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SCIENCE DIRECT

Metabolism Clinical and Experimental 55 (2006) 96–102

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Lipoprotein subclass and particle size differences in Afro-Caribbeans, African Americans, and white Americans: associations with hepatic lipase gene variation

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Received 2 May 2005; accepted 18 July 2005

Abstract

Despite a higher prevalence of coronary heart disease risk factors, men of African origin have less coronary atherosclerosis, as measured by coronary calcification, than whites. In part, this is thought to be because of the less atherogenic lipoprotein profile observed in men of African origin, characterized by lower triglycerides and higher high-density lipoprotein (HDL) cholesterol. We hypothesized that the −514C>T polymorphism in the hepatic lipase gene (LIPC) plays a significant role in determining a less atherogenic lipoprotein profile observed in men of African origin. Previously conducted studies of the LIPC −514C>T polymorphism in African Americans may have been confounded by a higher level of European admixture; in addition, the results from these studies do not necessarily apply to other African populations because gene-environment interactions may differ. Thus, we compared nuclear magnetic resonance spectroscopy–measured lipoprotein subclass patterns and LIPC −514C>T genotypes in population-based samples of older white American (n = 532) and African American (n = 97) men from the Cardiovascular Health Study to those among older, less admixed, Afro-Caribbean men (n = 205) from the Tobago Health Study. Men of African origin had a more favorable lipoprotein profile than whites. In addition, levels of low-density lipoprotein cholesterol, total cholesterol, and triglyceride, and large and small very low-density lipoprotein, small low-density lipoprotein, as well as very low-density lipoprotein particle size, were remarkably lower in Afro-Caribbean men than in either African American or white men. The frequency of the LIPC −514T allele was much higher in Afro-Caribbeans (0.57) and in African Americans (0.49) than in whites (0.20). The −514T allele in both populations of African origin, but not in whites, was associated with elevated large HDL and greater HDL size. Our findings indicate that the higher frequency of the LIPC −514T allele found in men of African origin living in different environments significantly contributes to the more favorable distribution of HDL subclasses compared with whites.

1. Introduction

Despite a higher prevalence of risk factors for coronary heart disease (CHD), men of African origin have less coronary atherosclerosis, as measured by coronary calcification, than whites [1,2]. In part, this is thought to be because of the less atherogenic, or more favorable, lipoprotein profile observed in men of African origin, characterized by lower levels of triglycerides (TGs) and higher levels of high-density lipoprotein cholesterol (HDL-C) [3-6]. The genetic and environmental factors that cause these differences in populations of African descent remain unclear. Higher HDL-C in African American men may be partly because of lower hepatic lipase (HL) activity [7]. HL is a lipolytic glycoprotein that hydrolyzes phospholipids and TG [8,9] and also assists the uptake of lipoproteins by specific receptors on the hepatocytes [8,9].

A single nucleotide polymorphism, the −514C>T, in the human hepatic lipase gene (LIPC) has been associated with lower postheparin HL activity [7,10] and increased levels of HDL-C [11-13], as well as large HDL [10,14,15] and large low-density lipoprotein (LDL) particles [10]. Furthermore,

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0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.07.011

the frequency of the LIPC −514T allele varies across ethnic groups, from 17% to 27% in whites [7,10,16-18], 44% to 54% in African Americans [7,14,17,19,20], 47% in US Hispanics [18], 37% in Koreans [21], and 50% in Japanese [12,17]. These observations are consistent with the hypothesis that the more favorable lipoprotein profile among men of African descent may be attributable to variation at the LIPC locus. Although the frequency of the LIPC 514C>T polymorphism [7,14,17,19,20] and the association between 514C>T genotypes and lipoprotein subclasses [14] have been previously investigated in African American samples, no studies have been conducted in populations of African descent outside the United States. Therefore, the results from previous studies do not necessarily apply to other African populations because gene-environment interactions may differ. In addition, the results may have been confounded by higher level of European admixture in African Americans. In the present study, we calculated the allele frequencies of the LIPC −514C>T promoter polymorphism among white, African American, and Afro-Caribbean older men from 2 large, population-based studies and determined whether these LIPC genotypes were associated with nuclear magnetic resonance (NMR) lipoprotein subclasses and particle sizes. LDL and HDL subclasses have been widely accepted as better predictors of atherosclerosis and CHD risk than lipids obtained by the standard tests (total cholesterol [TC], LDL cholesterol [LDL-C], and HDL-C) [22-24]. We hypothesized that the frequency of the putative protective T allele at position −514 of the LIPC is higher in men of African origin than in white men and that the −514T allele is associated with a more favorable lipoprotein profile characterized by higher concentrations of large HDL and large LDL.

