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The Cholesteryl Ester Transfer Protein (CETP) TaqIB Polymorphism in the Cholesterol and Recurrent Events Study: No Interaction With the Response to Pravastatin Therapy and No Effects on Cardiovascular Outcome

A Prospective Analysis of the CETP TaqIB Polymorphism on Cardiovascular Outcome and Interaction With Cholesterol-Lowering Therapy

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OBJECTIVES On the basis of quantitative coronary angiography data, the cholesteryl ester transfer protein (CETP) TaqIB gene polymorphism has been postulated to predict the progression of coronary atherosclerosis and response to cholesterol-lowering therapy.

BACKGROUND Cholesteryl ester transfer protein mediates the exchange of lipids between anti-atherogenic high-density lipoprotein (HDL) and atherogenic apolipoprotein B containing lipoproteins and therefore plays a key role in human lipid metabolism. Hence, CETP gene polymorphisms may alter susceptibility to atherosclerosis.

METHODS To investigate the significance of the CETP TaqIB gene polymorphism with respect to clinical end points, we used the Cholesterol And Recurrent Events (CARE) cohort. The CARE study was designed to investigate the effect of five years of pravastatin therapy on coronary events.

RESULTS We found that the odds ratios for the primary end point were not significantly different from unity for the three genetic subgroups after five years of placebo treatment. Furthermore, pravastatin induced similar changes in total cholesterol, low-density lipoprotein cholesterol, and HDL cholesterol among TaqIB genotypes, and both nonfatal myocardial infarction and deaths from coronary heart disease were reduced to the same extent in all three genotypes.

CONCLUSIONS In the CARE cohort, the CETP TaqIB polymorphism does not predict cardiovascular events or discriminate between those who will or will not benefit from pravastatin treatment. (J Am Coll Cardiol 2004;43:854–7) © 2004 by the American College of Cardiology Foundation

Because coronary heart disease (CHD) is an important cause of death, the timely identification of high-risk patients is essential for the prevention of mortality and morbidity. In the REGRESS study, we have shown that a cholesteryl ester transfer protein (CETP) gene polymorphism (denoted as "TaqIB") is associated with the progression of coronary atherosclerosis as assessed by computer-assisted quantitative angiography (1). In addition, a pharmacogenetic interaction between the TaqIB genotype and response to cholesterol-lowering therapy (pravastatin) was identified. Using angiographic outcome as a surrogate end point for cardiovascular events, it was hypothesized that this genetic marker could discriminate between those who will and those who will not benefit from pravastatin therapy with respect to cardiovascular end points. To test this hypothesis, we have studied the CETP TaqIB polymorphism and a CETP promoter polymorphism (−C629A) in the Cholesterol And Recurrent Events (CARE) cohort, a large prospective placebo-controlled secondary prevention trial with clinical end points as outcome (2). The −629 polymorphism was included in this analysis because it is in strong linkage

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Abbreviations and Acronyms

CARE = Cholesterol And Recurrent Events study
CETP = cholesteryl ester transfer protein
CHD = coronary heart disease
HDL-c = high-density lipoprotein cholesterol
LDL-c = low-density lipoprotein cholesterol
MI = myocardial infarction

disequilibrium with the TaqIB polymorphism and has been shown to directly affect CETP promoter activity.

Cholesteryl ester transfer protein mediates the exchange of lipids between anti-atherogenic high-density lipoprotein (HDL) and atherogenic apolipoprotein B containing lipoproteins, and therefore plays a key role in human lipid metabolism. Hence, CETP gene polymorphisms may alter susceptibility to atherosclerosis. In line with this concept, CETP gene variation has been shown to be associated with plasma CETP, HDL cholesterol (HDL-c) levels (3–5), and risk for CHD (3,4). Most studies indicate that the TaqIB B1B1 genotype is associated with higher plasma CETP and lower HDL-c levels than the B2B2 genotype.

We present TaqIB data on 2,764 men and 441 women of the CARE study, which was designed to investigate the effect of five years of pravastatin therapy on coronary events after a previous myocardial infarction (MI). The primary end point of the trial was death from CHD or nonfatal MI. For the present analysis, men and women were investigated separately.

