Polymorphism in the Cholesteryl Ester Transfer Protein Gene and the Risk of Early Onset Myocardial Infarction among Cigarette Smokers

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Background: Cigarette smoking and the common B1 allele of the *TaqI*B polymorphism have both been reported to be associated with increased cholesteryl ester transfer protein (CETP) activity and altered lipoprotein levels. Thus, it is possible that the combined presence of these two respective environmental and genetic factors may enhance cardiovascular risk. We hypothesized that susceptibility to early onset myocardial infarction (MI) among cigarette smokers may be related to the presence of *TaqI*B polymorphism in the CETP gene.

Methods: The age at onset of a first MI among current (n = 199), past (n = 345), and never (n = 270) smokers was related to the presence of the *TaqI*B1 and B2 alleles in a cohort of 814 first MI patients.

Results: Multivariate regression analysis demonstrated that cigarette smoking was associated with a significant increase in the risk for early onset MI only among carriers of the *TaqI*B1 allele: current smokers with the B1B1 and B1B2 genotypes displayed a respective 9.4 (P < 0.001) and 8.4 (P < 0.001) year reduction in the age at onset of a first MI compared with never smokers, and past smokers with these genotypes exhibited a respective 3.8 (P = 0.003) and 3.7 (P = 0.01) year reduction. By contrast, current and past smoking was not associated with a significant increase in the risk for early onset MI among B2B2 homozygotes (3.0 [P = 0.28] and 0.2 [P = 0.93] year reduction, respectively). The smoking x genotype interaction was statistically significant (P = 0.04).

Conclusions: The current findings suggest that genetic factors may modify susceptibility to early onset MI among cigarette smokers.

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smoking; single nucleotide polymorphism; myocardial infarction

Cigarette smoking is a major risk factor for coronary artery disease (CAD),¹⁻³ and has been shown to be associated with an earlier age at onset of a first myocardial infarction (MI).⁴⁻⁶ The association between smoking and adverse cardiovascular outcome has been suggested to occur through its effects on plasma lipids and lipoproteins, and potentially on atherogenesis and thrombosis.^{7,8} However, the cardiac risk conferred by cigarette smoking is not uniform. Thus, it is possible that genetic factors modulate susceptibility to an earlier onset of

atherothrombotic events among young individuals who smoke.

Low levels of high-density lipoprotein cholesterol (HDL-C) have been shown to be independently associated with cardiovascular disease.^{9,10} Cholesteryl ester transfer protein (CETP) plays a central role in HDL-C metabolism by shuttling cholesteryl esters from HDL-C particles to apolipoprotein B-containing particles in exchange for triglycerides,^{11,12} this results in smaller HDL-C particles that are more rapidly cleared from the

circulation. Thus, patients with mutations in CETP gene that increase its activity have abnormally low plasma HDL-C levels.^{13,14} A common polymorphism in intron 1 of the CETP gene, denoted *TaqIB*, was among the first genetic variations to be associated with HDL-C plasma levels.¹⁵ The more common B1 allele occurs at a frequency of 60% and is associated with higher CETP levels and reduced HDL-C levels compared with the less common B2 allele in most populations studied.¹⁶

Cigarette smoking was reported to enhance CETP activity,¹⁷ thereby possibly affecting HDL-C levels and the risk of CAD. We hypothesized that susceptibility for early onset MI among cigarette smokers may be related to the presence of the *TaqIB* polymorphism. We also examined the effect of smoking status on the association between *TaqIB* genotypes and plasma levels of lipoproteins, and HDL-C and low-density lipoprotein cholesterol (LDL-C) subfractions.

