European Heart Journal (2003) **24**, 1833–1842

ELSEVIER

EUROPEAN SOCIETY OF CARDIOLOGY

Clinical research

A polymorphism of the cholesteryl ester transfer protein gene predicts cardiovascular events in non-smokers in the West of Scotland Coronary Prevention Study

Dilys J. Freeman[a,†,*], Nilesh J. Samani[b], Valerie Wilson[a], Alex D. McMahon[c], Peter S. Braund[b], Suzanne Cheng[d], Muriel J. Caslake[e], Chris J. Packard[e], Dairena Gaffney[e], on behalf of the West of Scotland Study Group

[a] *Department of Biological Sciences, University of Durham, Durham, UK*
[†] *Present address: Department of Obstetrics and Gynaecology, University of Glasgow, Royal Infirmary, Glasgow, UK*
[b] *Division of Cardiology, University of Leicester, Glenfield Hospital, Leicester, UK*
[c] *Robertson Centre for Biostatistics, University of Glasgow, Glasgow, UK*
[d] *Department of Human Genetics, Roche Molecular Systems, Inc, Alameda, California, USA*
[e] *Department of Pathological Biochemistry, Glasgow Royal Infirmary University NHS Trust, Glasgow, UK*

Received 14 April 2003; received in revised form 22 July 2003; accepted 31 July 2003

KEYWORDS
Atherosclerosis;
Enzymes;
Genetics;
Lipoproteins;
CETP;
WOSCOPS

Aim The association of cholesteryl ester transfer protein (CETP) gene polymorphisms with risk of a cardiovascular event and whether any association was explained by an influence on high-density lipoprotein (HDL) levels or low-density lipoprotein (LDL) size was tested in the West of Scotland Coronary Prevention Study (WOSCOPS). Gene-smoking and gene-treatment interactions were investigated.

Methods and results Cases (n=498) and controls (n=1108) were typed for *Taq*IB, C(−631)A, C(−629)A, I405V and D442G CETP polymorphisms. Homozygotes for the *Taq*IB2 allele (B2B2) had a 30% reduced risk of a cardiovascular event (odds ratio [OR] 0.70, CI_{95} 0.51–0.96, P=0.03) compared to B1B1 homozygotes. Inclusion of HDL or LDL diameter in multivariate analysis only marginally attenuated the relationships. Non-smokers, but not smokers, showed a dose-dependent association of risk with *Taq*IB genotype. Treatment benefit was not significantly different in B1B1 (OR 0.71, pravastatin vs placebo), B1B2 (OR 0.68) and B2B2 (OR 0.61) individuals. The other CETP polymorphisms studied had no significant association with cardiovascular risk. Haplotype analysis did not add to the information given by the individual polymorphisms.

Conclusion The association between CETP *Taq*IB genotype and cardiovascular risk is primarily in non-smokers, is not fully explained by effects on HDL levels or LDL size, and the benefit of pravastatin treatment was not influenced by this polymorphism.

Introduction

There is interest in the role of genetic polymorphisms in predicting susceptibility to disease and responsiveness to dietary and drug interventions. Small-scale studies have looked at the effects of polymorphisms on physiological

* Correspondence to: Dr D. J. Freeman, University of Glasgow, Obstetrics and Gynaecology, 3rd Floor Queen Elizabeth Building Royal Infirmary, Glasgow G31 2ER, UK. Tel: +44 141 211 4706; Fax: +44 141 552 0873
E-mail address: d.freeman@clinmed.gla.ac.uk (D.J. Freeman).

0195-668X/03/$ - see front matter © 2003 Published by Elsevier Ltd on behalf of The European Society of Cardiology.
doi:10.1016/j.ehj.2003.07.001

or biochemical factors and have provided useful information on possible mechanistic links between variation at the gene level and risk factors for cardiovascular disease (CVD). Unfortunately these studies often lack power, have insufficient information on confounding lifestyle variables or are from ethnically non-homogenous populations. This has lead to confusion as to which polymorphisms have significant and clinically relevant influences on risk for disease that are independent of environmental variables.