2. Methods

The Tobago Health Study was derived from the Tobago Prostate Survey, a population-based prostate cancer screening survey of Afro-Caribbean men on the Caribbean island of Tobago. The target recruitment population for this study was all men aged 40 to 79 years (5121 men), of whom 3375 (66% of the cohort) participated in the survey. In 1990, the population of Tobago was estimated by self-report to be 92% African descent, 4.5% mixed, 2% Asian Indian, 0.4% white, and 1% other [25]. After obtaining written informed consent from each participant, a questionnaire was administered by trained interviewers. The questionnaire was designed to gather demographic and anthropometric data and to assess history of smoking and alcohol consumption, medical history, personal and family cancer history, and occupational history. Aliquots of frozen serum, drawn from fasting subjects, were shipped on ice packs by express courier and stored at the University of Pittsburgh −70°C freezers. The present study population includes a random sample of 205 Afro-Caribbean men older than 65 years, not diagnosed with prostate cancer, who reported 3 or more grandparents of African descent.

The Cardiovascular Health Study (CHS) is a population-based, longitudinal study of 5888 people older than 65 years, recruited from a stratified random sample of Medicare recipients from 4 US communities. A total of 5201 white CHS participants underwent baseline assessments of cardiovascular risk factors in 1989 to 1990 [26]. An additional 687 African Americans were recruited to CHS in 1992 to 1993 [27]. The CHS was designed to evaluate risk factors and noninvasive measures, and to describe and predict atherosclerotic events in older adults [26]. Participants had extensive baseline examinations, including anthropometric measurements, medical and lifestyle histories, and blood collection [26]. The present study population consists of a sample of 532 white men and 97 African American men who were assayed for NMR lipoproteins. All 629 CHS participants have provided informed consent for participation in genetic studies.

Serum samples, which had been stored at −70°C and were previously unthawed, were sent to LipoScience (Raleigh, NC) for the determination of lipoprotein concentrations and subclass distributions by NMR spectroscopy on freshly thawed aliquots. This method has been described in detail in reports by Otvos et al [28-30]. The NMR method uses the distinguishing signals broadcast by lipoprotein subclasses of different sizes as the base of their quantification. The intensities of signals are proportional to the lipid mass of the particles and are subsequently converted into units of milligrams per deciliter. NMR spectroscopy measures 11 subclasses of very low-density lipoprotein (VLDL), LDL, and HDL, as well as estimates of the concentrations of TC, total TG, LDL-C, HDL-C, LDL particle numbers, and average VLDL, LDL, and HDL particle size.

DNA isolation of Tobago participants' blood clots from a 15-mL coagulation tube was accomplished by mechanical disruption of the clot, protease K digestion, and isolation on a Qiagen column (Qiagen, Santa Clara, CA). The genotype at position −514 of LIPC promoter was determined by polymerase chain reaction–restriction fragment length polymorphism approach developed by Guerra et al [11]. After digestion with *Nla*III restriction endonuclease at 37°C overnight, electrophoresis was performed on 2% agarose gel stained with florescent dye ethidium bromide, using Tris-borate buffer, and photographs were made using UV illumination.

Allele frequencies were estimated by direct gene counting. The observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium by the goodness-of-fit χ^2 test. Comparison in allele frequencies between the 3 ethnic groups was evaluated using a χ^2 test. Initial descriptive data analysis showed that most of the NMR lipoprotein subclasses and sizes exhibited markedly skewed distributions. Before statistical analysis, we transformed the lipoprotein subclasses and sizes by square root (cholesterol, LDL-C, large HDL, small VLDL, medium VLDL, large LDL, and LDL particles), inverse (HDL, LDL, and VLDL size), or natural logarithms (TG) to reduce the

