GENETIC AND ENVIRONMENTAL DETERMINANTS OF LIPID PROFILE IN BLACK AND WHITE YOUTH: A STUDY OF FOUR CANDIDATE GENES

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Objective: To identify genotypes and gene-environment interactions, which may explain ethnic differences on lipid profile in Black and White youth.

Design, Setting, Participants: Healthy adolescents and young adults (N=413, 18.6 ± 2.8 yrs, 44% Black, 53% Male) drawn from a cardiovascular study.

Main Outcome Measures: Total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), and triglyceride (TG) concentrations were obtained from frozen plasma. The ApoB Glu4154Lys, LDL receptor (LDLR) T1773C, PPARγ Pro12Ala, and TNFα −308G/A polymorphisms were genotyped. Analyses adjusted for age, sex, ethnicity, body mass index (BMI), socioeconomic status (SES), and interactions.

Results: The ApoB Glu4154Lys polymorphism interacted with obesity and age to predict TC levels. As BMI increased, 4154Lys ApoB allele carriers had higher TC levels than 4154Glu homozygotes (difference=0.23 mmol/L at BMI=30 kg/m^2, 0.54 at BMI=40, P<.05). Juvenile, but not adult, ApoB 4154Lys allele carriers had higher TC (0.34 mmol/L, P<.01). Male −308A TNFα allele carriers had lower HDLC (0.10 mmol/L, P<.01). Carriers of the T1773 LDLR allele had higher TG (0.26 mmol/L, P<.01). No effect of the PPARγ Pro12Ala polymorphism was found; the 12Ala PPARγ allele was rare among Blacks (2%).

Conclusions: The ApoB, TNFα, and LDLR candidate genes influenced lipid profiles in youth independent of environmental factors. The T1773 LDLR allele, which is rare among Blacks (7%), may contribute to lower TG in Blacks. The −308A TNFα allele may contribute to lower HDLC in males. These gene effects and gene-environment interactions may inform prevention and treatment of atherosclerosis. (*Ethn Dis*. 2005;15:568–577)

Key Words: Risk Factors, Cardiovascular, Adolescents, Young Adults, Continental Population Groups, Cholesterol, HDL Lipoproteins, Triglycerides, Polymorphism, Body Mass Index

INTRODUCTION

Total cholesterol (TC) and high-density lipoprotein cholesterol (HDLC) are recognized as important risk factors for coronary heart disease. Triglyceride (TG) levels are a component of the metabolic syndrome and have recently been identified as an additional independent risk factor.[1] Prospectively, HDLC has been found to predict slower progression of atherosclerosis in the left main coronary artery.[2] Cholesterol levels in childhood predict carotid artery intimal-medial thickness in adulthood.[3,4] Dyslipidemia is associated with other aspects of the metabolic syndrome including obesity, visceral adiposity, hypertension and insulin resistance.[5,6] Triglyceride (TG) and HDLC are inversely correlated, with both genetic and environmental contributions to this link indicating a complex determination of coronary risk.[7] Greater understanding of the genetic and environmental determinants of lipid profile may improve our ability to quantify risk, prevent and treat metabolic syndrome and coronary heart disease. We chose to examine four candidate genes which have been linked to obesity and which have potential roles in regulating lipid profile.[8]

Interesting differences have been found between Blacks and Whites on lipid profile. Total cholesterol (TC) concentrations in Blacks and Whites tend to be similar, but HDLC concentrations are higher and TG concentrations lower in Blacks than Whites in the United States.[9] Longitudinal studies show that ethnic differences in lipid profile are already present in childhood.[10,11] Since plasma lipids in the young are strongly related to the initial stages of atherosclerosis, early ethnic differences in these levels may confer a protective effect in Blacks on coronary heart disease rates in adulthood, as seen in African populations.[12]

The puzzling advantage Blacks have for lipid profile stands in contrast to the higher prevalence of obesity, hypertension, and diabetes, and greater mortality from coronary heart disease among Blacks in the United States.[13] How do these closely interrelated risk factors interact to influence the development of atherosclerosis? Understanding of the determinants of early ethnic differences in lipids may help sort out genetic effects in a population not yet affected by advanced cardiovascular risk or secondary effects of disease.

Although a combination of environmental and genetic factors are assumed to contribute to these ethnic differences,[14] relatively little is known about the contribution of genetic factors. Most association studies relating plasma lipids and genetic markers involved only White adults. A polymorphism in the hepatic lipase promoter (−514C/T) has been consistently linked with higher HDLC in adult studies, and is more common in Black than White men.[15,16] Lower hepatic lipase and higher lipoprotein lipase activity may explain the higher HDLC in Black than White adults.[17] However, little is known about the factors responsible for lower TG levels in Blacks.

