ApoC-III gene polymorphisms and risk of coronary artery disease

Oliviero Olivieri,[1,*] Chiara Stranieri,[†] Antonella Bassi,[§] Barbara Zaia,[*] Domenico Girelli,[*] Francesca Pizzolo,[*] Elisabetta Trabetti,[†] Suzanne Cheng,[**] Michael A. Grow,[**] Pier Franco Pignatti,[*] and Roberto Corrocher[*]

Unit of Internal Medicine,[*] Department of Clinical and Experimental Medicine, Section of Biology and Genetics,[†] Department of Mother and Child, and Biology-Genetics,[§] Institute of Clinical Chemistry, University of Verona, Verona, Italy; and Department of Human Genetics,[**] Roche Molecular System, Alameda, CA, USA

Abstract **Several polymorphisms in the apolipoprotein C-III (apoC-III) gene have been associated with hypertriglyceridemia, but the link with coronary artery disease risk is still controversial. In particular, apoC-III promoter sequence variants in the insulin responsive element (IRE), constitutively resistant to downregulation by insulin, have never been investigated in this connection. We studied a total of 800 patients, 549 of whom had angiographically documented coronary atherosclerosis, whereas 251 had normal coronary arteriograms. We measured plasma lipids, insulin, apoA-I, apoB, and apoC-III and assessed three polymorphisms in the apoC-III gene, namely, T-455C in the IRE promoter region, C1100T in exon 3, and *Sst1* polymorphic site (S1/S2) in the 3′ untranslated region. Each variant influenced triglyceride levels, but only the T-455C (in homozygosity) and S2 alleles influenced apoC-III levels. In coronary artery disease (CAD) patients, 18.6% were homozygous for the −455C variant compared with only 9.2% in CAD-free group ($P < 0.001$). In logistic regression models, homozygosity for −455C variant was associated with a significantly increased risk of CAD (OR = 2.5 and 2.18 for unadjusted and adjusted models, respectively) suggesting that it represents an independent genetic susceptibility factor for CAD.—** Olivieri, O., C. Stranieri, A. Bassi, B. Zaia, D. Girelli, F. Pizzolo, E. Trabetti, S. Cheng, M. A. Grow, P. F. Pignatti, and R. Corrocher. **ApoC-III gene polymorphisms and risk of coronary artery disease. *J. Lipid Res.* 2002.** 43: **1450–1457.**

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Investigators have long disputed whether elevated serum triglyceride (TG) levels are an independent risk factor for coronary artery disease (CAD) (1). A major reason for this controversy stems from the heterogeneity of factors measured by triglyceride (TG) determination (TGs are carried in virtually all plasma lipoproteins) and from the high variance and collinearity of TGs with other recognized risk factors (1, 2). Alternative, and more reliable, markers of TG metabolism have therefore been proposed (1, 2). Perhaps the most important among them is apolipoprotein C-III (apoC-III), a 79 amino acid protein synthesized in the liver and in the intestine, which is an essential constituent of circulating particles rich in triacylglycerol, i.e., chylomicrons and VLDL (3). The results of large clinical studies have indicated that apoC-III levels are a better predictor of risk for the development and progression of CAD than the traditionally measured TG levels (4–9). ApoC-III inhibits the hydrolysis of TG-rich particles by lipoprotein lipase and their apoE–mediated hepatic uptake (10, 11). In vitro and transgenic animal studies have demonstrated that overexpression of apoC-III causes delayed clearance of TG-rich lipoproteins from plasma, resulting in overt hypertriglyceridemia (3).

The human apoC-III gene has been mapped on chromosome 11 and several variant alleles have been investigated as possible genetic markers of hypertriglyceridemia, an atherosclerosis-related "intermediate phenotype" (12). Biallelic polymorphisms have been described in the 5′ promoter region (five polymorphisms in strong linkage disequilibrium with one another: T-455C, C-482T, T-625 deletion, G-630A, C-641A) (13), in exon 3 (C1100T) and in the 3′ untranslated region (the so called *Sstl* polymorphic site S1/S2) (12). In particular, over the last decade, this latter polymorphism, which is also the one most extensively studied, has consistently been reported to be associated with hypertriglyceridemia (14, 15).

Despite the expected implications in terms of cardiovascular morbidity, the evidence of an association between S2 allelic variant and risk of CAD is still controversial (14–

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Abbreviations: CAD, coronary artery disease; IRE, insulin response element; TG, triglyceride.

