcription and the HL activity was indicated.

We did not find a significant association between any anthropometric phenotypes and the LIPC haplotype in any of five examinations. Although Cominacini et al. (46) reported that obese women and controls had significantly different HL activity, other studies did not find a significant relationship between anthropometric phenotypes and HL activity (47–49). The LIPC polymorphisms seem unlikely to be the important genetic factors for anthropometric phenotypes in the population at large.

Vega et al. (12) have tested the hypothesis that the higher frequency of the LIPC haplotype 2 in African American men explains their higher HDL-C levels compared with white American men. They found that black men had a lower HL activity compared with white men even within the same LIPC promoter haplotype. Thus the higher frequency of the LIPC promoter haplotype 2 in black subjects cannot totally account for the higher HDL-C level observed in black populations compared with white populations. In fact, the study by Despres et al. (50) compared the different lipid profiles between black and white subjects, and found that the racial difference in lipid profiles is mainly explained by HL activity, lipoprotein lipase activity, and anthropometric variables. This reflects the difficulty of interethnic comparison based on one haplotype variant, because complex traits are determined by oligogenic factors plus environment. Furthermore, the genetic effects on HL activity estimated from our previous metabolic ward study (41), where all subjects received tight dietary and other environmental control, is higher than in other studies (7, 12, 13, 17, 19), where participants did not receive any restriction in environmental factors. This also highlights the difficulty in comparing the genetic effects in different populations under different environmental influences.

In summary, the present study using longitudinal data from a large sample of black men confirms that the genetic effect of the LIPC promoter haplotype on HDL-C levels is primarily due to the effect on HDL_2-C levels. Other lipid phenotypes influenced by the LIPC promoter haplotype are likely due to an indirect effect. We also confirm the significant difference in haplotype frequencies between white and black subjects in the LIPC promoter polymorphisms. However, because we investigated only the promoter haplotypes, the genetic effects in our study may not reflect the total genetic effect of the LIPC gene.

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