APOC3/A5 haplotypes, lipid levels, and risk of myocardial infarction in the Central Valley of Costa Rica

Edward A. Ruiz-Narváez,*,† Yadong Yang,* Yukiko Nakanishi,* Jill Kirchdorfer,* and Hannia Campos[1,*,§]

Department of Nutrition,* Harvard School of Public Health, Boston, MA; Department of Epidemiology,† Harvard School of Public Health, Boston, MA; and Centro Centroamericano de Población,§ Universidad de Costa Rica, San Pedro, Costa Rica

Abstract **Genetic variation in the *APOC3* and *APOA5* genes has been associated with plasma triglyceride concentrations and may affect the risk of myocardial infarction (MI). To assess whether *APOC3/A5* haplotypes are associated with risk of MI, we examined three single-nucleotide polymorphisms (SNPs) in *APOC3* (3238C>G, −455T>C, and −482C>T) and six SNPs in the *APOA5* gene (−1131T>C, c.−3A>G, c.56C>G, IVS3+476G>A, c.553G>T, and c.1259T>C) in incident cases (n = 1,703) of a first nonfatal MI matched for gender, age, and area of residence with population-based controls (n = 1,703). Conditional logistic regression models, adjusted for potential environmental confounders, were used for analysis. The common *APOC3*222* haplotype was more frequent in cases than in controls (17.4% and 13.7%, respectively, $P < 0.001$) and was associated with increased risk of MI [odds ratio (OR) = 1.27; 95% confidence interval (95% CI), 1.09, 1.48] compared with *APOC3*111* wild-type haplotype. This association was independent of the *APOA5* SNPs. Although the *APOC3* 3238G, *APOA5* −1131C, *APOA5* c.−3G, and *APOA5* c.1259C alleles were associated with higher triglyceride plasma concentrations, these effects could not explain the associations with MI in this population. In summary, this study supports the hypothesis that haplotypes in the *APOC3* gene but not in the *APOA5* gene increase susceptibility to MI.**—Ruiz-Narváez, E. A., Y. Yang, Y. Nakanishi, J. Kirchdorfer, and H. Campos. **APOC3/A5 haplotypes, lipid levels, and risk of myocardial infarction in the Central Valley of Costa Rica.** ***J. Lipid Res.*** **2005.** 46: **2605–2613.**

Supplementary key words apolipoprotein • genetics • epidemiology • cardiovascular disease • risk factors

Apolipoprotein C-III (apoC-III) and A-V (apoA-V) regulate triglyceride metabolism in opposite ways (1–3). In mice, the overexpression of the human *APOC3* transgene (1) leads to severe hypertriglyceridemia, whereas knockout mice lacking the endogenous *Apoc3* gene have hypotriglyceridemia (2). In contrast, overexpression of the human *APOA5* transgene and the lack of the endogenous *Apoa5* gene show opposite triglyceride effects (3).

Several studies indicate that naturally occurring sequence variation in the *APOC3* and *APOA5* genes affects plasma triglyceride concentrations in humans (4–7). People with the G allele of the 3238C>G polymorphism (*SstI* site) in the 3′ untranslated region (3′ UTR) of *APOC3* tend to have higher plasma triglyceride concentrations (4, 5), as do individuals with two minor alleles, −482C>T and −455T>C (8–10), found in the insulin response element (IRE) of the *APOC3* promoter. Several minor alleles of *APOA5*, the −1131T>C (upstream of the proximal promoter) (3, 6, 7), c.−3A>G (in a putative Kozak sequence) (11), c.56C>G (amino acid change p.Ser19Trp in the signal peptide) (7, 11), IVS3+476G>A (3, 11), c.553G>T (amino acid change p.Gly185Cys in exon 3) (12), and c.1259T>C (located in the 3′ UTR) (3, 11), are also associated with increased plasma triglyceride concentrations.

Although it may be hypothesized that the deleterious effect of these polymorphisms on plasma lipids will increase the risk of coronary heart disease (CHD), evidence of such an association is scarce. The minor alleles of the *APOC3* gene that are associated with higher triglyceride concentrations have been related to increased CHD risk in some studies (9, 10, 13). It has also been suggested that the presence of the −455C allele interacts with the metabolic syndrome phenotype to affect CHD risk (10). But other studies have not found associations between *APOC3* polymorphisms and CHD risk (9, 14–16). Of five *APOA5* variants studied in the Framingham Heart Study, the

Abbreviations: apoC-III, apolipoprotein C-III; ASA, allele-specific assay; CHD, coronary heart disease; IRE, insulin response element; LD, linkage disequilibrium; MI, myocardial infarction; RORα, receptor-related orphan receptor-α; SNP, single-nucleotide polymorphism; 3′ UTR, 3′ untranslated region.

[1] To whom correspondence should be addressed.
e-mail: hcampos@hsph.harvard.edu

Manuscript received 1 February 2005 and in revised form 5 July 2005 and in re-revised form 22 September 2005.

Published, JLR Papers in Press, September 28, 2005.
DOI 10.1194/jlr.M500040-JLR200

−1131T>C polymorphism was associated with risk of CHD in women, although not in men (17). Increased risk of CHD was also found for carriers of the −1131C allele in Chinese (18) and Hungarian (19) populations, but other studies have failed to find such an association, either with CHD (7, 15, 20) or with CHD progression (21).

The emphasis on individual polymorphisms rather than haplotypes could explain some of these discrepancies. High linkage disequilibrium (LD) between the *APOC3* and *APOA5* genes exists (6), and the relative contribution of these polymorphisms to triglyceride changes and risk of CHD needs to be determined. In the present study, we used three single-nucleotide polymorphisms (SNPs) in the *APOC3* gene (3238C>G, −455T>C, and −482C>T) and six SNPs in the *APOA5* gene (−1131T>C, c.−3A>G, c.56C>G (p.Ser19Trp), IVS3+476G>A, c.553G>T (p.Gly185Cys), and c.1259T>C) to test whether haplotypes defined by these variants are associated with the risk of nonfatal myocardial infarction (MI) in the Central Valley of Costa Rica.

METHODS

Study population

The catchment area for this study consisted of 34 counties in the Central Valley of Costa Rica. Participants were adult patients that were survivors of a first acute MI as diagnosed by a cardiologist at any of the recruiting hospitals in the Central Valley of Costa Rica between 1994 and 2004. A study cardiologist confirmed all cases according to the World Health Organization criteria for MI, which requires typical symptoms plus either elevation in cardiac enzyme levels or diagnostic changes in the electrocardiogram. Enrollment was carried out in the step-down unit of the recruiting hospitals. Cases were not eligible if they *a*) died during hospitalization, *b*) were over 75 years of age on the day of their first MI, or *c*) were physically or mentally unable to answer the questionnaire and *d*) had a previous hospital admission related to CHD. For each case, one population-based control subject, matched for age (±5 years), sex, and area of residence (county) was recruited. The controls were randomly selected using data from the National Census and Statistics Bureau of Costa Rica. Because of the nationwide health system in Costa Rica, in which everyone has access to medical care regardless of income, the control subjects represent the base population that gave rise to the cases. Control subjects were ineligible if they had ever had an MI or if they were physically or mentally unable to answer the questionnaire.

