Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease

Oliviero Olivieri,[1,*] Antonella Bassi,[†] Chiara Stranieri,[§] Elisabetta Trabetti,[§] Nicola Martinelli,[*] Francesca Pizzolo,[*] Domenico Girelli,[*] Simonetta Friso,[*] Pier Franco Pignatti,[§] and Roberto Corrocher[*]

Unit of Internal Medicine, Department of Clinical and Experimental Medicine,[*] Institute of Clinical Chemistry,[†] Section of Biology and Genetics, Department of Mother and Child and Biology–Genetics,[§] University of Verona, Verona, Italy

Abstract **Apolipoprotein C-III (apoC-III) is a marker of triglyceride (TG)-rich lipoproteins, which are often increased in metabolic syndrome (MS). The T−455C polymorphism in the insulin-responsive element of the APOC3 gene influences TG and apoC-III levels. To evaluate the contribution of apoC-III levels and T−455C polymorphisms in the coronary artery disease (CAD) risk of MS patients, we studied 873 patients, 549 with CAD and 251 with normal coronary arteries. Patients were classified also as having or not having MS (MS, n = 270; MS-free, n = 603). Lipids, insulin, apolipoprotein levels, and APOC3 T−455C genotypes were evaluated. ApoC-III levels were significantly increased in MS patients, and the probability of having MS was correlated with increasing quartiles of apoC-III levels. MS patients with CAD had significantly higher apoC-III levels than did CAD-free MS patients. The carriership for the −455C variant multiplied the probability of CAD in MS in an allele-specific way and was associated with increased apoC-III and TG levels. Obesity was less frequent in MS carriers of the −455C allele than in MS noncarriers (21.6% vs. 34.8%, $P < 0.05$). In conclusion, apoC-III-rich lipoprotein metabolism and the APOC3 polymorphism have relevant impacts on the CAD risk of MS patents.**—Olivieri, O., A. Bassi, C. Stranieri, E. Trabetti, N. Martinelli, F. Pizzolo, D. Girelli, S. Friso, P. F. Pignatti, and R. Corrocher. **Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease.** ***J. Lipid Res.*** **2003.** 44: **2374–2381.**

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The metabolic syndrome (MS) is a clinical entity characterized by obesity, hypertension, hypertriglyceridemia, low serum HDL cholesterol, and either diabetes mellitus type 2 or glucose intolerance (1). The importance of this clinical clustering as a single pathological entity was recently defined (1), although the underlying etiological factors are as yet mostly unknown. Patients with MS have an enhanced propensity to develop premature arteriosclerosis and an increased cardiovascular disease mortality and morbidity rate (2), and they represent, depending on age, 24% to 42% of the US general population (3). Therefore, it is of paramount importance to identify causal and/or aggravating factors of MS, particularly in terms of cardiovascular disease prevention.

All of the key components of the syndrome have a genetic basis (4, 5). As a consequence, an interaction or multiplicative effects of polymorphisms in a number of different genes may potentially be involved in the pathogenesis (4). Gene mutations interfering with specific insulin or hormone-responsive elements in the regulatory regions have been regarded lately with particular attention (5).

In addition to the presence of hypertriglyceridemia and low serum HDL cholesterol, MS and/or insulin resistance are also characterized by an increase of small LDL particles and triglyceride (TG)-rich lipoproteins, features that also contribute to the cardiovascular disease risk (4–8). One of the most important and reliable markers of TG-rich lipoproteins levels is apolipoprotein C-III (apoC-III). ApoC-III is a 79-amino-acid protein synthesized by liver and intestine, which is an essential constituent of circulating particles rich in triacylglycerol, i.e., chylomicrons and VLDLs. ApoC-III inhibits the hydrolysis of TG-rich particles by the lipoprotein lipase and their hepatic uptake mediated by apoE (9, 10). Therefore, the overexpression of the APOC3 gene results in an overt hypertriglyceridemia [as reviewed in ref. (11)]. In spite of this important role of apoC-III in TG metabolism, relatively few data exist in the literature regarding the relationships between apoC-III and hypertriglyceridemia in MS patients (6–8). The atherogenetic role of apoC-III (12–17), as well as that of hypertriglyceridemia in MS for coronary artery disease

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

(CAD) risk (3–6), is well recognized. However, it is still unclear whether elevated levels of TG-rich lipoproteins and apoC-III are highly coexpressed in MS and what their specific contribution is to the higher risk for cardiovascular disease in MS patients.