nonnormality of the distributions of lipoproteins. Four white and 3 Afro-Caribbean men were excluded from further analyses because of missing body mass index (BMI), waist circumference, and smoking data. Analysis of variance (ANOVA) was performed to test any significant difference of baseline demographic (continuous) characteristics among 3 ethnic groups as well as among 3 genotypes within ethnic groups. Categorical variables were compared using χ^2 testing. Analysis of covariance (ANCOVA), which adjusted for age, waist circumference, and smoking, was conducted using the general linear model procedure to test for possible differences in mean levels of NMR lipoproteins among ethnic groups and among LIPC genotypes. We also used a trend analysis to test for linear trends across the genotypic means within each ethnic group. We considered $P < .05$ to be significant. For those traits that were significant, group means were compared by Fisher least significant difference test for multiple pairwise comparisons. Because the population distributions of small and medium LDL and large VLDL were highly skewed, we used the Kruskal-Wallis median test to test for possible differential effects among genotypes and ethnic groups. The Statistical Analysis System (version 8.2; SAS Institute, Cary, NC) and the Statistical Package for the Social Sciences (version 12.0.1; SPSS, Chicago, IL) were used for statistical analysis.

3. Results

Three populations were similar in age and BMI (Table 1). Waist circumference was significantly lower in Tobago men than either US population, whereas smoking was much more frequent in African American men (Table 1).

Significant differences across the 3 ethnic groups were found for all NMR lipoproteins, subclasses, and sizes, except for small HDL (Table 2). Afro-Caribbean men had a more favorable lipoprotein profile than African Americans or whites, characterized by lower TG, lower TC, lower LDL-C, as well as fewer LDL particles and lower small VLDL. Both populations of African men had similar levels of HDL-C, large HDL, and medium VLDL compared with each other, but when compared with white men, HDL-C and large HDL were higher, and medium VLDL was lower in men of African origin. Large LDL was considerably higher in African American men, even when compared with Tobago men. The average size of VLDL particles was smaller in Afro-Caribbeans when compared with whites and African Americans. Average LDL and HDL size were significantly greater in both groups of African men when compared with white participants.

None of the 3 ethnic groups showed significant deviation from Hardy-Weinberg equilibrium for the LIPC −514 genotypes. The LIPC −514T allele frequency was almost 3 times higher in Tobago men (57.3%) than in whites (19.8%, $P < .0001$) and was intermediate in African Americans (49.0%).

Within each ethnic group, 3 LIPC −514C>T genotypes were similar with respect to mean age, BMI, waist circumference, and smoking. Because of the differences in LIPC genotypic frequency among the 3 ethnic groups, we performed subsequent analyses within each group. First, we tested for mean differences in levels of the standard NMR lipoprotein measurements (TC, TG, LDL-C, and HDL-C) between the LIPC genotypes. No significant associations were found in any ethnic group, except for a difference between T allele carriers and noncarriers (CC genotype) on HDL-C in African Americans ($P = .014$, trend test $P = .04$) (Table 3).

We next tested for associations between the LIPC genotypes and VLDL, LDL, and HDL subclasses and observed no difference in mean VLDL subclasses between the LIPC genotypes in any of the ethnic groups. In white men,

Table 1
Participants' characteristics according to the ethnicity

	CHS whites (n = 528)	CHS African Americans (n = 97)	Tobago Afro-Caribbeans (n = 202)	P
Age (y)	73.1 ± 0.24	72.9 ± 0.57	73.1 ± 0.34	.95
BMI (kg/m^2)	26.4 ± 0.16	27.1 ± 0.4	26.8 ± 0.32	.17
Waist (cm)	98.0 ± 0.42	98.1 ± 1.14	93.9 ± 0.9	<.0001
Current smokers (%)	9.7	21.7	5.9	.0001

Values are means ± SEM. P value obtained in the comparison among ethnic groups (ANOVA test for means and χ^2 test for percentages).