Trained personnel visited all study participants in their homes for data collection. Anthropometrical measurements and biological specimens were collected from all subjects the morning after an overnight fast. The median time for collection of the blood specimens for lipid measurements was 24 days after the MI for cases and 28 days after enrollment for the matched controls. The definitions of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (22) were used to validate self-reported diabetes. Self-reported hypertension was validated using the recommended definitions of the Third Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (23). For this study, there were 1,703 case-control pairs, with genotype information and complete data on all the descriptive variables and potential confounders. Participation was 97% for cases and 89% for controls. All subjects gave informed consent on documents approved by the Human Subjects Committee of both the Harvard School of Public Health and the University of Costa Rica.

Selection of polymorphisms

Three SNPs in the *APOC3* gene, 3238C>G (rs5128), −455T>C (rs2854116), and −482C>T (rs2854117), were chosen on the basis of their function and published evidence about their potential role in MI (6, 24). In the *APOA5* gene, we selected the following six SNPs that have been associated with plasma triglyceride concentrations in several populations (3, 6, 7, 11, 12): −1131T>C (rs662799), c.−3A>G (rs651821), c.56C>G (p.Ser19Trp) (rs3135506), IVS3+476G>A (rs2072560), c.553G>T (p.Gly185Cys) (rs2075291), and c.1259T>C (rs2266788).

Genotyping

Genotyping was carried out using a variation of the allele-specific assay (ASA). The SNP genotyping procedure consisted of three steps: In step one, DNA fragments were obtained using PCR primers designed according to each SNP's vicinity sequence. The reverse primers contained an artificially introduced sequence (derived from the bacteriophage M13) at the 5′ end, which was identical across all SNPs. In step two, ASA, SNPs were genotyped in three different multiplex reactions, with allele-specific forward primers and a reverse primer whose sequence is universal for all the SNPs. Universal primers were labeled at the 5′ end with one of three fluorescent dyes (Fluorescein, Hexachlorofluorescein, or Tetrachlorofluorescein). In step three, ASA products were separated by capillary electrophoresis with the ABI Prism 310 genetic analyzer (Applied Biosystems, Perkin-Elmer) and analyzed using the Genotyper software. To confirm the accuracy of genotyping, 10 DNA samples for each SNP were subjected to direct DNA sequencing of the PCR products. There was 100% agreement between the two procedures. Eight control samples were genotyped for each plate throughout the study to assess the genotyping reproducibility. Reproducibility was 99.9%, and less than 1% of control and unknown samples had missing values. All samples, cases and controls, were double-blinded.

Dietary assessment

Dietary information was collected using a semiquantitative food-frequency questionnaire (FFQ) that was developed specifically for use in the Costa Rican population (25, 26). Trained interviewers administered the FFQ. Energy and nutrient intakes were estimated from the United States Department of Agriculture food composition tables. Dietary data were used to adjust for the potential effects of diet on lipid concentrations when examining the effects of polymorphisms on these lipids.

Lipid analysis

Plasma cholesterol, triglyceride, and HDL cholesterol concentrations were measured using an Abbott Diagnostics ABA-200 bichromatic analyzer and Abbott A-Gent enzymatic reagents. The Friedewald equation (27) was used to estimate LDL cholesterol concentrations. Total cholesterol, triglyceride, and HDL cholesterol assays were standardized through the Centers for Disease Control Lipid Standardization Program.

Data analysis

All data were analyzed with the Statistical Analysis Systems software version 9 (SAS Institute, Inc.; Cary, NC). Differences in health characteristics and potential confounders between cases and controls were assessed by Wilcoxon rank-sum tests for continuous variables and with χ^2 tests for categorical variables. Allele frequencies were estimated by the gene-counting method, and an exact test was performed to identify departures from Hardy-Weinberg proportions. The PROC HAPLOTYPE in the SAS/Genetics Soft-

ware was used to estimate maximum-likelihood haplotype relative frequencies. LD between pairs of SNPs was assessed using the standardized LD coefficient (D′) (28) that ranges between −1 and +1. A value of $D' > 0$ indicates a positive correlation between the major alleles of two SNPs, or an excess of haplotypes in *cis*-configuration. A value of $D' < 0$ denotes a negative correlation between the major alleles of two SNPs, or an excess of haplotypes in *trans*-configuration. A global test was used to assess differences in the overall distribution of haplotypes between cases and controls.

Two different approaches were used for evaluating the effect of *APOC3* and *APOA5* SNPs on the risk of MI. In the first approach, all the genotypes of the SNPs in the *APOC3* and *APOA5* genes were categorized as either noncarriers (homozygous of the wild-type allele) or carriers (heterozygous and homozygous of the minor allele), and all nine polymorphisms were included in a single conditional logistic model. In the second approach, haplotypes of the nine SNPs in the *APOC3* and *APOA5* genes were included in a single conditional logistic model. Odds ratios (ORs) of the *APOC3/A5* haplotypes were estimated using an expectation substitution approach (29, 30) that estimated the probabilities of all possible haplotype configurations of each individual in the sample, conditional on their genotype and case-control status. Haplotypes with an estimated relative frequency of <0.01 were pooled in one single group, and the most common haplotype (i.e., the triple wild-type haplotype) was used as the reference haplotype. All the models were conditional matched by sex, age (± 5 years), and county of residence and adjusted in the analysis for waist-to-hip ratio (quintiles based on the distribution in controls: ≤0.93, >0.93 to ≤0.96, >0.96 to ≤0.99, >0.99 to ≤1.02, >1.02), physical activity measured in metabolic equivalent tasks (quintiles based on the distribution in controls: ≤1.08, >1.08 to ≤1.34, >1.34 to ≤1.60, >1.60 to ≤2.01, >2.01), income (quintiles based on the distribution in controls), smoking (never, past smoker, current <10 cigarettes/day, current ≥10 to <20 cigarettes/day, current ≥20 cigarettes/day), alcohol consumption (never, past, and three tertiles of current drinkers), history of diabetes (no vs. yes), and history of hypertension (no vs. yes).

PROC GENMOD in the SAS software was used to estimate effects of SNPs and *APOC3/A5* haplotypes on lipid concentrations. Because MI and lipid-lowering medications affect plasma lipids and these measurements were determined after the MI among cases, the analyses of genetic effects on lipid concentrations were restricted to controls. All the potential confounders listed above, in addition to total energy intake (calories as a continuous variable), percent energy from carbohydrates, and percent energy from total fat, were used as covariates in the models. Plasma triglyceride, HDL, and LDL levels were log-transformed to obtain approximate normal distributions for performing statistical tests. Because both *APOC3* and *APOA5* genes affect triglyceride concentrations in opposite ways (31), we hypothesize that expression of both genes, as well as levels of both apolipoproteins, might be coregulated in order to maintain homeostasis of triglyceride metabolism. A corollary of this hypothesis is that the effect of *APOC3/A5* haplotypes on triglyceride concentrations would be stronger under conditions that might disrupt the coregulation of both genes, such as overexpression of the *APOC3* gene in a diabetic state (32, 33). To test this hypothesis, we evaluated the interaction between *APOC3/A5* haplotypes and presence of diabetes on triglyceride concentrations.