The *Taq*IB polymorphism of the CETP gene is consistently associated with high-density lipoprotein (HDL) levels and was associated with coronary heart disease risk in the Framingham Study[1] but not with risk of a myocardial infarction in the Physicians' Health Study.[2] The *Taq*IB polymorphism[3] is found in intron 1 and the rarer B2 allele has been found to be associated with raised plasma HDL cholesterol levels and reduced plasma CETP mass.[4–6] Lifestyle factors such as cigarette smoking, alcohol consumption and body mass index (BMI) interact with this association.[7,8] The relationship between cholesteryl ester transfer protein (CETP) genotype and HDL is strongest in non-smokers[5,7] and in those with high alcohol consumption.[8] The *TaqIB* polymorphism has not been shown to have direct effects on CETP levels or function.

Several other polymorphisms in the CETP gene have been identified. Some are rare, such as the D442G mutation close to the active site which leads to reduced plasma CETP mass and specific activity.[9] The more common I405V polymorphism, which affects CETP primary structure but not plasma CETP mass,[9] is associated with vascular disease.[10] Promoter polymorphisms at position −631 and −629 have been identified.[11] The C(−629)A polymorphism affects promoter activity and hence CETP mass and is in linkage disequilibrium with the *Taq*IB polymorphism.[12,13] A report of linkage between the CETP gene and low-density lipoprotein (LDL) particle size raises another possibility for the association between polymorphisms in the CETP gene and cardiovascular risk as both low HDL and increased small dense LDL particles are associated with cardiovascular risk.[14]

Clinical trials testing the efficacy of treatments for CVD provide a rich resource for studying genetic factors. These studies are large, collect detailed information on lifestyle factors and have clinically relevant endpoints. In the Regression Growth Evaluation Statin Study (REGRESS) the CETP *Taq*IB B1 allele was associated with higher CETP concentration, lower HDL cholesterol and greater progression of disease as assessed by a decrease in mean luminal diameter.[15] This association was abolished by pravastatin treatment; pravastatin appeared to slow progression in B1B1 but not B2B2 patients. Similarly, in the Veterans Affairs HDL Cholesterol Intervention Trial (VA-HIT) the CETP B1B1 genotype was associated with lower HDL, higher cardiovascular risk and greatest triglyceride-lowering response to gemfibrozil.[16]

The West of Scotland Coronary Prevention Study (WOSCOPS) was a primary prevention study in 45–64-year-old men that demonstrated a significant reduction of cardiovascular morbidity and mortality by pravastatin treatment.[17,18] During the 5-year course of the study, 580 cardiovascular events (fatal and non-fatal) occurred. We used a nested case control study design, matching two controls to every case by age and smoking habit, to assess the ability of the CETP *Taq*IB genotype, in addition to the C(−629)A, C(−630)A, I405V and D442G polymorphisms, to predict cardiovascular events and to examine interactions with smoking and pravastatin treatment. We also examined whether any effect of genotype on risk could be explained by its effect on HDL level or LDL diameter.

Methods

Study subjects

Baseline characteristics of the WOSCOPS participants have been detailed previously.[17] The study complies with the Declaration of Helsinki, was approved by the local Ethics Committee and participants gave informed consent. The WOSCOPS cohort comprised 6595 males age 45–65 (mean 55.2 years) with a mean baseline total cholesterol of 7.0 mmol/l, HDL cholesterol of 1.2 mmol/l and mean triglycerides of 1.8 mmol/l. Recruits had no history of myocardial infarction and had normal renal and hepatic function. Individuals who, during the course of the 5-year study, experienced a definite or suspect fatal or non-fatal myocardial infarction, sudden coronary death or required coronary artery bypass graft or angioplasty were defined as cases (n=580). Each patient was matched with two controls (also drawn from the original cohort of 6595 men), for a total of 1160 controls, on the basis of age (using 2-year age categories) and smoking status, with subject categorized as either non-smokers (those who had never smoked or who had quit smoking) or current smokers who were event free during the 5-year study as described previously.[19] A proportion of samples (6.7%, n=116) were missing from the Biobank and not available to prepare DNA.

Plasma lipids

Plasma lipids and lipoproteins were measured in fasting plasma samples according to the protocols of the Lipid Research Clinics.[20] LDL peak particle diameter, reported as the particle diameter of the major LDL fraction, was used as an index of LDL size distribution and was determined as described previously[21] with the exception that 2–10% polyacrylamide gel electrophoresis was used (Alamo Gels, San Antonio, Texas).

