Age-Related Effects of Genetic Variation on Lipid Levels: The Columbia University BioMarkers Study

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ABSTRACT. *Objectives.* **To examine the genotype: phenotype association in children compared with their parents.**

Methods. **Variations at 4 key gene loci, namely lipoprotein lipase (*LPL* S447X), hepatic lipase (*HL* −480C>T), cholesteryl ester transfer protein (*CETP Taq*IB), and apolipoprotein CIII (*APOC3* −455T>C and −482C>T), were examined in children (*n* = 495) and their parents (*n* = 353) in the Columbia University BioMarkers Study, 1994 to 1998.**

Results. **The frequencies of the rare alleles of the *HL* −480C>T and *APOC3* −455T>C and −482C>T (but not *LPL* S447X or *CETP Taq*IB) were significantly lower in non-Hispanic white participants compared with Hispanics. Overall, genotype effects seen in the adults were weaker in the children, although similar trends were seen. In an examination of the effect of body fat on the genotypic effects in the children, there was significant *HL* −480C>T:sum of skinfold interaction.**

Conclusions. **All genotypes were associated with clear relationships to plasma lipid levels in adults, but the effects were weaker in their children, unless stressed by body fat.** *Pediatrics* 2001;108(3). URL: http://www.pediatrics.org/cgi/content/full/108/3/e50; *atherosclerosis, cardiovascular disease, child, lipids, genetics.*

ABBREVIATIONS. LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; LPL, lipoprotein lipase; HL, hepatic lipase; CETP, cholesteryl ester transfer protein; TGRL, triglyceride-rich lipoproteins; CAD, coronary artery disease; apo, apolipoprotein; BMI, body mass index.

Atherosclerosis is a multifactorial disorder with origins in childhood.[1,2] Several lipid traits are among the identified risk factors, including elevated total and low-density lipoprotein (LDL) cholesterol,[3] reduced high-density lipoprotein (HDL) cholesterol, and elevated plasma triglyceride (TG) levels.[4] Both genetic and environmental factors contribute significantly to interindividual variability in these lipid traits,[5,6] and genotype:phenotype association studies in adults have shown that variations at several gene loci involved in lipid metabolism influence lipid levels. Very few such studies have been reported in children.

Lipoprotein lipase (LPL) and hepatic lipase (HL) are key enzymes in the hydrolysis of TG in adipose tissue and muscle and liver[7] and also act as ligands in the receptor-mediated uptake of lipoprotein (the bridging function).[8] Cholesteryl ester transfer protein (CETP) is involved primarily in the redistribution of lipids in reverse lipid transport pathways, transferring cholesteryl esters from HDL cholesterol to TG-rich lipoproteins (TGRL) in exchange for TG.[9–11] Biologically significant genotypic variation has been documented in all 3 of the genes that code for these proteins. Variation at residue 447 in the *LPL* gene (*LPL* S447X) results in premature termination of the protein by 2 amino acids.[12] The rare allele has been associated consistently in adult populations with lower plasma TG and higher HDL levels and seems to be protective in studies of coronary artery disease (CAD).[13,14] In the hepatic lipase gene (*HL*) promoter, the C to T change at site −480 is associated with lower HL activity and is more common in CAD patients compared with controls,[15,16] but it also is associated with higher HDL levels in healthy adults.[15,17,18] These seemingly contradictory findings may be explained by differential effects on HDL subclasses. The rare B2 allele of the *Taq*IB polymorphism in intron 2 in the *CETP* gene is associated with lower CETP mass[19] and activity[20] and with higher plasma HDL and apolipoprotein (apo) AI levels.[20–22] ApoCIII is a component of both very low-density lipoproteins and HDL,[23] and plasma apoCIII levels are correlated positively with plasma TG and cholesterol levels.[24–26] Elevated apoCIII levels have been found in hypertriglyceridemic adults[27] and in patients with carotid artery disease.[28] Two polymorphisms in the *APOC3* gene, −455T>C and −482C>T, have been shown in men to be associated with higher TG levels, particularly in smokers.[29] In young males who participated in the European Atherosclerosis Research Study II, the −482T allele was associated with greater insulin response after an oral glucose tolerance test,[30] whereas in the same study the −455C allele was associated with reduced TG clearance after an oral fat load (DM Waterworth and PJ Talmud, unpublished results). We therefore ana-

lyzed the relations of these variations in the *LPL*, *HL*, *CETP*, and *APOC3* genes to lipid levels in children and their parents. In this article, we report genotype: phenotype relations for these five genetic polymorphisms, focusing on evidence that there is less phenotypic expression in selected genes in children compared with adults.

