

APOA5 polymorphisms influence plasma triglycerides in young, healthy African Americans and whites of the CARDIA Study

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Abstract Genetic variation in the apolipoprotein A-V gene (*APOA5*) has been associated with variation in plasma triglyceride (TG) levels in African American and white females and males older than 40 years and/or at increased risk of coronary artery disease. We have examined whether plasma TG levels are associated with 16 *APOA5* polymorphisms in young (18–30 years) African American (1,075 females and 783 males) and white (1,041 females and 932 males) individuals of the Coronary Artery Risk Development in Young Adults (CARDIA) Study selected without regard to health. Plasma TG was significantly ($P < 0.01$) associated with markers 27376 and 28837 (–3A/G) in both white females and males, with 27709 (–1131T/C) and 29085 in white males, with 29009 (S19W) in African American females and white males, and with 30966 in African American females. No statistically significant associations were observed in African American males. These six single-nucleotide polymorphisms individually accounted for 0–0.78% of lnTG variation among white females, 0–2.46% among white males, and 0–0.69% among African American females. The results of our study suggest a small but replicable context-dependent influence of the *APOA5* gene region on plasma TG levels in young, healthy individuals.—Klos, K. L. E., S. Hamon, A. G. Clark, E. Boerwinkle, K. Liu, and C. F. Sing. *APOA5* polymorphisms influence plasma triglycerides in young, healthy African Americans and whites of the CARDIA Study. *J. Lipid Res.* 2005. 46: 564–570.

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Increased plasma triglyceride (TG) level has emerged as an independent risk factor for coronary artery disease (1, 2). Genetic sources of variation account for 21–40% of variation in plasma TG levels among individuals (3, 4). The *APOA1/C3/A4* gene cluster has received considerable attention for explaining variation in plasma TG levels among both healthy and hypertriglyceridemic subjects.

Recently, Pennacchio et al. (5) identified the apolipoprotein A-V gene (*APOA5*) as a new member of the *APOA1/C3/A4* gene cluster by comparative sequencing. Overexpression in transgenic mice leads to reduced plasma TG level, whereas increased TG levels are observed in knock-out mice (5). In humans, associations have been identified between plasma TG level and several *APOA5* polymorphisms, including –1131T/C, –3A/G, S19W, and 1259T/C (5–19). However, there has been some inconsistency in the reported race- and gender-specific associations of this limited set of *APOA5* polymorphisms with plasma TG levels [e.g., Pennacchio et al. (5, 7) and Evans, Buchwald, and Beil (10)].

The majority of studies have evaluated the influence of *APOA5* variation on interindividual variation in TG levels in older individuals (5, 6, 8, 10, 12, 16) and/or in individuals at increased risk of coronary artery disease based on increased lipid levels or family history (5, 9, 10–12, 14, 15). The purpose of our study was to evaluate 16 single-nucleotide polymorphisms (SNPs) in the *APOA5* gene for association with variation among individuals for plasma lnTG levels in healthy young (18–30 year old) African American and white females and males from the Coronary Artery Risk Development in Young Adults (CARDIA) Study.

MATERIALS AND METHODS

Sample and laboratory measurements

The details of the CARDIA Study have been described elsewhere (20). In brief, young adults aged 18–30 years were randomly recruited from the total community in Birmingham, AL; from selected census tracts for the field centers in Chicago, IL, and Minneapolis, MN; and from the Kaiser-Permanente health plan membership for the field center in Oakland, CA. Participants were recruited

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to represent proportionate racial, gender, age, and education groups from the four communities. Study participants were given six sequential examinations from the time of the study initiation (1985–1986); the results shown here pertain to the data collected at the first exam. Venous blood was drawn after a 12 h fast. Total plasma TG was determined using standard enzymatic methods (21). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. All participants gave written informed consent, and the study was approved by the institutional review boards of the four participating field centers.