The APOC3 gene is transcriptionally downregulated by insulin levels (18), and sequences in the promoter region with high affinity for the nuclear transcription factors mediating the insulin response are highly polymorphic (19). Variants at positions −455 and −482 have been shown to have a reduced affinity for the nuclear transcription factors mediating the insulin response (20), so that they appeared to be the first example of a genetic polymorphism in an insulin-responsive element and of "insulin resistance" at the gene level (20).

Recently, we reported that homozygosity for the APOC3 T−455C variant represents an independent factor for the susceptibility to CAD risk (21). Because homozygosity for the −455C allele is associated with increased levels of TG and apoC-III (21), we hypothesized a possible link between the −455C insulin-resistant variant and the MS phenotype, with a potential additive interaction for CAD risk. To this aim, in a large case-control study of subjects angiographically defined as either CAD or CAD-free, we analyzed TG and apoC-III levels according to the presence of both the MS phenotype and the −455C allele.

METHODS

Study population

We studied a total of 873 unrelated adult patients of both genders who were recruited consecutively 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 (the Verona Heart Project). Of these patients, 599 had angiographically documented, severe multivessel coronary atherosclerosis and were candidates for coronary artery bypass grafting (CAD group). As a control group, we considered 274 subjects with angiographically documented normal coronary arteries (CAD-free), examined for reasons other than possible CAD (in >90% of cases, valvular heart disease). The controls were required to have neither history nor evidence of atherosclerosis in other vascular beds. Because 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. At the time of blood sampling, a complete clinical and pharmacological history, including the presence or absence of the traditional cardiovascular disease risk factors, was obtained. According to these data, patients were classified as having MS (MS group) when at least three of the following elements were present: body mass index (BMI) ⩾30 kg/m^2, clinically documented history of hypertension or blood pressure >140/90, fasting glucose >110 mg/dl, plasma TG >150 mg/dl, and HDL cholesterol <40 (50 for females) mg/dl. All the remaining patients (including those with only one or two of the above described clinical features) were considered to be MS-free subjects.

The study was approved by our institutional review boards. Either written or oral informed consent was obtained from all patients.

Biochemical analysis

Samples of venous blood were drawn from each subject in the free-living state after an overnight fast. Serum lipids and the other routine biochemical parameters were determined as previously described (21). Insulin was measured by an immunometric "sandwich" assay (Immulite 2000 Insulin) from Diagnostic Products Corporation, Los Angeles, CA; intra- and interassay variation coefficients of the method were <5%. To obtain an estimate of insulin resistance, we applied the homeostasis model assessment (HOMA) of insulin resistance using the following formula: HOMA = fasting insulin (μIU/ml) × fasting glucose (mmol/l)/22.5 (22). ApoA-I, apoB, and apoE were measured by commercially available nephelometric immunoassays; antisera, calibrators and BNII nephelometer were from Dade Behring, Marburg, Germany. Intra-asssay variation coefficient was calculated on 10 control replicates and interassay on duplicates over 10 days. Imprecision was within manufacturer specifications, i.e., the intra-assay variation coefficients were 2.1%, 1.6%, and 1.98%, and interassay variation coefficients were 3.2%, 2.36%, and 3.98% for apoA-I, apoB, and apoE, 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 manufacturer was implemented on an RXL Dimension Analyzer (Dade International Inc., Newark, DE). Imprecision was assessed on three pools of control sera with low, medium, and high concentrations of apoC-III; intra-assay variation coefficients were 1.84%, 2.02%, and 1.98%, and interassay variation coefficients were 4.4%, 3.4%, and 2.29% for low, medium, and high concentration, respectively. In a subgroup of patients (CAD, n = 80; CAD-free, n = 36), apoC-III was measured in whole serum as well as heparin-Mn^{++} supernatants and heparin-Mn^{++} precipitates. In this way, apoC-III associated with HDL or with LDL+VLDL fractions was separately quantified.

Genotype analysis

Genomic DNA was extracted from whole blood samples by the phenol-chloroform procedure, and all subjects were genotyped for the APOC3 T−455C polymorphism as previously described (21).

Statistical analysis

All computations were performed using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL). Distributions of continuous variables were expressed as means ± SD. Logarithmic transformation was performed for skewed variables, i.e., for 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 clearness, nontransformed data are reported in the Results. Statistical significance for differences in quantitative variables was assessed by Student's unpaired *t*-test, and it was also tested by one-way ANOVA adjusted for age and/or sex (General Linear Model procedure). Qualitative data were analyzed by the χ^2 test. Correlation between log-transformed total apoC-III (measured in whole serum) and log-transformed apoC-III associated with HDL or associated with LDL-VLDL was evaluated by Pearson coefficient.