METHODS

Population

Subjects were drawn from a population of postinfarction patients enrolled in the Thrombogenic Factors and Recurrent Coronary Events (THROMBO) study. The study was a prospective, multicenter investigation that enrolled patients with acute MI from 13 participating hospitals between October 1, 1994, and June 30, 1997. A total of 1,161 patients were enrolled at the time of the index MI. The average follow-up was 28 months. The details of this study have been reported in the primary publication, and the clinical parameters that defined this study population included a full spectrum of traditional postinfarction risk factors.¹⁸ Blood was drawn for genotyping in 1,012 patients at the time of the index infarction. Since the aim of the present study was to evaluate the contribution of genetic and environmental factors to the early occurrence of a first atherothrombotic event, patients who experienced MI prior to the index event ($n = 192$) were excluded from the analysis. Six more patients were excluded because of relative lack of data regarding baseline clinical characteristics and medical history, leaving a population of 814 patients. Study patients comprised mainly three self-reported ethnic populations: white non-Latinos ($n = 622$), African-Americans ($n = 115$), and Latinos ($n = 64$) living in the United States; other populations including

Asian or Pacific Islanders, American Indians, native Alaskans and Indians comprised a relatively small proportion of study patients ($n = 13$).

Smoking Status

Smoking status was obtained at enrollment. Cigarette smokers were categorized as never smokers, past smokers, or current smokers with past smokers having quit ≥ 1 month before enrollment. Smoking exposure was further stratified by dose, categorized as >20 cigarettes per day or less.

End Points

The primary end point of this study was the age at onset of a first MI defined as (1) a continuous variable, analyzed as years of age reduction at presentation associated with the genetic risk factor compared to patients without the risk factor; and (2) a categorical variable, with younger age prespecified at the lowest age quartile of study patients (age <50 years).

Laboratory Methods

Biochemical Measurements

Blood (55 mL) was drawn in the fasting state at the baseline clinic visit 2 months after the index MI. Plasma and serum samples were each separated, frozen, and sent to Rochester, NY, for central storage in a -70°C freezer. Total cholesterol, HDL-C and triglyceride were measured by the use of colorimetric assays (Vitros Chemistry Products, Rochester, NY). LDL-C concentration was calculated by the Friedewald formula.¹⁹ Analyses were performed according to the manufacturer's specifications, and quality control was within the recommended precision for each test. LDL-C median diameter and HDL-C median diameter (defined as diameter where half the respective LDL-C and HDL-C absorbance is on larger, and half on smaller, particles) were determined by the use of non-denaturing composite gradient gel electrophoresis as described previously.²⁰ Lipoproteins in the gel were stained with Sudan black B, densitometry was performed, and absorbance profiles were analyzed using a self-developed software.²⁰

Genotyping

Buffy coats were isolated and stored at -70°C until extracted for DNA analysis. Genotyping of

the *TaqIB* polymorphism was performed using a melting curve analysis method (Light Cycler, Roche Diagnostics, IN) based on G-nucleobase quenching to determine binding affinity to polymerase chain reaction-amplified *CETP* sequence of 5'-FAM labeled probe (TCTGAACCCTAACTCGAAC) complementary to the *TaqIB1* allele. This method was validated on 134 samples by comparison with standard *TaqI* PCR-RFLP. The frequency of the *TaqIB1* allele in our population (0.6) was consistent with previously published reports, and the population was in Hardy-Weinberg equilibrium with respect to this polymorphism.

Statistical Analysis

Patients were categorized as never smokers, past smokers, or current smokers, and comparisons among groups were performed using the chi-squared categorical parameters and ANOVA for continuous variables.

Multivariate regression models were employed to analyze the end point of age as a continuous variable, and the effect on HDL-C and LDL-C subfractions, and multivariate logistic regression models were used to analyze the end point of the first age quartile (<50 years). Never smokers formed the reference group among smoking categories; and patients with the B2B2 genotype formed the reference group among the *TaqIB* genotypes. The relationship between the three smoking categories and the three *TaqIB* genotypes was studied using 3×3 interaction analysis: In a secondary analysis, 2×2 interaction was used to evaluate the effect of the *TaqIB1* allele (patients with either the B1B1 or the B1B2 genotype) among smokers at anytime (either current or past smokers).

Prespecified covariates in the multivariate models included gender, ethnicity, history of treated hypertension, history of treated diabetes mellitus, and body mass index. In an alternative analysis, HDL-C levels and treatment with lipid-lowering therapies were added to the multivariate models. The consistency of the results in the total population was validated in models that were assessed separately in white ($n = 622$) and nonwhite ($n = 192$) patients.

A significant level 0.05 was used for declaring statistical significance of 2-sided tests. The statistical software used for the analyses was SAS version 9.13.

