Contribution of Apolipoprotein C-III Gene Variants to Determination of Triglyceride Levels and Interaction With Smoking in Middle-Aged Men


Abstract—Variation within and around the apolipoprotein C-III (APOC3) gene has been associated with elevated triglyceride (Tg) levels and cardiovascular disease. The associations of 4 polymorphic variants in the APOC3 gene (3238>C in the 3′ untranslated region [SstI], 1100>C>T in exon 3, −482>C>T in the insulin-responsive element, and −2854>T>G in the APOC3-A4 intergenic region) with plasma Tg and cholesterol levels and their interaction with smoking have been investigated in the Second Northwick Park Heart Study (NPHSII), a large cohort of healthy men (n=2745). Analyzing the variants separately showed that 3238G, 1100T, and −482T alleles were all associated with raised Tg levels. For the 3238C>G and −482C>T sites, the Tg-raising effect appeared to depend on smoking status (test for interaction, P=0.042 and P=0.009, respectively), but for the 1100C>T site, the effect was constant irrespective of smoking status (test for interaction, P=0.27). The −2854>T>G site was not associated with effects on Tg levels in this sample. Because all of the variants showed significant allelic association, regression modeling was used to quantify the relative size of each effect and to assess whether the effects of the separate variants were independent. The 1100C>T variant had an independent effect on Tg levels that was not influenced by smoking status (increase of 8.2% in Tg with each T1100 allele), whereas the −482C>T variant had a separate effect that was dependent on smoking (increase of 13.7% in Tg for each −482T allele in current smokers, 8.6% in exsmokers, and −7.4% in those who never smoked). The 3238C>G variant did not show a separate independent effect on Tg concentration. Thus, by use of the regression model, it was possible to estimate how mean Tg levels would vary in groups of individuals with respect to APOC3 genotype and smoking information. Analysis in this large group of healthy men has allowed the identification of a statistically robust APOC3 genotype-smoking interaction, which now warrants further molecular study. (Arterioscler Thromb Vasc Biol. 2000;20:2663-2669.)

Key Words: APO A1-C3-A4 gene cluster ■ apolipoprotein B ■ linkage disequilibrium ■ insulin-responsive element

Apolipoprotein C-III (apoC-III), a 79-amino acid glycoprotein, is synthesized by the liver and small intestine and is a major component of triglyceride (Tg)-rich lipoproteins (TRLs) and HDL. ApoC-III is important in the regulation of plasma Tg concentration.1 Accumulating evidence has suggested that a subset of apoB-containing particles, such as VLDL and IDL, are related to the progression of coronary artery disease (CAD).2 ApoC-III and serum Tg levels are positively correlated.3,4 Furthermore, apoC-III acts as a marker of TRL metabolism, and the ratio of apoC-III HDL to VLDL has been found to be negatively associated with the progression of atherosclerosis in a number of studies.5,6

The gene for apoC-III has been mapped to chromosome 11q23.3 and is flanked by the genes for apoA-I and apoA-IV in a 15-kb gene cluster.8 The rare allele of the polymorphic SstI site (3238G) in the 3′-untranslated region of the apolipoprotein C-III (APOC3) gene has frequently been associated with raised apoC-III and Tg levels3,9 and with CAD.10 Because no function has been attributed to this polymorphism, it was thought that this effect was due to linkage disequilibrium with another functional variant. When variation in an insulin-responsive element (IRE) in the APOC3 promoter was identified, this was thought to be a strong candidate for the SstI effect, because loss of insulin regulation could conceivably result in overexpression of apoC-III.11 However, in a number of association studies, the IRE polymorphisms could not explain fully the effect observed at the SstI site.9,10,12

ApoC-III inhibits lipoprotein lipase (LPL) in vitro,13 and inhibition of LPL-activated lipolysis by VLDL-associated apoC-III may prolong the time that the arterial wall is exposed to this atherogenic particle.2 The precise function