Only a few studies have assessed the role of genetic markers on ethnic lipid

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differences in youth.[18–22] Among these studies, ethnic differences were observed in the allele frequencies of the common apolipoprotein E polymorphism, the adenosine triphosphate-binding cassette transporter 1 (ABCA1) gene R219K polymorphism, the C-514T promoter polymorphism of the hepatic lipase gene, and lipoprotein lipase serine 447 stop polymorphism. Similar effects of these polymorphisms on lipid profile were found in Blacks and Whites except for the ABCA1 gene R219K and the hepatic lipase C-514T polymorphisms, which only had effects in Whites. Apart from the hepatic lipase C-514T polymorphism in which the T allele associated with higher HDLC levels in Whites showed a higher frequency among Blacks, a common characteristic of the other studies is that the identified risk alleles that increased TG or lowered HDLC had higher allele frequencies in Blacks than in Whites. Given the same effect of these polymorphisms in Blacks and Whites, these genetic factors would tend to reduce early ethnic differences in HDLC and TG levels rather than explain them.

Considering the crucial role of the low-density lipoprotein receptor (LDLR) and apolipoprotein B (ApoB) in lipid homeostasis and common genetic variations at these two loci having been associated with altered circulating cholesterol and TG levels,[23–28] these two genes are worth investigating in a young US population. In addition, studies implicate tumor necrosis factor alpha (TNFα) and peroxisome proliferator-activated receptor gamma (PPARγ) in lipid regulation.[29,30] TNFα gene knockout mice exhibit lower TG levels than their wild-type littermates,[31] and the −308G/A TNFα promoter polymorphism was associated with plasma HDLC levels in obese subjects.[32] In vitro studies found that PPARγ can control the expression of key enzymes of lipid metabolism (lipoprotein lipase, fatty acid binding and transfer proteins, hormone-sensitive lipase) as well as secretory proteins (ie, leptin and TNFα).[33,34] Knoblauch et al found that the PPARγ gene locus was linked, and a biallelic variant in the PPARγ gene was associated with HDLC levels in healthy subjects.[35] In a Spanish study, the Ala12 allele of the PPARγ gene's Pro12Ala polymorphism was associated with lower TG levels.[36] Given these possible roles of TNFα and PPARγ on TG and HDLC metabolism, polymorphisms in these two genes may also contribute to variation in lipid profile.

The objectives of this study were: 1) to examine the allele frequencies and the effects of ApoB Glu4154Lys, LDLR T1773C, PPARγ Pro12Ala, and TNFα −308G/A polymorphisms on lipid profile in a sample of Black and White youth; and 2) to explore whether these polymorphisms partly explain early ethnic differences in lipid profile.

METHODS

Subjects

Subjects were 413 healthy adolescents and young adults (mean ± SD age, 18.6 ± 2.8 years; range: 12.3–26.9 years). Table 1 presents means by ethnicity and gender, including age-adjusted group differences. Twenty-two percent of subjects had a body mass index (BMI) of at least 25 and less than 30 kg/m^2 (20% of White males, 16% of White females, 32% of Black males, 22% of Black females) and 15% had a BMI of at least 30 (8% of White males, 14% of White females, 15% of Black males, 24% of Black females). They were participants in a longitudinal study of the development of cardiovascular risk factors in which evaluations have been conducted annually. This study includes data from one visit for each participant. Information on subject recruitment and evaluation has been previously described.[37,38] Subjects were children who were recruited based on a family history of CVD, including essential hypertension and/or premature myocardial infarction (<55 years of age) in parents or grandparents. All subjects were free of any acute or chronic illness based on parental report. The Human Assurance Committee at the Medical College of Georgia gave approval for the study. Informed consent was obtained from the child and a parent.

Subjects were classified as Black if 1) both parents reported being of African heritage; 2) they and the child were born and raised in the United States; and 3) parents considered themselves and their child to be African American. Subjects were classified as White if: 1) both parents reported that they were of European ancestry; 2) they and the child were born and raised in the United States; and 3) they considered themselves and their child to be European American and not of Hispanic, Native American, or Asian descent.