RESULTS

Clinical, genetic, and lipid characteristics of study patients by smoking status at the time of the first MI are shown in Table 1. Current smokers were younger and had a higher frequency of non-white patients as compared with past-smokers and never-smokers, whereas the frequency of treated hypertension was highest among never smokers (Table 1A). The frequencies of the *TaqIB* genotypes were similar among the smoking subgroups (Table 1B), and the frequency of the *TaqIB1* allele was similar in white and nonwhite patients (61% and 64%, respectively).

Analysis of lipid data showed that current smokers exhibited significantly higher levels of total- and LDL-cholesterol, and non significantly lower levels of HDL-C (Table 1C). Medical therapy with lipid lowering drugs was administered similarly among the 3 smoking categories (Table 1D).

Effect of the *TaqIB* Polymorphism on the Relationship between Cigarette Smoking and Early Onset Myocardial Infarction

In multivariate regression analysis (Table 2A), current and past smokers were demonstrated to experience their first MI 8.1 and 3.2 years earlier, respectively, than never smokers ($P < 0.001$ for the 2 comparisons). However, the relationship between cigarette smoking and the age at onset of a first MI was significant only among carriers of the *TaqB1* allele. Among patients with the B1B1 and B1B2 genotypes, current smokers experienced their first MI 9.4 and 8.4 years earlier, respectively, than never smokers ($P < 0.001$ for both comparisons), whereas among patients with the B2B2 genotype, the age at onset of a first MI among current smokers was non-significantly reduced by 3 years compared to never smokers. Similarly, past smokers with the B1B1 and B1B2 genotypes experienced their first MI 3.8 years ($P = 0.003$) and 3.7 years ($P = 0.01$) earlier, respectively, than never smokers with the respective genotypes, whereas among patients with the B2B2 genotype, past smoking was not associated with a significant increase in the risk for early MI onset compared with never smoking (0.2 year reduction, $P = 0.93$). The difference between the effects of the B1B1 and the B2B2 genotypes on the age of onset of a first MI among current smokers was statistically significant (P -value for smoking $\times$ genotype interaction = 0.04).

Table 1. Baseline Clinical Characteristics and Genetic Data by Smoking Status

	Current Smokers (n = 199)	Past Smokers (n = 346)	Never Smokers (n = 270)
A. Clinical Characteristics			
Age years (mean $\pm$ SD)	53.1 $\pm$ 10.6	58.8 $\pm$ 11.0	62.4 $\pm$ 12.4*
Diabetes mellitus (%)	15	17	19
Hypertension (%)	36	42	49*
White (%)	66	84	74*
Male (%)	72	80	69*
Body mass index, kg/m ² (mean $\pm$ SD)	27.9 $\pm$ 5.1	28.2 $\pm$ 5.1	28.0 $\pm$ 5.6
B. <i>TaqIB</i> genotypes			
B1B1 (%)	34	31	35
B1B2 (%)	55	53	50
B2B2 (%)	11	16	15
C. Laboratory[†]			
Total cholesterol, mg/dL (mean $\pm$ SD)	208 $\pm$ 46	192 $\pm$ 41	198 $\pm$ 46*
LDL-C, mg/dL (mean $\pm$ SD)	125 $\pm$ 38	114 $\pm$ 33	122 $\pm$ 41*
HDL-C, mg/dL (mean $\pm$ SD)	39 $\pm$ 11	39 $\pm$ 13	42 $\pm$ 11
Triglycerides, mg/dL (mean $\pm$ SD)	224 $\pm$ 127	199 $\pm$ 117	189 $\pm$ 107
D. Lipid Lowering Therapies			
Any type (%)	35	34	40
Statins (%)	30	29	35

*P < 0.05 for the comparison among the three smoking categories.

[†]Data were obtained 2 months after the index MI myocardial infarction in 744 patients.

HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol.

In a secondary analysis, we evaluated the effect of the *TaqIB1* allele (either the B1B1 or the B1B2 genotypes) among smokers at anytime (either current or past smokers). This analysis revealed that among *TaqIB1* allele carriers, smokers experienced a significant 5.7 (P < 0.001) year reduction in the age at onset of a first MI compared with nonsmokers, whereas among B2B2 homozygotes, smoking was not associated with a significant increase in the risk for early onset MI (0.9 year reduction; P = 0.62). Similar to the primary analysis, the results demonstrated a statistically significant smoking $\times$ genotype interaction (P = 0.04).