For the present study, genotypes were obtained for 3,831 individuals (1,075 African American females, 783 African American males, 1,041 white females, and 932 white males). SNPs were identified by resequencing the entire *APOA1/C3/A4/A5* gene cluster, including ~1,000 bp upstream of each gene, in 24 unrelated individuals from each of three populations: African Americans from Jackson, MS; Europeans from North Karelia, Finland; and European Americans from Rochester, MN (22). Sixteen SNPs in the *APOA5* gene region were polymorphic in the CARDIA samples and have been identified for the present study by their local *APOA5* sequence position (Fig. 1). Genotyping was performed using PCR amplification of genomic DNA, a short extension reaction across the polymorphic site, and mass spectrometry to detect allele-specific mass differences of the extension product. Allele detection and genotype calling were performed using the MassARRAY System from Sequenom® (San Diego, CA).

Statistical analyses

TGs were logarithmically transformed before analysis to reduce skewness. Transformation reduced skewness from 2.42 to 0.41 in African Americans and from 5.04 to 0.68 in whites. lnTG values were adjusted before analysis by fitting a race-, field center-, and gender-specific linear regression model containing age, age², age³, and BMI and adding the residual to the race- and gender-specific grand mean. Allele frequencies were obtained by direct counting. Hardy-Weinberg equilibrium was evaluated using

a Chi-square goodness-of-fit test. Pairwise composite linkage disequilibrium (LD) between SNPs was estimated as

$$\frac{\hat{\Delta}_{AB}^2}{P_A P_B P_A P_B} \quad (Eq. 1)$$

where $\Delta_{AB} = 2p_{AABB} + p_{AaBB} + 1/2p_{AaBb}$. (23). *P* values for the test of $\Delta_{AB} = 0$ were obtained using the Chi-square test statistic

$$\frac{\hat{\Delta}_{AB}^2}{\text{Var}(\hat{\Delta}_{AB})} \quad (Eq. 2)$$

Haplotype pairs were assigned to individuals based on SNP genotype data using the Bayesian algorithm of Stephens, Smith, and Donnelly (24) as implemented in PHASE 2.0.2. (25). The homogeneity of quantitative trait variances was evaluated using Levene's test (26). Differences in means were evaluated by the one-way ANOVA (27). Where variances were unequal, Welch's modified *F* statistic (28) was used to assess significance. Equivalent conclusions were obtained using the Welch technique and the standard ANOVA. The Boerwinkle and Sing (29) bias-corrected estimator of genetic variance was used to measure the proportion of plasma lnTG variance attributable to deviations of *APOA5* genotype means from the population mean. The bias-corrected estimates of variance attributable to *APOA5* haplotypes were obtained from one-way ANOVA in which individuals were identified as having zero, one, or two copies of a given three-SNP haplotype. Instead of applying a correction for multiple testing at $\alpha = 0.05$, statistical significance was defined as $P < 0.01$.

RESULTS

Characteristics of the CARDIA participants involved in the present study are summarized in Table 1. Within both