Table 2
Comparison of NMR lipoprotein distributions of Tobago and CHS men

	CHS whites (n = 528)	CHS African Americans (n = 97)	Tobago Afro-Caribbeans (n = 202)
CHOL[a,b]	225.6 ± 1.9	227.1 ± 3.5	179.2 ± 2.6
TG[a,b,c]	155.5 ± 3.3	129.2 ± 6.2	78.7 ± 2.1
LDL-C[a,b]	144.9 ± 1.7	139.9 ± 1.4	113.7 ± 2.2
HDL-C[a,b,c]	45.8 ± 0.7	54.0 ± 2.2	49.9 ± 0.96
Large HDL[a,c]	27.9 ± 0.8	36.2 ± 2.4	32.2 ± 0.98
Small HDL	18.0 ± 0.26	17.8 ± 0.7	17.7 ± 0.3
Small VLDL[a,b]	33.3 ± 0.7	32.0 ± 1.5	15.0 ± 0.7
Medium VLDL[a,c]	50.3 ± 1.6	31.6 ± 3.0	26.7 ± 1.2
Large VLDL[d]	33.2 ± 2.2	26.5 ± 3.8	3.4 ± 0.6
Small LDL[d]	38.7 ± 2.0	27.7 ± 4.2	14.7 ± 1.6
Medium LDL[d]	41.1 ± 1.7	30.5 ± 3.8	28.8 ± 1.4
Large LDL[c]	64.0 ± 2.0	81.0 ± 4.7	68.3 ± 2.6
LDL particles[a,b] (nmol/L)	1660 ± 22.0	1557 ± 42.7	1183 ± 26.5
HDL size[a,c] (nm)	8.95 ± 0.03	9.25 ± 0.07	9.4 ± 0.04
LDL size[a,c] (nm)	20.8 ± 0.03	21.1 ± 0.08	21.2 ± 0.04
VLDL size[a,b,c] (nm)	47.9 ± 0.4	45.3 ± 0.9	39.7 ± 0.3

Values are unadjusted means ± SEM (mg/dL). P value obtained by ANCOVA after adjustment for age, waist, and current smoking status showed significant differences across 3 ethnic groups for all NMR lipoproteins ($P < .0001$, large LDL $P = .002$) except for small HDL.

[a] Whites different from Tobago men.
[b] African Americans different from Tobago men.
[c] Whites different from African American men.
[d] Significant nonparametric Kruskal-Wallis P value.

Table 3
Associations between LIPC −514C>T polymorphism and NMR lipoproteins within ethnic groups

	Whites (n = 528)				African Americans (n = 97)				Tobago Afro-Caribbeans (n = 202)			
	TT (n = 28)	CT (n = 152)	CC (n = 348)	*P*	TT (n = 24)	CT (n = 47)	CC (n = 26)	*P*	TT (n = 62)	CT (n = 108)	CC (n = 32)	*P*
HDL-C	44.1 ± 2.6	46.9 ± 1.5	45.5 ± 0.8	.55	64.2 ± 5.4	53.5 ± 2.7	45.5 ± 3.5	.014[a,b,c]	51.6 ± 1.8	50.1 ± 1.3	45.9 ± 2.1	.13[a]
LDL-C	141.5 ± 8.0	145.5 ± 3.06	145.0 ± 2.02	.79	139.1 ± 9.3	137.6 ± 4.4	144.6 ± 5.1	.68	116.7 ± 4.4	110.7, 2.7	118.2 ± 6.2	.25
Small HDL	17.5 ± 1.05	17.1 ± 0.5	18.4 ± 0.3	.076[d]	16.2 ± 1.3	17.6 ± 1.1	19.6 ± 1.3	.25	17.1 ± 0.5	17.8 ± 0.5	18.5 ± 0.9	.45
Large HDL	26.6 ± 2.9	29.8 ± 1.7	27.1 ± 0.9	.26	48.0 ± 5.6	35.9 ± 2.8	25.9 ± 4.1	.003[a,b,d]	34.4 ± 1.8	32.4 ± 1.4	27.4 ± 2.0	.056[a,b,d]
Small LDL	44.5 ± 9.9	38.5 ± 3.7	38.4 ± 2.4	.72	11.6 ± 5.1	27.1 ± 6.3	43.7 ± 9.2	.03[e]	12.1 ± 2.8	13.9 ± 1.9	22.3 ± 6.0	.075
Large LDL	57.04 ± 8.6	63.7 ± 3.6	64.7 ± 2.5	.71	94.7 ± 9.9	79.0 ± 7.0	71.9 ± 7.9	.41	77.2 ± 5.1	64.6 ± 3.3	63.3 ± 6.3	.16
HDL size (nm)	8.94 ± 0.11	9.01 ± 0.05	8.93 ± 0.03	.26	9.5 ± 0.13	9.3 ± 0.1	8.9 ± 0.11	.024[a,b,d]	9.51 ± 0.07	9.42 ± 0.05	9.23 ± 0.08	.013[a,b,d]
LDL size (nm)	20.63 ± 0.16	20.8 ± 0.07	20.8 ± 0.04	.63	21.45 ± 0.11	21.1 ± 0.13	20.85 ± 0.16	.08[a,b]	21.4 ± 0.08	21.15 ± 0.06	21.06 ± 0.11	.055[a,b,d]

Values are unadjusted means ± SEM (mg/dL). *P* value obtained by ANCOVA after adjustment for age, waist circumference, and current smoking status ($P < .05$ was considered to be statistically significant).