METHODS

Study design and patients. The design of the CARE trial has been described elsewhere (2). In short, patients were recruited from 80 participating centers—13 in Canada and 67 in the U.S. Men and postmenopausal women were eligible if they had an acute MI between three and 20 months before randomization, were 21 to 75 years of age, and had plasma total cholesterol levels of <240 mg/dl (6.2 mmol/l), low-density lipoprotein cholesterol (LDL-c) levels of 115 to 174 mg/dl (3 to 4.5 mmol/l), fasting triglyceride levels of <350 mg/dl (4 mmol/l), fasting glucose levels of no more than 220 mg/dl (12 mmol/l), left ventricular ejection fractions of no less than 25%, and no symptomatic congestive heart failure. Diabetes and hypertension were either diagnosed by a physician or defined by patients' use of anti-diabetic or antihypertensive medication. This was verified in a sample of the medical records. Physical exercise was estimated on the basis of filled-out questionnaires. The primary end point of the trial was death from CHD (including fatal MI, either definite or probable; sudden death; death during a coronary intervention; and death from other coronary causes) or symptomatic (unless during noncardiac surgery) nonfatal MI confirmed by serum creatine kinase measurements. The protocol was approved by the safety and data monitoring committees and the institutional review boards of all participating centers.

Laboratory analyses. Plasma total cholesterol, HDL-c, and triglyceride levels were measured by the core laboratory, and LDL-c levels were calculated (2).

Genomic DNA was prepared from 1 ml of frozen blood with Purigene extraction kit (Gentra Systems, Minneapolis, Minnesota). All the genotyping was done with enzyme linked immunosorbent assay–based single base extension assay using SNPStreamTM 25K genotyping system (Orchid Bioscience, Princeton, New Jersey) (6). The sequences of the oligonucleotide primers used in these assays were as follows:

TaqIB PCR-forward primer:
CCAGGTATAGGGATTTGTGTTTG

TaqIB PCR-reverse primer:
CAAATATACACCAACCTCCTAATCTTTAC

TaqIB base extension primer:
GTCTGCGACCXAGAATCACTGGGGTTC

−629 PCR-forward primer:
CAGTTTCTCCCGGAGGCA

−629 PCR-reverse primer:
GTCCTCTATGTAGACTTTCCTTGATATGC

−629 base extension primer:
TTGATATGCATAAAATAACTCTGGG

Statistical analyses. A total of 3,274 of the 4,159 subjects in the CARE study had sufficient DNA for genotyping. Among the 3,274 subjects, 69 were excluded from the analysis because of failed genotyping. The total number of subjects used in the analyses was 3,205 (441 women and 2,764 men). Among these, 1,604 subjects (218 women and 1,386 men) were in the pravastatin treatment group, and 1,601 subjects (223 females and 1,378 males) were in the placebo group. All the statistical analyses were done separately for women and men.

The associations of TaqIB genotypic variation with quantitative lifestyle parameters, baseline plasma lipid levels, and changes of these lipid levels during the study were analyzed using analysis of variance and F tests. The TaqIB genotype associations with categorical lifestyle parameters were analyzed using contingency tables and chi-square tests. Logistic regression with likelihood ratio and Score tests were used with baseline HDL-c as a covariate to assess the interaction between TaqIB genotypic variation and the treatment effect on the clinical end points. The ratio of odds of the clinical event for subjects treated with pravastatin relative to that for subjects treated with placebo were calculated for each genotype. Logistic regression was also used with baseline HDL-c as a covariate to analyze the influence of TaqIB genotypic variation on clinical events in the placebo group alone. In these analyses, the ratios of the odds of the clinical event for subjects with the B1B1 or B1B2 genotype, each relative to those for subjects with the B2B2 genotype, were determined. All these analyses were

Table 1. Baseline Characteristics According to CETP TaqIB Genotype

Characteristic	Men				Women			
	B1B1 n = 907	B1B2 n = 1,390	B2B2 n = 467	p Value	B1B1 n = 177	B1B2 n = 203	B2B2 n = 67	p Value
General	Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	
Age (yrs)	57.8 (9.7)	58.2 (9.09)	58.1 (9.5)	0.65	62.0 (8.9)	61.3 (8.4)	59.3 (10.2)	0.11
Race: white (%)	93.1	94.3	93.1	0.41	90.1	93.1	90.1	0.49
Body mass index (kg/m^2)	27.7 (4.1)	27.5 (4.2)	27.6 (4.0)	0.75	27.4 (5.5)	27.4 (5.5)	27.5 (5.9)	0.99
Waist hip ratio	0.95 (0.1)	0.95 (0.1)	0.95 (0.1)	0.57	0.9 (0.1)	0.9 (0.1)	0.8 (0.1)	0.86
Systolic BP (mm Hg)	128.8 (18.0)	127.7 (17.8)	128.9 (18.3)	0.25	136.0 (19.7)	132.8 (19.1)	132.4 (20.6)	0.22
Diastolic BP (mm Hg)	79.0 (10.0)	78.6 (10.2)	78.8 (10.2)	0.67	77.7 (10.3)	77.8 (9.9)	76.9 (10.3)	0.82
Risk factors								
Smoker (%)	19.6	20.8	19.6	0.69	39.8	30.1	39.8	0.03
Exercise (%)	74.4	69.6	74.4	0.08	65.1	58.9	65.1	0.61
Hypertension (%)	41.7	39.1	41.7	0.24	56.1	50.7	56.1	0.49
Diabetes mellitus (%)	11.6	13.7	11.6	0.28	17.0	20.2	17.0	0.55
Family history of CAD (%)	39.5	38.8	39.5	0.84	44.1	41.9	44.1	0.08