The T−455C allele and genotype frequencies were compared, by χ^2 analysis, with the values predicted on the basis of the Hardy-Weinberg equilibrium. Lipid variables were compared among patients with different genotypes by ANOVA, using the Tukey procedure for post hoc multivariate comparison of the means. Odds ratio (OR) and 95% confidence interval (95% CI) for CAD or MS were calculated by logistic regression analysis. In particular, to assess the extent to which APOC3 genotypes and MS were associated with CAD, the population was stratified in six patient groups (TT, TC, and CC genotypes, with or without MS)

and OR with 95% CI was estimated by logistic-regression analysis. To provide separate ORs for each genotype, dummy variables were used, considering MS-free TT genotype as the reference group. Adjustment for the risk factors conventionally not associated with MS (age, gender, smoking status, and total cholesterol) was performed by including these covariates in a second set of multivariate logistic regression models. A regression model for formal interaction between MS and the T−455C genotype was also built to estimate the CAD risk proportion associated with the MS genotype term.

RESULTS

The clinical characteristics and the T−455C genotype frequencies of CAD and CAD-free patients are summarized in **Table 1**. CAD patients had more conventional risk factors and significantly higher apoC-III levels than did CAD-free patients (Table 1). Age (OR for CAD = 1.033; 95% CI, 1.016–1.05), male gender (OR = 1.96; 95% CI, 1.22–3.15), LDL cholesterol (OR = 1.456; 95% CI, 1.216–1.74), and smoking (OR = 2.62; 95% CI, 1.64–3.72) were the main predictors of CAD risk. Interestingly, the T−455C polymorphism and MS were also significantly associated with CAD risk. Both the −455C allele (0.403 vs. 0.341; OR for CAD = 1.304; 95% CI, 1.056–1.61) and genotype frequency (OR for CAD = 1.96; 95% CI, 1.22–3.15) were significantly higher in CAD than in CAD-free individuals (Table 1).

The presence of MS was the strongest predictor of risk: a total of 270 subjects presented the features of MS, and they were more represented in the CAD group than in the CAD-free group (38.1% vs. 15.3%; OR for CAD = 3.39; 95% CI, 2.35–4.90). The clinical characteristics and the T−455C genotype frequencies of patients according to MS and MS-free groups are reported in **Table 2**. The two groups were matched for age and total and LDL cholesterol levels, but they differed in several other aspects, both related and unrelated to the classic MS elements (see Table 2), and ~40% of MS patients presented overt insulin resistance (HOMA in the upper quartile of the distribution). MS patients were characterized by increased levels of apoC-III and apoE (Table 2). In particular, 74% of MS patients had apoC-III values higher than the median distribution value of the MS-free population (=10.2 mg/dl). To evaluate whether the increase in apoC-III is a common feature in MS patients, we then computed the risk for MS associated with apoC-III levels divided in quartiles distribution of the study population as a whole. As shown in **Fig. 1**, OR for MS was directly related with apoC-III levels divided in quartiles; this relationship reached statistical significance for apoC-III levels >10.58 mg/dl (values corresponding to the third or fourth quartile), and was confirmed even after adjustment for age, sex, total cholesterol, apoA-I, apoB, apoE, TG, and the other MS elements. In the patient samples analyzed by means of heparin-Mn^{++} centrifugation, total apoC-III was strongly correlated with non-HDL apoC-III concentration ($R = 0.93$; $P < 0.0001$) and much more weakly correlated with HDL-associated apoC-III ($R = 0.38$; $P < 0.001$).

TABLE 1. Characteristics of patients with or without coronary artery disease[a]