[1] To whom correspondence should be addressed.
e-mail: oliviero.olivieri@univr.it

17). Less information is available regarding the possible coronary risk linked to the other polymorphic variants (18, 19). This relatively limited interest may be particularly surprising if one considers the expression studies concerning the apoC-III promoter sequence variants in the insulin responsive element (IRE) and their interaction with insulin (20). In animals and in cultured cells, the apoC-III gene is transcriptionally downregulated by insulin (20). In 1995, Li et al. showed that, unlike the wild-type promoter, the promoter containing variants at positions –455 and –482 remains constitutively active over a 10^8-fold range of insulin concentrations inasmuch as polymorphic sequences have a reduced affinity for the nuclear transcription factors mediating the insulin response (21). The variation in the promoter of the apoC-III gene is the first reported example of a genetic polymorphism in an insulin responsive element and of "insulin resistance" at the gene level (21). Such considerations prompt speculation as to the possible links between these genetic variants and hypothetical related "intermediate" phenotypes, characterized by increased synthesis of apoC-III- and TG-rich lipoproteins, which in turn may imply an increased risk of CAD.

In the light of all these elements, we designed a large case-control study in patients with or without angiographically documented CAD to evaluate: *i*) a possible association between apoC-III gene polymorphisms and circulating levels of apoC-III and/or plasma lipids, and *ii*) whether the distribution of these polymorphisms was in turn associated with an increased risk of CAD.

METHODS

Study population

The criteria for selection of the study population have already been described in detail elsewhere (22). In brief, we studied a total of 800 unrelated adult patients of both sexes who were recruited from those referred to the Institute of Cardiovascular Surgery or to the Cardiovascular-Hypertension Unit of the Department of Internal Medicine of the University of Verona in Italy. Of these patients, 549 had angiographically documented, severe, often multivessel coronary atherosclerosis and were candidates for coronary-artery bypass grafting. The disease severity was evaluated by counting the number of major epicardial coronary arteries (left anterior descending, circumflex, and right) affected with $\geq$1 significant stenosis ($\geq$50%). As a control group, we considered 251 subjects with angiographically documented normal coronary arteries (CAD-free), examined for reasons other than possible coronary artery disease (in 90% of cases valvular heart disease; the remaining cases were studied for miscellany conditions including atypical chest pain of uncertain origin, congenital heart disease, etc.). The controls were required to have no stenosis in angiogram, no history of atherosclerosis, nor evidence of atherosclerosis in other vascular beds. Since the primary aim of our selection was to provide an objective and clear-cut definition of the atherosclerotic phenotype, subjects with nonsignificant coronary stenosis (<50%) were not included in the study. The angiograms were assessed by two cardiologists unaware that the patients were to be included in the study. All the study participants came from northern Italy and had similar socioeconomic and ethnic backgrounds.

At the time of blood sampling, a complete clinical and pharmacological history, including the presence or absence of cardiovascular risk factors such as smoking, hypertension, and diabetes mellitus, was obtained from the patients. Patients who were taking statins or fibrates (n = 266) were excluded from the genotype-phenotype correlation studies because of the documented lowering effects of these drugs on apoC-III and lipids levels (5, 9, 23).

The study was approved by our institutional review boards. Either written or oral informed consent was obtained from all the patients after a full explanation of the study.

Biochemical analysis

Samples of venous blood were drawn from each subject after an overnight fast within 10 days from the angiographic procedures. Serum lipids and the other common biochemical parameters were determined as previously described (22). Insulin was measured by an immunometric sandwich assay (Immulite 2000 Insulin) from Diagnostic Products Corporation, Los Angeles, CA; intra- and interassay CVs of the method were <5%. ApoA-I and apoB were measured by commercially available nephelometric immunoassays; antisera, calibrators, and BNII nephelometer were from Dade Behring, Marburg, Germany. Intraassay CV was calculated on 10 control replicates and interassay on duplicates over 10 days. Imprecision was within manufacturer specifications, i.e., the intraassay CVs were 2.1 and 1.6% and interassay CVs were 3.2 and 2.36 for apoA-I and apoB, respectively.