RESULTS

Table 1 shows selected characteristics of the study participants. Traditional risk factors were more frequent in MI cases as compared with controls. Relative frequencies

TABLE 1. General characteristics in myocardial infarction (MI) cases and population-based controls from the Central Valley of Costa Rica

Characteristics[a]	Controls (n = 1,703)	Cases (n = 1,703)
Age (years)	58 (11)	58 (11)
Sex (% female)	26	26
Waist-to-hip ratio[b]	0.95 (0.07)	0.97 (0.07)
Body mass index (kg/m^2)[b]	26.4 (4.0)	25.8 (4.0)
Physical activity (METS)[b]	1.55 (0.72)	1.50 (0.72)
Smoking status (%)[b]		
Never smoked	39	30
Past smoker	40	30
Current smoker (<10 cigs/day)	9	8
Current smoker (≥10 to <20 cigs/day)	5	8
Current smoker (≥20 cigs/day)	7	24
History of diabetes (%)[b]	15	24
History of hypertension (%)[b]	29	38
Alcohol status (%)[b]		
Never drank	21	20
Past drinker	26	30
Current drinker	53	50
Cholesterol-lowering medication use (%)[c]	5.7	5.6
Alcohol among users only (g/day)[b]	11 (18)	15 (25)
Total energy intake (kcal/day)[b]	2,448 (769)	2,718 (950)
Plasma cholesterol (mg/dl)[b,d]	223 (47)	202 (45)
HDL cholesterol (mg/dl)[b,d]	36 (8)	32 (8)
LDL cholesterol (mg/dl)[b,d]	145 (46)	125 (46)
Plasma triglycerides (mg/dl)[b,d]	210 (116)	223 (111)

METS, metabolic equivalent tasks.
[a] Mean (SD) for continuous variables.
[b] Cases significantly different from controls, $P < 0.05$.
[c] For cases, use of cholesterol-lowering medications before MI.
[d] Lipid levels measured after MI among cases.

TABLE 2. Allelic relative frequencies of nine polymorphisms in the *APOC3* and *APOA5* genes in 1,703 case-control pairs in the Central Valley of Costa Rica

Minor Allele	Controls (n = 1,703)[b]	Cases (n = 1,703)[b]
APOC3 3238G[a]	0.201 (0.006)	0.229 (0.007)
APOC3 −455C	0.422 (0.009)	0.432 (0.009)
APOC3 −482T	0.396 (0.008)	0.408 (0.008)
APOA5 −1131C	0.032 (0.003)	0.031 (0.003)
APOA5 c.−3G	0.139 (0.006)	0.141 (0.006)
APOA5 c.56G	0.102 (0.005)	0.096 (0.005)
APOA5 IVS3+476A[a]	0.090 (0.005)	0.105 (0.005)
APOA5 c.553T	0.022 (0.002)	0.021 (0.002)
APOA5 c.1259C	0.095 (0.005)	0.102 (0.005)

[a] Allelic relative frequencies significantly different between cases and controls, $P < 0.05$.
[b] Allelic relative frequency (SE).

of the variant alleles of all the polymorphisms studied are shown in **Table 2**. The substitutions in the *APOC3* promoter (−455T>C and −482C>T) were the most variable. Hardy-Weinberg proportions were observed for all the SNPs. Minor alleles of both *APOC3* 3238C>G ($P = 0.01$) and *APOA5* IVS3+476G>A ($P = 0.05$) SNPs were more frequent in cases as compared with controls. **Table 3** shows D′ values estimated in controls. D′ value greater than 0.50 was observed between the *APOC3* 3238C>G and *APOA5* IVS3+476G>A, the two polymorphisms associated with MI shown in Table 2. In general, LD was also observed more strongly between SNPs within each gene than between the two genes.

Table 4 shows maximum-likelihood estimates of the haplotypic relative frequencies and the estimated ORs for haplotypes. When only *APOC3* haplotypes were taken into account, the overall distribution of estimated relative frequencies was different between MI cases and controls ($P = 0.001$). In particular, the *APOC3*222* haplotype was more frequent in cases compared to controls ($P < 0.001$). In contrast, the *APOC3*121* ($P = 0.04$), *APOC3*122* ($P = 0.06$), and *APOC3*212* ($P = 0.03$) haplotypes were in the opposite direction. The global distribution of estimated relative frequencies of *APOA5* haplotypes was not significantly different between MI cases and controls ($P = 0.97$), and no single *APOA5* haplotype showed statistical differences in the estimated relative frequencies between both groups. When *APOC3/A5* haplotypes were analyzed together, individual haplotype case-control differences in estimated relative frequencies were observed, although the global test was not statistically significant ($P = 1.0$).

We explored whether the effects of *APOC3/A5* haplotypes on the risk of MI could be mediated by lipid concentrations (**Table 5**). It is clear that the association between increased triglyceride concentrations and the *APOC3* 3238G allele was independent of the other polymorphisms. The *APOC3/A5*211111111* haplotype was associated with increased (30 mg/dl) triglyceride concentrations relative to the *APOC3/A5*111111111* haplotype ($P < 0.05$), but the effect of the *APOC3* 3238G allele on triglyceride concentrations was absent in the presence of both minor alleles of the *APOC3*-promoter sites and the *APOA5* wild-type background (*APOC3/A5*222111111* haplotype). The *APOA5*121212* haplotype was correlated with a modest increase in triglyceride concentration (15 mg/dl), and its effect was strongest in the presence of the *APOC3*222* haplotype (*APOC3/A5*222121212* haplotype, $P < 0.01$). Two other *APOA5* haplotypes (*APOA5*121112*, $P < 0.001$ and *APOA5*211111*, $P < 0.001$) were also associated with higher triglyceride concentrations; however, their frequencies were less than 1% when considering *APOC3* haplotypes.

Lower HDL concentrations were associated with *APOC3/A5*112111111* ($P < 0.001$), *APOC3/A5*122111111* ($P < 0.05$), and *APOC3/A5*211111111* ($P < 0.01$) haplotypes. Higher HDL concentrations were associated with the *APOC3/A5*222121212* haplotype ($P < 0.01$). Although both *APOC3*121* ($P < 0.05$) and *APOC3*212* ($P < 0.05$) haplotypes were associated with higher LDL concentrations, their effect was weakened when taking into account the *APOA5* haplotypes. No significant effect on total cholesterol concentrations was observed.

For individual SNPs, minor alleles of the 3238C>G SNP ($P < 0.001$) in the *APOC3* gene and of the −1131T>C ($P < 0.01$), c.−3A>G ($P < 0.001$), and c.1259T>C ($P < 0.001$)

TABLE 3. Standardized linkage disequilibrium coefficient (D′)[a] among nine *APOC3/A5* SNPs

	APOC3		*APOA5*					
SNPs	−455T>C	−482C>T	−1131T>C	c.−3A>G	c.56C>G	IVS3+476G>A	c.553G>T	c.1259T>C
3238C>G	0.458[d]	0.641[d]	0.283[d]	0.355[d]	−0.134	0.524[d]	−0.315	0.593[d]
−455T>C		0.758[d]	0.181	0.190[d]	−0.100	0.281[d]	0.174	0.280[d]
−482C>T			0.167	0.226[d]	−0.047	0.296[d]	0.306[b]	0.359[d]
−1131T>C				0.437[d]	−0.736[b]	0.449[d]	0.073[c]	0.419[d]
c.−3A>G					−1.000[d]	0.791[d]	−0.518	0.877[d]
c.56C>G						−0.759[d]	−0.533	−0.871[d]
IVS3+476G>A							0.037	0.818[d]
c.553G>T								−0.352

SNP, single-nucleotide polymorphism.
[a] A value of D′ > 0 indicates a positive correlation between the major alleles of two SNPs, or an excess of haplotypes in *cis*-configuration. A value of D′ < 0 denotes a negative correlation between the major alleles of two SNPs, or an excess of haplotypes in *trans*-configuration.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

TABLE 4. Estimated haplotypic relative frequencies (SE) and haplotypic odds ratios of nine polymorphisms in the *APOC3* and *APOA5* genes in MI cases and population-based controls in the Central Valley of Costa Rica