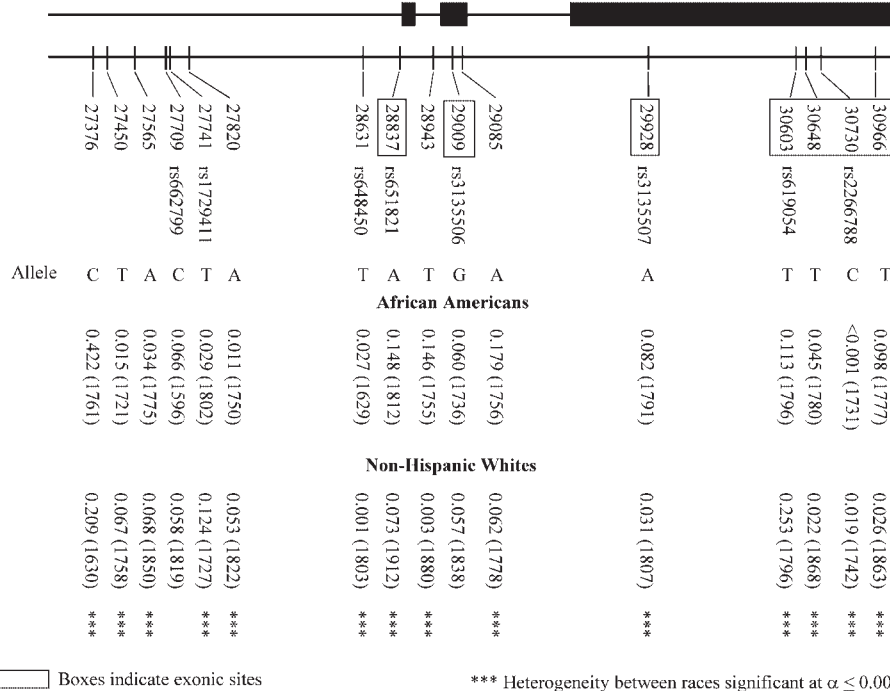


Fig. 1. Gene locations and relative frequencies (N) of 16 single-nucleotide polymorphisms (SNPs) in the apolipoprotein A-V gene (*APOA5*) in African American and white samples.

TABLE 1. Characteristics and tests of homogeneity among races for 1,858 African Americans and 1,973 whites of the Coronary Artery Risk Development in Young Adults (CARDIA) Study

Variable	African American		White		PValues from Tests of Homogeneity between Races	
	Females	Males	Females	Males	Female Means (Variance)	Male Means (Variance)
N	1,075	783	1,041	932		
Age	24.5 ± 3.9	24.3 ± 3.7	25.6 ± 3.4	25.5 ± 3.4	<0.0001 (<0.0001)	<0.0001 (<0.0001)
Body mass index	25.8 ± 6.4	24.6 ± 4.4	23.1 ± 4.4	24.3 ± 3.5	<0.0001 (0.0101)	0.0601 (0.0003)
TG	62.9 ± 29.6	70.7 ± 40.2	69.4 ± 38.8	87.68 ± 66.8	<0.0001 (<0.0001)	<0.0001 (0.0067)

TG, triglyceride. Values shown are means ± SD.

racess, females and males differed significantly for average BMI and plasma TG levels. African American and white females differed for age, BMI, and plasma TG, and males differed between races for average age and plasma TG. Variances differed between races for all traits in both genders. Covariates accounted for 3.88% of total lnTG variation in African American females, 10.89% in African American males, 11.65% in white females, and 17.53% in white males (data not shown).

Figure 1 shows the gene location, the relative allele frequency, and tests of homogeneity of the relative allele frequencies between races for each of the 16 *APOA5* markers. The relative frequency of the rare allele differed between races for 14 of the 16 SNPs. Because of the small relative frequency of the rare allele 30730 in African Americans, this marker was excluded from the analyses of this sample. Relative frequencies of 27709 (-1131T/C), 29009 (S19W), and 30730 (1259T/C) have been reported for other African American and white samples. The relative frequencies of 27709 and 30730 in our white sample (0.058 and 0.019, respectively) were generally lower than

the frequencies reported in the literature (-1131C = 0.06-0.085, 1259C = 0.083) (5-7, 14, 17, 19). The relative frequency of 27709 in this African American sample (0.066) was lower than that reported for the African American sample (0.118) by Pennacchio et al. (7). Allele frequencies of 29009 were similar to those reported for African American and white samples in the literature (5-7, 14, 16, 19).

A summary of the estimates of a composite measure of pairwise LD is presented in Fig. 2. Patterns of LD were similar in both races, apart from those involving SNP 30730, which was not tested in African Americans. Strong LD was observed between 27450 and 27565, between 27709 (-1131T/C) and 28837 (-3A/G), between 29009 (S19W) and 29085, and among 29928, 30648, and 30966.