ApoC-III was measured by a fully automated turbidimetric immunoassay. The reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan) and the procedure recommended by the manifacturer was implemented on a RXL Dimension Analyzer (Dade International Inc. Newark, DE). Sample values were determined by interpolation of two spectrophotometric wavelengths measurements on a logit, five-points, calibration curve, covering the range 0.0–20.0 mg/dl; for concentrations of 20–30 mg/dl, a smaller sample volume was automatically rerun by the instrument, whereas for concentrations >30 mg/dl, the sample was diluted manually. Imprecision was assessed on three pools of control sera with low, medium and high concentrations of apoC-III; intraassay CV was 1.84, 2.02, and 1.98% and interassay CV 4.4, 3.4, and 2.29% for low, medium, and high concentration, respectively.

Mutation analysis

Mutation analysis (as well as routine biochemical analysis) was conducted as a study that was blinded as to whether the sample came from CAD or a CAD-free subject.

Three polymorphic variants mapping on the promoter (T-455C), on exon 3 in the coding region (C1100T), and on the 3′ untranslated region (S1/S2), were evaluated (**Fig. 1**).

Genomic DNA was prepared from whole blood samples by phenol-chloroform extraction and was then used according to a recently described multilocus genotyping assay protocol (24). Briefly, each sample was amplified by two 33 cycle Multiplex Polymerase Chain Reactions (32 ng of genomic DNA each) and the PCR products were then hybridized to an array of immobilized oligonucleotide probes. The colorimetric detection was based upon streptavidin-horseradish peroxidase method.

Statistical analysis

All computations were performed by using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL). Distributions of continuous variables in groups were expressed as means ± SD. Logarithmic transformation was performed for skewed variables, i.e., apoC-III and TG, and the statistical differences concerning these parameters were also computed on the corresponding log-transformed values, although, for the sake of simplicity and clarity, crude data are reported in the Results. Statistical significance of

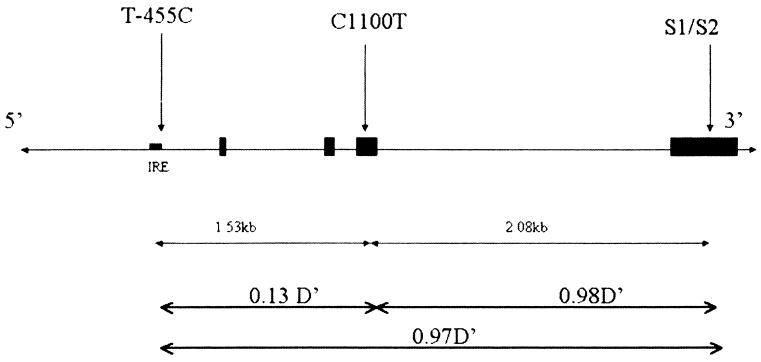

Fig. 1. ApoC-III polymorphisms physical distance and linkage disequalibrium.

differences in quantitative variables was assessed by Student's *t*-test, and it was also tested by one-way ANOVA adjusted for age and sex (General Linear Model procedure). Qualitative data were analyzed using the chi-square test. Pearson coefficient was used for correlation between variables.

In each of the two patient groups (with or without CAD), genotype frequencies were compared by chi-square analysis with the values predicted on the basis of Hardy-Weinberg equilibrium. Intragenic haplotypes for multiple markers within the apoC-III locus were estimated using the EH program (25) and were used to detect pairwise linkage disequilibrium values (D′). Insulin and lipid variables were compared among patients with different polymorphisms by ANOVA, using the Tukey procedure for post hoc multivariate comparison of the means (ANOVA). For T-455C and C1100T polymorphisms, computation was also performed considering the less frequent allele as recessive (homozygous for the less frequent variant vs. all other patients).

To assess the extent to which the various genotypes were associated with coronary artery disease, odds ratios with 95% confidence intervals were estimated by logistic-regression analysis. To provide separate odds ratios for each genotype, dummy variables were used, with wild-type genotype used as the reference group. Adjustment for the patients' conventional risk factors (age, gender, smoking status, presence of diabetes and hypertension, cholesterol, triglycerides, apoA-I and apoB) was done by including these covariates in a second set of logistic-regression models.

RESULTS

The clinical and biochemical characteristics of the population studied are summarized in **Table 1**. CAD patients had more conventional cardiovascular risk factors and significantly higher levels of apoC-III than control patients. There were no statistical differences in insulin plasma levels between the two groups (Table 1). In the population as a whole, apoC-III was statistically correlated with total ($R = 0.40$, $P < 0.001$), LDL ($R = 0.238$, $P < 0.001$), and HDL cholesterol (HDL-C) ($R = -0.08$, $P < 0.05$) and, more strongly, with TG levels ($R = 0.68$, $P < 0.001$).