Measures of anthropometry and family socioeconomic status (SES) were obtained during each annual examination. Body mass index (BMI) was calculated (weight/height^2). Socioeconomic status (SES) was assessed by parental education level, ie, mother's and father's years of education. Parental education level was measured on a 7-point scale, ranging from less than high school to postgraduate education and subsequently divided into three categories: 1) low (<12 yrs, 10% of sample); 2) medium (≥12 and

Table 1. Sample characteristics (mean ± SD)

	Whites		Blacks	
	Males	Females	Males	Females
N	126	107	92	88
Age, years	18.2 ± 3.0*	18.5 ± 2.7*	19.2 ± 2.9	18.7 ± 2.5
BMI, kg/m^2	23.4 ± 4.6*	24.0 ± 5.9*	25.8 ± 5.4	26.4 ± 7.8
SES (father's education), yrs	14.7 ± 2.4†	14.6 ± 2.2†	13.1 ± 2.4	13.6 ± 1.8
TC, mmol/L	3.54 ± 0.92*	3.93 ± 0.98	3.94 ± 0.84	3.78 ± 1.22
TG, mmol/L	1.17 ± 0.65†	1.06 ± 0.57†	0.86 ± 0.37	0.75 ± 0.36
HDLC, mmol/L	1.04 ± 0.34*	1.21 ± 0.37	1.21 ± 0.42	1.22 ± 0.42

* Differ from other groups, $P<.01$.
† Differ from other groups, $P<.001$.

<16 yrs, 59%); and 3) high (≥16 yrs, 31%). Five subjects had missing values for father's education and they were omitted from analyses in which this variable was included.

The fact that 70 of the total of 413 subjects in this study were siblings may have affected the significance of observed effects, because they share genes and environment. However, when the relationship between siblings was accounted for in the analyses by using generalized estimating equations, results were virtually unchanged, so results for the entire sample are reported here. Generalized estimating equations is a regression technique that provides valid estimators of regression parameters and their standard errors in related individuals.[39]

Plasma Lipid Measurements

The Cholestech LDX system (Cholestech, Hayward, Calif) was used to measure levels of TC, HDLC, and TG from frozen plasma. Subjects were not required to fast or queried regarding fasting status. Product literature indicates that between 95% and 98% of LDX values for TC, HDLC, and TG were in complete agreement with the reference method according to the National Cholesterol Education Program criteria, and coefficients of variation (CVs) for the LDX lipid profile tests were 2%–3% for TC, 3%–6% for HDLC, and 2%–4% for TG. With respect to the current study, the interassay CVs for TC, HDLC and TG were 3.7%–4.9%, 4.0%–6.6% and 3.7%–4.6%, respectively. One limitation of this method is that it only gives results within a certain range. The range for TC is 2.59–12.93 mmol/L (100–500 mg/dL). For HDLC and TG, the measurement range is 0.39–2.59 mmol/L (15–100 mg/dL) and 0.51–6.77 mmol/L (45–600 mg/dL), respectively.

DNA Analysis

Genomic DNA was extracted from plasma buffy coats using QiaAmp DNA Blood Mini Kit (Qiagen, Valencia, Calif). The extracted DNA was stored at −72°C until analyzed. The genotypes for ApoB Glu4154Lys, LDLR T1773C, PPARγ Pro12Ala, and TNFα −308G/A polymorphisms were detected by polymerase chain reaction (PCR) followed by restriction enzyme digestion assays, as previously described elsewhere with minor modifications (Appendix is available from author).[40–43] PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, Calif, USA) with a 15 μl reaction volume containing 30–50 ng of genomic DNA, 20 pmol of each primer, and 1× Taq PCR master mix (Qiagen, Valencia, Calif). The Taq PCR master mix provides a final concentration of 2.5 unit of Taq DNA Polymerase, PCR Buffer (1.5 mM $MgCl_2$), and 200μM each dNTPs. Digested fragments were separated by electrophoresis on 1.5%–3% agarose gel and identified by ethidium bromide. The total number of subjects genotyped for each polymorphism varied slightly and was somewhat lower than 413. This was due to unavailable genomic DNA for seven subjects and failure to amplify the target sequences for some samples (5.6% for ApoB, 1.2% for LDLR, 5.6% for PPARγ, and 2.5% for TNFα, respectively). The primers, lengths of PCR products, related restriction endonuclease, as well as digested bands are shown in the appendix.

Statistical Analysis

Hardy-Weinberg equilibrium was tested separately in Blacks and Whites by a χ^2 test with 1 *df*. Ethnic differences in allele and genotype frequencies were tested with χ^2 tests of 1 and 2 *df*, respectively (Table 1). To prevent inflated significance, these tests were performed in data including just one of the siblings, chosen at random.

Tobit regression is an analytic technique specifically designed for censored data.[44] Censored variables can be considered to be a mixture of discrete and continuous distributions. The Tobit regression model is a single equation that models the relationship of one or more independent variables with the probability of being uncensored and, if uncensored, the level of the continuous variable. The technique is similar to survival analysis in that it uses a censoring value to stand in for an indeterminate quantity. In the present analyses, subjects with values below the detection limit for TC (<2.59 mmol/L), HDLC (<0.39 mmol/L), or TG (<0.51 mmol/L) were treated as left-censored at values of 2.59, 0.39, or 0.51 mmol/L, respectively. No subjects had values above the detection limit in our data. Tobit regression was used to analyze these censored lipid data. To calculate means, censored values were set equal to the lower limit for each variable. Traditional linear regression was used for all other uncensored variables.

