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

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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.

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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 (StuI 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, −455T>C 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.553G>T (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; OR, receptor-related orphan receptor; SNP, single-nucleotide polymorphism; 3′ UTR, 3′ untranslated region.

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−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+4-cent 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 χ² 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-
software 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 into 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

| Characteristics | Controls (n = 1,703) | Cases (n = 1,703) |
|-----------------|---------------------|------------------|
| Age (years)     | 58 (11)             | 58 (11)          |
| Sex (% female)  | 26                  |                  |
| Waist-to-hip ratio b | 0.95 (0.07)         | 0.97 (0.07)      |
| Body mass index (kg/m²) 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 <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)\(^a\) | Cases (n = 1,703)\(^b\) |
|--------------|-----------------|-----------------|
| APOC3 3238G<sup>a</sup> | 0.201 (0.006) | 0.229 (0.007) |
| APOC3 455G | 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.5G | 0.139 (0.006) | 0.141 (0.006) |
| APOA5 c.56G | 0.102 (0.005) | 0.096 (0.005) |
| APOA5 IVS3+476G<sup>a</sup> | 0.090 (0.005) | 0.105 (0.005) |
| APOA5 c.553C | 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).

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SNPs in the APOA5 gene were associated with increased triglyceride concentrations. The strongest effect on triglyceride was observed in the APOA5 c.1259T>G polymorphism; the minor allele c.1259G 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*2221111111) 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, is 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 abolishes 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*2221111111 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 expression, these findings suggest that the APOC3/A5*2221111111 haplotype may be associated with reduced triglyceride synthesis or increased triglyceride clearance.

### 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 | APOC3 | APOC3 | APOA5 | APOA5 | APOA5 | APOA5 | IVS3+476 | APOA5 | c.12509 | Controls | Cases | OR (95% CI)* |
|-------|-------|-------|-------|-------|-------|-------|----------|-------|---------|-----------|-------|-----------|
| 0.328 | −0.455| −0.482| −0.1131| c.−3   | c.56   | c.56   | IVS3+476 | c.553  | c.1259  |           |       |           |
| 1     | 1     | 1     |       |       |       |       |          |       |         | 0.487 (0.008) | 0.489 (0.008) | 1.00 (ref.)  |
| 1     | 2     | 1     |       |       |       |       |          |       |         | 0.054 (0.003) | 0.054 (0.003) | 1.07 (0.82, 1.42) |
| 1     | 2     | 2     |       |       |       |       |          |       |         | 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) |
| 1     | 1     | 1     |       |       |       |       |          |       |         | 0.713 (0.008) | 0.714 (0.008) | 1.00 (ref.)  |
| 1     | 1     | 2     |       |       |       |       |          |       |         | 0.014 (0.002) | 0.013 (0.002) | 0.87 (0.55, 1.38) |
| 1     | 2     | 1     |       |       |       |       |          |       |         | 0.095 (0.005) | 0.093 (0.005) | 1.02 (0.86, 1.21) |
| 1     | 2     | 2     |       |       |       |       |          |       |         | 0.050 (0.004) | 0.046 (0.004) | 0.93 (0.73, 1.17) |
| 2     | 1     | 2     |       |       |       |       |          |       |         | 0.011 (0.002) | 0.010 (0.002) | 0.62 (0.36, 1.06) |
| 2     | 1     | 2     |       |       |       |       |          |       |         | 0.058 (0.004) | 0.066 (0.004) | 1.12 (0.90, 1.39) |
| 2     | 2     | 2     |       |       |       |       |          |       |         | 0.011 (0.002) | 0.010 (0.002) | 0.90 (0.75, 1.18) |
| 2     | 2     | 2     |       |       |       |       |          |       |         | 0.014 (0.002) | 0.016 (0.002) | 0.97 (0.76, 1.28) |

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.

*1, major allele; 2, minor allele.
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-erba 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 3288G 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 3288G 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*I11111 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

| APOC3 | APOC3 | APOC3 | APOC3 | APOC3 | APOC3 | APOC3 | APOC3 | APOC3 | APOC3 | HDL cholesterol | LDL cholesterol |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------------|----------------|
| 3288  | −455  | −482  | −1131 | c. −3 | c.56  | IVS+476| c.553 | c.1259 |       |                |                |
|       |       |       |       |       |       |       |       |       |       |                |                |
| APOC3 haplotypes |       |       |       |       |       |       |       |       |       |                |                |
| 1 1   | 1 1   |       |       |       |       |       |       |       |       | 0.0 (ref.)     | 0.0 (ref.)     |
| 1 1   | 1 2   |       |       |       |       |       |       |       |       | 4.9 (−12, 22) | −5.3 (−7.3, −3.2)d |
| 1 2   | 1 1   |       |       |       |       |       |       |       |       | 5.8 (−6.7, 18) | −1.1 (−2.6, 0.4) |
| 1 2   | 2 2   |       |       |       |       |       |       |       |       | 0.5 (−7.5, 8.5) | −1.4 (−2.3, −0.4)c |
| 2 1   | 1 1   |       |       |       |       |       |       |       |       | 27 (7.1, 48)  | −4.8 (−7.2, −2.4)c |
| 2 1   | 2 2   |       |       |       |       |       |       |       |       | 38 (15, 61)   | −5.9 (−8.8, −3.0)d |
| 2 2   | 1 2   |       |       |       |       |       |       |       |       | 6.6 (−3.6, 17) | 0.8 (−0.4, 2.0) |