Genotype frequencies of the apoC-III polymorphic variants for CAD and CAD-free groups are described in **Table 2**. All three polymorphisms were in the Hardy-Weinberg equi-

TABLE 1. Characteristics of the study patients and controls[a]

Parameters	CAD Patients (n = 549)	CAD-free (n = 251)	*P* value
Age (years)	60.4 ± 9.4	57.6 ± 12.6	<0.01
Male sex (%)	81.8	66.9	<0.001
BMI (kg/height^2)[b]	26.5 ± 3.3	25.3 ± 3.4	<0.001
Cholesterol[b]			
Total (mmol/l)	5.83 ± 1.12	5.51 ± 1.05	0.001
LDL (mmol/l)	3. 88 ± 0.98	3.53 ± 0.93	<0.001
HDL (mmol/l)	1.20 ± 0.32	1.42 ± 0.43	<0.001
Triglycerides (mmol/l)[b]	2.01 ± 1.13	1.50 ± 0.71	<0.001
ApoA-I (g/l)[b]	1.31 ± 0.24	1.42 ± 0.31	<0.001
ApoB (g/l)[b]	1.22 ± 0.30	1.06 ± 0.25	<0.001
ApoC-III (mg/dl)[b]	12.31 ± 4.4	10.7 ± 3.25	<0.001
Insulin (μIU/ml)[b]	14.64 ± 7.7	15.75 ± 12.2	NS
Smoking (%)	69.7	41.4	<0.001
Hypertension (%)	58.3	30.8	<0.001
Diabetes (%)	21.9	13.3	<0.01
Patients taking lipid-lowering drugs	266	—	

[a] Plus-minus values are means ± SD.

[b] Age- and sex-adjusted values.

Statistical significance for differences was tested by Student's unpaired *t*-test or by χ^2 test when appropriate. *P* value was considered significant when <0.05.

To convert the values for cholesterol to milligrams per deciliter, divide by 0.02586. To convert the values for triglycerides to milligrams per deciliter, divide by 0.01129.

TABLE 2. ApoC-III genotypes in the study patients and controls

Genotype (% of Patients)	CAD-free Patients (n = 251)	CAD Patients (n = 549)	Chi-Square	*P* value
T-455C				
−455 TT(%)	43.8	35.3		
−455 TC (%)	47	46.1		
−455 CC(%)	9.2	18.6	13.07	0.001
C1100T				
1100 CC (%)	50.2	54.3		
1100 CT (%)	43	37.3		
1100 TT (%)	6.8	8.4	2.529	NS
S1/S2				
S1/S1 (%)	85.2	82.4		
S1/S2 (%)	14.8	17.6	0.95	NS

librium in both groups of patients. No homozygous individuals for S2 allele were found. The distribution of C1100T and S1/S2 polymorphisms was similar in CAD and CAD-free patients. In contrast, the frequency of −455C homozygous subjects in CAD group was significantly higher than that observed in individuals free of coronary artery disease (18.6 vs. 9.2%, $P < 0.001$; Table 2).

Strong linkage disequilibrium between S1/S2 and both T-455C and C1100T (D' = 0.98, D' = 0.97, respectively) was observed, but not between T-455C and C1100T polymorphisms (D' = 0.13) (Fig. 1). It should be noted that, out of a total of 125 homozygotes for −455 variant, there were 55 (44%) subjects with S1/S2 and 13 (10.4%) with 1100TT genotype.

In order to estimate the impact of the polymorphisms on apoC-III and/or other lipid metabolism parameters, genotype-phenotype analysis was performed with data from the entire study population. Due to the well known lowering effect of statins and fibrates on apoC-III (5, 9, 23), patients taking these drugs (n = 266, all CAD patients) were excluded from the analysis. Results for the levels of apoC-III, triglycerides, and insulin, according to each genotype in the remaining population (n = 534), are reported in **Table 3**. Different genotype groups of such population were similar for age and sex distribution (data not shown). Homozygous individuals for the promoter variant had increased levels of apoC-III as compared with carriers of other genotypes, but a statistical significance was evident only upon assuming recessive allelic transmission as the model (Table 3). Homozygotes for −455C had significantly higher triglyceride values than those observed in heterozygous and wild-type individuals (whether considered separately or as a single group, Table 3).