B1 denotes the presence of a restriction site for TaqIB in intron 1 of the CETP gene, and B2 its absence.
CETP = cholesteryl ester transfer protein; SD = Lower 95% confidence level, Upper 95% confidence level; BP = blood pressure.

also performed for the −629 variant. The analyses were carried out with SAS version 8.1 (7).

RESULTS

There were no significant differences in lifestyle parameters or CHD risk factors among these genotypes at baseline except for a lower percentage of smokers in female B1B2 carriers than in B1B1 and B2B2 genotypes (Table 1).

Table 2 gives lipid and lipoprotein concentration in the three genetic subgroups. A significant and apparently dose-dependent relationship between the number of copies of the B1 allele and lower HDL-c at baseline was observed ($p < 0.001$ in men and $p < 0.002$ in women), whereas no relationship with other lipids or lipoproteins was present.

The odds ratios for the primary end point were not significantly different from unity for the three genetic subgroups after five years of placebo treatment. Furthermore, pravastatin induced similar changes in total cholesterol, LDL-c, and HDL-c among TaqIB genotypes. We found that nonfatal MI and deaths from CHD were reduced to the same extent in all three genotypes (Fig. 1). Similar results were obtained with other end points, including coronary revascularization, unstable angina, and stroke. This was also true when the −629 polymorphism was used to define genotypes.

DISCUSSION

In accordance with our results from the REGRESS and numerous other studies (3–5), we observed a significant and apparently dose-dependent relationship between the number of copies of the B1 allele and lower HDL-c at baseline in the CARE cohort, whereas no relationship with other lipids or lipoproteins was present. We could also confirm that pravastatin induced similar changes in total cholesterol, LDL-c, and HDL-c among TaqIB genotypes. Unexpectedly, however, the odds ratios for the primary end points (i.e., death from CHD or nonfatal MI) were not significantly different from unity for the three genetic subgroups after five years of placebo treatment. Accordingly, in the pravastatin treated group, nonfatal MI and deaths from CHD were reduced to the same extent in all three genetic subgroups.

These findings are in agreement with those found for a cohort of healthy U.S. physicians (5), but they contrast with

Table 2. Baseline Plasma Lipids and Lipoproteins and CHD Events According to the CETP TaqIB Genotype

	Men				Women			
	B1B1 (n = 907)	B1B2 (n = 1,390)	B2B2 (n = 467)	p Value	B1B1 (n = 171)	B1B2 (n = 203)	B2B2 (n = 67)	p Value
Total cholesterol (mmol/l)	5.35 (0.46)	5.35 (0.45)	5.40 (0.43)	0.10	5.54 (0.40)	5.55 (0.39)	5.66 (0.36)	0.09
HDL cholesterol (mmol/l)	0.95 (0.20)	0.98 (0.22)	1.03 (0.20)	< 0.0001	1.12 (0.27)	1.15 (0.27)	1.26 (0.32)	0.002
LDL cholesterol (mmol/l)	3.60 (0.39)	3.57 (0.38)	3.55 (0.38)	0.09	3.61 (0.37)	3.61 (0.35)	3.62 (0.34)	0.97
Log_e (triglycerides)	1.33 (0.38) (n = 449)	1.31 (0.39) (n = 695)	1.34 (0.39) (n = 234)	0.52	1.31 (0.38) (n = 89)	1.29 (0.4) (n = 97)	1.27 (0.4) (n = 37)	0.77
CHD events (OR*)	0.78 (0.47, 1.29)	1.12 (0.71, 1.76)	1.0	0.17*	0.72 (0.22, 2.31)	1.36 (0.46, 4)	1.0	0.34

*Odds ratio of CETP TaqIB genotypes (B1B1 and B1B2 each relative to B2B2) for cardiovascular end points in the placebo group.
B1 = the presence of a restriction site for TaqIB in intron 1 of the CETP gene, and B2 its absence. CETP = cholesteryl ester transfer protein; CHD events = coronary heart disease death or nonfatal myocardial infarction; HDL = high-density lipoprotein; LDL = low-density lipoprotein; OR = odds ratio (lower 95% confidence level; upper 95% confidence level).