| APOA5 haplotypes |       |       |       |       |       |       |       |       |       |                |                |
| 1 1   |       |       |       |       |       |       |       |       |       | 0.0 (ref.)     | 0.0 (ref.)     |
| 1 1   |       |       |       |       |       |       |       |       |       | 17 (−10, 44)  | −3.3 (−6.6, 0.0)b |
| 1 2   |       |       |       |       |       |       |       |       |       | 3.1 (−7.5, 14) | 0.7 (−0.6, 2.0) |
| 1 2   |       |       |       |       |       |       |       |       |       | 9.8 (−4.2, 24) | 0.0 (−1.7, 1.7) |
| 2 1   |       |       |       |       |       |       |       |       |       | 82 (33, 111)d | 1.4 (−2.1, 5.0) |
| 2 1   |       |       |       |       |       |       |       |       |       | 15 (1.7, 28)d | 3.2 (1.5, 4.8)d |
| 2 2   |       |       |       |       |       |       |       |       |       | 51 (24, 78)d | −2.6 (−5.9, 0.8) |
| 2 2   |       |       |       |       |       |       |       |       |       | 11 (−16, 38) | −0.7 (−4.1, 2.6) |

| APOC3/A5 haplotypes |       |       |       |       |       |       |       |       |       |                |                |
| 1 1   | 1 1   |       |       |       |       |       |       |       |       | 0.0 (ref.)     | 0.0 (ref.)     |
| 1 1   | 1 1   |       |       |       |       |       |       |       |       | 2.9 (−14, 20) | 0.5 (−1.6, 2.5) |
| 1 1   | 1 2   |       |       |       |       |       |       |       |       | 15 (−7.5, 38) | 0.5 (−2.2, 3.3) |
| 1 1   | 1 2   |       |       |       |       |       |       |       |       | 17 (−11, 45) | 2.0 (−1.4, 5.5) |
| 1 2   | 1 1   |       |       |       |       |       |       |       |       | 4.9 (−15, 24) | −5.6 (−8.0, −3.3)d |
| 1 2   | 1 2   |       |       |       |       |       |       |       |       | 4.6 (−9.7, 19) | −0.1 (−1.9, 1.6) |
| 1 2   | 1 1   |       |       |       |       |       |       |       |       | 5.0 (−5.1, 15) | −1.3 (−2.6, −0.1)b |
| 2 1   | 1 1   |       |       |       |       |       |       |       |       | 4.9 (−32, 23) | 2.0 (−1.4, 5.3) |
| 2 1   | 1 1   |       |       |       |       |       |       |       |       | 30 (3.2, 57)d | −4.3 (−7.6, −1.0)c |
| 2 2   | 1 1   |       |       |       |       |       |       |       |       | 1.4 (−16, 13) | 0.7 (−1.1, 2.4) |
| 2 2   | 1 2   |       |       |       |       |       |       |       |       | 24 (6.2, 42)c | 3.6 (1.4, 5.8) |

* 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.
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 c.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 c.−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

### 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 | APOC3 | APOC3 | APOA5 | APOA5 | APOA5 | APOA5 | APOA5 | Difference in triglyceride concentrations (95% CI) |
|-------|-------|-------|-------|-------|-------|-------|-------|-----------------------------------------------|
| mg/dl |       |       |       |       |       |       |       |                                              |
| 1 1 1 1 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0.0 (ref) | 13 (−14, 41) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 5.6 (−12, 25) | −9.8 (−63, 44) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 15 (−9.2, 39) | 25 (−37, 87) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 1 | 2 | 3.4 (−27, 34) | 113 (38, 187) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | −2.6 (−23, 18) | 68 (14, 123) |
| 1 1 1 1 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7.6 (−7.7, 25) | −1.6 (−38, 35) |
| 1 1 1 1 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 4.6 (−6.4, 16) | 18 (−7.0, 43) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 10 (−40, 20) | 37 (−36, 100) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 32 (36, 61) | 26 (−48, 100) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | −0.1 (−16, 15) | 5.1 (−30, 41) |
| 1 1 1 1 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 16 (−4.4, 36) | 20 (70, 109) |

* Change in plasma triglycerides associated with substitution of one variant haplotype for one wild-type haplotype (reference haplotype).

Interaction between haplotypes and diabetes.
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 triglyceride levels 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.

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