Polymorphism detection

DNA was extracted from packed blood cells stored at −70 °C .[22] The *Taq*IB polymorphism was detected as described.[8] The C(−631)A, C(-629)A, I405V, and D442G CETP gene polymorphisms were simultaneously genotyped using a novel multilocus assay, essentially as described by Cheng et al.[23] DNA was amplified using a multiplex PCR reaction with biotinylated primers. The amplified PCR products were detected colorimetrically by temperature sensitive hybridization to immobilized sequence specific oligonucleotide probes arrayed in a line format on nylon membrane strips and correct allele identification was confirmed by direct sequencing. For the rarest alleles [(−631)A,(−629)A] synthetic oligonucleotide templates were used to validate the probes. Due to the proximity of the C(−631)A and C(−629)A polymorphisms, the two-site haplotypes were detected directly.

Table 1 Baseline characteristics of case and control groups. Cases and controls were defined as described in the Methods section. Data given for continuous variables are mean (standard deviation), and for categorical variables are the number of subjects (%) with the stated attribute

Characteristic	Case (*n*=498)	Control (*n*=1108)	*P*
Age (years)	56.9 (5.1)	56.7 (5.2)	[a]
Body mass index (kg/m^2)	26.0 (3.2)	25.6 (3.2)	0.04
Smokers (%)[a]	266 (53%)	606 (55%)	[a]
Alcohol consumption (units/week)	11 (13)	11 (13)	0.55
Total cholesterol (mmol/l)	7.08 (0.61)	7.02 (0.57)	0.09
Triglycerides (mmol/l)	1.96 (0.83)	1.84 (0.77)	0.05
LDL cholesterol (mmol/l)	5.02 (0.46)	4.95 (0.44)	0.04
HDL cholesterol (mmol/l)	1.07 (0.22)	1.14 (0.25)	<0.001
LDL diameter (nm)	26.33 (0.85)	26.40 (0.89)	0.17

[a]Cases and controls were matched for age and smoking.

Genotypes were not obtained in 1.1% of the samples due to failed polymerase chain reaction.

Statistical methods

Differences in baseline characteristics were assessed using conditional logistic regression. Linkage disequilibrium coefficients were estimated using the ASSOCIATE vs 2.35 programme (J. Ott, http://linkage.rockefeller.edu/ott/linkutil.htm#ASSOCIATE). Coefficients were reported as D′ where $D' = D_{AB}/D_{max}$[$D_{max} = \min\{p_A q_B, q_A p_B\}$ if D>0 and $D_{max} = \min\{p_A p_B, q_A q_B\}$ if D<0]. Haplotypes were reconstructed using the PHASE program (version 1.0).[24] Differences in haplotype frequency were tested using a chi-squared test. Differences between cases and controls were compared using a *t*-test or Wilcoxon test. The effects of the covariates, including CETP genotype, were examined both univariately and simultaneously by conditional logistic regression. Statistical significance was calculated using Wald tests and Wald's confidence intervals are quoted. Tests for interactions between CETP genotype and each of smoking and pravastatin treatment were carried out using likelihood-ratio tests. Additionally, the mean levels of HDL were compared amongst the three genotypes by analysis of variance. All analyses were carried out using the SAS statistical package (version 8.02).

Results

Study subjects

The study comprised 498 case and 1108 control males, matched for age and smoking habit, and baseline characteristics are shown in Table 1. Cases had higher body mass index, triglyceride and LDL cholesterol and lower HDL cholesterol than controls. There was no difference in alcohol consumption, total cholesterol and LDL diameter between the groups.

Genotype frequencies

For the D442G polymorphism no individuals were found with the rare allele. The rare allele frequencies for the CETP *Taq*IB, C(−631)A, C(−629)A and I405V polymorphisms were 0.44, 0.08, 0.48 and 0.30 respectively. Genotype frequencies in the total group, cases and controls were in Hardy–Weinberg equilibrium for each of the polymorphisms. The CETP *Taq*IB, C(−631)A and C(−629)A polymorphisms were in strong linkage disequilibrium (Table 2).