APOC3 3238	*APOC3* −455	*APOC3* −482	*APOA5* −1131	*APOA5* c.−3	*APOA5* c.56	*APOA5* IVS3+476	*APOA5* c.553	*APOA5* c.1259	Controls (n = 1,703)	Cases (n = 1,703)	OR (95% CI)[a]
APOC3 haplotypes[b] (*P* of global test = 0.001)											
1	1	1	—	—	—	—	—	—	0.487 (0.008)	0.489 (0.008)	1.00 (ref.)
1	1	2	—	—	—	—	—	—	0.034 (0.003)	0.034 (0.003)	1.07 (0.82, 1.42)
1	2	1	—	—	—	—	—	—	0.074 (0.004)	0.061 (0.004)	0.80 (0.64, 0.99)
1	2	2	—	—	—	—	—	—	0.205 (0.007)	0.187 (0.007)	0.88 (0.77, 1.01)
2	1	1	—	—	—	—	—	—	0.036 (0.003)	0.031 (0.003)	0.76 (0.55, 1.06)
2	1	2	—	—	—	—	—	—	0.020 (0.002)	0.014 (0.002)	0.58 (0.38, 0.91)
2	2	2	—	—	—	—	—	—	0.137 (0.006)	0.174 (0.006)	1.27 (1.09, 1.48)
APOA5 haplotypes (*P* of global test = 0.97)											
—	—	—	1	1	1	1	1	1	0.713 (0.008)	0.714 (0.008)	1.00 (ref.)
—	—	—	1	1	1	1	2	1	0.014 (0.002)	0.013 (0.002)	0.87 (0.55, 1.38)
—	—	—	1	1	2	1	1	1	0.095 (0.005)	0.093 (0.005)	1.02 (0.86, 1.21)
—	—	—	1	2	1	1	1	1	0.050 (0.004)	0.046 (0.004)	0.93 (0.73, 1.17)
—	—	—	1	2	1	1	1	2	0.011 (0.002)	0.010 (0.002)	0.62 (0.36, 1.06)
—	—	—	1	2	1	2	1	2	0.058 (0.004)	0.066 (0.004)	1.12 (0.90, 1.39)
—	—	—	2	1	1	1	1	1	0.011 (0.002)	0.010 (0.002)	0.90 (0.55, 1.48)
—	—	—	2	2	1	2	1	2	0.014 (0.002)	0.016 (0.002)	0.97 (0.64, 1.48)
APOC3/A5 haplotypes (*P* of global test = 1.0)											
1	1	1	1	1	1	1	1	1	0.372 (0.008)	0.384 (0.008)	1.00 (ref.)
1	1	1	1	1	2	1	1	1	0.050 (0.004)	0.052 (0.004)	1.08 (0.83, 1.42)
1	1	1	1	2	1	1	1	1	0.026 (0.003)	0.018 (0.002)	0.69 (0.49, 1.00)
1	1	1	1	2	1	2	1	2	0.014 (0.002)	0.011 (0.002)	0.73 (0.44, 1.22)
1	1	2	1	1	1	1	1	1	0.024 (0.003)	0.026 (0.003)	1.06 (0.77, 1.45)
1	2	1	1	1	1	1	1	1	0.059 (0.004)	0.044 (0.004)	0.70 (0.54, 0.90)
1	2	2	1	1	1	1	1	1	0.153 (0.006)	0.139 (0.006)	0.81 (0.68, 0.97)
1	2	2	1	1	2	1	1	1	0.020 (0.002)	0.018 (0.002)	0.97 (0.61, 1.55)
2	1	1	1	1	1	1	1	1	0.023 (0.003)	0.019 (0.002)	0.71 (0.46, 1.11)
2	2	2	1	1	1	1	1	1	0.071 (0.004)	0.093 (0.005)	1.40 (1.12, 1.74)
2	2	2	1	2	1	2	1	2	0.037 (0.003)	0.049 (0.004)	1.28 (0.97, 1.68)

95% CI, 95% confidence interval; OR, odds ratio.

[a] Conditional models matched by sex, age, and county of residence and adjusted in the analysis for waist-to-hip ratio, physical activity, smoking, income, alcohol consumption, history of diabetes, and history of hypertension.

[b] 1, major allele; 2, minor allele.

SNPs in the *APOA5* gene were associated with increased triglyceride concentrations. The strongest effect on triglyceride was observed in the *APOA5* c.1259T>C polymorphism; the minor allele c.1259C was associated with a triglyceride concentration increase of 28 mg/dl. Lower HDL concentrations were associated with the minor alleles of the *APOC3* −482C>T ($P < 0.05$) and *APOA5* c.553G>T ($P < 0.05$) polymorphisms. On the other hand, the presence of minor alleles of the c.−3A>G ($P < 0.01$), IVS3+476G>A ($P < 0.001$), and c.1259T>C ($P < 0.05$) SNPs in the *APOA5* gene resulted in increased HDL concentrations. No individual variant by itself was associated with either LDL or total cholesterol concentrations.

We examined the interaction between *APOC3/A5* haplotypes and presence of diabetes on the determination of triglyceride concentrations. As shown in **Table 6**, both *APOC3/A5*111121212* ($P = 0.02$) and *APOC3/A5*222121212* ($P = 0.05$) haplotypes showed interactions with diabetes on differences in triglyceride concentrations. In diabetics, these haplotypes were associated with differences of 113 mg/dl and 70 mg/dl in triglyceride concentrations, respectively, compared with nondiabetics with the wild-type haplotype. In contrast, these haplotypes were not associated with triglyceride concentrations among nondiabetics (3.4 and 16 mg/dl, respectively). The *APOC3* triple mutant with wild-type *APOA5* background (*APOC3/A5*222111111*) was not associated with triglyceride in diabetics and nondiabetics (−0.1 and 5.1 mg/dl, respectively).

DISCUSSION

We conducted a population-based, case-control study to assess the relationship between genetic variation in the *APOC3* and *APOA5* genes, lipid levels, and the risk of MI in the Central Valley of Costa Rica. Our results show that genetic variation in the *APOC3* gene, specifically the *APOC3*222* haplotype, was associated with increased risk of MI. This association between the *APOC3*222* haplotype and risk of MI was independent of the *APOA5* gene, which was not related to MI.

The association between the *APOC3*222* haplotype and risk of MI was not mediated by triglyceride concentrations, despite the association between the 3238G allele (located in the 3′ UTR of the *APOC3* gene) and plasma triglyceride observed in this and previous studies (5, 34, 35). Minor alleles of both the −455 and −482 sites in an IRE that abolish in vitro insulin regulation of *APOC3* gene expression (8) were not associated with triglyceride concentrations. Thus, the effect of the 3238G allele on triglyceride was not due to LD with other functional mutations in the *APOC3* promoter, and it disappeared in the presence of these variants in an *APOA5* wild-type background.