Since LDL levels were calculated rather than measured, and to limit type I error, analyses were limited to measured fractions (TC, HDLC, and TG).

Type I error was limited and environmental control maximized by first building the most parsimonious environmental model for each lipid variable, then building on these final environmental models to test all four candidate genes for each of the three lipid variables. Thus, a total of 12 gene-environment models were tested. Models 1–5 are the steps in building the environmental model, and Model 6 is the full gene-environment model for each lipid variable.

We first explored the effects of age, ethnicity, gender, BMI, SES and their interactions on TC, HDLC, and TG by using hierarchical Tobit regression analyses in order to arrive at the most parsimonious full 'environmental' model for each lipid variable. To this end, only significant terms were kept in the intermediate models (models 2, 3, 4). Since obesity and low SES are more common in Blacks than in Whites and associate with high TG and low HDLC levels, we explored and adjusted for the effects of BMI and SES separately. First, in our basic model (Model 1), we included age, gender and ethnicity. Next, the two- and three-way interactions among these three variables were added to Model 1 separately to get an age, ethnicity, and gender interaction model (Model 2). Next, we tested separately for significant contributions of BMI (Model 3) and SES (Model 4) by comparing these models with the ethnicity and gender interaction model (Model 2). More specifically, in Model 3, we added BMI and its interactions with the terms in Model 2 (age, ethnicity, gender) to get a BMI model. In Model 4, SES and its interactions with the variables in Model 2 were assessed to get an SES model; father's and mother's education category were each tested separately using natural coding (vector 1 coded for medium, and vector 2 coded for high). Finally, BMI, SES, BMI $\times$ SES, and their interaction with predictors in Model 2 were simultaneously entered in the full environmental model (Model 5). In Model 6, by entering the gene effect and its interactions with the terms in the full environmental model, we arrive at a model including genetic and environmental effects for each polymorphism and each lipid variable.

This method has a number of advantages. First, accounting for main and interaction effects of demographic and environmental variables increases the power to detect the role of genetic factors, including gene-environment interactions, in complex traits such as lipids.[45] Second, the hierarchical sequence avoids the spurious multicollinearity caused by interactions, permitting the investigator to detect interaction effects uncontaminated by such artifacts. Third, including both ethnic groups in the same analyses allowed us to investigate ethnic heterogeneity by assessing the significance of ethnicity-environment and ethnicity-gene interactions.

A likelihood ratio test was used to determine the significance of the variables that were added to the model in each analysis step. This test yields the deviance of the model which is defined as $-2 \times$ log-likelihood. The deviance difference (between 2 models) is asymptotically distributed as χ^2, with degrees of freedom equal to the difference in number of estimated parameters. Where necessary due to missing data, models were repeated with lower n.

TC, HDLC, and TG were logarithmically transformed before analysis to ensure normally distributed residuals. Untransformed values are presented in Table 1 and figures. All statistical analyses considered *a* $P<0.05$ statistically significant and were conducted using STATA 8.0 (StataCorp, College Station, Tex).

RESULTS

Sample characteristics are presented by ethnicity and gender in Table 1. Regression analyses adjusted for age assessing gender and ethnic differences are also presented. Blacks were a little older and had a higher BMI, whereas Whites had higher father's education levels. No significant ethnicity or gender differences were found for mother's education level (mean $\pm$ SD$=14 \pm$ 2 yrs). Blacks had lower TG levels. For TC and HDLC, interactions between ethnicity and gender were identified because White males had lower TC and HDLC levels than the other three ethnicity by gender categories ($P<$ 0.001), as has been found in other investigations.[11] Twenty-seven subjects had lipid values below the detection limit for TC, 13 for HDLC, and 66 for TG. Though we cannot verify that subjects were fasting, the observed TG range is within normal limits for fasting status.

Table 2 shows the genotype and allele distributions of the four polymorphisms in Whites and Blacks. No significant deviation from Hardy-Weinberg equilibrium was observed for any polymorphism whether in Whites or in Blacks. Significantly different allele and genotype frequencies for LDLR T1773C and PPARγ Pro12Ala polymorphisms ($P<0.001$), and significantly different allele frequencies for TNFα -308G/A ($P<0.05$) were found between Blacks and Whites. The T1773 allele and PPARγ 12Ala allele were more common and the TNFα -308A allele was less common in Whites (45%, 12%, and 12%, respectively) than in Blacks (7%, 2%, and 19%, respectively). For the association analyses with lipid phenotypes, heterozygotes and homozygotes for rare alleles were combined into one group to increase statistical power. The effect of PPARγ was only explored in the Whites because the 12Ala allele frequency was too low in Blacks (2%).