METHODS

Participants

The study children were participants in the Columbia University BioMarkers Study, conducted from 1994 to 1998. Families were characterized as recruited either from a high-risk setting at the Columbia Presbyterian Medical Center or from the population at large. High-risk recruitment settings included the Children's Cardiovascular Health Center, to which children were referred for diagnosis and management of lipid disorders; the adult cardiac catheterization laboratory; and private adult cardiology practices. In addition, families of hospitalized patients with early onset ischemic heart disease, identified using the New York Presbyterian Hospital computerized information system, were classified as high risk. Families that were recruited from general pediatric practices or through fliers were classified as recruited from the population at large (low-risk settings). Families with at least 1 healthy child, 4 to 21 years of age, were eligible for participation. Siblings 21 years of age or younger ($n = 220$) were included in the analysis as well as 3 siblings aged 22 to 24 years who entered the study. Participation of at least 1 parent also was required. Race/ethnicity was categorized on the basis of the mother's self-report, following definitions used in the US census of 1990,[31] as Hispanic, black but not of Hispanic heritage, non-Hispanic white, Asian, or Pacific Islander. The race/ethnicity of children was based on that of the mother.

A total of 352 eligible families that comprised 612 children were recruited for the study. We excluded from this analysis children with race/ethnicity other than Hispanic or non-Hispanic white (17 blacks, 5 Asians, and 11 race unknown), those with missing lipid data ($n = 12$), and those with no genotype data ($n = 72$), leaving 495 children in analyses. Anthropometric data were obtained from 1 child in each family, specifically, the oldest age-eligible child who was willing to participate, and were available for 322 children. A total of 445 adults were recruited. Exclusions on the basis of race/ethnicity (16 blacks, 5 Asians, and 17 race unknown), missing lipid data ($n = 2$), and missing genotype data ($n = 52$) left 353 adults available for analyses. Because inclusion into the analyses for both children and adults required available data for lipid levels and at least 1 genotype, the number with available data varied from trait to trait. This is reflected in the analyses and presented in the tables. The study was approved by the Institutional Review Board of Columbia University and the New York Presbyterian Hospital.

Measures of Family History, Obesity, and Other Clinical Characteristics

Medical histories were obtained through individualized interviews conducted in English or Spanish using structured questionnaires. Information on CAD in family members was verified when possible by review of medical records. Family history of early onset of CAD was classified as positive if 1 or both parents had experienced early onset (55 years of age for men and 65 years of age for women) of clinical CAD, as indicated by a history of bypass surgery, coronary angioplasty, or sudden death (either documented in medical records or self-reported) or by coronary arteriographic documentation of 50% narrowing of the luminal diameter of 1 or more major epicardial coronary arteries. Family history was classified as indeterminate when family members were unsure of their medical history or when there was a history of early onset of CAD in 1 or more grandparents. Family history was categorized as negative when the self-reported medical history was negative for early onset of CAD in both parents and all 4 grandparents.

Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured to the nearest 0.1 kg using a calibrated balance scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Five skinfolds were measured on the right side of the body with calipers (Lafayette Instrument Co., Lafayette, IN) as previously described[32]: triceps, subscapular, suprailiac, abdominal, and thigh.[33] Each skinfold was measured twice, and the mean value was recorded. If the 2 values differed by more than 2 mm, then a third measure was taken and the mean of the 2 closest measurements was recorded. Sum of skinfolds was calculated as the sum of the value for the 5 skinfolds. Circumferences at the waist (the narrowest part of the torso) and the hip (the maximum extension of the buttocks) were measured with a tape measure.[33]

Biochemical and Genetic Analyses

Participants were instructed to fast after dinner the night before the interview, except for water, and blood samples were obtained at the start of the interview. Plasma levels of total cholesterol and TG were measured using standardized enzymatic procedures with a Hitachi 705 automated spectrophotometer (Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was measured by the precipitation of plasma apoB-containing lipoproteins and with phosphotungstic acid.[33,34] LDL cholesterol levels were calculated using the Friedewald equation.[35] Genotyping for the *LPL* S447X,[14] *HL* −480C>T,[36] *CETP* *Taq*IB,[20] and *APOC3* −482C>T[37] were conducted as reported previously. The PCR product used for −482C>T genotyping incorporated the −455 polymorphic site. Genotyping of the −455T>C was achieved by FokI digestion.