The normal triglyceride concentrations observed in the *APOC3/A5*222111111* haplotype are consistent with the normal triglyceride concentrations observed in transgenic mice expressing both *APOC3* and *APOA5* human transgenes. Because apoC-III and apoA-V regulate triglyceride

TABLE 5. Effect of *APOC3*, *APOA5*, and *APOC3/A5* haplotypes on plasma lipid concentrations in 1,703 population-based controls in the Central Valley of Costa Rica

APOC3 3238	*APOC3* −455	*APOC3* −482	*APOA5* −1131	*APOA5* c.−3	*APOA5* c.56	*APOA5* IVS3+476	*APOA5* c.553	*APOA5* c.1259	Difference in plasma lipid concentrations (95% CI)[a]		
									Triglycerides	HDL cholesterol	LDL cholesterol
										mg/dl	
						APOC3 haplotypes					
1	1	1	—	—	—	—	—	—	0.0 (ref.)	0.0 (ref.)	0.0 (ref.)
1	1	2	—	—	—	—	—	—	4.9 (−12, 22)	−5.3 (−7.3, −3.2)[d]	1.9 (−4.1, 8.0)
1	2	1	—	—	—	—	—	—	5.8 (−6.7, 18)	−1.1 (−2.6, 0.4)	5.7 (1.1, 10)[b]
1	2	2	—	—	—	—	—	—	0.5 (−7.5, 8.5)	−1.4 (−2.3, −0.4)[c]	2.7 (−0.3, 5.6)
2	1	1	—	—	—	—	—	—	27 (7.1, 48)[c]	−4.8 (−7.2, −2.4)[d]	2.5 (−4.8, 9.8)
2	1	2	—	—	—	—	—	—	38 (15, 61)[c]	−5.9 (−8.8, −3.0)[d]	11 (2.5, 20)[b]
2	2	2	—	—	—	—	—	—	6.6 (−3.6, 17)	0.8 (−0.4, 2.0)	−3.3 (−7.1, 0.5)
						APOA5 haplotypes					
—	—	—	1	1	1	1	1	1	0.0 (ref.)	0.0 (ref.)	0.0 (ref.)
—	—	—	1	1	1	1	2	1	17 (−10, 44)	−3.3 (−6.6, 0.0)[b]	9.1 (−0.9, 19)
—	—	—	1	1	2	1	1	1	3.1 (−7.5, 14)	0.7 (−0.6, 2.0)	2.5 (−1.5, 6.4)
—	—	—	1	2	1	1	1	1	9.8 (−4.2, 24)	0.0 (−1.7, 1.7)	0.3 (−4.9, 5.5)
—	—	—	1	2	1	1	1	2	82 (53, 111)[d]	1.4 (−2.1, 5.0)	−3.6 (−15, 8.2)
—	—	—	1	2	1	2	1	2	15 (1.7, 28)[b]	3.2 (1.5, 4.8)[d]	−2.2 (−7.3, 2.8)
—	—	—	2	1	1	1	1	1	51 (24, 78)[d]	−2.6 (−5.9, 0.8)	2.6 (−7.6, 13)
—	—	—	2	2	1	2	1	2	11 (−16, 38)	−0.7 (−4.1, 2.6)	−7.1 (−17, 3.0)
						APOC3/A5 haplotypes					
1	1	1	1	1	1	1	1	1	0.0 (ref.)	0.0 (ref.)	0.0 (ref.)
1	1	1	1	1	2	1	1	1	2.9 (−14, 20)	0.5 (−1.6, 2.5)	1.3 (−4.9, 7.5)
1	1	1	1	2	1	1	1	1	15 (−7.5, 38)	0.5 (−2.2, 3.3)	0.7 (−7.6, 9.1)
1	1	1	1	2	1	2	1	2	17 (−11, 45)	2.0 (−1.4, 5.5)	−2.8 (−14, 8.1)
1	1	2	1	1	1	1	1	1	4.9 (−15, 24)	−5.6 (−8.0, −3.3)[d]	3.2 (−3.9, 10)
1	2	1	1	1	1	1	1	1	4.6 (−9.7, 19)	−0.1 (−1.9, 1.6)	5.2 (−0.1, 10)
1	2	2	1	1	1	1	1	1	5.0 (−5.1, 15)	−1.3 (−2.6, −0.1)[b]	1.6 (−2.1, 5.4)
1	2	2	1	1	2	1	1	1	−4.9 (−32, 23)	2.0 (−1.4, 5.3)	8.5 (−1.6, 19)
2	1	1	1	1	1	1	1	1	30 (3.2, 57)[b]	−4.3 (−7.6, −1.0)[c]	−2.2 (−12, 7.8)
2	2	2	1	1	1	1	1	1	−1.4 (−16, 13)	0.7 (−1.1, 2.4)	−4.1 (−9.5, 1.3)
2	2	2	1	2	1	2	1	2	24 (6.2, 42)[c]	3.6 (1.4, 5.8)[c]	−2.7 (−9.4, 4.1)

[a] Change in plasma lipids associated with substitution of one variant haplotype for one wild-type haplotype (reference haplotype).
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

metabolism in opposite directions (3, 31), it is plausible that the expression of both *APOC3* and *APOA5* genes is coregulated, where overexpression of *APOC3* as a result of the mutant alleles in the promoter (8) can result in upregulation of the *APOA5* gene. It has been shown that expression of both genes is downregulated by insulin (32, 36, 37), that transcription is upregulated by the retinoic acid receptor-related orphan receptor α (RORα) (38, 39), and that activation of peroxisome proliferator-activated receptor α stimulates transcription of the *APOA5* gene (40) and suppresses *APOC3* expression through both displacement of the hepatic nuclear factor-4 from the *APOC3* promoter (41) and induction of the expression of the orphan nuclear hormone receptor Rev-erbα that displaces to RORα from the *APOC3* promoter (42). Because downregulation of *APOA5* results in higher triglyceride concentrations, coregulation would also explain the finding of higher triglycerides among carriers of the mutation in the c.−3 site that resides in a putative Kozak sequence that potentially lowers apoA-V concentrations (43) (*APOC3/A5*222121212* haplotype). Our data also show that the triglyceride-raising effect of the *APOC3* 3238G allele was not counteracted by upregulation of the *APOA5* gene (*APOC3/A5*211111111* haplotype), probably because this triglyceride increase did not depend on transcription. The main effect of the 3238G allele in the *APOC3* 3′ UTR could be attributed to stabilization of the *APOC3* mRNA, as suggested by studies showing that the 3′ UTR may influence gene regulation (44, 45) and mRNA turnover in eukaryotes (46).

Overexpression of *APOC3* in noninsulin-dependent diabetes mellitus patients is partly responsible for their increased plasma apoC-III and triglyceride concentrations (32, 33). Under our working hypothesis, hypertriglyceridemia induced by *APOC3* overexpression in a diabetic state would be diminished by counterregulatory upregulation of *APOA5* expression. The protective effect of *APOA5* would be abolished by mutations that decrease either *APOA5* expression or apoA-V concentrations. Consistent with the discussion above, diabetic individuals carrying the *APOA5*111111* wild-type haplotype were less likely to have high triglyceride concentrations as compared with diabetic individuals carrying the *APOA5*121212* haplotype. The *APOA5*121212* haplotype might be associated with decreased apoA-V concentrations resulting from the presence of a mutation in c.−3 (43). It should be noted that, relative to apoC-III, very low concentrations of apoA-V are probably sufficient to maintain triglyceride homeostasis. Human plasma apoC-III concentrations (50–140 mg/l) are 1,000 to 2,000-fold higher than plasma apoA-V concentrations (31, 47–50), but an approximately 20-fold

TABLE 6. Effect of interaction between *APOC3/A5* haplotypes and diabetes on changes in triglyceride plasma levels in 1,703 population-based controls in the Central Valley of Costa Rica