The mean plasma TG levels for six *APOA5* SNP polymorphisms with $P \leq 0.01$ in tests of association with plasma lnTG in at least one race/gender are summarized in Table 2. Significant associations were observed between plasma lnTG level and single-site genotype variation for 27376, 27709, 28837, 29009, and 29085 in white males. P values for

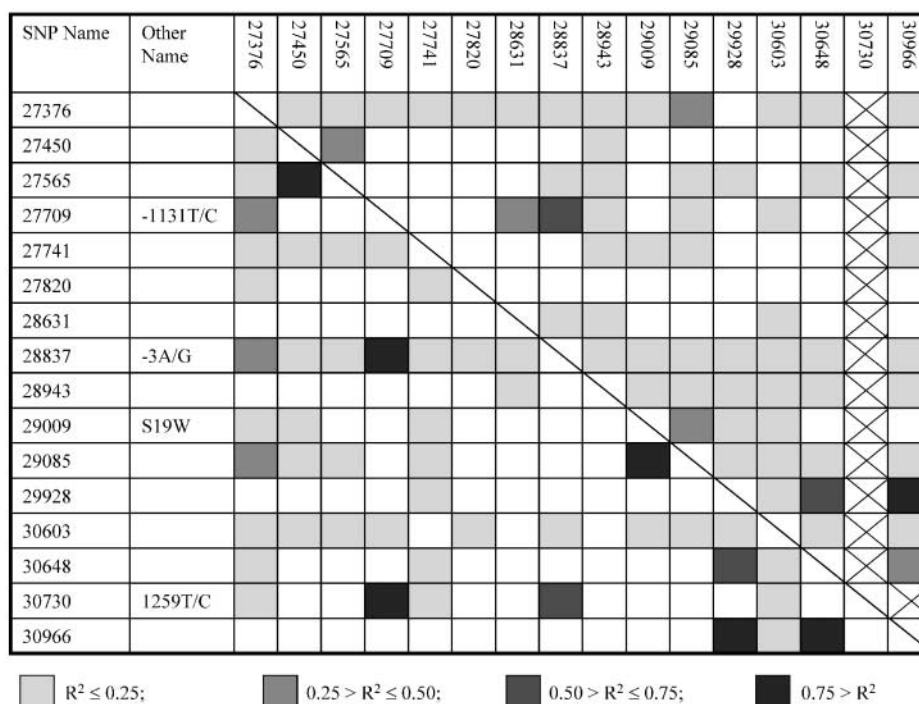


Fig. 2. Significant ($P < 0.01$) estimates of pairwise composite linkage disequilibrium (R^2) for 15 *APOA5* SNPs in African Americans (upper cells) and 16 *APOA5* SNPs in whites (lower cells).

TABLE 2. Plasma TG levels for genotypes of six *APOA5* polymorphisms and estimates of the percentage of total plasma lnTG variance among individuals associated with the measured genotypes

Local Position		African American		White	
		Females	Males	Females	Males
27376	T/T	62.26 ± 26.32	71.68 ± 40.67	66.70 ± 35.00	83.99 ± 58.32
	C/T and C/C	63.07 ± 31.39	69.46 ± 40.14	71.54 ± 36.10	93.86 ± 83.15
	σ^2_{27376}	0.00	0.07	0.78 ^a	1.61 ^b
27709 (-1131T/C)	T/T	62.57 ± 30.27	69.34 ± 39.16	68.69 ± 38.53	85.24 ± 64.16
	T/C and C/C	65.54 ± 31.64	75.59 ± 41.56	75.86 ± 43.74	108.28 ± 92.56
	σ^2_{27709}	0.00	0.00	0.39	1.57 ^b
28837 (-3A/G)	G/G	62.72 ± 30.18	69.47 ± 39.98	68.18 ± 38.29	85.08 ± 64.21
	A/G and A/A	63.47 ± 28.28	74.05 ± 41.00	75.72 ± 43.01	104.56 ± 82.99
	σ^2_{28837}	0.00	0.00	0.47 ^a	1.64 ^b
29009 (S19W)	C/C	61.75 ± 29.17	70.35 ± 41.00	68.39 ± 37.74	84.99 ± 59.93
	C/G and G/G	69.46 ± 61.75	68.96 ± 33.04	73.44 ± 42.20	114.21 ± 113.96
	σ^2_{29009}	0.66 ^c	0.00	0.29	2.46 ^b
29085	C/C	62.50 ± 29.35	72.17 ± 43.21	68.57 ± 36.25	84.92 ± 59.98
	A/C and A/A	63.74 ± 30.64	68.46 ± 35.38	72.93 ± 42.21	107.67 ± 107.49
	σ^2_{29085}	0.16	0.00	0.13	2.63 ^b
30966	C/C	62.21 ± 27.63	70.95 ± 40.37	69.48 ± 39.43	87.76 ± 68.28
	C/T and T/T	65.84 ± 37.19	71.91 ± 42.36	72.27 ± 35.74	77.52 ± 35.14
	σ^2_{30966}	0.69 ^c	0.00	0.00	0.00