Statistical Analysis

Allele frequencies were determined using the gene counting method, and 95% confidence intervals were calculated using the z transformation. In calculating the allele frequencies, only 1 child from families that contributed multiple children was considered, with the oldest participant being selected. Analyses were conducted using Intercooled STATA version 6 (Stata Corp, College Station, TX). From initial analyses and in agreement with Jansen et al,[36] lipid values for carriers of the rare alleles for the *HL* and the *APOC3* polymorphisms were similar, and for all subsequent analyses heterozygotes were combined with rare homozygotes. To determine the allelic association between the 2 *APOC3* variants, we calculated linkage disequilibrium (Δ) using the method of Chakravarti et al.[38] For *LPL*, the frequency of the rare allele was low and rare allele carriers were pooled for all analyses. For the *CETP* *Taq*IB polymorphism, because a "recessive effect" has been described, carriers of the common allele were compared with rare allele homozygotes.[20,30] Dependent variables considered included total, HDL, and LDL cholesterol and TG. Covariates included in analyses of gene-lipid level relations included age, gender, race/ethnicity, recruitment source, and family history and BMI in the children.

Family history was grouped as positive versus negative or indeterminate. Natural logarithm transformations were used to improve normality of the distributions for HDL and LDL cholesterol, TG, and BMI. For a more familiar presentation of results, for variables that were log-transformed, means presented are the anti-log of log-transformed means together with approximate standard deviations. For testing for differences between children and parents in the magnitude of the gene-lipid trait relation, linear regression models were fitted with the logarithmic transformation of the lipid trait as the dependent variable and terms for the genetic polymorphism, adult–child status, and the interaction between the genetic polymorphism and adult–child status. The statistical test on the coefficient for the interaction term was used to test for a difference in gene expression in the children versus adults. Interaction terms between race/ethnicity and genotypes also were considered in all models to check for the possibility of differences in gene lipid trends by race/ethnicity. In these models, other covariates were not included because not all of the same covariates were measured in the children and adults. Separate multivariate linear regression models were fitted in the children and the parents to estimate each gene-lipid level relation adjusting for measured covariates in each group. In all analyses, we estimated the standard errors using the Huber-White correction and the cluster option in STATA to adjust for the fact that the error terms for children within the same family are not independent. Model checking was done via Cook's distance and diagnostic plots. Sum of skinfolds and genotype interactions were tested a

priori on the basis of previous studies.[39] No adjustments for multiple comparisons were made, following the argument of Rothman[40] and Perneger,[41] because analyses were based on a priori hypotheses from previous reports of genotype associations. Statistical significance was taken as $P < .05$.

RESULTS

Lipid Levels in Different Groups

The baseline measures for age, BMI, and lipid levels in the children, considering gender, race/ethnicity, and family history of early CAD, are presented in Table 1. In the male children from families with no family history of CAD, levels of total and LDL cholesterol were higher in the non-Hispanic white children compared with the Hispanic children, as previously reported.[42] This also was evident when comparing the male children on the basis of family history of CAD, reflecting the differential recruitment of more non-Hispanic white families from the high-risk settings and more Hispanic families from the low-risk settings. Similarly, in the female children from families with no family history of CAD, levels of total and LDL cholesterol and TG were higher in the non-Hispanic white children compared with the Hispanic children. Overall, in all of the children, irrespective of race/ethnicity, those with a positive family history of early CAD (33 Hispanics and 39 non-Hispanic whites) compared with those with a negative (271 Hispanics and 43 non-Hispanic whites) or indeterminate (85 Hispanics and 24 non-Hispanic whites) family history had significantly higher levels of cholesterol (9.2%; $P < .01$), LDL-cholesterol (18.0%; $P < .001$), and TG (19.3%; $P < .001$) and lower levels of HDL cholesterol (7.5%; $P = .01$). Results for the adults are presented in Table 2. None of the lipid values were significantly different between Hispanic males compared with non-Hispanic white males. In the female adults, cholesterol levels alone were significantly higher in the non-Hispanic white women compared with the Hispanic women.