Table 2. Genotype and allele frequencies by ethnicity

Polymorphism	Whites					Blacks				
	Genotype*				Allele Frequency	Genotype*				Allele Frequency
	11	12	22	*N*		11	12	22	*N*	
ApoB Glu4154Lys	144	(61	8)	213	0.82 / 0.18	121	(42	7)	170	0.84 / 0.16
LDLR T1773C†	(53	97)	77	227	0.45 / 0.55	(2	21)	151	174	0.07 / 0.93
PPARγ Pro12Ala†	169	(45	4)	218	0.88 / 0.12	157	8	0	165	0.98 / 0.02
TNFα –308G/A‡	173	(42	6)	221	0.88 / 0.12	118	(49	8)	175	0.81 / 0.19

* 11=homozygote for the 1st allele; 12=heterozygote; 22=homozygote for the 2nd allele.
† Genotype and allele frequencies differ significantly by ethnicity, $P<.001$.
‡ Allele frequencies differ significantly by ethnicity, $P<.05$.
Parentheses indicate genotypes combined into one group for association analyses.

Table 3 shows the results of hierarchical modeling of TC, HDLC, and TG with demographic and environmental variables. As shown in BMI models (model 3), BMI had a significant effect on each of the lipid values, showing a negative effect on HDLC (b=−0.016, $P<.01$) and a positive effect on TC (b=0.007, $P<.01$) and TG (b=0.085, $P<.01$). In addition, BMI showed a significant interaction with gender on the levels of TG (b=−0.027, $P<0.01$) and HDLC (b=0.013, $P<.05$), indicating that males showed a stronger linear increase in TG and a smaller linear decrease in HDLC with BMI than did females. Compared with the demographic models, the BMI models explained an additional 10.2%, 2.3%, and 9.2% of variances in TC, HDLC, and TG, respectively. For SES models, no significant effects were found for mother's education on any lipid outcome. Father's education showed a significant positive effect only on HDLC (b=0.02, $P<.01$), reflecting that subjects whose fathers have education beyond high school have higher HDLC levels. Father's education showed no significant interactions with age, gender or ethnicity. Compared with the demographic model (model 2), this SES model explained an addi-

Table 3. Environmental models predicting lipid variables

	Model	Predictors	−2 × log-likelihood	χ^2	*df*	vs Model	*P*<	Explained Variance %
TC	1	Age, Ethnicity, Gender	95.1					
	2	Age, Ethnicity, Gender, Ethnicity × Gender	82.8	12.3	1	1	.001	28.0
	3	Obesity model: BMI	71.0	11.8	1	2	.001	38.2
	4	SES model*	...	...	...	...	...	...
	5	Environmental Model: Age, Ethnicity, Gender, Ethnicity × Gender, BMI	71.0	11.8	1	2	.001	38.2
HDLC	1	Age, Ethnicity, Gender	389.0					
	2	Age, Ethnicity, Gender, Age × Ethnicity × Gender	384.6	4.4	1	1	.05	4.0
	3	Obesity model: BMI, BMI × Gender	375.5	9.1	2	2	.05	6.3
	2′	Model 2†	382.9					3.8
	4	SES model: father's education category	373.4	9.5	2	2′	.01	6.2
	5	Environmental Model: Age, Ethnicity, Gender, Age × Ethnicity × Gender, father's education category§	373.4	9.5	2	2′	.01	6.2
TG	1	Age, Ethnicity, Gender	640.1					
	2	Age, Ethnicity, Gender‡	640.1	0	0	1	...	7.9
	3	Obesity model: BMI, BMI × Gender§	575.8	64.3	2	2	.001	17.1
	4	SES model*	...	...	...	...	...	...
	5	Environmental Model: Age, Ethnicity, Gender, BMI, BMI × Gender|	575.8	64.3	2	2	.001	17.1

* Not significant.
† Model repeated with $n=408$ (cases with missing father's education excluded for comparison with model 4).
‡ No interactions entered the model, so model 2 equals model 1.
§ BMI and BMI × Sex interaction were not significant after father's education entered, and were omitted from model 5. Model 5 is equal to model 4.
| The contribution of age was no longer significant after including BMI and BMI × Sex.