Table 2 Linkage disequilibrium coefficients between the *Taq*IB, C(−631)A, C(−629)A and I405V polymorphisms of the CETP gene

Polymorphism	D′[b] *Taq*IB	C(−631)A	C(−629)A	I405V
Taq IB		−0.98[a]	0.97[a]	0.16[a]
C(−631)A			−1.00[a]	−0.58[a]
C(−629)A				−0.47[a]

[a]*P*<0.001.
[b]The sign denotes whether linkage disequilibrium is positive [rare (or common) alleles preferentially associated] or negative [rare allele preferentially associated with common allele].

The CETP genotype frequencies for case and control groups are shown in Table 3. Homozygotes for the rare CETP *Taq*IB allele (B2B2) were less common in the case (15%) than in the control group (20%). Accordingly, using the B1B1 common allele homozygote as referent, the B2B2 genotype was associated with a 30% reduced risk of a cardiovascular event (odds ratio 0.70, 95% confidence interval [CI_{95}] 0.51–0.96, *P*=0.03). There was no reduction in risk of event in the B1B2 heterozygotes. None of the other CETP genotypes were associated with a significant reduction in risk for a cardiovascular event, although the CETP C(−631)A AA genotype and the CETP C(−629)A AA genotype were associated with a trend towards an increase and decrease in cardiovascular risk respectively (Table 3). The opposite direction of these effects is explained by the respective negative and positive linkage disequilibrium of the C(−631)A and C(−629)A polymorphisms with the *Taq*IB polymorphism (Table 2).

Lipid parameters

In both cases and controls there were significant differences in HDL cholesterol between the CETP *Taq*IB, C(−629)A and I405V genotype groups (Table 4). The *Taq*IB

Table 3 Genotype frequencies of cholesteryl ester transfer protein (CETP) polymorphisms in cases and controls; univariate analysis

	Number (%)		Univariate analysis		
Frequency	Case	Control	Odds ratio	Confidence interval	*P*-value
CETP *TaqI*B[a]					
B1B1	164 (33)	339 (31)	1.00	referent value	
B1B2	259 (52)	541 (49)	1.00	0.79–1.26	0.97
B2B2	76 (15)	225 (20)	0.70	0.51–0.96	0.03
CETP C(−631)A[b]					
CC	420 (84)	955 (86)	1.00	referent value	
CA	71 (14)	146 (13)	1.11	0.82–1.51	0.50
AA	7 (1.4)	6 (0.5)	2.60	0.86–7.82	0.09
CETP C(−629)A[b]					
CC	139 (28)	286 (26)	1.00	referent value	
CA	261 (52)	551 (50)	0.98	0.77–1.26	0.89
AA	98 (20)	270 (24)	0.75	0.55–1.02	0.06
CETP I405V[c]					
CC	238 (48)	543 (49)	1.00	referent value	
CT	211 (42)	473 (43)	1.02	0.82–1.27	0.88
TT	49 (10)	92 (8.3)	1.21	0.83–1.76	0.33

[a] 1604
[b] 1605 or
[c] 1606 individuals.

B2B2, C(−629)A AA and I405V TT individuals had higher HDL levels than B1B1, CC and CC individuals respectively, with B1B2, CA and CT individuals having intermediate levels. A reverse trend in HDL concentration was seen among the C(−631)A genotypes, again explained by the opposite sign of linkage disequilibrium. However the contribution of CETP genotype to variation in HDL levels was very small (*TaqI*B cases r^2=0.03, controls r^2=0.02; C(−629)A cases r^2=0.04, controls r^2=0.02, I405V cases r^2= 0.01, controls r^2= 0.01). Only in CETP C(−631)A cases were plasma triglycerides different between the genotype groups.