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and mechanism of action of apoC-III in lipid metabolism is still uncertain, but some recent insight has come from apoC-III transgenic mice studies. The apoc3 knockout mice exhibited a 70% reduction in fasting Tg levels, and postprandial hypertriglyceridemia was abolished.14 Human APOC3 transgenic mice had a 40% increase in apoC-III levels, which resulted in a doubling of plasma Tg.15 This effect is thought to be due to substitution of apoE by apoC-III on VLDL particles, affecting LDL receptor or LDL receptor–related protein recognition. However, this assumption was contradicted by the absence of severe hypertriglyceridemia in apoE knockout mice.16 An investigation into APOC3 transgenic/apoe null mice17 showed a marked decrease in VLDL glycosaminoglycan binding that was independent of apoE. The investigators suggested that the predominant mechanism of apoC-III–induced hypertriglyceridemia appeared to be decreased lipolysis at the cell surface. In addition, hepatic production of VLDL-Tg was moderately increased, and increased nonesterified fatty acid (NEFA) levels were also observed. Smokers are insulin resistant and show impaired lipid metabolism, with a reduced Tg clearance after a mixed meal.18 Smoking also acutely increases the activity of the sympathetic nervous system and thus raises the concentrations of circulating catecholamines.19 Therefore, smoking will effect the function of hormone sensitive lipase (HSL) and LPL, both of which are under the control of insulin and catecholamines.20 Elevated catecholamine concentrations activate HSL, leading to energy mobilization and increased levels of NEFAs in the circulation, and downregulate LPL.21

To determine which APOC3 polymorphism(s) have the greatest impact on lipid concentrations, we investigated 4 different polymorphic sites that span the APOC3 gene region. In addition to the 3238C>G (SsrI) and −482C>T (IRE) sites, we examined 2 other sites. The first is a 1100C>T base change in exon 3, where the T allele has been reported to be associated with higher apoC-III levels in healthy subjects22 and with elevated concentrations of apoC-III, plasma Tgs, VLDL, and LDL particles in patients with familial combined hyperlipidemia.23 The second is a novel base-change −2854T>G,24 which extends our investigation further upstream into the APOC3-A4 region. Because the effects of smoking on insulin resistance and lipid intolerance are recognized, we investigated the possibility of an interactive effect between genotype and smoking on the various lipid traits available.

**Methods**

**Sample Population**

The Second Northwick Park Heart Study (NPHSII) is a large prospective study of healthy middle-aged (50- to 61-year-old) men from 9 UK general practices. Of the initial cohort of 3052 men, 2745 were white and had DNA available for genotyping. The study has been ongoing for 9 years, and men were followed up annually for lipids (5 years) and throughout the study for CAD events. Details of the study have been described previously.25 Briefly, 9 general practices participated in the study, and all men with a history of unstable angina, myocardial infarction, cerebrovascular disease, life-threatening malignancy, or regular medication with aspirin or anticoagulants were excluded. Each respondent attended in the nonfasting state after having been instructed to avoid a cooked breakfast or a heavy meal before examination. They had refrained from smoking and vigorous exercise from midnight beforehand. Baseline characteristics and demographic information were ascertained by means of a questionnaire completed at entry to the study. From this, subjects were classified as never smokers, exsmokers (cessation for minimum of 1 year), or current smokers. Serum Tg and cholesterol concentrations were determined by automated enzymatic procedures with reagents from Sigma Chemical Co. ApoB and apoa-I were measured by immunoturbidimetry with reagents from Incstar.

**Polymorphism Detection**

All variants analyzed were restriction fragment length polymorphisms, and assays were performed as described previously.25 The fragments were separated by using 5% to 10% polyacrylamide microtiter array diagonal gel electrophoresis.26

**Data Analysis**

Data were entered onto an EXCEL spreadsheet (Microsoft) and tested for deviation from Hardy-Weinberg equilibrium by using a χ² test. Linkage disequilibrium (Δ) was calculated by EXCEL and the method of Chakravarti et al.27 Statistical analysis was performed by using STATA (Intercooled STATA Version 5.0, STATA Corp). Cholesterol, Tg, apoA-I, and apoB were available at baseline, and cholesterol and Tg were available for 5 years. ApoB and apoA-I were only available in a subsample for technical reasons (there were no significant differences in any of the characteristics between those with and those without apoA-I and apoB measures). Tg and apoB levels were logarithmically transformed. Differences in clinical and biochemical characteristics according to smoking status were analyzed by 1-way ANOVA. The Welch test was used when there was evidence of unequal group variances. Associations between genotype and Tg, cholesterol, apoB, and apoa-I were initially examined by ANOVA and included tests for smoking interactions.