C1100T polymorphism was significantly associated with plasma triglycerides (with the raising effect confined to the homozygotes), but this site was not associated with variations in apoC-III levels (Table 3).

Different levels of apoC-III and triglycerides were also associated with the S1/S2 polymorphism, with higher values being observed in S2 carriers (Table 3).

No substantial effect on fasting insulin values due to the different apoC-III gene polymorphisms was evident. Similarly, none of the other lipid variables analyzed (total, LDL, and HDL-C, apoA-I, apoB) showed any statistically relevant differences according to apoC-III genotype (data not shown).

To estimate the disease risk associated with apoC-III genotypes, logistic regression analysis was performed. Crude and adjusted odds ratios for coronary disease in relation to each apoC-III genotype are shown in **Table 4**. The greatest risk was conferred by homozygosity for the −455C variant, which was associated with a more than 2-fold increased probability of disease. Adjustment for the main vascular risk factors produced no change in the result, indicating that this polymorphism is an independent determinant of CAD risk.

The other apoC-III gene polymorphisms were not associated with a significantly increased risk of CAD (Table 4). The multiple regression model including all the polymorphisms showed identical results, suggesting a genetic risk specifically associated with −455C but not with the other variants (data not shown).

TABLE 3. Levels of apoC-III, TGs, and insulin in the study population, according to the ApoC-III genotypes (by ANOVA)

ApoC-III Genotypes	No. of Patients (n = 534)	ApoC-III (mg/dl)	Triglycerides (mmol/l)	Insulin (μIU/ml)
−455TT	206	11.47 ± 3.9	1.79 ± 0.94[a]	15.7 ± 11
−455TC	252	11.30 ± 3.7	1.73 ± 0.86[a]	14.6 ± 8.8
−455CC	76	12.65 ± 5.1	2.12 ± 1.40	14.8 ± 7.2
−455TT and −455TC	458	11.38 ± 3.8[a]	1.76 ± 0.89[a]	15.1 ± 9.9
−455CC	76	12.65 ± 5.1	2.12 ± 1.40	14.8 ± 7.2
1100CC	274	11.3 ± 3.6	1.73 ± 0.85[a]	15.3 ± 10.6
1100CT	218	11.8 ± 4.5	1.85 ± 1.05	14.9 ± 8.7
1100TT	42	12.1 ± 4.5	2.13 ± 1.40	14.1 ± 5.7
1100CC and 1100CT	492	11.5 ± 4.0	1.79 ± 0.94[a]	15.1 ± 9.8
1100TT	42	12.1 ± 4.5	2.13 ± 1.40	14.1 ± 5.7
S1/S1	438	11.3 ± 3.8[b]	1.75 ± 0.92[b]	14.9 ± 9.9
S1/S2	96	12.8 ± 4.8	2.10 ± 1.22	15.6 ± 7.7

[a] Statistically different from patients homozygous for the less common allele.
[b] Statistically different from S1/S2 patients.

TABLE 4. Odds ratios for coronary artery disease according to apoC-III genotypes (by multiple logistic regression analysis)

ApoC-III Genotypes	Unadjusted Model		Adjusted Model[a]	
	Odd Ratio (95% CI)[b] n = 800	*P* value[c]	Odd Ratio (95% CI)[b] n = 753	*P* value[c]
−455TT	1		1	
−455TC (−455TT as reference)	1.21 (0.88–1.67)	NS	1.19 (0.81–1.76)	NS
−455CC (−455TT as reference)	2.51 (1.51–4.18)	<0.001	2.18 (1.21–3.91)	0.001
1100CC	1		1	
1100CT (1100CC as reference)	0.80 (0.59–1.1)	NS	0.79 (0.54–1.15)	NS
1100TT (1100CC as reference)	1.14 (0.63–2.07)	NS	1.11 (0.56–2.21)	NS
S1/S1	1		1	
S1/S2 (wild type as reference)	1.22 (0.81–1.85)	NS	0.97 (0.59–1.59)	NS

[a] The multiple logistic-regression model was adjusted for age, gender, smoking status, presence of diabetes and hypertension, cholesterol, triglycerides, apoA-I, and apoB. The analysis was performed on total of 753 cases because of missing data in 47 cases.

[b] CI, confidence interval.