Consistent with the results of the multivariate regression analysis, the mean age at onset of a first MI was significantly younger among current smokers with the B1B1 and B1B2 genotypes (52 and 53 years, respectively) than among current smokers with the B2B2 genotype (59 years; P < 0.001), whereas never smokers experienced their first MI at a similar age regardless of genotype (Fig. 1).

Similar results were obtained when the effect of the *TaqIB* polymorphism on the relationship

between cigarette smoking and MI age was analyzed using multivariate logistic regression analysis for the end point of early onset MI, defined as the first age quartile (age <50 years; Table 2B). In this analysis current smoking was associated with an odds ratio of 3.74 (P < 0.001) for early onset MI. However, consistent with the regression analysis results, the association was significant only among current smokers with the B1B1 (odds ratio = 4.91, P < 0.001) and B1B2 (odds ratio = 4.64, p < 0.001) genotypes, but not among current smokers who had the B2B2 genotype (odds ratio = 0.45, p = 0.28), with a significant interaction effect (P-value for current smoking $\times$ genotype interactions <0.01).

The effect of the *TaqIB* polymorphism on the association between smoking and early onset MI was consistent in white-patients (smokers who are carriers of the of *TaqIB1* allele: 6.0 year reduction [P < 0.001]; smokers who are non-*TaqIB1* allele carriers [B2B2 homozygotes]: 0.7 year reduction [P = 0.80]) and nonwhite-patients (smokers who are carriers of the *TaqIB1* allele: 5.5 year reduction [P = 0.03]; smokers who are non-*TaqIB1* allele carriers: 1.1 year reduction [P = 0.96]).

Table 2. Risk of Early Myocardial Infarction by *TaqI*B Genotypes Among Smoking Categories

A. Multivariate Regression Analysis: Age Difference between Smokers and Never Smokers at First Myocardial Infarction*					
	Current Smokers		Past Smokers		Never Smokers
	Age Difference, Years	P-Value	Age Difference, Years	P-Value	Age Difference, Years (Reference)
Overall	−8.1	<0.001	−3.2	0.01	0.00
By <i>TaqI</i> B genotypes [†]					
B1B1 (n = 270)	−9.4	<0.001	−3.8	0.003	0.00
B1B2 (n = 428)	−8.4	<0.001	−3.7	0.01	0.00
B2B2 (n = 142)	−3.0	0.28	−0.2	0.93	0.00

*Adjusted for ethnicity, diabetes mellitus, hypertension and gender; in the overall model adjusted also for genotype; similar findings were obtained after further adjustment for HDL-C levels and treatment with lipid lowering drugs.

[†]Among current smokers: P-value for the difference between the effects of the B1B1 and B2B2 genotypes = 0.04; P-value for the difference between the effects of the B1B2 and B2B2 genotypes = 0.06. Among past smokers: P-value for the difference between the effects of the B1B1 and B2B2 genotypes = 0.17. P-value for the difference between the effects of the B1B2 and B2B2 genotypes = 0.19.

B. Multivariate Logistic Regression Analysis: Odds Ratio for Early Myocardial Infarction Defined as First Age Quartile (<50 Years) among Smokers (Reference Group: Never Smokers).*					
	Current Smokers		Past Smokers		Never Smokers
	Odds Ratio (95% CI)	P-Value	Odds Ratio (95% CI)	P-Value	(Reference)
Overall	3.74 (2.38–5.88)	<0.001	1.33 (0.86–2.07)	0.20	1.00
By <i>TaqI</i> B genotypes [†]					
B1B1 (n = 270)	4.91 (2.58–9.31) [‡]	<0.001	1.83 (0.89–3.77)	0.10	1.00
B1B2 (n = 428)	4.64 (2.16–9.99) [‡]	<0.001	1.09 (0.56–2.12)	0.79	1.00
B2B2 (n = 142)	0.45 (0.10–1.95)	0.28	1.05 (0.38–2.91)	0.92	1.00

*Adjusted for ethnicity, diabetes mellitus hypertension and gender; in the overall model adjusted also for genotype; similar findings were obtained after further adjustment for HDL-C levels and treatment with lipid lowering drugs.