APOC3 3238	*APOC3* −455	*APOC3* −482	*APOA5* −1131	*APOA5* c.−3	*APOA5* c.56	*APOA5* IVS3+476	*APOA5* c.553	*APOA5* c.1259	Difference in triglyceride concentrations (95% CI)[a]		P^b
									Nondiabetic	Diabetic	
									mg/dl		
1	1	1	1	1	1	1	1	1	0.0 (ref.)	13 (−14, 41)	
1	1	1	1	1	2	1	1	1	5.6 (−12, 23)	−9.8 (−63, 44)	0.34
1	1	1	1	2	1	1	1	1	15 (−9.2, 39)	25 (−37, 87)	0.91
1	1	1	1	2	1	2	1	2	3.4 (−27, 34)	113 (38, 187)	0.02
1	1	2	1	1	1	1	1	1	−2.6 (−23, 18)	68 (14, 123)	0.07
1	2	1	1	1	1	1	1	1	7.6 (−7.7, 23)	−1.6 (−38, 35)	0.27
1	2	2	1	1	1	1	1	1	4.6 (−6.4, 16)	18 (−7.0, 43)	0.99
1	2	2	1	1	2	1	1	1	−10 (−40, 20)	37 (−36, 109)	0.39
2	1	1	1	1	1	1	1	1	32 (3.6, 61)	26 (−48, 100)	0.64
2	2	2	1	1	1	1	1	1	−0.1 (−16, 15)	5.1 (−30, 41)	0.69
2	2	2	1	2	1	2	1	2	16 (−4.4, 36)	70 (30, 109)	0.05

[a] Change in plasma triglycerides associated with substitution of one variant haplotype for one wild-type haplotype (reference haplotype).
[b] Interaction between haplotypes and diabetes.

lower concentration of apoA-V can abolish the in vitro LPL-inhibitory effect of apoC-III (51).

Despite their lack of effect on MI, *APOA5* SNPs and haplotypes were associated with triglyceride and HDL concentrations in the current study. The −1131C, c.−3G, and c.1259C alleles were associated with increased triglyceride concentrations, as reported previously (3, 6, 11). However, our results did not confirm previous observations of higher triglyceride concentrations among carriers of minor alleles of the c.56C>G (7, 11), IVS3+476G>A (3, 11), and c.553G>T (12) polymorphisms. It is noteworthy that the relationship between the c.56C>G SNP and triglyceride concentrations is not clear, inasmuch as some studies (7, 11) but not all (21, 52) have found an association. The lack of association between the IVS3+476G>A SNP and triglycerides in our study may be due to the genetic structure of the trihybrid Costa Rican population. In Caucasians, almost complete LD exists among the −1131, c.−3, IVS3+476, and c.1259 sites (3, 11), and the triglyceride-raising effect of the IVS3+476A allele has been found within the haplotypic background defined by the minor alleles of these sites (*APOA5*2* haplotype definition by Pennacchio et al.) (11). But in Costa Rica, LD among these sites is not complete. The lack of an association can also be explained by the low frequency of the *APOA5*2* haplotype in Costa Rica (1.4%) compared with that in Caucasians from the Berkeley Lipid Study Population (8%) (11), Caucasians of northern European origin (4%) (53), and East Asian populations (∼17%) (54). On the other hand, the haplotype defined by the minor alleles of the c.−3, IVS3+476A, and c.1259 sites (*APOA5*121212*) was relatively frequent (∼6%) and associated with triglyceride concentrations in the Costa Rican population. Thus, the effect of the IVS3+476A allele on triglycerides may depend on the presence of other variants in the *APOA5* gene. Our study is the first to report allele frequencies of the c.553G>T SNP in a Hispanic population, and the low relative allele frequency (∼2.1%) suggests that this SNP may have low variability in Hispanics.

A potential limitation of our study is that we did not correct for multiple testing in our analyses, even when it involved the testing of various hypotheses (for example, association of specific genetic variation with MI and triglyceride concentrations) at different levels (individual SNPs and haplotypes). The need for such corrections is a topic of current debate, but it is difficult to justify any given approach for one correction compared with another. The classical Bonferroni method does not offer an optimal solution when it is being used for genetic association studies of complex diseases, because it is unclear how to define the number of hypotheses that are being tested. For example, should the study evaluate the number of hypotheses being tested in one particular study, the worldwide number of tests being performed by the research community in a particular disease, or all the tests being conducted across the genome. It is also possible that if individual polymorphisms have small effects on the risk of disease, the stringent criteria under the Bonferroni correction may lead to failure to detect true causal genetic variants. It is also difficult to determine the appropriate level of analysis. Most of the current genetic association studies are performed either at the SNP level or at the haplotype level, but Neale and Sham (55) have proposed the gene as the appropriate level of analysis. Under their approach, the significance level is adjusted to take into account all the variants within a candidate gene. By assuming an average of 30 different haplotypes per gene (56), Neale and Sham (55) have suggested a Bonferroni correction of 30 when testing a single SNP.

In the context of genetic epidemiology studies, it seems reasonable to follow the use of a Bayesian framework that takes into account the *prior* credibility of the hypotheses being tested (57–59). Given the known functions of the *APOC3* and *APOA5* genes and their known effects on lipid metabolism, the finding of an association between *APOC3* and MI is not surprising and may be interpreted as noteworthy (59). In particular, we found that no single site by itself was associated with risk of MI, but haplotypic analysis

revealed that the *APOC3*222* haplotype conferred an increased risk of MI that was independent of other SNPs in the *APOA5* gene. Our study rejected the hypothesis that plasma triglycerides mediated the effect of *APOC3* polymorphisms on MI, but alternative mechanisms should be explored. Recently, it has been shown that apoC-III increases the ability of apoB-containing lipoproteins to bind to vascular proteoglycans, which may affect the risk of development of atherosclerosis (60). Furthermore, a recent study showing that apoC-III promotes apoptosis of β cells in type 1 diabetes (61) raises the question of whether apoC-III may also affect cell death of macrophages during the atherosclerotic process. The mechanisms for the observed association between *APOC3* haplotype and MI are unknown and deserve further research.

This study was supported by Grants HL-49086 and HL-60692 from the National Institutes of Health.