Table 4. Individual predictors for significant genetic models

	Predictors	b	*P*<
TC	Age	0.02	.001
	Ethnicity	0.11	.01
	Gender	0.10	.01
	BMI	0.01	.05
	Ethnicity × Gender	−0.22	.001
	ApoB	0.34	NS
	ApoB × Age	−0.03	.01
	ApoB × BMI	0.01	.05
HDLC	Age	0.02	.05
	Ethnicity	0.15	.01
	Gender	0.09	NS
	Father's education (vector 1)	0.17	.01
	Father's education (vector 2)	0.14	.05
	Age × Ethnicity × Gender	−0.01	.05
	TNFα	−0.15	.01
	TNFα × Gender	0.24	.01
TG	Age	0.01	NS
	Ethnicity	−0.35	.001
	Gender	0.52	.01
	BMI	0.05	.001
	BMI × Gender	−0.03	.001
	LDLR	−0.15	.01

NS=not significant.

tional 2.4% of HDLC variance. Body mass index (BMI), SES and their interactions were simultaneously entered into model 2 to obtain a full demographic and environmental model that explained the most variance for TC, HDLC, and TG. Their interactions with the variables in the demographic model were also assessed. For TC and TG, the full environmental model was the same as the BMI model. For HDLC, BMI, and BMI × gender interaction were no longer significant after inclusion of father's education and therefore omitted from the model, so the full environmental model for HDLC was equal to the SES model.

To explore the gene effects, a full genetic model for each polymorphism and each lipid variable was constructed by entering the gene effect and its interactions with the terms of the full environmental model. Table 4 shows the individual predictors for genetic models that showed significant gene effects. A significant negative interaction between apoB and age and a significant positive interaction between apoB and BMI were observed for TC levels. Compared with the full environmental model, the full genetic model explained an additional 12.2% variance of TC ($\chi^2(3)=10.8$, $P<.05$). The negative interaction of apoB and age ($P<.01$) implies that 4154Lys allele carriers had a smaller linear increase in TC with increasing age (Figure 1a). Stratification of the sample in a young and older group with mean age as the cutoff showed that the gene effect was only significant in younger subjects (≤18.2 y; GluGlu: 3.44 mmol/L (133 mg/dL) vs GluLys and LysLys: 3.79 mmol/L (147 mg/dL), $P<.01$). The positive interaction of apoB with BMI ($P<.05$) indicated that 4154Lys allele carriers showed a stronger linear increase in TC with BMI than did 4154Glu allele homozygotes, translating in larger TC differences between carriers and non-carriers of the Lys allele with increasing BMI (Figure 1b).

A significant interaction of TNFα −308G/A and gender on HDLC levels was observed ($P<.01$). Figure 2 shows that the −308G/A polymorphism had a significant effect on HDLC only in males, with significantly lower HDLC levels in the −308A allele carriers. Compared with the full environmental model, the full genetic model explained an additional 2.3% variance of HDLC ($\chi^2(2)=8.7$, $P<.05$).

A main effect of LDLR T1773C on TG was found, with T allele carriers having higher TG levels. Compared with the full environmental model, the full genetic model explained an additional 1.1% variance of TG ($\chi^2(1)=7.3$, $P<.01$). The average difference in TG between T allele carriers and C allele homozygotes was 0.27 mmol/L (23 mg/dL; Figure 3). None of the polymorphisms showed a significant interaction with ethnicity, that is, the effects of these polymorphisms were the same in Whites and Blacks.

DISCUSSION

This study examined the allele frequencies and the effects of ApoB gene Glu4154Lys, LDLR T1773C, PPARγ Pro12Ala, and TNFα −308G/A polymorphisms on lipid profile in a sample of Black and White youth. The main findings of the study were as follows: The ApoB Glu4154Lys polymorphism interacted with obesity and age to predict TC levels. The TNFα −308G/A polymorphism was associated with HDLC only among males; this gene-gender interaction may contribute to the lower levels of HDLC among males. The main effect of the LDLR T1773C polymorphism on TG, along with ethnic differences in allele frequency, may contribute to lower TG levels found in US Blacks. No effect of the PPARγ Pro12Ala polymorphism was found among Whites. The rarity of this polymorphism among Blacks in this study did not permit its evaluation in this group.