Parameters	CAD Patients (n = 599)	CAD-Free (n = 274)	*P*
Age (years)	60.3 ± 9.4	57.9 ± 12.4	<0.01
Male sex (%)	81.1	66.4	<0.001
BMI (kg/height^2)[b]	26.6 ± 3.4	25.3 ± 3.5	<0.001
Cholesterol[b]			
Total (mmol/l)	5.83 ± 1.11	5.52 ± 1.04	<0.001
LDL (mmol/l)	3.89 ± 0.98	3.55 ± 0.92	<0.001
HDL (mmol/l)	1.21 ± 0.32	1.44 ± 0.42	<0.001
TGs (mmol/l)[b]	2.01 ± 1.12	1.47 ± 0.67	<0.001
ApoA-I (g/l)[b]	1.30 ± 0.24	1.43 ± 0.30	<0.001
ApoB (g/l)[b]	1.22 ± 0.29	1.06 ± 0.25	<0.001
ApoC-III (mg/dl)[b]	12.34 ± 4.5	10.65 ± 3.17	<0.001
ApoE (mg/dl)[b]	4.80 ± 3.70	4.33 ± 3.87	NS
Insulin (μIU/ml)[b]	15.80 ± 24.2	15.24 ± 11.6	NS
Uric acid (mmol/l)[b]	0.36 ± 0.08	0.37 ± 0.1	NS
Current smoking (%)	68.7	41.5	<0.001
Hypertension (%)	58.3	31	<0.001
Diabetes (%)	20.7	9.8	<0.01
MS patients	228	42	<0.001
(%)	38.1	15.3	
−455C allele frequency	0.403	0.341	<0.02
(95% CI)	0.375–0.431	0.30–0.38	
T−455C genotypes			
−455 TT (%)	222 (37.1)	115 (42)	
−455 TC (%)	271 (45.2)	131 (47.8)	
−455 CC (%)	106 (17.7)	28 (10.2)	<0.02

ApoA-I, apolipoprotein A-I; BMI, body mass index; TG, triglyceride; MS, metabolic syndrome; CAD, coronary artery disease; 95% CI, 95% confidence interval. Statistical significance for differences was tested by Student's unpaired *t*-test or by χ^2 test when appropriate.

[a] ± Values are means ± SD.

[b] Age- and sex-adjusted values.

TABLE 2. Characteristics of patients with or without MS[a]

Parameters	MS Patients (n = 270)	MS-Free (n = 603)	*P*
Age (years)	58.7 ± 9.6	59.9 ± 10.8	NS
Male sex (%)	80.7	74.6	<0.05
BMI (kg/height^2)[b]	28.4 ± 3.0	24.8 ± 3.1	<0.001
Glucose (mmol/l)[b]	7.95 ± 6.4	5.3 ± 0.95	<0.05
Cholesterol[b]			
Total (mmol/l)	5.81 ± 1.17	5.76 ± 1.06	NS
LDL (mmol/l)	3.84 ± 1.03	3.75 ± 0.94	NS
HDL (mmol/l)	1.09 ± 0.26	1.45 ± 0.36	<0.001
TG (mmol/l)[b]	2.41 ± 1.23	1.53 ± 0.76	<0.001
ApoA-I (g/l)[b]	1.27 ± 0.22	1.43 ± 0.27	<0.001
ApoB (g/l)[b]	1.24 ± 0.31	1.13 ± 0.27	<0.001
ApoC-III (mg/dl)[b]	13.6 ± 5.12	11 ± 3.40	<0.001
ApoE (mg/dl)[b]	4.93 ± 3.65	4.79 ± 3.79	<0.05
Insulin (μIU/ml)[b]	17.57 ± 35.2	14.4 ± 8.96	0.01
Uric acid (mmol/l)[b]	0.37 ± 0.09	0.35 ± 0.09	<0.001
HOMA, upper quartile (%)	39.8	18.8	<0.001
Current smoking (%)	66.7	57	<0.01
Hypertension (%)	76.1	37.7	<0.001
−455C allele frequency	0.380	0.381	NS
(95% CI)	(0.33–0.42)	(0.35–0.41)	
T−455C genotypes			
−455 TT (%)	99 (36.7)	238 (39.5)	
−455 TC (%)	130 (48.1)	272 (45.1)	
−455 CC (%)	41 (15.2)	93 (15.4)	NS

HOMA, homeostasis model assessment. 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.

[a] ± Values are means ± SD.

[b] Sex-adjusted values.

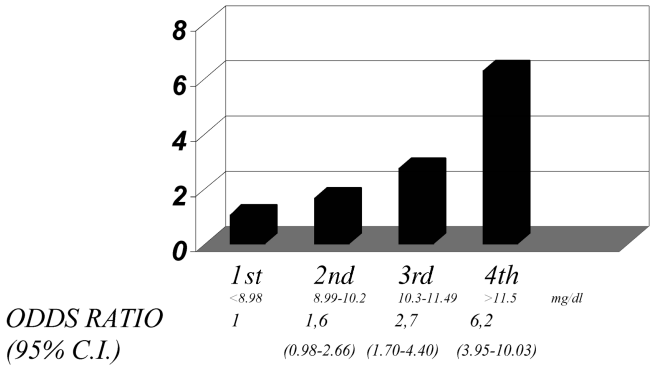


Fig. 1. Odds ratio for metabolic syndrome (MS) in relation to the percentiles distribution of apolipoprotein C-III in the whole population. 95% CI, 95% confidence interval.