[†]Among current smokers: P-value for the difference between the effects of the B1B1 and B2B2 genotypes = 0.005; P-value for the difference between the effects of the B1B2 and B2B2 genotypes = 0.007. Among past smokers: P-value for the difference between the effects of the B1B1 and B2B2 genotypes = 0.07; P-value for the difference between the effects of the B1B2 and B2B2 genotypes = 0.12.

Effect of the *TaqI*B Polymorphism on the Relationship between Smoking Dose and the Age at Onset of a First Myocardial Infarction

When smoking status was further stratified by the numbers of cigarettes smoked per day, multivariate regression analysis demonstrated a graded relationship between cigarette smoking and the age of a first MI (Table 3): current- and past- smokers who smoked >20 cigarettes per day experienced their first MI 8.9 and 3.4 years earlier, respectively,

than never smokers, whereas the effect of smoking status on the onset of a first MI was attenuated in current and past smokers who smoked ≤20 cigarettes per day (4.9 year and 1.5 year reduction, respectively). However, the relationship between smoking dose and early onset MI was consistent only among patients with the B1B1 and B1B2 genotypes, whereas among patients with the B2B2 genotype no significant association between smoking dose and MI onset was demonstrated (Table 3). Consistent with these results, when the mean age of a first MI was related to smoking

Figure 1. Mean age at onset of a first MI by *TaqIB* genotypes within smoking categories.

dose and the *TaqIB* polymorphism (Fig. 2), current smokers with the B1B1 and B1B2 genotypes who smoked >20 cigarettes per day were shown to experience their first MI at a significantly early age (mean age: 52 years) than patients with the B2B2 genotype who smoked the same amount of cigarettes (mean age: 56 years; $P < 0.001$). Similarly, among current smokers with the B1B1 and B1B2 genotypes who smoked ≤ 20 cigarettes per day, the mean age at onset of a first MI was 54 years and 55 years, respectively, whereas the mean age of a first MI was 65 years among patients with B2B2 genotype who smoked the same amount ($P < 0.001$).

Effect of the *TaqIB* Polymorphism on the Relationship between Smoking Status and Serum Lipids

Since the mechanism that underlies the possible relationship between the *TaqIB* polymorphism and CAD possibly relates to its effect on lipid levels,^{13–16} we evaluated, in a further exploratory analysis, the association between *TaqIB* genotypes and serum lipids within the smoking subgroups.

Current smokers who carried the *TaqB1* allele exhibited significantly lower levels of HDL-C, and nonsignificantly higher levels of total and LDL cholesterol, compared with current smokers with the B2B2 genotype (Supplementary Appendix Table).

When lipid particle size was analyzed, current smokers who carried the *TaqIB1* allele were shown to have also a significantly lower mean particle diameter of LDL-C than the respective B2B2 homozygotes. Furthermore, the proportion of HDL_{2A} and LDL₁ was significantly lower, and that of LDL₃ levels was significantly higher, among the former subgroup (Supplementary Appendix Table). Accordingly, multivariate regression analysis demonstrated that the association between the *TaqIB1* allele and a respective lower and higher proportion of HDL_{2A} and LDL₃ was most prominent among current smokers (Fig. 3A and B, respectively).

DISCUSSION

The primary finding of this study is that there is a dose-dependent interaction between cigarette smoking and *TaqIB* polymorphism in the CETP gene that results in a significant increase in the risk of early onset MI among smokers who carry the *TaqIB1* allele. These findings may have important clinical and epidemiological implications considering that the fact that the frequency of this common allele in the general population is between 55% and 60%.^{16,21,22}

The *TaqIB1* allele was shown to be associated with increased circulating levels of CETP,²¹ which is known to signal HDL-C to transfer cholesterol ester to very low density lipoprotein cholesterol in exchange for triglycerides. In addition to its effect

Table 3. Age Difference at First Myocardial Infarction by *TaqIB* Genotypes Among Smoking Dose Categories*