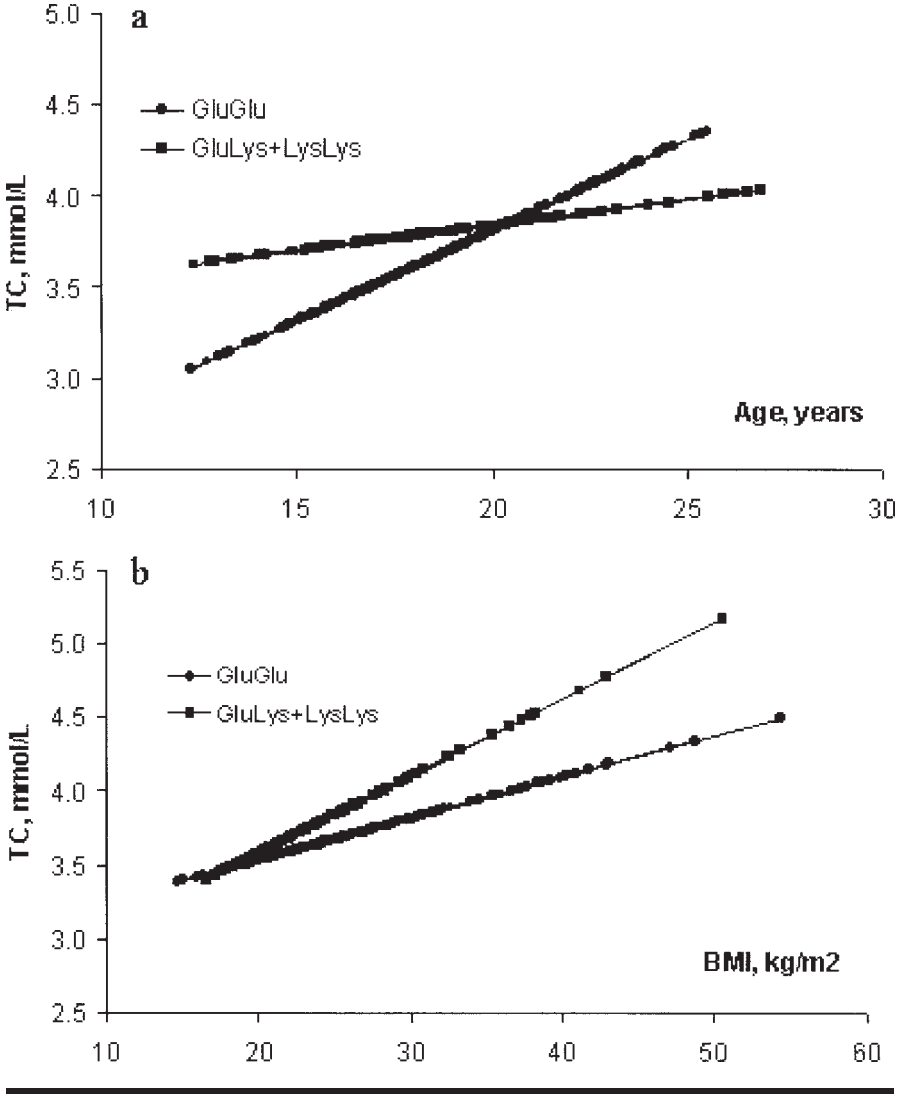

Fig 1a. Interaction of ApoB Glu4154Lys with age on predicted total cholesterol level

Fig 1b. Interaction of ApoB Glu4154Lys with body mass index on predicted total cholesterol level

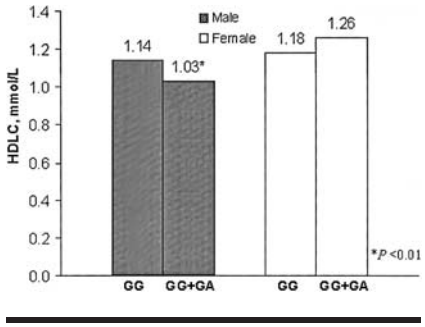

Fig 2. Interaction of TNFα gene −308G/A polymorphism with gender on high density lipoprotein cholesterol

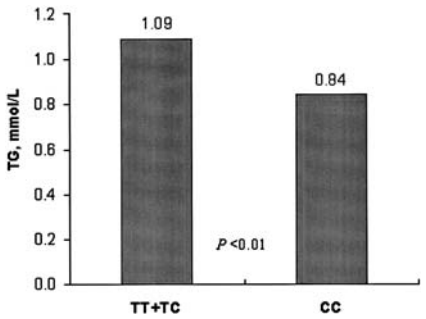

Fig 3. LDLR gene T1773C polymorphism and triglyceride level

About a quarter of the variance of TC was attributable to age, ethnicity, and gender. Obesity explained an additional 10.2% variance in TC after accounting for these constitutional factors. After adjusting for significant environmental predictors, the ApoB 4154Lys allele was associated with greater vulnerability to obesity-induced hypercholesterolemia in Black and White youth. For example, the average TC level of 4154Lys allele carriers was 0.23 mmol/L (9 mg/dL) higher than that of 4154Glu allele homozygotes at a BMI of 30 kg/m^2 and this difference increased to 0.54 mmol/L (21 mg/dL) at a BMI of 40 (Figure 1b). Since HDLC and LDL are components of TC, but no effect of this polymorphism was shown on HDLC, we believe this result implicates LDL levels, which were not directly measured in this investigation. The interaction of this gene with age meant that the effect was only significant in younger subjects; we speculate that this might contribute to elevated cholesterol levels in adolescence which remit in adulthood.[46] The LDLR, PPARγ, and TNFα genotypes were not associated with TC.