There was no difference in T−455C genotype distribution between MS and MS-free patients, and no increase of MS risk was associated with the mutant allele (Table 2). Differences between MS patients with or without CAD were also analyzed (**Table 3**). The two groups of MS patients were well matched for most parameters, including apoA-I and HDL cholesterol levels, but differed for lipid variables such as total cholesterol, TG, apoC-III, apoB, and apoE levels (Table 3).

When the T−455C genotype distribution was reanalyzed, separating the population into different subgroups based on both MS/MS-free and CAD/CAD-free status, an evident asymmetry was observed (**Table 4**): a very high proportion of MS patients, heterozygous or homozygous for the −455C variant, were affected by CAD (87% and 92.7%, respectively), as compared with 58.1% and 73.1% in the corresponding genotype groups without MS (Table 4). On the basis of this observation, in order to assess the extent to which APOC3 genotypes and MS could interact in determining CAD risk, we estimated the OR for CAD after stratifying the whole population in six groups (TT, TC, and CC genotypes, with or without MS) by logistic regression analysis. The results obtained using MS-free patients with a TT genotype as the reference group are detailed in **Fig. 2**. Homozygosity for the −455C allele was by itself associated with CAD risk in both MS and MS-free conditions. Similarly, the presence of MS appeared to be significantly associated with an increased OR for CAD, regardless of the genotype (OR for −455TT MS group = 2.24; 95% CI, 1.30–3.85). The most striking finding, however, was the strong graded increase of risk associated with the carriership of the −455C allele in cases of coexisting MS phenotype, with one mutant allele associated with a factor 2 of multiplication of risk (Fig. 2). Adjustment for the variables not included in the MS definition (age, sex, total cholesterol, and smoking) did not change the results (for the MS-free −455CC subgroup, OR = 1.69; for the MS −455TT subgroup, OR = 2.17; for the MS −455TC subgroup, OR = 3.93; for the MS −455CC subgroup, OR = 8.5). To confirm the interaction between MS and apoC3 genotype, a formal model including the interaction term between these variables was also carried out, and results are reported in **Table 5**.

TABLE 3. Characteristics of MS patients, separated into two groups according to the angiographic evidence of CAD

Parameters	CAD Patients (n = 228)	CAD-Free (n = 42)	*P*
Age (years)	58.7 ± 9.4	59.1 ± 10.6	NS
Male sex (%)	82	73.8	NS
BMI (kg/height^2)[a]	28.5 ± 3.1	28.9 ± 2.4	NS
Cholesterol[a]			
Total (mmol/l)	5.90 ± 1.17	5.21 ± 1.05	<0.001
LDL (mmol/l)	3.92 ± 1.03	3.49 ± 0.97	<0.02
HDL (mmol/l)	1.03 ± 0.25	1.09 ± 0.32	NS
TGs (mmol/l)[a]	2.59 ± 1.28	2.01 ± 0.69	0.001
ApoA-I (g/l)[a]	1.22 ± 0.21	1.26 ± 0.26	NS
ApoB (g/l)[a]	1.27 ± 0.31	1.07 ± 0.26	<0.001
ApoC-III (mg/dl)[a]	14.03 ± 5.2	12.2 ± 4.2	<0.02
ApoE (mg/dl)[a]	5.23 ± 3.86	4.24 ± 1.86	<0.01
HOMA, upper quartile (%)	40.1	39.5	NS
Uric acid (mmol/l)[a]	0.37 ± 0.09	0.40 ± 0.1	NS
Current smoking (%)	68.5	57.1	NS
Hypertension (%)	76	69.2	NS
Diabetes (%)	41	35.1	NS

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.

[a] ± Values are means ± SD.

To evaluate whether the higher risk for CAD related to the presence of the APOC3 −455C variant was to be ascribed to the abnormal lipid metabolism in subject carriers of the mutation, we performed further analyses. After exclusion of individuals treated with statins or fibrates at the time of blood sampling (n = 310, most of them CAD patients), lipid and apolipoprotein profiles in the different genotype groups with or without MS were, therefore, compared. A total of 563 patients were then analyzed, and the results are reported in **Table 6**. As expected, MS patients had less apoA-I and HDL cholesterol and higher TG and apoC-III levels than did MS-free subjects, but homozygous mutants for T−455C also had a more pronounced increase in TG and apoC-III in comparison with the other MS genotype groups (Table 6). The difference between groups for apoC-III did not, however, reach statistical significance, probably due to the relatively small sample size. In spite of a clear TG- and apoC-III-raising trend for the −455CC group, the genotype effects considered separately in the subgroup of MS-free subjects were statistically null; in MS subjects, on the contrary, −455C homozygosity was associated with significantly higher TG but not with higher apoC-III levels (Table 6). The further reduction of sample size, however, limited the value of both of these results.