Polymorphism modeling was used to investigate the effect of any interaction between APOC3 variants and smoking on levels of baseline Tg. Initial investigations concentrated on each polymorphism separately. A codominant model was assumed for each of the 4 variants such that for each variant, the effect of possessing 2 rare alleles would be twice that of possessing 1 rare allele. In other words, the effect on mean Tg associated with the possession of a rare allele would be independent of the second allele. To incorporate this structure into the regression modeling, each variant was coded as 0, 1, or 2 according to the number of rare alleles present. The variants were then included in the models as continuous variables, which enabled the parameter estimates and standard errors associated with the rare alleles to be calculated directly. As coded, the effect of each allele on mean Tg corresponded exactly with that of a 1-unit increase in each variable. For each polymorphism, the assumption of codominance was checked by including a second variable, coded 1 for homozygous carriers of the rare allele and 0 for others, in the regression model. Assessing the significance these additional terms produced no evidence against a codominant structure. The significance of all the parameters in the resulting models, including the interaction between smoking and genotype, was assessed with an F test. Smoking was included as a categorical variable with 3 levels (see Sample Population). Adjustment was also made for possible confounding factors, which included body mass index (BMI, as a logged continuous variable) and clinic (as a fixed categorical variable with 9 levels).

Finally, to quantify the relative size of the effects observed in the separate models and to assess whether the 4 variants were acting on Tg independently, all the polymorphisms were included in 1 regression model by use of a stepwise procedure. The significance of the variables was again assessed by F test. Raw parameter estimates (on the additive log scale) and 95% CIs from the final model are given in Table 3. For the purposes of the model, it was assumed that there was no allelic interaction between the 4 variants.

**Results**

Baseline clinical and biochemical characteristics are shown in Table 1. Smoking status was ascertained for 2715 of the participants: 867 men (32%) had never smoked, 1075 (40%) were exsmokers, and 773 (28%) were current smokers. The 3
groups did not differ significantly in mean age, cholesterol, and apoA-I or apoB levels. However, current smokers had the lowest mean BMI compared with exsmokers, who had the highest, and never smokers, who had intermediate measures (ANOVA, \( P<0.0001 \)). Exsmokers had mean Tg concentrations with a magnitude similar to that of current smokers, with never smokers having much lower levels in comparison (ANOVA, \( P<0.0001 \)). Clinic (general practice) was also found to be associated with smoking status (\( \chi^2 \) test, \( P=0.032 \); data not tabulated). In addition, both BMI (correlation, \( P<0.0001 \)) and clinic (1-way ANOVA, \( P<0.0001 \)) were highly significantly associated with Tg levels. As a result, BMI and clinic were included in the regression modeling as possible confounders.

The locations of the 4 polymorphic sites in the APOC3 gene region and the allele frequencies are shown in Figure 1. The genotype distribution did not deviate from the expected Hardy-Weinberg equilibrium for any of the polymorphisms studied. The rare allele frequencies (and CI) were as follows: 0.24 (0.23 to 0.25) for 3238G, 0.21 (0.20 to 0.22) for 1100T, and 0.33 (0.32 to 0.35) for 2854G. The linkage disequilibrium between the markers is found to suggest that smoking modified the Tg-raising effects of the 1100T allele (test for interaction, \( P=0.096 \)). Because all 4 APOC3 variants are in allelic association, it is unclear which polymorphisms were more likely to be etiologic and which were merely markers for these effects. Therefore, additional regression modeling was performed to ascertain whether any of these polymorphisms were having independent effects on Tg and to characterize the smoking interaction in more detail.

