ApoC-III gene polymorphisms and risk of coronary artery disease

Oliviero Olivieri,1,* Chiara Stranieri,1 Antonella Bassi,3 Barbara Zaia,* Domenico Girelli,* Francesca Pizzolo,* Elisabetta Trabetti,1 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.

Supplementary key words lipids • risk factors • insulin

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 SstI 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–

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

† To whom correspondence should be addressed.

e-mail: oliviero.olivieri@univr.it

Manuscript received 29 March 2002.
DOI 10.1194/jlr.M200145-JLR200

1450 Journal of Lipid Research Volume 43, 2002
whether the distribution of these polymorphisms was in
dence of atherosclerosis in other vascular beds. Since the pri-
stenosis in angiogram, no history of atherosclerosis, nor evi-
tal heart disease, etc.). The controls were required to have no
tions including atypical chest pain of uncertain origin, congeni-
disease; the remaining cases were studied for miscellany condi-
possible coronary artery disease (in 90% of cases valvular heart

coronary arteries (CAD-free), examined for reasons other than
ated by counting the number of major epicardial coronary arter-
coronary-artery bypass grafting. The disease severity was evalu-
mated from those referred to the Institute of Cardiovascular
ory to the Cardiovascular-Hypertension Unit of the Depart-
ent of Internal Medicine of the University of Verona in Italy. Of
these patients, 549 had angiographically documented, severe, of-
ten multivessel coronary atherosclerosis and were candidates for
coronary-artery bypass grafting. The disease severity was evalu-
ated by counting the number of major epicardial coronary arter-
es (left anterior descending, circumflex, and right) affected
with \( \geq 1 \) significant stenosis (\( \geq 50\% \)). As a control group, we con-
sidered 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 condi-
tions including atypical chest pain of uncertain origin, congeni-
tal heart disease, etc.). The controls were required to have no
stenosis in angiogram, no history of atherosclerosis, nor evi-
dence of atherosclerosis in other vascular beds. Since the pri-
mary 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 un-
naware that the patients were to be included in the study. All the
study participants came from northern Italy and had similar so-
socioeconomic and ethnic backgrounds.

At the time of blood sampling, a complete clinical and phar-
macological history, including the presence or absence of car-
diovascular 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 proce-
dures. Serum lipids and the other common biochemical param-
ters 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 nephelomet-
ic immunonephelometry; antisa, calibrators, and BNII nephelome-
ter were from Dade Behring, Marburg, Germany. Intraassay CV
was calculated on 10 control replicates and interassay on duplica-
tes over 10 days. Imprecision was within manufacturer specifica-
tions, 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 im-
monoassay. The reagents were obtained from Wako Pure Chemi-
cal Industries (Osaka, Japan) and the procedure recommended
by the manufacturer was implemented on a RXL Dimension Ana-
lyzer (Dade International Inc. Newark, DE). Sample values were
determined by interpolation of two spectrophotometric wave-
lengths 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 concentra-
tion, respectively.

Mutation analysis

Mutation analysis (as well as routine biochemical analysis) was
conduct 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-
545C), 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 immobi-
lized oligonucleotide probes. The colorimetric detection was
based upon streptavidin-horseradish peroxidase method.

Statistical analysis

All computations were performed by using the SPSS 10.0 sta-
tistical package (SPSS Inc., Chicago, IL). Distributions of contin-
uous variables in groups were expressed as means \( \pm \) SD. Loga-
rithmic 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-trans-
formed values, although, for the sake of simplicity and clarity,
crude data are reported in the Results. Statistical significance of
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-

![Fig. 1. ApoC-III polymorphisms physical distance and linkage disequilibrium.](image)

**TABLE 1.** Characteristics of the study patients and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAD Patients (n = 549)</th>
<th>CAD-free (n = 251)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.4 ± 9.4</td>
<td>57.6 ± 12.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>81.8</td>
<td>66.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/height²)</td>
<td>26.5 ± 3.3</td>
<td>25.3 ± 3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.83 ± 1.12</td>
<td>5.51 ± 1.05</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.88 ± 0.98</td>
<td>3.53 ± 0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.20 ± 0.32</td>
<td>1.42 ± 0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.01 ± 1.13</td>
<td>1.50 ± 0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoA-I (g/l)</td>
<td>1.31 ± 0.24</td>
<td>1.42 ± 0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>1.22 ± 0.30</td>
<td>1.06 ± 0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoC-III (mg/dl)</td>
<td>12.31 ± 4.4</td>
<td>10.7 ± 3.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (μIU/ml)</td>
<td>14.64 ± 7.7</td>
<td>15.75 ± 12.2</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>69.7</td>
<td>41.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>58.3</td>
<td>30.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>21.9</td>
<td>13.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Patients taking lipid-lowering drugs</td>
<td>266</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

* Plus-minus values are means ± SD.

* Age- and sex-adjusted values.

Statistical significance for differences was tested by Student’s unpaired t-test or by χ² 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.
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 (D² = 0.98, D² = 0.97, respectively) was observed, but not between T-455C and C1100T polymorphisms (D² = 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-Ι, 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 2. ApoC-III genotypes in the study patients and controls

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

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

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

*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)

<table>
<thead>
<tr>
<th>ApoC-III Genotypes</th>
<th>Odd Ratio (95% CI)</th>
<th>P-value</th>
<th>Odd Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>−455TT</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>−455TC (−455TT as reference)</td>
<td>1.21 (0.88–1.67)</td>
<td>NS</td>
<td>1.19 (0.81–1.76)</td>
<td>NS</td>
</tr>
<tr>
<td>−455CC (−455TT as reference)</td>
<td>2.51 (1.51–4.18)</td>
<td>&lt;0.001</td>
<td>2.18 (1.21–3.91)</td>
<td>0.001</td>
</tr>
<tr>
<td>1100CC</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1100CT (1100CC as reference)</td>
<td>0.80 (0.59–1.1)</td>
<td>NS</td>
<td>0.79 (0.54–1.15)</td>
<td>NS</td>
</tr>
<tr>
<td>1100TT (1100CC as reference)</td>
<td>1.14 (0.63–2.07)</td>
<td>NS</td>
<td>1.11 (0.56–2.21)</td>
<td>NS</td>
</tr>
<tr>
<td>S1/S1</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S1/S2 (wild type as reference)</td>
<td>1.22 (0.81–1.85)</td>
<td>NS</td>
<td>0.97 (0.59–1.59)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*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.

CI, confidence interval.

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 constitutively 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 (SstI-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 (SstI-restricted S1/S2) regions. Previous studies concerning SstI 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 ethnic 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 yielded 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 heterogeneous 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 Sedberg 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.}

Olivieri et al. ApoC-III gene and coronary disease 1455
REFERENCES