	Current Smokers: >20 Cigarettes/Day		Current Smokers: ≤20 Cigarettes/Day		Past Smokers >20 Cigarettes/Day		Past Smokers ≤20 Cigarettes/Day		Never Smokers	
	Age Difference, Years	P-Value	Age Difference, Years	P-Value	Age Difference, Years	P-Value	Age Difference, Years	P-Value	Age Difference, Years	P-Value (Reference)
Overall	-8.9	<0.001	-4.9	0.01	-3.4	<0.001	-1.5	0.3	0.00	
By <i>TaqIB</i> genotypes										
B1B1 (n = 270)	-10.5†	<0.001	-6.1	0.01	-4.2	0.002	-1.9	0.39	0.00	
B1B2 (n = 428)	-8.1	<0.001	-6.2	0.11	-3.7	0.03	-1.9	0.58	0.00	
B2B2 (n = 142)	-5.2	0.10	2.2	0.68	-0.5	0.84	2.5	0.45	0.00	

* Adjusted for ethnicity, diabetes mellitus, hypertension and gender; in the overall model adjusted also for genotype; similar findings were obtained after further adjustment for HDL-C levels and treatment with lipid lowering drugs.

† Among current smokers >20 cigarettes/day: P-value for the difference between the effects of the B1B1 and B2B2 genotypes = 0.045; P-value for the difference between the effects of the B1B2 and B2B2 genotypes = 0.11. Among current smokers ≤20 cigarettes/day: P-value for the difference between the effects of the B1B1 and B2B2 genotypes = 0.09; P-value for the difference between the effects of the B1B2 and B2B2 genotypes = 0.12. Interactions in past smokers had P-values >0.10.

on HDL-C, a linkage of the CETP gene to LDL-C particle size has been reported.²³ Increased small, dense LDL-C concentrations are associated with increased risk of cardiovascular disease. Thus, this may also be a potential mechanistic link between variation at the CETP gene and the risk of CAD (23). Prior studies (21,22), and a meta-analysis comprising 13,677 subjects,¹⁶ have shown that the presence of the *TaqIB*1 allele is associated with alterations in lipid levels and a higher risk of CAD. Notably, the relationship of the *TaqIB* polymorphism to HDL-C was shown to be modified by several metabolic and environmental factors known to determine HDL-C levels, including age, sex, oral contraceptive use, body mass index, alcohol consumption, and cigarette smoking.^{16,24}

Our data suggest that the association between cigarette smoking and atherothrombotic disease may be genetically dependent and related to the metabolism of lipids, as determined by the CETP gene. The fact that the risk of early onset MI among smokers is associated with the presence of the *TaqIB*1 allele suggests that environmental and genetic proatherogenic factors are interacting. Dullaart et al.¹⁷ have shown that cigarette smoking is associated with increased CETP activity and lower HDL-C levels in general, and a reduction in the levels of the HDL_{2a} subfraction, specifically. In addition, cigarette smoking was demonstrated to be associated with an increase in oxidatively modified LDL-C, as expressed by a lower proportion of LDL₁ and a higher proportion of LDL₃.^{25,26} These metabolic effects may attenuate the cardioprotective effects of HDL-C and increase the atherogenic effects of LDL-C.²³ Thus, the reduction in CETP activity in individuals with the *TaqIB*2 allele may protect against the lipid modulating effects of smoking. Alternatively, in *TaqIB*1 carrying subjects who have higher CETP activity, susceptibility to the additional effects of cigarette smoking on CETP activity and serum lipids may be enhanced. Consistent with this possible mechanism, we have shown that smokers who carry the *TaqIB*1 allele have several important alterations in serum lipids, including lower HDL-C levels that are associated with a lower proportion of the cardioprotective HDL_{2a} subfraction, and higher LDL-C levels that are associated with a higher proportion of the atherogenic LDL₃ subfraction. Thus, it is possible that convergence of environmental and genetic effects on atherogenic and protective metabolic factors predisposes to premature atherothrombotic

Figure 2. Mean age at onset of a first MI by *TaqIB* genotypes within smoking categories further stratified by smoking dose.

events among cigarette smokers who carry the *TaqIB1* allele.