When the 3238C>G (SsrI) variant was included in a model with the -482C>T (IRE) variant, the interaction between smoking and -482C>T genotype remained significant (\( P=0.0045 \)). However, there was no evidence for further interaction between 3238C>G and smoking (\( P=0.48 \)), although the main Tg-raising effect of the G3238 allele was still observed (\( P=0.001 \)), irrespective of smoking status. After addition of the (exon3) 1100C>T (IRE) variant, the evidence for interaction between -482C>T and smoking was still strong (\( P=0.005 \)), and there was also strong evidence for a smoking-independent raising effect of the 1100T allele on Tg levels (\( P=0.001 \)). However, the effect of the 3238G allele on Tg was no longer present (\( P=0.15 \)). Therefore, the effect of 3238C>G on Tg levels was not found to be Independent of -482C>T or 1100C>T, but the -482C>T and 1100C>T sites were found to be acting independently of each other. The

![Figure 1. APO A1-C3-A4 gene region.](image-url)

**TABLE 1. Clinical and Biochemical Characteristics of Subjects**

<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>Never Smokers</th>
<th>Exsmokers</th>
<th>Current Smokers</th>
<th>( P ) (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>867 ± 3.37</td>
<td>1075</td>
<td>772</td>
<td>0.32</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.0 ± 3.13</td>
<td>26.8 ± 3.63</td>
<td>27.5 ± 3.53</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.71 ± 0.97</td>
<td>5.77 ± 1.01</td>
<td>5.70 ± 1.05</td>
<td>0.22</td>
</tr>
<tr>
<td>Tgs, mmol/L</td>
<td>1.65 ± 0.83</td>
<td>1.85 ± 0.99</td>
<td>1.87 ± 0.98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>1.62 ± 0.35</td>
<td>1.61 ± 0.34</td>
<td>1.61 ± 0.33</td>
<td>0.57</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>0.88 ± 0.27</td>
<td>0.88 ± 0.27</td>
<td>0.87 ± 0.25</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*Geometric means and approximate SD.
†Welch test, equality of group variances not assumed.

In determining Tg levels. Significant interaction was found between smoking and the -482C>T (\( P=0.009 \)) and 3238C>G (\( P=0.042 \)) sites (see Table 2). Possession of the 1100T allele was significantly associated with raised mean Tg levels (\( P<0.0001 \)). However, no additional evidence was found to suggest that smoking modified the Tg-raising effects of the 1100T allele (test for interaction, \( P=0.26 \)). There was no statistical evidence to suggest that the -2854T>G variant was associated with Tg levels (\( P=0.096 \)). Because all 4 APOC3 variants are in allelic association, it is unclear which polymorphisms were more likely to be etiologic and which were merely markers for these effects. Therefore, additional regression modeling was performed to ascertain whether any of these polymorphisms were having independent effects on Tg and to characterize the smoking interaction in more detail.

When the 3238C>G (SsrI) variant was included in a model with the -482C>T (IRE) variant, the interaction between smoking and -482C>T genotype remained significant (\( P=0.0045 \)). However, there was no evidence for further interaction between 3238C>G and smoking (\( P=0.48 \)), although the main Tg-raising effect of the G3238 allele was still observed (\( P=0.001 \)), irrespective of smoking status. After addition of the (exon3) 1100C>T (IRE) variant, the evidence for interaction between -482C>T and smoking was still strong (\( P=0.005 \)), and there was also strong evidence for a smoking-independent raising effect of the 1100T allele on Tg levels (\( P=0.001 \)). However, the effect of the 3238G allele on Tg was no longer present (\( P=0.15 \)). Therefore, the effect of 3238C>G on Tg levels was not found to be independent of -482C>T or 1100C>T, but the -482C>T and 1100C>T sites were found to be acting independently of each other. The
The 1100C>T site, irrespective of smoking status, was to raise Tg levels by 8.2% \[\exp(0.079)\] for each 1100T allele compared with the 1100C genotype. The effect on Tg levels of the -482T allele was dependent on smoking habit. The main effect at the -482C>T site is given for the baseline group of never smokers, for whom possession of each -482T allele lowered Tg levels by 7.4% \[\exp(-0.077)\]. Smoking modified this result such that in exsmokers the effect on Tg levels was 8.6% \[\exp(0.083)\] greater than this baseline estimate, giving an overall raising effect of 0.6% \[\exp(-0.077+0.083)=1.006]\] for each -482T allele, and in current smokers, it was 13.7% greater \[\exp(0.128)=1.137]\], indicating an elevation of 5.2% for each -482T allele \[\exp(-0.077+0.128)=1.052]\]. Because genotype is fixed but smoking status may be altered, the effect of smoking on Tg levels with use of the model is shown for each -482C>T genotype (Table 4).