Allele Frequencies

We examined the genotype frequencies for each of the polymorphic sites of the loci of interest in the Hispanic and non-Hispanic white groups separately and found no evidence of departure from Hardy-Weinberg equilibrium. Allele frequencies for the *LPL* S447X and the *CETP Taq*IB polymorphisms did not differ between Hispanics and non-Hispanic whites among either the children or the adults (Table 3). The frequency of the *HL* −480T allele was higher in adult Hispanics compared with non-Hispanic whites (0.36 vs 6.17; $P < .0001$). A similar but nonsignificant trend was seen in the children (0.26 vs 6.36). The *APOC3* −455C and *APOC3* −482T alleles were more frequent in Hispanics compared with non-Hispanic whites (Table 3). The frequencies of these 5 polymorphisms did not differ significantly between children with a positive versus negative family history of CAD or when categorized on the basis of race/ethnicity (Table 4). For the *APOC3* gene, there was a strong positive allelic association between the −455 and the −482 variants that was of similar magnitude in the non-Hispanic white and Hispanic adults ($\Delta = 0.72$ [$P < .001$] and $\Delta = 0.77$ [$P < .001$], respectively).

Genotype Associations With Lipid Levels

LPL S447X

In the children, mean total cholesterol level was lower in carriers of the *LDL* 447X allele than in individuals who were homozygous for the 447S allele, a difference that was not significant in the adults (Table 5). In the adults, carriers of the 447X allele had lower mean plasma TG levels compared with 447S homozygotes, an effect that was not significant in the children. Tests for adult/child status:genotype interaction were not significant for either lipid trait (Table 5).

HL −480C>T

In the children, carriers of the *HL* −480T allele had 5.9% higher mean HDL cholesterol level than indi-

TABLE 1. Mean (SD) Age, BMI, and Serum Lipid Levels in Children

	Positive Family History		P Value	Negative or Indeterminate Family History		P Value	P Value for FH
	Hispanic	Non-Hispanic Whites		Hispanic	Non-Hispanic Whites		
Males							
Age (y)	13.68 (4.07) [19]	13.50 (4.48) [24]	NS	8.6 (3.68) [170]	9.49 (3.73) [35]	NS	<.0000
BMI (kg/m)	22.84 (3.80) [14]	21.43 (4.54) [15]	NS	20.80 (5.32) [109]	18.43 (3.08) [22]	.04	NS
Cholesterol (mg/dL)	170.62 (45.77) [19]	190.82 (4808) [24]	NS	153.07 (30.80) [171]	189.52 (49.25) [35]	<.000	<.001
TG (mg/dL)	105.47 (55.57) [19]	118.21 (49.01) [24]	NS	92.29 (37.77) [171]	116.46 (41.34) [35]†	NS	.01
LDL (mg/dL)	99.2 (34.49) [19]	136.3 (36.87) [23]	.002	88.19 (28.03) [169]	112.8 (29.75) [35]	<.000	<.0000
HDL (mg/dL)*	41.86 (10.06) [19]	33.26 (7.65) [24]	.002	41.82 (9.94) [169]	42.56 (10.63) [35]	NS	.001
Females							
Age (y)	12.79 (4.77) [14]	13.30 (4.43) [15]	NS	8.88 (3.93) [185]	10.09 (3.61) [32]	NS	<.0000
BMI (kg/m)	23.70 (6.38) [10]	22.64 (6.90) [10]	NS	19.86 (4.97) [111]	18.75 (2.63) [20]	NS	.005
Cholesterol (mg/dL)	165.63 (32.57) [14]	168.08 (36.74) [15]	NS	158.97 (34.43) [185]	185.4 (45.46) [32]	<.0003	NS
TG (mg/dL)	115.89 (50.83) [14]	112.76 (27.20) [15]	NS	92.67 (28.93) [185]	113.36 (45.86) [32]	.001	007
LDL (mg/dL)	90.66 (26.53) [14]	106.08 (31.51) [14]	NS	90.0 (30.27) [184]	115.10 (28.67) [31]	<.0001	NS
HDL (mg/dL)*	40.64 (7.55) [14]	40.90 (5.70) [15]	NS	42.38 (10.22) [181]	41.67 (7.39) [30]	NS	NS

Numbers in brackets are the numbers of participants in each category for whom data are available for each variable.
* Means computed as the anti-log of the mean of log transformed values; standard deviations given are appropriate (see "Methods").
† Overall not considering family history, there is a significant difference by race/ethnicity.