Haplotype analysis

Haplotype reconstruction for the four CETP polymorphisms under study resulted in 11 haplotypes (Table 5). The small difference in haplotype frequency distribution between cases and controls was significant (P<0.001). Plasma HDL and triglyceride levels and LDL diameter in homozygotes, heterozygotes and non-carriers of the most frequent haplotype (1111) were compared in case and control groups. Only plasma HDL levels differed significantly between the haplotype carrier groups (controls: 1111 homozygote 1.06 (0.24) vs 1111 heterozygote 1.13 (0.25) vs 1111 non-carrier 1.18 (0.24) mmol/l, P<0.001; cases: 1111 homozygote 1.01 (0.18) vs 1111 heterozygote 1.04 (0.21) vs 1111 non-carrier 1.13 (0.25) mmol/l, P<0.001). Mean HDL levels in the most frequent haplotypes (minimum group size=20) for cases and controls are shown in Fig. 1. In all haplotypes mean HDL concentration in cases is less than or equal to that in controls. No one frequent haplotype is characterised by markedly low HDL concentration or a marked difference in HDL level between cases and controls. Furthermore, the haplotypes with the highest HDL concentration (2121, 2121 and 2122, 2121) carried two *TaqI*B B2 alleles and had equivalent HDL concentrations, in both cases and controls, to those of the *TaqI*B B2B2 homozygotes as a whole (Fig. 1 and Table 4). Similarly, the haplotypes with the lowest HDL concentrations (1111, 1211 and 1111, 1111) carried two *TaqI*B B1 alleles and had equivalent HDL concentrations to those of *TaqI*B B1B1 homozygotes as a whole (Fig. 1 and Table 4). Thus the haplotypes did not add any further information to that provided by the *TaqI*B polymorphism alone.

Multivariate analysis

Univariate odds ratios for baseline variables for risk of a cardiovascular event were calculated (Table 6). BMI, plasma triglycerides and LDL cholesterol were significant univariate predictors of increased risk of an event. Treatment with pravastatin and HDL cholesterol levels were associated with a significantly lower risk of a cardiovascular event. In a multivariate model, including potential confounding variables other than plasma HDL cholesterol, the *TaqI*B B2B2 genotype remained significantly associated with a reduced risk of a cardiovascular event (odds ratio 0.69, CI_{95} 0.50–0.95, P=0.02). Adding HDL cholesterol into the model altered the odds ratio to 0.78 (CI_{95} 0.56–1.09) and the P value was no longer significant. In similar multivariate models, the C(−631)A and C(−629)A and I405V polymorphisms were not significantly

Table 4 Plasma HDL cholesterol and triglyceride levels in case and controls subdivided by CETP genotype. Plasma lipids were measured in baseline samples for case and controls. Mean (standard deviation) and geometric mean (standard deviation) are given for HDL cholesterol and triglyceride respectively

	HDL cholesterol (mmol/l)		Triglyceride (mmol/l)	
	Case	Control	Case	Control
CETP *Taq*IB				
B1B1	1.02 (0.20)	1.10 (0.27)	1.82 (0.02)	1.71 (0.02)
B1B2	1.09 (0.23)	1.15 (0.24)	1.82 (0.02)	1.68 (0.02)
B2B2	1.12 (0.22)[b]	1.19 (0.25)[b]	1.73 (0.02)	1.73 (0.02)
CETP C(-631)A				
CC	1.07 (0.22)	1.14 (0.25)	1.78 (0.02)	1.69 (0.02)
CA	1.09 (0.27)	1.13 (0.25)	1.87 (0.02)	1.76 (0.02)
AA	0.93 (0.10)	1.06 (0.13)	2.68 (0.02)[b]	1.64 (0.01)
CETP C(-629)A				
CC	1.00 (0.19)	1.08 (0.24)	1.87 (0.02)	1.73 (0.02)
CA	1.09 (0.23)	1.15 (0.25)	1.78 (0.02)	1.66 (0.02)
AA	1.12 (0.22)[b]	1.19 (0.25)[b]	1.76 (0.02)	1.73 (0.02)
CETP I405V				
CC	1.05 (0.21)	1.12 (0.24)	1.82 (0.02)	1.69 (0.02)
CT	1.08 (0.23)	1.16 (0.26)	1.78 (0.02)	1.69 (0.02)
TT	1.14 (0.24)[a]	1.16 (0.24)[a]	1.82 (0.02)	1.73 (0.02)

[a] $P<0.05$.
[b] $P<0.001$. ANOVA common homozygote vs heterozygote v rare homozygote in cases and controls separately.