APOA5, apolipoprotein A-V gene. Values shown are means ± SD.

^aSignificant at $\alpha \leq 0.05$.

^bSignificant at $\alpha \leq 0.001$.

^cSignificant at $\alpha \leq 0.01$.

27376 and 28837 were also marginally significant ($0.01 \leq P < 0.05$) in white females. In African Americans, significant association between plasma lnTG and genotypes defined by 29009 and by 30966 were observed in females. No significant associations were observed in the African American male sample. In all cases of significant association, carriers of the rare allele had higher plasma TG than did noncarriers.

The percentage of adjusted plasma lnTG variation attributable to single-site SNP variation is also presented in Table 2. Variation in SNPs 27376 and 28837 (-3A/G) accounted for 0.78% and 0.47% of variation in plasma lnTG level in white females and 1.61% and 1.64% in white males, respectively. Variation in 27709 (-1131T/C) and 29085 accounted for 1.57% and 2.63%, respectively, of the total variation in plasma lnTG among white males. Variation at 29009 (S19W) accounted for 0.66% of variation in African American females and 2.46% in white males. In African American females, variation in SNP 30966 accounted for 0.69% of total adjusted plasma lnTG variance.

To compare the combined effects of SNPs evaluated in this sample with those reported for other samples, haplotypes were constructed with SNPs 27709 (-1131T/C), 28837 (-3A/G), and 29009 (S19W), which were each significantly associated with plasma lnTG in one or more race/gender in our study and which were also reported in haplotype analyses elsewhere. Four of six assigned haplotypes (111, 221, 121, and 112) accounted for 99.65% of the chromosomes in African Americans, and three of six assigned haplotypes (111, 221, and 112) accounted for 99.57% of the chromosomes in whites. The frequency of individuals with haplotype pairs containing two, one, or zero copies of each of these four common haplotypes is reported in Table 3. Haplotype 111 represents the common allele

at all three SNPs. Haplotype 112 represents the rare 19W allele with the common alleles of the two other SNPs and accounted for 5.9% of chromosomes in African Americans and 5.6% of chromosomes in whites. The 221 haplotype accounted for 8.8% of chromosomes in African Americans and 6.9% in whites. The 121 haplotype accounted for 4.0% in African Americans but only 0.2% in whites and so was evaluated for association in African Americans only. Haplotype pairs within individuals were associated with plasma lnTG level in white males ($P < 0.0001$). Table 4 summarizes the association of *APOA5* variation, defined by the number of 111, 112, 121, and 221 haplotypes in an individual, with variation in plasma lnTG level. In this sample, the 111 haplotype explained

TABLE 3. Number of individuals with *APOA5* genotypes defined by zero, one, or two copies of three SNP haplotypes (based on markers -1131T/C, -3A/G, and S19W) from among 1,858 African American and 1,973 white individuals of the CARDIA Study

Haplotype Pair	African American		White	
	Females	Males	Females	Males
111/111	687	505	798	726
111/X ^a	331	245	220	180
X/X	57	33	23	26
221/221	11	2	9	12
221/X	212	154	131	106
X/X	852	627	901	814
121/121	6	1	0	0
121/X	76	60	5	3
X/X	993	722	1,036	929
112/112	10	2	4	7
112/X	99	83	103	84
X/X	966	698	934	841

SNP, single-nucleotide polymorphism.