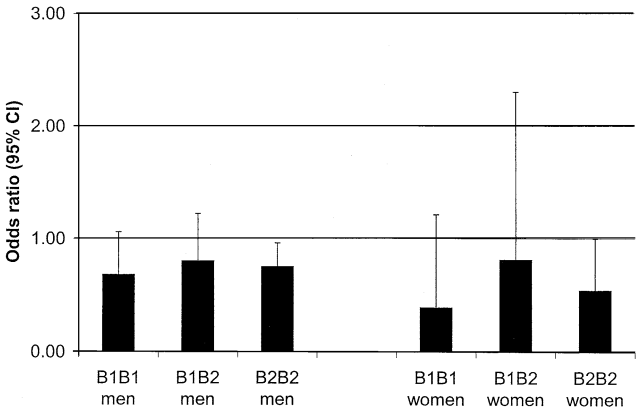

Figure 1. Gender-TaqIB genotype-specific odds ratios for death from cardiovascular disease or nonfatal myocardial infarction in the pravastatin relative to placebo group after five years of treatment. The bars represent the upper limit of the 95% confidence interval of the odds ratio.

data from the Framingham Offspring Study (FOS) (4) and the Veterans Affairs HDL-c Intervention Trial (VA-HIT) (3), in which significant associations between TaqIB and cardiovascular end points were observed. However, upon adjustment for either: 1) age, body mass index, systolic blood pressure, diabetes, smoking, alcohol consumption, beta-blocker use, and total and HDL-c (FOS); or 2) age, diabetes, smoking, and hypertension (VA-HIT), this association was reduced in magnitude, losing statistical significance. In conclusion, the association between CETP genotype and CHD is as yet unclear. Nevertheless, with respect to our previous analysis, we are confronted with a disconnection between surrogate end points in the REGRESS study and cardiovascular end points in the CARE study. Maybe angiographic end points are not equivalent to clinical events in every respect, perhaps for CETP in particular. In this respect, we would like to address that angiography can assess the atherosclerotic process only locally, whereas an atherothrombotic event is ultimately defined by additional factors, including plaque stability, coagulation, and fibrinolysis.

Thus, in the CARE cohort, the CETP TaqIB and −C629A polymorphisms do not predict cardiovascular events, nor do they discriminate between those who will or will not benefit from pravastatin treatment. Finally, unpublished REGRESS data on TaqIB and risk question the concept that a single genetic marker can predict the clinical outcome of a complex, chronic disease such as atherosclerosis. Instead, the effects of variation at multiple gene loci should be studied simultaneously in order to unravel the highly complex genetic component of atherogenesis and cardiovascular disease.

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REFERENCES

1. Kuivenhoven JA, Jukema JW, Zwinderman AH, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. N Engl J Med 1998;338:86–93.
2. Sacks FM, Pfeffer MA, Moye LA, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 1996;335:1001–9.
3. Brousseau ME, O'Connor JJ Jr., Ordovas JM, et al. Cholesteryl ester transfer protein TaqI B2B2 genotype is associated with higher HDL cholesterol levels and lower risk of coronary heart disease end points in men with HDL deficiency: Veterans Affairs HDL Cholesterol Intervention Trial. Arterioscler Thromb Vasc Biol 2002;22:1148–54.
4. Ordovas JM, Cupples LA, Corella D, et al. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. Arterioscler Thromb Vasc Biol 2000;20:1323–9.
5. Liu S, Schmitz C, Stampfer MJ, et al. A prospective study of TaqIB polymorphism in the gene coding for cholesteryl ester transfer protein and risk of myocardial infarction in middle-aged men. Atherosclerosis 2002;161:469–74.
6. Nikiforov TT, Rendle RB, Goelet P, et al. Genetic Bit Analysis: a solid phase method for typing single nucleotide polymorphisms. Nucleic Acids Res 1994;22:4167–75.
7. SAS Institute, Inc. SAS/STAT User's Guide, Version 8, Cary NC: SAS Institute, Inc., 1999.