The regression model allows an estimate to be made of the effect of a particular genetic profile combined with smoking information for calculation of the effects in groups of subjects most at risk of high Tg and the subsequent risk of CAD. For example, the most advantageous genotype and smoking combination in men would be the following: possession of the APOC3 genotype 1100CC and of -482TT and to have never smoked; their estimated mean Tg would be lowered by 7.4% for each -482T allele \[\exp(-0.077+0.077)=0.86]\], a total Tg-lowering effect of 14% \(\frac{7.4+7.4}{2}=8.6\%\) (compared with the combination of 1100CC, -482CC, and never having smoked). The most harmful combination would be the APOC3 genotype 1100TT, -482TT, and current smoking; their estimated mean Tg will be raised by 8.2% for each 1100T allele, lowered by 7.4% for each -482T allele, raised by 7.6% for being current smokers, and raised by an additional 13.3% for each -482T allele plus being current smokers \[\exp(0.079+0.079-0.077-0.077+0.074+0.128+0.128)=1.40\]. Thus, the estimated effect of this harmful genotype and smoking status is a Tg-raising effect of \(\approx 40\%\) (compared with 1100CC, -482CC, and never having smoked).

**Discussion**

The SstI site (3238C>G) in the 3' untranslated region of the APOC3 gene was first described in 1983, and its association with hypertriglyceridemia has been confirmed in the majority of studies and a review. We have confirmed this relationship between the APOC3 SstI polymorphism and serum Tg concentration in this large study of healthy middle-aged men (NPHSII). However, other polymorphisms both within the APOC3 gene itself and in the promoter region also show associations with Tg levels. This may be due, at least partly, to the allelic association between the rare alleles of the 4 APOC3 variants. We have confirmed these genetic effects and observed that they are modified by smoking. Failure to allow for smoking would have obscured the association with the -482C>T variant and in part explain why other studies

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**TABLE 2. Geometric Mean (Approximate SD) Tg Levels by APOC3 Genotype and Smoking Status**

| Genotype | Never Smokers | Exsmokers | Current Smokers | P
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean±SD</td>
<td>n</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>3238C&gt;G</td>
<td>CC</td>
<td>670</td>
<td>1.52±0.72</td>
<td>844</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>133</td>
<td>1.59±0.81</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>1</td>
<td>0.99</td>
<td>9</td>
</tr>
<tr>
<td>1100C&gt;T</td>
<td>CC</td>
<td>502</td>
<td>1.51±0.71</td>
<td>635</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>278</td>
<td>1.55±0.77</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>40</td>
<td>1.68±0.79</td>
<td>53</td>
</tr>
<tr>
<td>-482C&gt;T</td>
<td>CC</td>
<td>434</td>
<td>1.58±0.76</td>
<td>549</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>283</td>
<td>1.51±0.74</td>
<td>329</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>39</td>
<td>1.39±0.47</td>
<td>57</td>
</tr>
<tr>
<td>-2854T&gt;G</td>
<td>TT</td>
<td>364</td>
<td>1.53±0.73</td>
<td>437</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>351</td>
<td>1.53±0.77</td>
<td>451</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>95</td>
<td>1.53±0.59</td>
<td>121</td>
</tr>
</tbody>
</table>

Values are mean±approximate SD.