TABLE 2. Mean (SD) Age, BMI, and Serum Lipid Levels in the Adults

Parameter	Males		Females	
	Hispanic	Non-Hispanic Whites	Hispanic	Non-Hispanic Whites
Age (yr)	41.5 (7.56) [39]	44.9 (6.08) [41]	36.6 (7.30) [220]	42.0 (5.45) [52]†
Total cholesterol (mg/dL)	206.9 (40.31) [40]	212.3 (61.96) [41]	174.2 (35.77) [220]	191.8 (44.15) [52]*
TG (mg/dL)	170.2 (87.16) [40]	181.8 (133.15) [41]	108.4 (43.30) [220]	108.7 (55.18) [52]
LDL (mg/dL)	144.6 (30.62) [40]	149.0 (57.18) [41]	106.2 (33.94) [218]	117.5 (39.76) [51]
HDL (mg/dL)	36.3 (8.56) [40]	34.7 (9.25) [41]	44.3 (10.44) [220]	47.7 (16.07) [52]

Numbers in brackets are the numbers of participants in each category for whom data were available for each variable. Means computed as the anti-log of the mean of log-transformed values; standard deviations given are approximate (see "Methods").
* P < .01.
† P < .001.

TABLE 3. Numbers of Participants, Frequencies, and 95% CI of Rare Alleles in the *LPL, HL, CETP,* and the *APOC3* Genes in Index Children and Their Parents, by Race/Ethnicity

Polymorphism	Group	Hispanic			Non-Hispanic Whites		
		n	Frequency	(95% CI)	n	Frequency	(95% CI)
LPL S447X	Adults	260	0.08	(0.05, 0.10)	93	0.11	(0.06, 0.15)
	Children	225	0.07	(0.04, 0.09)	55	0.07	(0.02, 0.12)
HL −480C>T	Adults	234	0.36	(0.32, 0.41)	75	0.17	(0.11, 0.23)‡
	Children	199	0.36	(0.31, 0.41)	48	0.26	(0.17, 0.35)
*CETP Taq*IB	Adults	223	0.38	(0.33, 0.42)	79	0.38	(0.32, 0.46)
	Children	191	0.39	(0.34, 0.44)	44	0.35	(0.25, 0.45)
APOC3 −455T>C	Adults	212	0.55	(0.50, 0.59)	72	0.40	(0.32, 0.48)*
	Children	180	0.53	(0.48, 0.58)	39	0.37	(0.26, 0.48)*
APOC3 −482C>T	Adults	210	0.44	(0.39, 0.49)	77	0.27	(0.20, 0.34)†
	Children	180	0.45	(0.40, 0.50)	39	0.26	(0.16, 0.36)*

Both parents but only 1 child from each family were included. CI indicates confidence interval.
* P < .01 for Hispanic versus non-Hispanic whites.
† P < .001 for Hispanic versus non-Hispanic whites.
‡ P < .0001 for Hispanic versus non-Hispanic whites.

TABLE 4. Frequencies and 95% CI of Rare Alleles in the *LPL, HL, CETP,* and the *APOC3* Genes in Index Children

Polymorphism	Frequency in Children With Positive Family History of CAD			Frequency in Children With Negative Family History of CAD			P Value
	n	Frequency	95% CI	n	Frequency	95% CI	
LPL S447X	40	0.08	(0.02, 0.13)	184	0.06	(0.04, 0.09)	NS
HL −480C>T	37	0.35	(0.24, 0.46)	158	0.35	(0.30, 0.40)	NS
CETP Taq/B	35	0.41	(0.30, 0.53)	152	0.36	(0.30, 0.41)	NS
APOC3 −455T>C	35	0.46	(0.33, 0.57)	144	0.50	(0.45, 0.56)	NS
APOC3 −482C>T	32	0.36	(0.24, 0.48)	143	0.42	(0.36, 0.47)	NS

viduals who were homozygous for the −480C allele (P = .04), an association that was not significant in the adults (Table 5). In the adults, carriers of the −480T allele had higher mean TG and LDL cholesterol levels than individuals who were homozygous for the common −480C allele, effects not seen in the children. Tests for adult/child:genotype interactions were not significant (Table 5).