Our findings are consistent with a recently reported interaction between the effects of cigarette smoking and the *TaqIB* polymorphism on lipids levels in Turkish subjects,²⁷ and contrast with the results of two reports that have shown an association between the *TaqIB* polymorphism and the risk of CAD among nonsmokers.^{28,29} Of note, the two latter studies employed a case control design, in which controls subjects were matched to cases with CAD based on smoking habit, thereby potentially limiting the analysis of possible interactions between smoking and the *TaqIB* polymorphism, whereas in the current study we assessed the risk of early onset MI using age as a quantitative end point in a case-only cohort. This methodology facilitated a comprehensive analysis of possible genotype-smoking interactions, including a dose-response effect.

Study Limitations

The present results provide evidence for an association between the *TaqIB* polymorphism and the risk of early onset MI among smokers. However, CETP levels were not assessed in the current study, and therefore the results do not demonstrate the direct mechanism by which the interaction between smoking and the polymorphism affects outcome. Nevertheless, the significant alternations in plasma lipids among smokers who carried the *TaqIB1* allele

provide indirectly a possible mechanism by which the combined presence of the two respective environmental and genetic factors may affect cardiovascular risk. It should also be noted that finding an association in one or more individual candidate single nucleotide polymorphism variants (single-marker associations) with the age-related end point does not necessarily indicate causality, since the identified single nucleotide polymorphism may be in linkage disequilibrium with an unidentified causal genetic variant. Further evaluation is required to determine whether *TaqIB* polymorphism mediates coronary risk directly or acts as a marker of other gene polymorphisms.

Lipid levels tend to be unstable during the acute phase of MI. For this reason, lipid analyses were performed on blood samples that were obtained 2 months after the index MI among 744 (91%) study subjects. The interpretation of the results regarding the effects of smoking and the *TaqIB* polymorphism on serum lipids should be regarded as preliminary and exploratory. Further studies are required to identify possible environmental-genetic interactions on metabolic factors that predispose to early onset MI.

Despite the fact that the frequency of the *TaqIB1* allele in study patients was similar among ethnic groups, the distribution of white and non-white patients among smoking categories was significantly different. All models in the current study included ethnicity as a covariate. Furthermore, all findings were validated in separate analyses that were

Figure 3. Multivariate regression analysis. Changes in the proportion of (A) HDL_{2a} subfraction; and (B) LDL₃ subfraction by *TaqI*B genotypes within smoking categories (analysis was carried out in 744 patients in whom laboratory data were obtained 2 months after the index MI. *Findings were adjusted for age, gender, ethnicity, history of treated hypertension, history of treated diabetes mellitus, and body mass index, and treatment with lipid lowering therapies).

carried out in white and nonwhite patients. However, it is still possible that important ethnic-specific clinical and genetic risk factors were not measured and unadjusted for in the current analy-

sis. As with any genetic association study, the validity of our findings will be significantly strengthened if replicated in an independent population and among separate ethnic groups.

CONCLUSIONS

We have shown that cigarette smokers have a significant, dose-dependent, increase in the risk of early onset MI if they carry the common *TaqIB1* allele in the CETP gene. These findings suggest a genetic contribution to the effect of cigarette smoking on the risk of premature atherothrombotic disease, and may be important in understanding the interaction between genetic and environmental factors in the development of premature CAD. The high-frequency of *TaqIB1* allele in the population further stresses the importance of complete smoking cessation for the prevention of early onset atherothrombotic disease.

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Supplementary Appendix Table. Lipids Levels and LDL and HDL Subfractions by *TaqI*B Genotypes within Smoking Categories*