*Codominant models were first fitted for each polymorphism, assuming no interaction with smoking, and the effect of each genotype was examined by F test (models were adjusted for smoking, clinic, and BMI). Initial results appeared to show strong association between Tg and 3238C>G and 1100C>T genotypes only.

†The 4 polymorphisms were tested separately for interaction with smoking status by F test, assuming a codominant model (models were adjusted for clinic and BMI). Results show evidence that the effect of the 3238C>G genotype on Tg levels depends on smoking status. The effect of the -482C>T genotype is also dependent on smoking status but would have been missed entirely if interaction terms had not been included in the model.

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-2854T>G variant did not appear to affect Tg levels and was not included in the final model shown in Table 3.

The regression model allows an estimate to be made of the effect of a particular genetic profile combined with smoking information for calculation of the effects in groups of subjects most at risk of high Tg and the subsequent risk of CAD. For example, the most advantageous genotype and smoking combination in men would be the following: possession of the APOC3 genotype 1100CC and of -482TT and to have never smoked; their estimated mean Tg would be lowered by 7.4% for each -482T allele \[\exp(-0.077+0.077)=0.86\], a total Tg-lowering effect of 14% \(\frac{7.4+7.4}{2}=8.6\%\) (compared with the combination of 1100CC, -482CC, and never having smoked). The most harmful combination would be the APOC3 genotype 1100TT, -482TT, and current smoking; their estimated mean Tg will be raised by 8.2% for each 1100T allele, lowered by 7.4% for each -482T allele, raised by 7.6% for being current smokers, and raised by an additional 13.3% for each -482T allele plus being current smokers \[\exp(0.079+0.079-0.077-0.077+0.074+0.128+0.128)=1.40\]. Thus, the estimated effect of this harmful genotype and smoking status is a Tg-raising effect of \(\approx 40\%\) (compared with 1100CC, -482CC, and never having smoked).

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**Figure 2.** Effect of APOC3 genotype and smoking status on mean Tg levels. Adjusted mean Tg values correspond with those in Table 2.
have failed to show significant and separate effects associated with this variant over and above that of the 3238C
(\texttt{I}). 10,12 In addition, the inclusion of smoking as a covariate
with this variant over and above that of the 3238C
have failed to show significant and separate effects associated
in the plasma and circulates as NEFA complexed to
hormone-sensitive lipase (HSL), the rate-limiting step for mobilization of adipose tissue Tg, which is released
by the liver, leading to an increased levels of VLDL31 and, consequently, elevated
with an impaired Tg clearance after a
mixed meal.18 The increase in circulating catecholamines
from the sympathetic nervous system and the subsequent
hyperinsulinemia will affect the regulation of both HSL and
enzymes that regulate Tg metabolism.19
The mechanism of the Tg-raising effect associated with the
1100C>T variant is unknown. The 1100C
in exon 3 and does not encode an amino acid change.
Therefore, it is not likely to be functional and may be in
linkage disequilibrium with another variant, possibly situated
in the \texttt{APOAI-C3} intergenic region (none have so far been
reported).
There are numerous reports and a review30 of the association
between smoking and elevated Tg concentrations. However,
the mechanisms involved in this process remain unclear.
Smoking has been shown to elicit insulin resistance and poor
 handling of dietary fat, with an impaired Tg clearance after a
mixed meal.18 The increase in circulating catecholamines
from the sympathetic nervous system and the subsequent
hyperinsulinemia will affect the regulation of both HSL and
enzymes that regulate Tg metabolism.19
Smoking influences Tg metabolism directly through activation
of HSL by catecholamines. HSL is the rate-limiting
The model also allowed us to estimate the effect on
hormone-sensitive lipase (HSL), the rate-limiting step for mobilization of adipose tissue Tg, which is released
by the liver, leading to a decreased levels of VLDL31 and, consequently, decreased
serum Tg levels.