SES explained 2.4% variance in HDLC after accounting for constitutional factors. Compared to those whose fathers had no high school diploma, individuals whose fathers had at least a high school education had the advantage of 0.20 mmol/L (8 mg/dL) higher HDLC. These data add to the studies linking low SES with lower HDLC and higher cholesterol among women[47,48] and scant literature linking childhood poverty with adverse adult cholesterol levels.[49,50] Obesity explained 2.3% variance in HDLC after accounting for constitutional factors, but was no longer significant when SES was added to the model. SES showed no effect on TC or TG.

After adjusting for environmental factors, lower HDLC levels were associated with the TNFα −308A allele only in males. This extends the findings

from a prior study in Australians.[32] This allele was fairly common (12%–19%) in our sample. Inclusion of this gene-gender interaction effect in the model along with the main effect of TNFα resulted in the expected main effect of gender becoming nonsignificant. This observation, along with the similar HDLC levels found among male and female −308G homozygotes, suggests that this gene-environment interaction may contribute to the lower levels of HDLC typically found among postpubertal males. Alternatively, its negative effect in females may be masked by the female advantage for HDLC. However, in this sample Black females and males had similar HDLC levels. The ApoB, PPARγ, and LDLR genotypes showed no effect on HDLC.

Obesity explained 9.2% variance in TG after accounting for constitutional factors. Significantly lower TG levels were observed in LDLR 1733C allele homozygotes compared to T allele carriers (0.27 mmol/L or 23 mg/dL mean difference) after adjusting for environmental factors. Considering the higher frequency of the C allele in Blacks (93% vs 55% in Whites), this polymorphism may contribute substantially to the paradoxically lower TG levels found in Blacks compared to Whites in the United States The ApoB, PPARγ and TNFα genotypes showed no effect on TG.

Postprandial samples might be expected to show a higher mean and variability of TG than fasting samples, based on adult studies,[51] though TC and HDL results would not be affected.[52] These data were not verified fasting samples, yet the TG values are in a fasting range, showed expected ethnic variation (0.28 mmol/L higher in Whites than Blacks) and yielded robust results, suggesting that the postprandial effect was minimal. We suspect that TG levels among healthy young people may be less affected by eating than those of adults with more advanced cardiovascular risk. Even in adults, several investigations have demonstrated that nonfasting triglycerides independently predict coronary heart disease.[53–56] In the context of the current concern about a childhood obesity epidemic and increased cardiovascular risk in adolescents,[57,58] routine nonfasting screening of lipid profile in childhood or adolescence may be a practical strategy, with fasting tests reserved as follow-up for cases with elevated total cholesterol or triglycerides.

Information about this multiethnic study sample is a novel aspect of the study, since most prior studies include only one ethnic population. Examination of a young, healthy, unmedicated study sample eliminates secondary effects of pathophysiology and treatment. Other strengths include excellent control of environmental factors in the analysis. Ethnicity and gender along with associated factors, BMI and SES, were included in the analysis, allowing assessment of their independent as well as interactive effects. No significant effects were found for the PPARγ polymorphism in our study. Its rarity among Blacks (allele frequency of 2%), also recently observed in the Bogalusa Heart study,[59] limited our ability to evaluate its effect among Blacks and reduced the power in our overall sample.

Self-reported ethnicity is neither purely biological nor measured with precision. It represents a mixture of genetic, social, economic, behavioral (eg, diet), psychological (eg, identity), and other environmental factors. Despite this, use of self-reported ethnicity has been advocated in biomedical and genetic research.[60] It is a useful tool to follow up on known ethnic variation and identify possible explanatory disease mechanisms. Methods able to distinguish genetic from environmental aspects of ethnicity, along with development of excellent measures of social factors and behavior, will advance the understanding of these underlying factors in cardiovascular risk development and may enable our society to more effectively remedy ethnic health disparities.[61]

In sum, the ApoB, TNFα, and LDLR candidate genes, which have previously been associated with obesity, were associated with lipid profiles in youth after accounting for constitutional factors, body habitus, and socioeconomic influence. These gene effects and gene-environment interactions may inform prevention and treatment of coronary heart disease.

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