[c] *P* values are for the overall comparison among patients with a given polymorphism and were calculated by chi-square analysis.

DISCUSSION

ApoC-III is probably the most important marker of the levels of circulating TG-rich lipoproteins, that in turn have been demonstrated to be strongly atherogenic (2, 4–9). Clinical cross-sectional and perspective studies have shown an elevated potential for the development and progression of coronary artery disease associated with the levels of apoC-III- and TG-rich lipoproteins (4–9). The results of our study, in which we examined the relationships between the CAD phenotype and three polymorphisms in the apoC-III gene, confirm and extend these previous observations, providing some major new findings suggestive of a role of the promoter polymorphic variant −455C as an independent determinant of CAD risk and as a genetic marker reflecting the overall risk associated with the metabolism of TG- and apoC-III-rich lipoproteins.

The pathophysiological sequence of events suggested by our results is: *i*) homozygotes for −455C variant are constitively resistant to insulin-mediated down-regulation of apoC-III gene transcription; *ii*) lack of this physiological inhibitory modulation increases the synthesis of apoC-III in liver and intestine; *iii*) from liver and intestine, apoC-III-enriched lipid particles enter in the circulation and have a prolonged residence time because of reduced hepatic and peripheral clearance; *iv*) the resulting hypertriglyceridemia favors the atherosclerotic process and individual susceptibility to the development and progression of CAD.

In this respect, the role of the other apoC-III gene variants appears less important. Although all the variants examined were associated with TG levels, only S1/S2 and T-455C were associated with apoC-III levels (Table 3). Worthy of note is the fact that −455C was the only functional variation of the three sites evaluated, and its effect of increasing the apoC-III concentration was restricted to homozygotes. These results agree with the previous observation that the polymorphisms in the promoter and 3′UTR (*Sstl*-restricted S1/S2) regions are in linkage disequilibrium (12, 13) and, in turn, strictly associated with the apoC-III phenotype (14, 15, 26). In contrast, the TG-raising effect associated with C1100T variant, also observed by other investigators (18, 27), remains to some extent unexplained, because the base change in exon 3 does not code for an amino acid change (28); the polymorphism may be in linkage disequilibrium with another, unrecognized functional variant.

To the best of our knowledge, this is the first study reporting an increased risk of CAD associated with the T-455C variant, with the result that direct comparison with other similar studies is impossible. Nevertheless, a comparison is suggested by the recognized linkage disequilibrium between the polymorphisms in the IRE promoter and 3′UTR (*Sstl*-restricted S1/S2) regions. Previous studies concerning *Sstl* polymorphic site and CAD risk have yielded controversial results, possibly related to differences in methodological aspects such as the selection of cases and controls, their ethnical composition, and the sample size of the study (14–17, 29). In a multifactorial pathology such as CAD, assessment of the phenotype may greatly influence the results of any genetic analysis. For example, apparently healthy "control subjects", randomly selected from the general population (14–16) may have substantial (though not clinically manifest) coronary atherosclerosis, thus contributing to negative conclusions regarding genetic association. Similarly, using survivors of myocardial infarction as "cases", the patients more severely affected by CAD, who died as a result of thrombotic complication of the disease, may be excluded a priori (16).

One of the most important features of the present study was represented by the rigorously objective definition of the clinical phenotype, according to a clear-cut stratification by coronary angiography for both CAD and CAD-free patients (including patients with or without previous myocardial infarction). In this way, we are confident that we minimized the chance of spurious results in the analysis of the association with apoC-III genotypes.

Collaborative studies recruiting ethnically heterogenous populations from different countries have also

yielded negative results for the possible association between apoC-III gene polymorphisms and CAD (16). The power of these studies to detect a significant genetic association may be relatively weak because of possible different distributions of the variant of interest in ethnically heterogenous populations. As all the subjects in our population had similar ethnic background, population substructure would not be expected.

Another important methodological is sample size. It has been postulated that an adequate analysis of the polymorphic variants of the apoA-I/apoC-III/apoA-IV gene complex requires a sample of at least 600 subjects to allow the detection of a twice the risk of disease (30). Only two previous studies (16, 17) evaluated a sufficient number of subjects to avoid a type-II statistical error. In this regard, our study had a power of 98–99% (at a 5% of significance level) to detect a difference in genotype frequency distribution such as the one observed between our CAD and CAD-free patients.