The insulin responsiveness at the \texttt{APOC3} locus is ablated in vitro in those individuals who carry the \(-482T\) (IRE) rare
allele, resulting in inappropriate expression of apoC-III.11
Postprandial insulin production normally downregulates

TABLE 3. Final Regression Model of \texttt{APOC3} Variants and Smoking Status on Log(Tg)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1100T allele*</td>
<td>0.079</td>
<td>0.042–0.115</td>
</tr>
<tr>
<td>(-482T) allele†</td>
<td>-0.077</td>
<td>-0.138–0.015</td>
</tr>
<tr>
<td>Exsmoker‡</td>
<td>0.031</td>
<td>-0.031–0.094</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.074</td>
<td>0.006–0.141</td>
</tr>
<tr>
<td>Interaction terms/effect modifiers§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-482T) allele × exsmoker</td>
<td>0.083</td>
<td>0.001–0.164</td>
</tr>
<tr>
<td>(-482T) allele × current smoker</td>
<td>0.128</td>
<td>0.042–0.214</td>
</tr>
</tbody>
</table>

Coefficients are additive, and model includes variables to adjust for confounding effects of BMI and clinic.
*Coefficient given for possession of 1100T allele applies to all men irrespective of smoking status.
†Coefficient given for possession of \(-482T\) allele applies directly to never smokers.
‡Coefficients given for smoking are also directly applicable to noncarriers of \(-482T\) allele. To estimate effect of smoking in \(-482T\) carriers, these must also be modified by appropriate interaction term.
§To calculate effect of \(-482T\) allele in exsmokers or current smokers, the appropriate interaction term must also be used.

to the risk of CAD,2 this model will be a useful component in
the generation of a comprehensive risk profile for CAD. This
extension of the regression model approach will also be a
useful paradigm for the investigation of other loci where fine
mapping of variants in allelic association is required and
gene-environment interactions may be crucial. Furthermore,
compared with family studies, the present study illustrates the
advantages of using large cohorts of sex- and age-specific
unrelated individuals to determine the effect of etiologic
variants on quantitative phenotypes. We believe this method
to be superior to a haplotype analysis, because in unrelated
subjects, haplotypes can only be inferred, making assump-
tions about the data that may not be correct. A haplotype
analysis would also not allow us to distinguish separate
effects of different variants, which is our primary question.
The mechanism of the Tg-raising effect associated with the
1100C>T variant is unknown. The 1100C>T base change is
in exon 3 and does not encode an amino acid change.
Therefore, it is not likely to be functional and may be in
linkage disequilibrium with another variant, possibly situated
in the \texttt{APOAI-C3} intergenic region (none have so far been
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<table>
<thead>
<tr>
<th>Genotype</th>
<th>Smoking Status</th>
<th>Percentage Rise in Tg (95% CI)</th>
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<tbody>
<tr>
<td>CC</td>
<td>Exsmoking</td>
<td>3.2% (-3.1–9.8%)</td>
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TABLE 4. Effect of Smoking Status on Tg Levels for Each
\(-482C>T\) Genotype Group

have failed to show significant and separate effects associated
with this variant over and above that of the 3238C>G
(S\texttt{SlI}).10,12 In addition, the inclusion of smoking as a covariate
(as is usually the case), as opposed to testing for an interaction,
would also result in the concealment of any effect. Other
reasons for the disparity between the different studies may be
due to differences in either age or sex.

Because this cohort is composed of unrelated individuals,
family-based mapping methods are not feasible. To help to
determine the etiologic variants, we have used regression
modeling to distinguish the separate effects on Tg levels. This
approach also allowed us to include environmental informa-
tion, such as smoking and BMI, rendering the model more
realistic. We have established that the 1100T allele has a
raising effect on serum Tg concentrations and that the \(-482T\)
algebraic addition of \(-482T\) allele and \(1100T\) allele has a separate raising effect that varied according to
smoking habit. We have also shown that the effect of the
3238G allele on Tg is not independent of the 1100T and
\(-482T\) allele effects, whereas the \(-2854T>G\) site does
not effect Tg levels in this sample of men. In attempting
to ascertain the specific contribution of \texttt{APOC3} variants
in hypertriglyceridemia, this approach has proved useful in
indicating which variants are more likely to be important in
determining Tg levels and therefore reduce the number of
variants that require further investigation in functional stud-
ies. Furthermore, the interaction with smoking has been
localized to the \(-482C>T\) variant, a site in an IRE already
known to be functional,11 lending support to the present
findings. The model also allowed us to estimate the effect on
Tg concentration of a particular \texttt{APOC3} genotype and smok-
ning status. Because Tg concentration is thought to be related