REFERENCES

1. Ito, Y., N. Azrolan, A. O'Connell, A. Walsh, and J. L. Breslow. 1990. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science.* **249:** 790–793.
2. Maeda, N., H. Li, D. Lee, P. Oliver, S. H. Quarfordt, and J. Osada. 1994. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J. Biol. Chem.* **269:** 23610–23616.
3. Pennacchio, L. A., M. Olivier, J. A. Hubacek, J. C. Cohen, D. R. Cox, J. C. Fruchart, R. M. Krauss, and E. M. Rubin. 2001. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* **294:** 169–173.
4. Ordovas, J. M., F. Civeira, J. Genest, Jr., S. Craig, A. H. Robbins, T. Meade, M. Pocovi, P. M. Frossard, U. Masharani, P. W. Wilson, et al. 1991. Restriction fragment length polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus. Relationships with lipids, apolipoproteins, and premature coronary artery disease. *Atherosclerosis.* **87:** 75–86.
5. Surguchov, A. P., G. P. Page, L. Smith, W. Patsch, and E. Boerwinkle. 1996. Polymorphic markers in apolipoprotein C-III gene flanking regions and hypertriglyceridemia. *Arterioscler. Thromb. Vasc. Biol.* **16:** 941–947.
6. Talmud, P. J., E. Hawe, S. Martin, M. Olivier, G. J. Miller, E. M. Rubin, L. A. Pennacchio, and S. E. Humphries. 2002. Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. *Hum. Mol. Genet.* **11:** 3039–3046.
7. Hubacek, J. A., Z. Skodova, V. Adamkova, V. Lanska, and R. Poledne. 2004. The influence of APOAV polymorphisms (T-1131>C and S19>W) on plasma triglyceride levels and risk of myocardial infarction. *Clin. Genet.* **65:** 126–130.
8. Li, W. W., M. M. Dammerman, J. D. Smith, S. Metzger, J. L. Breslow, and T. Leff. 1995. Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. *J. Clin. Invest.* **96:** 2601–2605.
9. Olivieri, O., C. Stranieri, A. Bassi, B. Zaia, D. Girelli, F. Pizzolo, E. Trabetti, S. Cheng, M. A. Grow, P. F. Pignatti, et al. 2002. ApoC-III gene polymorphisms and risk of coronary artery disease. *J. Lipid Res.* **43:** 1450–1457.
10. Olivieri, O., A. Bassi, C. Stranieri, E. Trabetti, N. Martinelli, F. Pizzolo, D. Girelli, S. Friso, P. F. Pignatti, and R. Corrocher. 2003. Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease. *J. Lipid Res.* **44:** 2374–2381.
11. Pennacchio, L. A., M. Olivier, J. A. Hubacek, R. M. Krauss, E. M. Rubin, and J. C. Cohen. 2002. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. *Hum. Mol. Genet.* **11:** 3031–3038.
12. Kao, J. T., H. C. Wen, K. L. Chien, H. C. Hsu, and S. W. Lin. 2003. A novel genetic variant in the apolipoprotein A5 gene is associated with hypertriglyceridemia. *Hum. Mol. Genet.* **12:** 2533–2539.
13. Ferns, G. A., J. Stocks, C. Ritchie, and D. J. Galton. 1985. Genetic polymorphisms of apolipoprotein C-III and insulin in survivors of myocardial infarction. *Lancet.* **2:** 300–303.
14. Russo, G. T., J. B. Meigs, L. A. Cupples, S. Demissie, J. D. Otvos, P. W. Wilson, C. Lahoz, D. Cucinotta, P. Couture, T. Mallory, et al. 2001. Association of the Sst-I polymorphism at the APOC3 gene locus with variations in lipid levels, lipoprotein subclass profiles and coronary heart disease risk: the Framingham offspring study. *Atherosclerosis.* **158:** 173–181.
15. Wong, W. M., E. Hawe, L. K. Li, G. J. Miller, V. Nicaud, L. A. Pennacchio, S. E. Humphries, and P. J. Talmud. 2003. Apolipoprotein AIV gene variant S347 is associated with increased risk of coronary heart disease and lower plasma apolipoprotein AIV levels. *Circ. Res.* **92:** 969–975.
16. Chhabra, S., R. Narang, R. Lakshmy, S. Vasisht, D. P. Agarwal, L. M. Srivastava, S. C. Manchanda, and N. Das. 2004. Apolipoprotein C3 SstI polymorphism in the risk assessment of CAD. *Mol. Cell. Biochem.* **259:** 59–66.
17. Lai, C. Q., S. Demissie, L. A. Cupples, Y. Zhu, X. Adiconis, L. D. Parnell, D. Corella, and J. M. Ordovas. 2004. Influence of the APOA5 locus on plasma triglyceride, lipoprotein subclasses, and CVD risk in the Framingham Heart Study. *J. Lipid Res.* **45:** 2096–2105.
18. Bi, N., S. K. Yan, G. P. Li, Z. N. Yin, and B. S. Chen. 2004. A single nucleotide polymorphism -1131T>C in the apolipoprotein A5 gene is associated with an increased risk of coronary artery disease and alters triglyceride metabolism in Chinese. *Mol. Genet. Metab.* **83:** 280–286.
19. Szalai, C., M. Keszei, J. Duba, Z. Prohaszka, G. T. Kozma, A. Csaszar, S. Balogh, Z. Almassy, G. Fust, and A. Czinner. 2004. Polymorphism in the promoter region of the apolipoprotein A5 gene is associated with an increased susceptibility for coronary artery disease. *Atherosclerosis.* **173:** 109–114.
20. Lee, K. W., A. F. Ayyobi, J. J. Frohlich, and J. S. Hill. 2004. APOA5 gene polymorphism modulates levels of triglyceride, HDL cholesterol and FERHDL but is not a risk factor for coronary artery disease. *Atherosclerosis.* **176:** 165–172.
21. Talmud, P. J., S. Martin, M. R. Taskinen, M. H. Frick, M. S. Nieminen, Y. A. Kesaniemi, A. Pasternack, S. E. Humphries, and M. Syvanne. 2004. APOA5 gene variants, lipoprotein particle distribution, and progression of coronary heart disease: results from the LOCAT study. *J. Lipid Res.* **45:** 750–756.
22. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 2003. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care.* **26 (Suppl.):** 5–20.
23. Rose, G. A., H. Blackburn, R. F. Gillum, and R. J. Prineas. 1982. Cardiovascular Survey Methods. World Health Organization, Geneva.
24. Groenendijk, M., R. M. Cantor, T. W. de Bruin, and G. M. Dallinga-Thie. 2001. The apoAI-CIII-AIV gene cluster. *Atherosclerosis.* **157:** 1–11.
25. Kabagambe, E. K., A. Baylin, D. A. Allan, X. Siles, D. Spiegelman, and H. Campos. 2001. Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. *Am. J. Epidemiol.* **154:** 1126–1135.
26. Baylin, A., E. K. Kabagambe, X. Siles, and H. Campos. 2002. Adipose tissue biomarkers of fatty acid intake. *Am. J. Clin. Nutr.* **76:** 750–757.
27. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
28. Lewontin, R. C. 1964. The interaction of selection and linkage. II. Optimum models. *Genetics.* **50:** 757–782.
29. Zaykin, D. V., P. H. Westfall, S. S. Young, M. A. Karnoub, M. J. Wagner, and M. G. Ehm. 2002. Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. *Hum. Hered.* **53:** 79–91.
30. Stram, D. O., C. Leigh Pearce, P. Bretsky, M. Freedman, J. N. Hirschhorn, D. Altshuler, L. N. Kolonel, B. E. Henderson, and D. C. Thomas. 2003. Modeling and E-M estimation of haplotype-specific relative risks from genotype data for a case-control study of unrelated individuals. *Hum. Hered.* **55:** 179–190.
31. Baroukh, N., E. Bauge, J. Akiyama, J. Chang, V. Afzal, J. C. Fruchart, E. M. Rubin, J. Fruchart-Najib, and L. A. Pennacchio. 2004. Analysis of apolipoprotein A5, c3, and plasma triglyceride concentrations in genetically engineered mice. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1297–1302.