^aX indicates a combined class including all remaining haplotypes.

TABLE 4. Plasma TG levels for genotypes defined by zero, one, or two copies of three-SNP *APOA5* haplotypes (based on markers -1131T/C, -3A/G, and S19W) and estimates of the percentage of total plasma lnTG variance among individuals associated with the assigned haplotype pairs

Haplotype Pair	African American		White	
	Females	Males	Females	Males
111/111	61.80 ± 29.79	69.91 ± 40.88	67.81 ± 37.72	81.31 ± 52.36
111/X ^a	64.15 ± 29.04	70.86 ± 38.24	73.39 ± 40.38	102.82 ± 76.80
X/X	68.95 ± 30.65	81.18 ± 42.88	84.22 ± 54.02	158.42 ± 190.39
σ^2_{111}	0.34	0.36	0.84 ^b	3.99 ^c
112/112	69.20 ± 31.07	52.00 ± 14.14	89.75 ± 41.06	291.00 ± 339.79
112/X	69.48 ± 31.83	69.37 ± 33.29	72.81 ± 42.31	99.48 ± 53.66
X/X	62.16 ± 29.31	70.89 ± 40.97	68.88 ± 38.39	84.74 ± 58.86
σ^2_{112}	0.48 ^d	0.00	0.34	2.53 ^c
121/121	57.83 ± 12.58	56.00	–	–
121/X	64.82 ± 29.43	78.55 ± 47.02	–	–
X/X	62.79 ± 29.71	70.05 ± 39.54	–	–
σ^2_{121}	0.00	0.00	–	–
221/221	61.27 ± 34.64	91.50 ± 68.59	88.22 ± 64.10	111.75 ± 54.92
221/X	64.11 ± 28.44	71.83 ± 37.62	75.22 ± 41.51	104.67 ± 87.11
X/X	62.62 ± 29.86	70.34 ± 40.75	68.31 ± 38.01	85.04 ± 63.49
σ^2_{221}	0.00	0.00	0.41 ^d	1.45 ^c

Values shown are means ± SD.

^a X indicates a combined class including all remaining haplotypes.

^b Significant at $\alpha \leq 0.01$.

^c Significant at $\alpha \leq 0.001$.

^d Significant at $\alpha \leq 0.05$.

3.99% of variation in plasma lnTG, the 221 haplotype explained 2.53% of plasma lnTG variation, and the 112 haplotype explained 1.45% of variation. Copy number of the 121 haplotype was not tested in whites, because of rarity, and did not account for significant plasma TG variation in African Americans. The estimates of plasma lnTG variation explained in these three analyses of haplotype effect are not independent as a result of sample overlap. Trends in mean plasma TG level for haplotype pairs (Table 4) were remarkably consistent across race and gender given the small number of individuals homozygous for the rare haplotypes. Where haplotype had a significant, or marginally significant, effect on plasma lnTG, mean plasma TG levels decreased with increasing copies of the common 111 haplotype and increased with copy number of the rare haplotypes, except for 221 homozygotes in African American females.

DISCUSSION

In this study, we provide evidence that variation in the *APOA5* gene (or variation in LD with it) influences variation in plasma TG levels in young (18–30 years), healthy African American females and white females and males of the CARDIA Study. Tests of association with six *APOA5* polymorphisms indicate an influence of the gene on plasma TG levels in these young, healthy individuals, dependent on both race and gender. However, the amount of plasma lnTG variation attributable to any single polymorphism is small (<3%) in any race- or gender-specific sample.