Making due allowance for all these possible methodological limitations, we can say that the conclusions of most single previous reports tend to exclude an association between *Sstl* polymorphic site and CAD risk (14–17, 28), which clearly contrasts with a substantial body of data showing an increased risk of hypertriglyceridemia (14, 15, 17, 31, 32). Our findings for S1/S2 polymorphism lead to similar conclusions: it is in linkage disequilibrium with T-455C and is associated with the apoC-III phenotype, but does not confer an increased risk of CAD. The simplest explanation of this apparent discrepancy may be that −455C is the true functional "culprit" allele and S2 "the innocent" co-segregated variation. However, a number of functional aspects not fully investigated in the present work may also be involved.

Recent reports have shown important postprandial effects of IRE polymorphic sites such as C-482T on insulin levels. It should be stressed that in humans the −482T sequence rarely appears without the −455C and that the two IRE variants possess and equally low affinity for the nuclear transcription factors mediating the insulin response (21). In a considerable number of healthy young subjects in the EARS group, Waterworth et al. (33) recognized an association between increased insulin and glucose levels during and after an oral glucose tolerance test (OGTT) and IRE C-482T variant. Qualitatively similar data for the insulin response after OGTT were reported by Salas et al. (34) in a smaller number of S2 carriers following specific diets with different lipid composition. All the differences observed in both studies were independent of fasting levels of insulin.

These findings agree with our failure to detect statistical differences in fasting insulin levels by separating the patients according to apoC-III genotypes, which may be better associated with post-prandial insulin concentrations. It appears plausible that, as insulin levels increase postprandially, a condition of "insulin resistance" conferred by IRE polymorphisms may increase CAD risk regardless of fasting levels of insulin. In the same way, it is also conceivable that fasting levels of TG or apoC-III do not fully reflect the increased CAD risk associated with IRE −455C variant (or, conversely, not associated with *Sstl* polymorphic site). Of concern for this interpretation, we measured apoC-III as total plasma protein without distinguishing between subfractions according to the types of lipoproteins. The best predictor of CAD progression seems to be the apoC-III fraction in VLDL + LDL or in lipoproteins with Svedberg flotation unit (Sf) 12 to 60 (IDL + small VLDL) or even remnants lipoproteins (2), that require separation by analytical ultracentrifugation or by an immunological method, respectively (9, 35). It is therefore conceivable that the differences in apoC-III of fractionated apoB-containing particles may also be more important than those observed as total apoC-III in our CAD/CAD-free patients. The concentration of atherogenic remnant lipoproteins may also contribute to a better discrimination between genotype-related effects on CAD risk (2). A recent report has demonstrated that the levels of plasma remnant particles are preferentially determined by the presence of −482T IRE variant rather than *Sstl* polymorphic site (36). In parallel with our results on the −455C, the raising effect of −482T IRE variant was confined to homozygous carriers (36) in whom the loss of insulin-mediated downregulation of apoC-III gene is physiologically more important (21). Thus, the demonstrated association with the effects on the atherogenic apoC-III-rich particles and on glucose and insulin postprandial metabolism may better account for the increased CAD risk carried by −455C homozygosity rather than S2 carrier status.

Although the design of the study (which was not prospective but case-control) suggests a need for caution, the potential value of our results is important. If genetic markers express a cumulative lifelong effect, better representing the actual individual risk than single measures of phenotypic variables, evaluation of T-455C polymorphism may be clinically useful as an "integrated" predictor of risk, particularly for that portion of risk that is related to TG-rich lipoproteins (VLDL or remnant lipoproteins) and not to LDL-C.

Advances in therapeutic strategies might also derive from pharmacogenomic considerations, with patients at higher genetic risk treated early with fibrates and/or statins. In this regard, it is worthy of note that statin therapy lowers LDL-C and VLDL-C levels to a similar extent in percentage terms (37); thus, the positive results of clinical trials of statin therapy may reflect a benefit from lowering apoC-III-rich lipoproteins as well as LDL level. Fibrates are agents which are well known for their lowering effects on apoC-III rich lipoproteins. However, the choice of statins or fibrates treatment is currently based on fasting TG and LDL/HDL-C levels, which are not sufficiently specific predictors of favorable responses to any class of drugs (38). A fascinating working hypothesis raised from our results is that apoC-III genotype may improve this predictive power and serve as useful tool for achieving a better future pharmacological approach.

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