32. Chen, M., J. L. Breslow, W. Li, and T. Leff. 1994. Transcriptional regulation of the apoC-III gene by insulin in diabetic mice: correlation with changes in plasma triglyceride levels. *J. Lipid Res.* **35:** 1918–1924.
33. Venkatesan, S., H. Imrie, S. Read, and D. Halliday. 1995. Apo C subclasses from non-insulin-dependent diabetic patients—a quantitative comparison with control subjects. *Biochem. Soc. Trans.* **23 (Suppl.):** 278.
34. Dammerman, M., L. A. Sandkuijl, J. L. Halaas, W. Chung, and J. L. Breslow. 1993. An apolipoprotein CIII haplotype protective against hypertriglyceridemia is specified by promoter and 3′ untranslated region polymorphisms. *Proc. Natl. Acad. Sci. USA.* **90:** 4562–4566.
35. Shoulders, C. C., T. T. Grantham, J. D. North, A. Gaspardone, F. Tomai, A. de Fazio, F. Versaci, P. A. Gioffre, and N. J. Cox. 1996. Hypertriglyceridemia and the apolipoprotein CIII gene locus: lack of association with the variant insulin response element in Italian school children. *Hum. Genet.* **98:** 557–566.
36. Dallinga-Thie, G. M., M. Groenendijk, R. N. Blom, T. W. De Bruin, and E. De Kant. 2001. Genetic heterogeneity in the apolipoprotein C-III promoter and effects of insulin. *J. Lipid Res.* **42:** 1450–1456.
37. Nowak, M., A. Helleboid-Chapman, H. Jakel, G. Martin, D. Duran-Sandoval, B. Staels, E. M. Rubin, L. A. Pennacchio, M. R. Taskinen, J. Fruchart-Najib, et al. 2005. Insulin-mediated down-regulation of apolipoprotein A5 gene expression through the phosphatidylinositol 3-kinase pathway: role of upstream stimulatory factor. *Mol. Cell. Biol.* **25:** 1537–1548.
38. Raspe, E., H. Duez, P. Gervois, C. Fievet, J. C. Fruchart, S. Besnard, J. Mariani, A. Tedgui, and B. Staels. 2001. Transcriptional regulation of apolipoprotein C-III gene expression by the orphan nuclear receptor RORalpha. *J. Biol. Chem.* **276:** 2865–2871.
39. Genoux, A., H. Dehondt, A. Helleboid-Chapman, C. Duhem, D. W. Hum, G. Martin, L. A. Pennacchio, B. Staels, J. Fruchart-Najib, and J. C. Fruchart. 2005.Transcriptional regulation of apolipoprotein A5 gene expression by the nuclear receptor RORalpha. *Arterioscler. Thromb. Vasc. Biol.* **25:** 1186–1192.
40. Prieur, X., H. Coste, and J. C. Rodriguez. 2003. The human apolipoprotein AV gene is regulated by peroxisome proliferator-activated receptor-alpha and contains a novel farnesoid X-activated receptor response element. *J. Biol. Chem.* **278:** 25468–25480.
41. Hertz, R., J. Bishara-Shieban, and J. Bar-Tana. 1995. Mode of action of peroxisome proliferators as hypolipidemic drugs. Suppression of apolipoprotein C-III. *J. Biol. Chem.* **270:** 13470–13475.
42. Coste, H., and J. C. Rodriguez. 2002. Orphan nuclear hormone receptor Rev-erbalpha regulates the human apolipoprotein CIII promoter. *J. Biol. Chem.* **277:** 27120–27129.
43. Pennacchio, L. A., and E. M. Rubin. 2003. Apolipoprotein A5, a newly identified gene that affects plasma triglyceride levels in humans and mice. *Arterioscler. Thromb. Vasc. Biol.* **23:** 529–534.
44. Beutler, B., and T. Brown. 1993. Polymorphism of the mouse TNF-alpha locus: sequence studies of the 3′-untranslated region and first intron. *Gene.* **129:** 279–283.
45. Zaidi, S. H., R. Denman, and J. S. Malter. 1994. Multiple proteins interact at a unique cis-element in the 3′-untranslated region of amyloid precursor protein mRNA. *J. Biol. Chem.* **269:** 24000–24006.
46. Sachs, A. B. 1993. Messenger RNA degradation in eukaryotes. *Cell.* **74:** 413–421.
47. Marz, W., G. Schenk, and W. Gross. 1987. Apolipoproteins C-II and C-III in serum quantified by zone immunoelectrophoresis. *Clin. Chem.* **33:** 664–669.
48. Sakurabayashi, I., Y. Saito, T. Kita, Y. Matsuzawa, and Y. Goto. 2001. Reference intervals for serum apolipoproteins A-I, A-II, B, C-II, C-III, and E in healthy Japanese determined with a commercial immunoturbidimetric assay and effects of sex, age, smoking, drinking, and Lp(a) level. *Clin. Chim. Acta.* **312:** 87–95.
49. Noma, A., Y. Hata, and Y. Goto. 1991. Quantitation of serum apolipoprotein A-I, A-II, B, C-II, C-III and E in healthy Japanese by turbidimetric immunoassay: reference values, and age- and sex-related differences. *Clin. Chim. Acta.* **199:** 147–157.
50. O'Brien, P. J., W. E. Alborn, J. H. Sloan, M. Ulmer, A. Boodhoo, M. D. Knierman, A. E. Schultze, and R. J. Konrad. 2005. The novel apolipoprotein A5 is present in human serum, is associated with VLDL, HDL, and chylomicrons, and circulates at very low concentrations compared with other apolipoproteins. *Clin. Chem.* **51:** 351–359.
51. Schaap, F. G., P. C. Rensen, P. J. Voshol, C. Vrins, H. N. van der Vliet, R. A. Chamuleau, L. M. Havekes, A. K. Groen, and K. W. van Dijk. 2004. ApoAV reduces plasma triglycerides by inhibiting very low density lipoprotein-triglyceride (VLDL-TG) production and stimulating lipoprotein lipase-mediated VLDL-TG hydrolysis. *J. Biol. Chem.* **279:** 27941–27947.
52. Martin, S., V. Nicaud, S. E. Humphries, and P. J. Talmud. 2003. Contribution of APOA5 gene variants to plasma triglyceride determination and to the response to both fat and glucose tolerance challenges. *Biochim. Biophys. Acta.* **1637:** 217–225.
53. Olivier, M., X. Wang, R. Cole, B. Gau, J. Kim, E. M. Rubin, and L. A. Pennacchio. 2004. Haplotype analysis of the apolipoprotein gene cluster on human chromosome 11. *Genomics.* **83:** 912–923.
54. Lai, C. Q., E. S. Tai, C. E. Tan, J. Cutter, S. K. Chew, Y. P. Zhu, X. Adiconis, and J. M. Ordovas. 2003. The APOA5 locus is a strong determinant of plasma triglyceride concentrations across ethnic groups in Singapore. *J. Lipid Res.* **44:** 2365–2373.
55. Neale, B. M., and P. C. Sham. 2004. The future of association studies: gene-based analysis and replication. *Am. J. Hum. Genet.* **75:** 353–362.
56. Crawford, D. C., C. S. Carlson, M. J. Rieder, D. P. Carrington, Q. Yi, J. D. Smith, M. A. Eberle, L. Kruglyak, and D. A. Nickerson. 2004. Haplotype diversity across 100 candidate genes for inflammation, lipid metabolism, and blood pressure regulation in two populations. *Am. J. Hum. Genet.* **74:** 610–622.
57. Efron, B., and R. Tibshirani. 2002. Empirical bayes methods and false discovery rates for microarrays. *Genet. Epidemiol.* **23:** 70–86.
58. Sabatti, C., S. Service, and N. Freimer. 2003. False discovery rate in linkage and association genome screens for complex disorders. *Genetics.* **164:** 829–833.
59. Wacholder, S., S. Chanock, M. Garcia-Closas, L. El Ghormli, and N. Rothman. 2004. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J. Natl. Cancer Inst.* **96:** 434–442.
60. Olin-Lewis, K., R. M. Krauss, M. La Belle, P. J. Blanche, P. H. Barrett, T. N. Wight, and A. Chait. 2002. ApoC-III content of apoB-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan. *J. Lipid Res.* **43:** 1969–1977.
61. Juntti-Berggren, L., E. Refai, I. Appelskog, M. Andersson, G. Imreh, N. Dekki, S. Uhles, L. Yu, W. J. Griffiths, S. Zaitsev, et al. 2004. Apolipoprotein CIII promotes Ca2+-dependent beta cell death in type 1 diabetes. *Proc. Natl. Acad. Sci. USA.* **101:** 10090–10094.