In other studies, plasma TG levels have been associated with one or more of the SNPs -1131T/C, -3A/G, 1259T/C,

-12238T/C, and IVS3+476G/A in white females (10, 16, 19) and males (5, 6, 7, 10, 14, 16, 19), in Chinese men (18) and with genders combined (12, 13), in Japanese (17), and in Hispanics of both genders (7). S19W has been associated with TG variation in African American females and males (7) and white females (7, 16, 19) and males (6, 7, 14, 16, 19). Statistical analyses cannot predict function, and the presence of as-yet unidentified functional polymorphisms in LD with these cannot be ruled out; however, the potential function(s) of these SNP markers have been discussed elsewhere (6, 7, 9).

Evaluation of measured genotype effects in large population-based studies is important for determining the impact of genetic variation on cardiovascular health in the population at large. Five other large population-based samples of African Americans and/or whites have been evaluated for associations between plasma TG levels and *APOA5* markers (Table 5). SNPs -1131T/C and S19W are the only markers in the current study that can be directly compared with results from several other population-based studies. For this reason, the remainder of this discussion will focus on these two markers.

No statistically significant association between plasma lnTG level and -1131T/C among African American females or males was observed in our study. Nor was an effect at this marker detected in the African American individuals of the Dallas Heart Disease Prevention Project (DHDPP) (7). Evaluation of additional population-based samples of African Americans would be useful to confirm this race-specific context dependency of the *APOA5* gene effect on plasma lnTG level.

Significant association of the -1131T/C variation with lnTG variation in white males of the CARDIA Study was consistent with the results from four of five other popula-

TABLE 5. Summary of the association between the *APOA5* SNPs –1131T/C and S19W and plasma TG levels in five population-based studies

Study and Data	African American		White	
	Females	Males	Females	Males
Talmud et al. (6)				(N = 2,808)
Age (years)	–	–	–	50–61
<i>P</i> for –1131T/C	–	–	–	0.002
<i>P</i> for S19W	–	–	–	0.003
Pennacchio et al. (7)	(N = 1,392)		(N = 848)	
Age (years)	18–65	18–65	18–65	18–65
<i>P</i> for –1131T/C	0.30	0.24	0.39	0.02
<i>P</i> for S19W	0.0023	0.024	0.012	0.0012
Martin et al. (14)				(N = 415)
Age (years)	–	–	–	18–28
<i>P</i> for –1131T/C	–	–	–	NS
<i>P</i> for S19W	–	–	–	0.008
Hubacek et al. (16)			(N = 1,368)	(N = 1,191)
Age (years)	–	–	Average 48.8	Average 49.2
<i>P</i> for –1131T/C	–	–	0.001	0.001
<i>P</i> for S19W	–	–	0.001	0.001
Lai et al. (19)			(N = 1,262)	(N = 1,129)
Age (years)	–	–	28–79	28–79
<i>P</i> for –1131T/C	–	–	0.005	0.001
<i>P</i> for S19W	–	–	0.006	0.002

tion-based studies (Table 5). Interestingly, association was not identified in the European Atherosclerosis Study II (EARSII), the sample most similar in age (415 healthy white males aged 18–28 years) to our sample (14). A significant association ($P = 0.026$), however, was observed in a sample of 407 males from the EARSII population whose fathers had suffered a myocardial infarction before age 55. In the CARDIA sample, 148 white males reported a known incidence (but not age) of heart attack in one or both parents. No statistically significant association between lnTG level and the –1131T/C marker in white males with a self-reported family history of heart attack was detected, nor did –1131T/C genotype predict family history in a logistic regression analysis (data not shown).

No statistically significant association between plasma lnTG and either –1131T/C or S19W was detected in white females in our study, in contrast with two of the three other studies (16, 19). Both of these studies were of individuals older than those in our study. The study by Pennacchio et al. (7) of 359 females who ranged in age from 18 to 65 years found association with S19W but not –1131T/C. Sample size differences and sampling error, or differences in population structure, may be responsible for the variable results among these studies. There may also be additional genetic or environmental factors that play a role in modifying the gene effect identified by these SNPs.