CETP TaqIB

In the adults, homozygotes for the rare B2 allele had 14.5% higher HDL cholesterol levels (P = .001) compared with carriers of the B1 allele, an effect not seen in the children (test for child/adult status:genotype interaction, P = .01; Table 5).

APOC3 −455T>C and −482C>T

In the adults, the *APOC3* −455T>C allele was associated with 14.8% lower mean TG level (P = .01), an effect that was not significant in the children (Table 5). The test for adult/child:genotype interac-

tion was not significant. No relation of the *APOC3* −482C>T polymorphism with any of the lipid traits was found in either the children or the adults.

Effects of Race/Ethnicity, Gender, Recruitment Source, Family History of CAD, and BMI

There was no evidence, from the multivariate models, of interactions between genotype and either race/ethnicity, gender, or family history in relation to lipid levels. There was no heterogeneity of effect of genotype on lipid levels by race/ethnicity, consequently, as presented in Table 5; Hispanics and non-Hispanic whites were considered together.

Obesity: Genotype Interaction in Children

Measures of obesity in the children, including skinfold measures, BMI, and waist/hip ratio, were strongly correlated to each other.[39] Using tertiles of the sum of the skinfold measures as the classification variable, the interactions of genotype with obesity on lipid levels were examined by multiple linear regres-

TABLE 5. Unadjusted Geometric Means and Appropriate Standard Errors for Lipid Traits in Children and Adults, by Allele at Selected Polymorphisms

Lipid Trait	Children			Adults			Adult/Child:Genotype Interaction (P Value)†
	SS [434]	SX+XX [61]	P Value*	SS [303]	SX+XX [50]	P Value*	
LPL S447X							
Total cholesterol (mg/dL)	163.7 (0.8)	155.7 (1.8)	.04	185.4 (1.0)	178.5 (20.9)	NS	.08
TG (mg/dL)	97.4 (1.0)	95.4 (1.0)	NS	123.0 (1.0)	104.7 (1.1)	.03	.08
	CC [188]	CT+TT [229]		CC [149]	CT+TT [160]		
HL −480C>T							
HDL-cholesterol (mg/dL)	41.5 (0.6)	43.0 (0.7)	.04	44.4 (0.9)	43.9 (0.9)	NS	NS
TG (mg/dL)	95.9 (1.0)	100.6 (1.0)	NS	112.2 (1.0)	125.8 (1.0)	<.001	NS
LDL cholesterol (mg/dL)	102.3 (2.8)	104.73 (3.0)	NS	113.72 (1.0)	118.21 (3.4)	.02	NS
	B1B1+B1B2 [356]	B2B2 [62]		B1B1+B1B2 [252]	B2B2 [50]		
*CETP Taq*IB							
HDL cholesterol (mg/dL)	42.4 (0.5)	43.8 (1.3)	NS	42.5 (0.7)	50.2 (2.0)	.001	.01
	TT [81]	C+CC [306]		TT [68]	TC+CC [216]		
APOC3 −455T>C							
TG (mg/dL)	103.4 (2.1)	96.2 (0.81)	NS	137.4 (3.8)	116.8 (1.47)	.01	NS

Numbers in brackets are the numbers of participants with gene and lipid trait data available for analysis.
* P values from bivariate comparisons.
† P value testing the interaction term parent/child status:genotype, after adjustment for parent/child status and genotype.

sion analysis with adjustment for other covariates. There was significant interaction between tertiles of sum of skinfold thickness and the *HL* −480C>T genotype in determining HDL cholesterol levels (P = .02; Fig 1). In the lowest tertile, carriers of the −480T allele had lower mean HDL cholesterol level, whereas in the two upper tertiles, −480T carriers had higher mean HDL cholesterol levels when compared with the CC homozygotes. The findings were similar when BMI tertiles were examined. Interactions with measures of obesity were not seen with the other genotypes and lipid levels. Measures of obesity were not obtained in the adults, and equivalent analyses could not be conducted.