[a] Test for linear trend across genotypes was significant.

[b] TT different than CC.

[c] TT different than CT.

[d] CT different than CC.

[e] *P* value obtained by nonparametric Kruskal-Wallis test.

we observed no significant associations between LIPC genotypes and LDL or HDL subclass or mean diameter. In African American men, however, carriers of the T allele had higher levels of large HDL than noncarriers ($P = .003$, P for trend = .0006) and lower levels of small LDL ($P = .03$) (Table 3). A similar pattern was observed in Tobago men, but the difference between genotype groups was of borderline significance ($P = .056$, P for trend = .024) (Table 3).

Analyses of HDL and LDL mean diameters differed among the LIPC genotypes in 2 groups of African ancestry. HDL particle diameter was greater in both African American ($P = .024$, P for trend = .007) and in Afro-Caribbean ($P = .013$, P for trend = .005) carriers of the 514T allele (Table 3). In addition, LDL size tended to be greater in carriers of the T allele in both men of African ancestry (in African Americans, P for trend = .033; in Afro-Caribbeans, P for trend = .022) (Table 3).

Because BMI and waist circumference were highly correlated, we have not included BMI as a covariate in the ANCOVA model. However, BMI might confound associations of the LIPC and lipoproteins. When considering an interaction of BMI with the LIPC −514C>T polymorphism, BMI was analyzed as a categorical variable, grouped into tertiles (<24.4, 24.4-26.9, >27.9). The interaction between the LIPC −514C>T polymorphism and BMI in determining level of large HDL and HDL size was tested by 2-way ANOVA. No significant interaction between the LIPC genotypes and BMI was found.

4. Discussion

A number of well-designed epidemiological studies reported an increased level of TG as an independent CHD risk factor [31]. The inverse association between HDL-C and the development of atherosclerosis and CHD is also well established [32,33]. However, the beneficial or harmful effect of VLDL-C, LDL-C, and HDL-C on the process of atherosclerosis may depend on particle size. It has been shown that the presence of CHD was more strongly associated with HDL particle size distribution than with the low HDL-C level [34,35], and that small HDL had a positive association [34] and large HDL a negative association [36] with the development of CHD. In addition, the growing body of evidence suggests that large VLDL is also a predictor of the risk of atherosclerosis, independent of TG levels or other VLDL subclasses [22].

As far as we are aware, this study is the first to investigate NMR-measured lipoprotein subclasses in the population of African origin, which is much less admixed than African Americans. The present study has shown that older men of African origin, from both US and Caribbean environments, have a more favorable distribution of the NMR lipoprotein particle numbers and sizes than white men. In addition, levels of LDL-C, TC, and TG, large and small VLDL, small LDL, as well as VLDL particle size were remarkably lower in Afro-Caribbean men than in either CHS African American or CHS white men. Our findings are consistent with results from several previous studies that reported people of African origin across all geographic regions have lower TG levels and higher HDL-C than whites [4-6]. Two factors that affect lipoprotein measures, central obesity and smoking, were also lower in older men from Tobago compared with CHS men. However, even after taking into consideration some of the risk factors in our analyses, differences in lipoprotein subclasses among the 3 ethnic groups were still present in our study. We speculate that Afro-Caribbeans live in a different environment than African Americans, which could potentially explain some of the observed differences. However, the lipoprotein profile in African Americans in our study was also less atherogenic, when compared with whites, and similar to the one observed in Tobago men. This observation suggests that genetic factors may play an important role in determining a more favorable lipoprotein profile in African men from our study.

One of the genes that may affect lipoprotein distribution is HL. In the present study, we observed significant differences in the LIPC −514T allele frequencies among 3 populations. The observed frequencies in CHS African American and CHS white men are consistent with the findings from previous studies [7,10,12,16-21,37], although the frequency observed in Tobago men is the highest reported to date. This is the first report of associations between the LIPC genotype frequencies and the NMR-measured lipoprotein subclasses in a non-American population of African origin.

In our study, in men of African ancestry, the LIPC −514T allele was associated with a more cardioprotective lipoprotein profile, characterized by higher levels of HDL-C, higher large HDL, and greater diameter of HDL and LDL particles. In contrast, in CHS white men, there was no significant association between the LIPC genotypes and LDL or HDL distributions. However, given the small sample size of whites with the TT genotype (N = 28), the effects of the LIPC genotype in this ethnic group must be interpreted with caution.