An association of lnTG level with S19W variation was observed in African American females and white males. The lack of significant association in African American males is in contrast to findings by Pennacchio et al. (7) of significant associations for S19W with plasma TG levels in both genders of African Americans from the DHDPP. The age at recruitment was broader (18–65 years) in the DHDPP than in CARDIA, suggesting a role for gender-specific age and age-related factors. The association of lnTG


level with variation in S19W in white males is consistent across population-based studies (Table 5).

In the Framingham Heart Study, variation in –1131T/C explained 0.51% of lnTG variance in females and 0.78% in males, and S19W explained 0.45% of lnTG variance in females and 0.78% in males (19). This was similar to the amount of lnTG variation accounted for by these SNPs in females of our study (<0.40%) but less than that accounted for in males (<2.50%). Given the relatively low proportion of variance explained by measured *APOA5* genotype effects, the level of consistency among population-based studies is surprising. Replicated observations of the influence of *APOA5* gene variation across samples suggests that some common variations in or near this gene play an important role in plasma TG regulation. Yet, the variable results among samples of African American and white females and males indicate that there may be additional unidentified genetic and/or environmental factors that are important modifiers of gene effect. Additionally, larger proportions of TG variation (7–14%) explained by S19W in individuals diagnosed with familial combined hyperlipidemia compared with their healthy spouses (4%) suggest that variation at or near the *APOA5* gene may play a greater role in TG metabolism within certain strata of the population (9). It should be kept in mind, however, that the validity of comparisons among samples depends on uniformity of population structure, especially with regard to the percentage of variability explained, and caution should be exercised when drawing conclusions.

The proportion of plasma TG variance explained by measured variation in the *APOA5* gene appears to be in agreement with that reported for other apolipoproteins. Kaprio et al. (30) examined associations between plasma TG and measured genotypic variation in *APOE*, *APOA4*, and *APOH* in white males and females of the Rochester Family Heart Study. *APOE* variation explained 2.5% of plasma TG variation in males, a similar proportion of variation to that explained by *APOA5* in the white males of this study (1.45–3.99%). They also found gender specificity in the proportion of TG explained by *APOE*, but in that case the proportion explained in females was much larger (22.76%). They reported a proportion of plasma TG variance explained by the *APOA4* and *APOH* genetic variations of less than 1% in both genders. This was not significant in their sample of 226 females and 227 males.

Pennacchio et al. (7), evaluating LD and association in African Americans, Hispanics, and whites, identified a pattern consistent with two functional sites in the *APOA5* gene. The rare alleles of SNPs –1131T/C (27709), –3A/G (28837), and 1259T/C (30730), as well as the *APOA5* markers –12238T/C and IVS3+476G/A not measured in our study, were shown to represent a single haplotype, whereas S19W (29009) had an independent effect on TG levels (5–7). Similar haplotype effects have been reported in analyses of other samples (13, 17, 19). The haplotype effects observed in our study were consistent with this pattern, but they were only significant in white males. Interestingly, greater lnTG variation was explained by copy number of the common haplotype (3.99% in white males) than

by any single SNP. Mean plasma TG level decreased with increasing copy number of that haplotype in all samples.

The purpose of our study was to evaluate the influence of *APOA5* polymorphisms on TG variation in a young, healthy population-based sample. Single-site and three-SNP haplotype effects on plasma lnTG were identified without attempting to identify a functional site(s). Comparisons with other population-based studies suggest that the influence of the *APOA5* gene on lipid metabolism may be dependent on both race and gender and that such effects are present in young adults before the onset of clinical manifestations of cardiovascular disease. Finally, the consistency of findings among samples for a gene that accounts for less than 5% of risk factor variation demonstrates the potential for genetic association studies to identify genes that are involved in the pathogenesis of a complex multifactorial disease. 

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