Finally, we examined the relative expression of MS elements in subjects either carrying or not carrying one mutant allele. There was no significant difference in the degree of hyperglycemia, hypertension, low HDL cholesterolemia, or hypertriglyceridemia between the two groups, but obesity was significantly less frequent in −455C carriers as compared with the noncarrier group (21.6% vs. 34.8%; $P < 0.05$). The groups were well matched for all other clinical and biochemical characteristics, but −455C carri-

TABLE 4. Apo C-III genotype distribution in patients with or without MS, with or without CAD

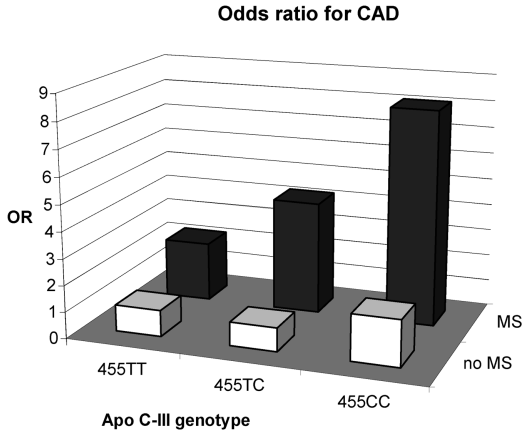
Diagnosis	Apo CIII Genotype (No MS)			ApoC III Genotype (With MS)			Total Patients
	−455TT	−455TC	−455CC	−455TT	−455TC	−455CC	
CAD-free patients	93	114	25	22	17	3	274
Within DG (%)	33.9	41.6	9.1	8	6.2	1.1	
Within genotype (%)	39	41.9	26.9	22.2	13	7.3	
CAD patients	145	158	68	77	113	38	599
Within DG (%)	24.2	26.3	11.3	12.8	18.9	6.3	
Within genotype (%)	61	58.1	73.1	77.8	87	92.7	
Total patients	238	272	93	99	130	41	873

ers had BMIs statistically lower than did patients with the −455TT genotype (28.02 ± 2.93 kg/m^2 vs. 28.92 ± 2.9 kg/m^2; $P < 0.05$).

DISCUSSION

The results of the present study suggest that increased apoC-III levels are a common feature of the MS phenotype. ApoC-III levels were, indeed, significantly increased in the majority of MS patients, and the probability of having MS was directly correlated with apoC-III levels divided into quartiles (Fig. 1). Moreover, a relevant interaction able to modulate CAD risk was observed between MS phenotype and the carriership of an APOC3 variant, predisposing to an insulin resistant, abnormal apoC-III production.

Genetic and/or acquired mechanism(s) leading to the development of MS may, directly or indirectly, imply an increased apoC-III synthesis, due either to gene activation and/or to stabilization of its transcript, or to a decreased catabolism of the protein. The former mechanism may reasonably be invoked in the case of carriership of the −455C genetic variant. It has been shown that the most frequent promoter variant, −455T, is associated with a 40–50% insulin-mediated downregulation of APOC3 gene expression (20). In contrast, the less common −455C variant seems associated with the complete loss of the insulin-mediated suppression of APOC3 gene transcription (20). Based on these findings, −455C carriership should lead, in vivo, to an increased synthesis of apoC-III in a proportion ranging from 25% to 50%, depending on the presence of the variant in heterozygosis and homozygosis, even in the presence of an effective cell insulin action. A recent study reported for the first time the reference limits for apoC-III in a large, healthy, French Caucasian population, in which the level corresponding to the 50th percentile for middle-aged men was 10 mg/dl (23). Considering the French values as a reference, our MS Italian Caucasians with either the −455TC or −455CC genotype had apoC-III levels 29% and 53% higher, respectively. Patients with the same genotypes but without MS showed, however, increments of apoC-III by 7% and 11.3%, respectively. Using the apoC-III mean values observed for −455TT



	-455TT	-455TC	-455CC	
No MS	1 (Reference)	0.89 (0.62-1.26)	1.74 (1.03-2.95)	OR for CAD (95% C.I.)
MS	2.24 (1.30-3.85)	4.26 (2.40-7.56)	8.12 (2.44-27.1)	OR for CAD (95% C.I.)