There are a number of explanations for observing the significant associations only in men of African ancestry. One possibility is that the LIPC−514C>T polymorphism may not be the causal modulator of the observed differences in lipoprotein phenotypes and that some other causal polymorphism is in strong linkage disequilibrium with the −514C>T variant in men of African origin, but not in white men. Sequencing of the whole LIPC gene and flanking regions in our populations could potentially clarify our findings. Furthermore, the data on alcohol consumption, diet, and physical activity were incomplete in our study. These environmental factors, which were not included as covariates in the ANCOVA analyses, could affect our results. We believe this possibility is not likely, in that the CHS whites share a more similar environment with the CHS African Americans than either CHS groups do with the Tobago Afro-Caribbeans; yet, we observe similar associations with the LIPC in both groups with African ancestry.

It is further important to emphasize that Afro-Caribbean population of Tobago has considerably less European admixture (~6%; unpublished data) compared with the more genetically heterogeneous CHS African American population (estimated at ~21% [38]). Higher degree of European admixture and smaller size of the CHS African American sample may be an alternative explanation for positive associations found in this group of CHS men. However, we believe that it is an unlikely scenario because the same significant associations and significant gene-dose trend were also observed in less admixed Afro-Caribbean sample.

The NMR analysis in the present study was performed on previously unthawed serum samples that were kept frozen at −70°C. The use of frozen samples theoretically has the potential to cause alterations to lipoproteins [39]. The effect on the results of our study, however, can be expected to be small. First, any changes attributable to freezing can be assumed to be randomly distributed across participants and across populations. As a result, freezing may lead to effect dilution, but it cannot lead to the introduction of spurious relations. Furthermore, we have found a high correlation between the chemically obtained and NMR estimates of cholesterol ($r = 0.88$, $P < .01$), TG ($r = 0.94$, $P < .01$), and HDL-C ($r = 0.85$, $P < .01$). In addition, the NMR lipoprotein subclass values in the present study are consistent with those reported in other studies [40,41]. Finally, in the recent study by Suter et al [42], it was shown that the NMR analyses of fresh and frozen (−70°C) plasma specimens produced highly similar results, as confirmed by the observed very high correlation ($r = 0.96$, $P < .0001$) between chemically determined TG concentration (immediately after the blood was drawn) and that estimated from the NMR subclass measurements on samples that had been frozen.

Further studies are needed to clarify whether the LIPC −514C>T variant is associated with lipoprotein profiles in individuals with African ancestry. Future studies should not only include larger samples of African populations and other candidate genes involved in lipoprotein metabolisms but also the haplotype profiles in the LIPC in different ethnic groups. In whites, the −514T allele is 2- to 3-fold less common than in Africans or Asians. It is possible that because of the high frequency of the T allele in African populations, this polymorphism, together with other polymorphisms, could be variously combined in haplotypes, and these haplotypes are likely to be differently distributed in populations of white and African descent [43].

In conclusion, the present study demonstrates significant ethnic differences in NMR lipoprotein subclass distributions, with similar patterns of more favorable, less atherogenic lipoprofile in older African American and Afro-Caribbean men compared with older white men. In addition, the present investigation shows a significant association of the LIPC −514C>T polymorphism with HDL subclasses and sizes, and possibly LDL size, in older men of African origin, living in different environments, but not in older white men. The current findings confirm that further evaluation of the effects of the LIPC gene polymorphisms on lipoprotein levels should consider ethnicity as a potential moderator of those effects. Our findings may have important implications for the understanding of ethnic differences in lipoprotein distributions and susceptibility to atherosclerosis.

Acknowledgment

This work was supported by the National Heart, Lung, and Blood Institute grants for the Cardiovascular Health Study (N01-HC-85079 through N01-HC-85086, N01-HC-35129, and N01 HC-15103) and, in part, by funding or in-kind services from the Division of Health and Social Services, Tobago House of Assembly, the University of Pittsburgh Cancer Institute, US Department of Defense (contact DAMD 17-99-1-9015), US National Cancer Institute (grant R01 CA84950), and the National Institutes of Diabetes and Digestive and Kidney Diseases (grant DK046204). A full list of participating CHS investigators and institutions can be found at the CHS web site (http://www.chs-nhlbi.org).

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