DISCUSSION

We examined the relations of well-characterized polymorphisms in the *LPL*, *HL*, *CETP*, and *APOC3* gene loci to lipid levels and compared genotype:

phenotype relations in children versus their parents. In an earlier study of the relation of a polymorphism in the β-fibrinogen gene promoter to plasma fibrinogen level, confirmed by additional statistical analysis here, we found a genotype effect in the parents but not in the children.[39] The findings for the *CETP Taq*IB gene variant in relation to serum HDL cholesterol level reported here also are consistent with lesser expression in childhood.

CETP plays a major role in the remodeling of HDL and the determination of HDL cholesterol levels.[43–45] CETP also plays a role in reverse cholesterol transport in the transfer of cholesterol from HDL to TGRL in exchange for TG, thus influencing the TG content of TGRL particles. In adults, the *CETP Taq*IB polymorphism (B2 allele) is associated with higher HDL cholesterol levels,[46–48] and CETP activity has been shown to have a positive correlation with total cholesterol levels.[49,50] In agreement, adults in the present

Fig 1. Interaction between sum of skinfolds and *HL* −480C>T genotype in relation to HDL cholesterol levels in children in the Columbia University BioMarkers Study.
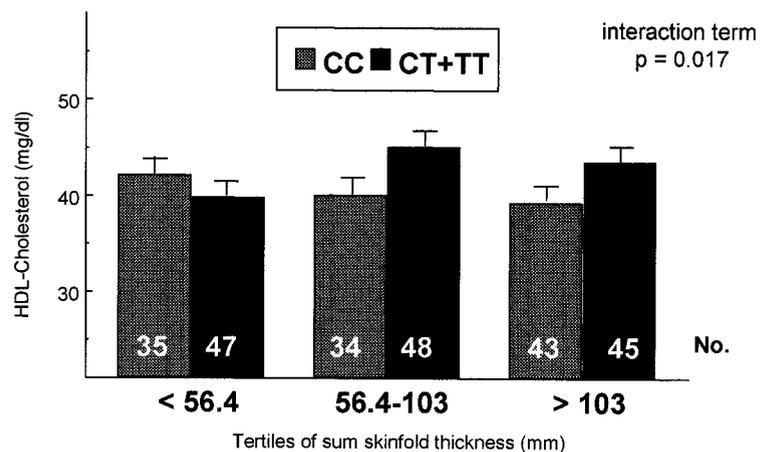
study with the B2/B2 genotype at the *CETP Taq*IB locus had higher mean HDL cholesterol levels, by approximately 14.5% ($P < .001$), whereas no such effect was found in the children.

The *APOC3* −455C allele was associated in our study with lower TG levels in the adults. This finding differs from previous results in which the same allele was associated with elevated TG levels after an oral fat tolerance test in young healthy men. This difference may be explained by differences between the sample groups; the previous study was composed of young healthy European men, whereas the majority of the adults in the present study are Hispanic women. Unlike previous studies,[30,51] the *APOC3* −482C>T polymorphism showed no effect on TG levels in the adults or the children in this sample.

Children with the rare *LPL* 447X allele had slightly lower (approximately 5%) mean total cholesterol than children who were homozygous for the common allele. In the adults, the relation of the 447X allele to total cholesterol level was not apparent, but this allele was associated with lower TG levels, an effect not present in the children. The 447X allele has been shown to be associated in adults with lower TG levels in several studies (reviewed in reference 52). In the European Atherosclerosis Research Study of 18- to 26-year-old university students with or without a family history of CAD, carriers of the 447X allele had lower TG and had a relative risk of 0.7 for family history of myocardial infarction.[13] This protective effect against myocardial infarction was confirmed recently in the Framingham Offspring Study.[14] The mechanism behind this association remains unclear, but in vitro studies suggest greater production of *LPL* 447X leading to higher levels of LPL activity.[53] In the present study, no frequency difference was seen between children with a family history of CAD and those without.