Fig. 2. Odds ratio for coronary artery disease in different genotype groups of patients with or without MS.

TABLE 5. Interaction model[a] between T−455C genotype and MS on CAD risk

Covariates	β Coefficient	Significance	OR (95% CI)
T−455C genotype		0.04	
−455TC	−0.145	0.40	0.86 (0.61–1.21)
−455CC	0.522	0.05	1.65 (1.0–2.75)
MS	0.694	0.023	2 (1.1–3.63)
Interaction term		0.03	
−455TC MS[a]	1.26	0.01	3.51 (1.33–9.24)
−455CC MS[a]	0.91	0.26	2.49 (0.5–12.5)

OR, odds ratio.
[a] By logistic regression.

MS-free subjects in our population as a reference (10.6 mg/dl, see Table 4), we observed that percent increases of apoC-III for MS patients were substantially similar to those reported in the French study (22% and 44% of increment for heterozygous and homozygous, respectively). There were none or only marginal differences (6.6%) in comparison with the corresponding genotype groups of MS-free patients. Therefore, the comparison of the data predicted by in vitro gene expression studies with those obtained in our human study suggests that the apoC-III-raising effect inherent in the genetic variant cannot be expressed in the absence of MS and becomes detectable only with the coexistence of MS. The opposite situation does not necessarily occur. In fact, increased apoC-III levels in MS do not require the presence of the −455C variant. Indeed, apoC-III was increased also in noncarriers with MS (Table 6), and no difference in distribution of T−455C genotypes was observed between MS and MS-free patients (Table 2). This observation excludes an etiologic role for the gene variant in MS and implies that other independent apoC-III-raising mechanisms, such as a reduced protein catabolic rate, also have to be activated in MS (8). In this context, it should be interesting to verify the role of other recently discovered TG-raising genetic variants on the same apoA3/A4/A5 genes cluster (24–26). Similarly, we cannot exclude the possibility that the −455C mutation is in linkage disequilibrium with these new variants, resulting in being merely a marker for some of them. Our findings indicate, however, that the interaction between the insulin-resistant T−455C gene polymorphism and MS seems to play a synergic role in the expression of MS-associated lipid abnormality and in its impact on CAD risk.

In our study, MS patients affected or not by CAD differed exclusively in lipid metabolism parameters, i.e., total and LDL cholesterol, apoB, apoC-III, and apoE levels (Table 3), suggesting a primary role for lipid abnormalities in CAD risk associated with MS. Furthermore, no differences were observed in apoA-I and HDL cholesterol levels between the two groups. Increased apoB and cholesterol in circulating lipid particles is a well-known feature in CAD patients, but the notion of increased apoC-III and apoE levels in MS patients with CAD is not generally accepted. Sparse information concerning the different MS elements, such as obesity (7, 8, 27) or type II diabetes (28, 29), exist, generally confirming the relation between hypertriglyceridemia and increase of apoC-III and apoE, but there are no specific studies reporting on MS as a unique complex. To the best of our knowledge, there are no studies evaluating the CAD risk of MS patients in relation to the extent of apoC-III increase. Several reports have independently confirmed the role of apoC-III in increasing CAD risk without distinguishing between patients with or without MS (12–17).

It is possible that more accurate information on CAD risk could be obtained by evaluation of non-HDL apoC-III fraction (17), and the lack of availability of these parameters for analysis in our study is certainly a limitation of the present work. However, in a subgroup of patients, total apoC-III concentration was much more strongly correlated with non-HDL fraction ($R = 0.93$) than with HDL apoC-III ($R = 0.38$), suggesting that the informative power of the total concentration of the apolipoprotein should be similar to that given by the fraction not associated with HDL.

In our population, MS was associated per se with an odds ratio for CAD of 3.39 (95% CI, 2.35–4.90). Consistent with our previous report (21), −455C homozygosity was also associated with CAD per se, regardless of coexisting MS (see Fig. 2, −455CC no MS group). The new and

TABLE 6. Lipid and apolipoprotein profile in the different genotype groups of patients with or without MS, and free of hypolipidemic therapy (n = 563)[a]