	Current Smokers				Past Smokers				Never Smokers			
	B1B1	B1B2	B2B2		B1B1	B1B2	B2B2		B1B1	B1B2	B2B2	
Total cholesterol	209 ± 46	209 ± 46	199 ± 47		193 ± 43	192 ± 40	188 ± 44		193 ± 43	192 ± 40	188 ± 44	
Triglycerides	247 ± 145	214 ± 117	205 ± 113		197 ± 134	206 ± 112	184 ± 95		190 ± 100	196 ± 99	185 ± 143	
LDL-C	127 ± 40	126 ± 38	114 ± 39		117 ± 34	113 ± 30	113 ± 39		121 ± 46	124 ± 40	119 ± 31	
LDL MED	26.2 ± 0.8	26.5 ± 0.63	26.7 ± 0.6		26.3 ± 0.7	26.4 ± 0.6	26.4 ± 0.6		26.3 ± 0.6	26.4 ± 0.7	26.7 ± 0.6*	
HDL-C	38 ± 12	39 ± 10	44 ± 9		38 ± 14	40 ± 14	41 ± 11		42 ± 10	41 ± 11	42 ± 10	
HDL MED	8.7 ± 0.3	8.7 ± 0.2	8.9 ± 0.2		8.8 ± 0.3	8.8 ± 0.3	8.7 ± 0.2		8.9 ± 0.3	8.9 ± 0.3	8.9 ± 0.3	
<i>LDL subfractions</i>												
LDL ₁	0.47 ± 0.24	0.55 ± 0.21	0.62 ± 0.20		0.52 ± 0.21	0.51 ± 0.20	0.52 ± 0.20		0.50 ± 0.18	0.55 ± 0.23	0.60 ± 0.21*	
LDL ₂	0.25 ± 0.13	0.25 ± 0.13	0.23 ± 0.12		0.24 ± 0.12	0.26 ± 0.12	0.25 ± 0.13		0.26 ± 0.11	0.23 ± 0.13	0.21 ± 0.11	
LDL ₃	0.20 ± 0.16	0.14 ± 0.12	0.10 ± 0.09		0.16 ± 0.14	0.15 ± 0.13	0.15 ± 0.13		0.16 ± 0.13	0.15 ± 0.14	0.13 ± 0.11	
LDL ₄	0.08 ± 0.08	0.06 ± 0.05	0.06 ± 0.04		0.08 ± 0.06	0.08 ± 0.05	0.07 ± 0.04		0.08 ± 0.05	0.07 ± 0.06	0.07 ± 0.04	
<i>HDL subfractions</i>												
HDL ₁	0.04 ± 0.03	0.05 ± 0.03	0.04 ± 0.02		0.05 ± 0.03	0.05 ± 0.03	0.04 ± 0.03		0.05 ± 0.03	0.05 ± 0.03	0.04 ± 0.03	
HDL _{2A}	0.22 ± 0.04	0.22 ± 0.04	0.24 ± 0.04		0.21 ± 0.04	0.22 ± 0.04	0.22 ± 0.04		0.21 ± 0.04	0.22 ± 0.04	0.22 ± 0.04	
HDL _{2B}	0.21 ± 0.10	0.23 ± 0.08	0.24 ± 0.09		0.23 ± 0.08	0.23 ± 0.07	0.23 ± 0.06		0.23 ± 0.08	0.23 ± 0.07	0.23 ± 0.06	
HDL _{3A}	0.25 ± 0.05	0.25 ± 0.04	0.25 ± 0.05		0.24 ± 0.04	0.25 ± 0.04	0.25 ± 0.04		0.24 ± 0.04	0.25 ± 0.04	0.25 ± 0.04	
HDL _{3B}	0.18 ± 0.06	0.17 ± 0.05	0.17 ± 0.05		0.17 ± 0.05	0.17 ± 0.05	0.17 ± 0.05		0.17 ± 0.05	0.17 ± 0.05	0.17 ± 0.05	
HDL _{3C}	0.09 ± 0.03	0.09 ± 0.04	0.08 ± 0.03		0.10 ± 0.04	0.09 ± 0.03	0.10 ± 0.04		0.10 ± 0.04	0.09 ± 0.03	0.10 ± 0.04	

* Lipid levels were obtained 2 months after the index myocardial infarction in 744 patients; data for particle size are in nanometer, and for HDL and LDL subfractions as a proportion (±SD) of the respective lipoprotein in each genotype.

P < 0.05 for the comparison among the 3 genotypes in each smoking category subgroup.

HDL-C = high-density lipoprotein cholesterol; HDL MED = high-density lipoprotein median diameter; LDL-C = low-density lipoprotein cholesterol; LDL MED = low-density lipoprotein median diameter.