*Coefficient given for possession of \(1100T\) allele applies to all men irrespective of smoking status.
†Coefficient given for possession of \(-482T\) allele applies directly to never smokers.
‡Coefficients given for smoking are also directly applicable to noncarriers of \(-482T\) allele. To estimate effect of
smoking in \(-482T\) carriers, these must also be modified by appropriate interaction term.
§To calculate effect of \(-482T\) allele in exsmokers or current smokers, the appropriate interaction term must also be used.

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apoC-III expression and consequently removes its inhibitory effect on LPL, but this downregulation will be reduced in carriers of the −482T allele. Therefore, constitutive expression of apoC-III will lead to reduced activity of LPL, and the increase in circulating NEFA in individuals who smoke would more fully elucidate the relative contributions of these polymorphisms. We have recently performed such a study by including a smoking-specific effect at the −482C>T variant. In middle-aged men, a group at high risk for CAD, regression modeling has allowed us to determine a useful predictive model for estimating Tg levels, which also incorporates smoking information, known to be one of the strongest risk factors for CAD.

In interpreting these results, it is important to consider the details of the study design. Subjects were requested to consume only a light breakfast; therefore, they were not fasting but postprandial to a varying degree. However, they will not have consumed a substantial amount of fat or carbohydrate, thus limiting postprandial metabolic sequelae. Furthermore, this increase in variability of the Tg measure (compared with fasting) will consequently add “noise” and therefore make associations more difficult to find. Thus, given that we did find robust associations, the validity of our results are not in question. We see no obvious reason why this situation should make interpretation of the data more difficult, and one might even argue that the fasting situation is more artificial and our study design is more realistic.

When the smoking interactions are interpreted, it is important to remember that lifestyle habits also vary between smokers and nonsmokers (eg, physical activity, diet, and alcohol consumption); it is possible that some of these factors may be contributing to these effects attributed to smoking. Likewise, the intermediate effect on Tgs observed in exsmokers may be confounded by lifestyle differences that are maintained (or started) after smoking cessation.

As indicated by the transgenic mouse studies, the majority of the effect of apoC-III can be observed only postprandially. Therefore, it is possible that other polymorphic sites may contribute to the regulation of apoC-III on lipid levels postprandially. In particular, because insulin levels are high postprandially, it is reasonable to expect the IRE polymorphism of Tg-rich lipoproteins is a highly variable and dynamic process according to both time and level of nutrition. A full postprandial study with the inclusion of smoking information would more fully elucidate the relative contributions of these polymorphisms. We have recently performed such a study by use of the European Atherosclerosis Research Study (EARS) II cohort, a group of young healthy male subjects (n = 800) from an offspring study, in whom both an oral glucose tolerance test and an oral fat tolerance test have been performed. These results showed that the −2854T>G variant modulates response to an oral fat tolerance test and that the −482C>T variant modulates response to an oral glucose tolerance test, indicating substrate-specific effects from distinct polymorphic sites. It is interesting that no association with fasting Tgs was observed in this group of young healthy men, suggesting that insulin resistance may be the primary defect coded for by the −482C>T and that the effect on Tgs seen in this middle-aged group may be secondary. In addition, no significant associations were observed with the −2854T>G variant in this cohort, but the effect of this variant was only seen in the stimulated situation (after an oral fat tolerance test).

Thus, we have established that genetic variations in the APOC3 promoter and coding region influence serum Tg concentrations under different physiological conditions, including a smoking-specific effect at the −482C>T variant. In middle-aged men, a group at high risk for CAD, regression modeling has allowed us to determine a useful predictive model for estimating Tg levels, which also incorporates smoking information, known to be one of the strongest risk factors for CAD.

Acknowledgments
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