Children with the rare *HL* −480C>T allele had higher mean HDL cholesterol levels than those who were homozygous for the common allele, a relation not present in the adults (Table 5). However, the adults with the rare allele had higher mean TG and LDL cholesterol levels than adults who were homozygous for the common allele, and neither of these relations was apparent in the children. Other studies have shown an association in adults of the *HL* −480T allele with higher HDL cholesterol levels.[15,17,18,36]

We found frequency differences for the *HL* −480C>T and *APOC3* −455C>T and 482C>T polymorphisms between Hispanic and non-Hispanic whites, with the Hispanics having higher frequencies of the rare allele in both genes. We previously identified a highly significant frequency difference of the *APOC3* −482C>T between whites, Asians from the Indian subcontinent, and Africans, all living in South London, suggesting that this polymorphism shows a high degree of ethnic variation.[54] Similarly, ethnic frequency differences have been reported for the *HL* −480C>T[55] For the *HL* −480C>T, the effect on plasma HDL cholesterol was modest in the children with the rare allele associated with higher levels. In the adults, a significant but modest effect was seen on plasma TG levels and LDL cholesterol levels; carriers of the rare T allele had significantly higher levels than individuals who were homozygous for the *HL* −480 T allele. However, the frequency differences did not explain the differences in lipid levels seen between Hispanic and non-Hispanic whites, and there were no significant race/ethnicity:genotype interactions in relation to any of the traits.

As previously reported, despite the similar mean BMI, the non-Hispanic white children had higher mean total cholesterol, LDL cholesterol, and TG levels and lower HDL cholesterol than the Hispanic children.[42] Lipid levels also were significantly higher in children with a family history of CAD compared with those without. In the CATCH school-based study of children of Latino, white, and African American origin, the white children had the lowest HDL cholesterol levels, but no difference in total cholesterol levels were seen.[56] We examined whether variation in several candidate genes could explain these non-Hispanic white/Hispanic differences.

In the children, we found a significant interaction between body fat measures with *HL* genotype on HDL levels. In children in the bottom tertile of skinfold thickness, carriers of the *HL* −480T allele had lower mean HDL cholesterol levels than individuals who were homozygous for the −480C allele. Among children in the upper 2 tertiles of skinfold thickness, this effect was reversed, and HDL cholesterol levels were higher among *HL* −480T carriers (Fig 1). HL plays a key role in the receptor-mediated removal of TGRL by acting as a ligand and "bridging" the uptake. HL also plays a role in the hydrolysis of phospholipids and the remodeling of HDL particles. In both the children and the adults, overall, the −480T allele was associated with higher TG levels, confirming previous results.[36] When obesity is taken into account in the children, the partitioning of this effect is such that among children in the higher tertiles of skinfolds, in whom TG levels are higher (because obesity and TG are positively correlated), the −480T allele was associated with higher HDL levels. Thus, our findings suggest that at the higher TG level, *HL* −480T allele is having a larger effect on HDL raising. HL plays a role in the conversion of HDL_2 to HDL_3, and the −480T allele has been associated with lower HL activity. Thus, this elevation in HDL levels in −480T children with higher body fat may reflect impaired HDL metabolism. This supports the general hypothesis that obesity in childhood increases the expression of certain pro-atherogenic genetic polymorphisms.

One limitation of our study is that we did not ascertain the children's Tanner stage because many of the participants found disrobing unacceptable; we also did not measure sex hormone levels. Sexual maturation is known to influence lipid levels in children.[57,58] We included adjustments for age and gender in multivariate analyses, but our data cannot exclude an interaction between sexual maturity and the effects of allelic variation on lipid levels. Conversely, lipid levels in children, by comparison to adults, are influenced less by certain environmental factors, including medications, alcohol consumption,

and smoking. A second limitation is that the Friede-wald equation, which was used to calculate LDL cholesterol level,[35] has not been validated in children. We acknowledge that the sample is not representative but instead was designed to include families from high- and low-risk groups and that this may limit generalizability of the findings pending other studies of these genotype:phenotype associations in other population samples. We did not adjust for multiple statistical comparisons. We recognize that different authorities take differing approaches to the potential for false-positive inference, and this issue should be considered in interpreting the results of our analyses.

The availability of genetic and lipid measures from both parents and their children is a major strength of our study. The data presented here show associations of genotypes with lipid traits in adults that are less strongly present in their children. A previous study reported that the −455G>A polymorphism in the fibrinogen gene promoter was associated with higher plasma fibrinogen levels in the adults but not in their children.[39] Confirmation of the differential gene effects in childhood compared with adulthood that we described will require longitudinal studies for confirmation. The apparent lack of expression in childhood of several genetic polymorphisms that are known to influence adult lipid levels potentially may help to explain the relatively modest tracking of lipid levels from childhood to adulthood.

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