	Without MS				With MS			
APOC3 Genotype	−455TT (n = 155)	−455TC (n = 182)	−455CC (n = 54)	P^b	−455TT (n = 63)	−455TC (n = 86)	−455CC (n = 23)	P^b
LDL cholesterol (mmol/l)	3.82 ± 1.02	3.66 ± 0.84	3.78 ± 0.92	NS	3.82 ± 1.06	3.98 ± 1.05	3.64 ± 0.99	NS
HDL cholesterol (mmol/l)	1.39 ± 0.39	1.43 ± 0.39	1.40 ± 0.32	NS	1.04 ± 0.26[c]	1.03 ± 0.31[c]	0.95 ± 0.15[c]	NS
TGs (mmol/l)	1.48 ± 0.69	1.51 ± 0.77	1.68 ± 1.09	NS	2.39 ± 0.99[c]	2.25 ± 0.89[c]	3.04 ± 1.55[c,d]	<0.05
ApoA-I (g/l)	1.37 ± 0.32	1.39 ± 0.24	1.43 ± 0.24	NS	1.23 ± 0.22[c]	1.23 ± 0.22[c]	1.16 ± 0.18[c]	NS
ApoB (g/l)	1.14 ± 0.28	1.10 ± 0.25	1.15 ± 0.30	NS	1.24 ± 0.28[e]	1.24 ± 0.31[e]	1.21 ± 0.38	NS
ApoC-III (mg/dl)	10.6 ± 3.03	10.7 ± 3.15	11.3 ± 4.43	NS	12.9 ± 4.74[c]	12.9 ± 4.60[c]	15.3 ± 5.63[c]	NS
ApoE (mg/dl)	4.58 ± 4.0	4.25 ± 1.26	4.55 ± 2.72	NS	5.63 ± 6.56	4.65 ± 1.72	6.00 ± 3.12	NS

[a] Comparison between all six groups of patients:
[b] Comparison between genotype groups in MS or MS-free patients, respectively (by ANOVA, post hoc Tukey test).
[c] Significantly different ($P < 0.05$) from every group of patients without MS (by ANOVA, post hoc Tukey test).
[d] Significantly different ($P < 0.05$) from every other group of patients (by ANOVA, post hoc Tukey test).
[e] Significantly different ($P < 0.05$) from −455TC group of patients without MS (by ANOVA, post hoc Tukey test).

interesting finding was that a graded, strong interaction in determining CAD risk emerged when both MS and carriership of the −455C gene variant coexist in the same individual (Fig. 2, Table 5). This result is important for at least two reasons: *i*) it strongly supports the preeminent role of apoC-III-rich lipoproteins in increasing CAD risk in MS patients, because the mathematical relation (a factor 2) we observed between the graded ORs for CAD risk and −455C heterozygosity or homozygosity is difficult to explain without accepting the apoC-III-raising effect of the gene variant on an allelic basis; and *ii*) a strong interaction was also demonstrated in the cases of heterozygosis for the −455C allele, thus extending the potential impact of the gene variant in terms of general population risk. Because in the general population, ~50–70% of individuals are −455C carriers (30–33), the risk deriving from the interaction of this polymorphism with acquired or lifestyle factors favoring the development of MS is potentially very relevant.

Among all factors, one of the most important is probably obesity (34) or, more generally, a condition of increased calorie availability derived from excess alimentary intake. Its role has been particularly stressed in the case of the association of hyperlipidemia with MS and insulin resistance, in which a liver abundance of free fatty acids flux stimulates the assembly and secretion of TG-rich lipoproteins (35). Recently, obese men with insulin resistance (mean BMI, 33.6 ± 4.1 kg/m^2) were demonstrated to have higher plasma apoC-III and TG-rich lipoprotein levels and a lower estimated fractional catabolic rate of these particles (8). Both increased synthesis and reduced catabolic rate of apoC-III rich particles seem, therefore, to be involved in hyperlipidemia observed in MS patients, although the relative contribution of each mechanism remains to be specified. In the case of our MS patients, obese individuals (BMI ⩾30 kg/m^2) were significantly less numerous in −455C carriers than in noncarriers (21.6% vs. 34.8%), and carriers had significantly lower BMIs than did noncarriers. There was no apparent reason for this difference (particularly considering that it is the result of a post hoc analysis) unless a causal effect is attributed to −455C carriership. If a reduced catabolic rate of apoC-III-rich lipoproteins is the prevailing mechanism leading to hyperlipidemia in obesity (8), then obese individuals should be preferentially found in the group of −455C noncarriers rather than in the −455C carriers. Another reasonable interpretation is that in −455C carriers, a relatively smaller BMI increase and liver fatty acid flux are required to trigger the cascade of metabolic events peculiar to MS, i.e., an increased synthesis of TG and apoC-III. As a consequence, a generally lower threshold of risk for MS (and, in turn, for CAD) may be hypothesized for −455C carriers, with all the obvious results in terms of cardiovascular disease prevention.

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