Table 5 Reconstructed haplotype frequencies for *Taq*IB, C(-631)A, C(-629)A and I405V CETP polymorphisms

Haplotype[a]	Control *n* (%)	Case *n* (%)
1111	846 (38.2)	388 (39.0)
2121	524 (23.6)	193 (19.4)
2122	449 (20.3)	211 (21.2)
1211	131 (5.9)	80 (8.0)
1112	95 (4.3)	62 (6.2)
1122	79 (3.6)	30 (3.0)
1121	41 (1.9)	23 (2.3)
1212	27 (1.2)	3 (0.3)
2111	17 (0.8)	1 (0.1)
2112	7 (0.3)	3 (0.3)
2211	0 (0)	2 (0.2)

[a] Haplotype order: *Taq*IB, C(−631)A, C(−629)A, I405V [2216 (2×1108) haplotypes were assigned in the controls and 996 (2×498) were assigned in the cases]. P <0.001, chi-squared test for differences in frequency distribution between cases and controls.

associated with risk of a cardiovascular event whether or not HDL was included in the model though analysis of the less frequent polymorphisms had limited power. A multivariate analysis model including confounding variables, HDL and LDL diameter was carried out (Table 6). The inclusion of LDL diameter only marginally attenuated the relationship between CETP genotype and cardiovascular risk for each of the genotypes.

Interaction with smoking

In a univariate model the association of *Taq*IB genotype with cardiovascular events was only seen in non-smokers (odds ratio for B2B2 compared to B1B1 0.50, CI_{95} 0.31–0.81, P=0.005) and was not apparent in smokers (odds ratio 0.93, CI_{95} 0.60–1.43, P=0.74). A likelihood-ratio test for interaction suggested there was evidence of an interaction with smoking (P=0.096). Similarly, the association of the C(−631)A and C(−629)A polymorphisms and cardiovascular events was only seen in non-smokers [C(−631)A odds ratio 8.68, CI_{95} 0.97–78.0, P=0.054: C(−629)A odds ratio 0.54, CI_{95} 0.34–0.85, P=0.007) and not in smokers (C(−631)A odds ratio 1.34, CI_{95} 0.31–5.72, P=0.69: C(−629)A odds ratio 1.00, CI_{95} 0.66–1.52, P=1.00]. There was no association between the I405V polymorphism and risk of cardiovascular disease in either smokers or non-smokers. A multivariate analysis of the association between CETP *Taq*IB genotype and risk of a cardiovascular event is shown in Fig. 2. In non-smokers there is a clear dose-dependent association of cardiovascular risk with CETP genotype which is only marginally attenuated by the inclusion of HDL in the model. In smokers, there is no association between cardiovascular risk and CETP genotype whether or not HDL is included in the multivariate model. Similar dose-dependent relationships were observed in non-smokers for the C(−629)A and C(−631)A polymorphisms.

Interaction with pravastatin treatment

Treatment with pravastatin significantly reduced the risk of a cardiovascular event in this subgroup of WOSCOPS (odds ratio 0.66, CI_{95} 0.54–0.82, P=0.0002). In a univariate analysis, pravastatin treatment had similar benefit in all CETP genotype groups: B2B2 odds ratio 0.61 (CI_{95} 0.36–1.05, P=0.08), B1B2 odds ratio 0.68 (CI_{95} 0.50–0.91, P=0.01) and B1B1 odds ratio 0.71 (CI_{95} 0.48–1.04, P=0.07). A likelihood-ratio test for interaction indicated that there was no interaction between any of the CETP genotypes studied and pravastatin therapy; P=0.92 for *Taq*IB, P=0.84 for C(−631)A, P=1.00 for C(−629)A and P=0.46 for I405V.

Discussion

In this case control study derived from the WOSCOP Study the CETP *Taq*IB genotype was predictive of cardiovascular events in a univariate analysis. Individuals with B2B2 genotype had a significantly reduced risk of 30% (CI_{95} 4–49%) of suffering a cardiovascular event that was independent of alcohol consumption and BMI. The previously recognised interaction of the *Taq*IB genotype with smoking was observed.[5,7] The association between *Taq*IB genotype and cardiovascular risk was limited to non-smokers and, in this subgroup, a dose-dependent relationship between CETP *Taq*I B genotype and cardiovascular risk emerged. The analysis of the C(−631)A,

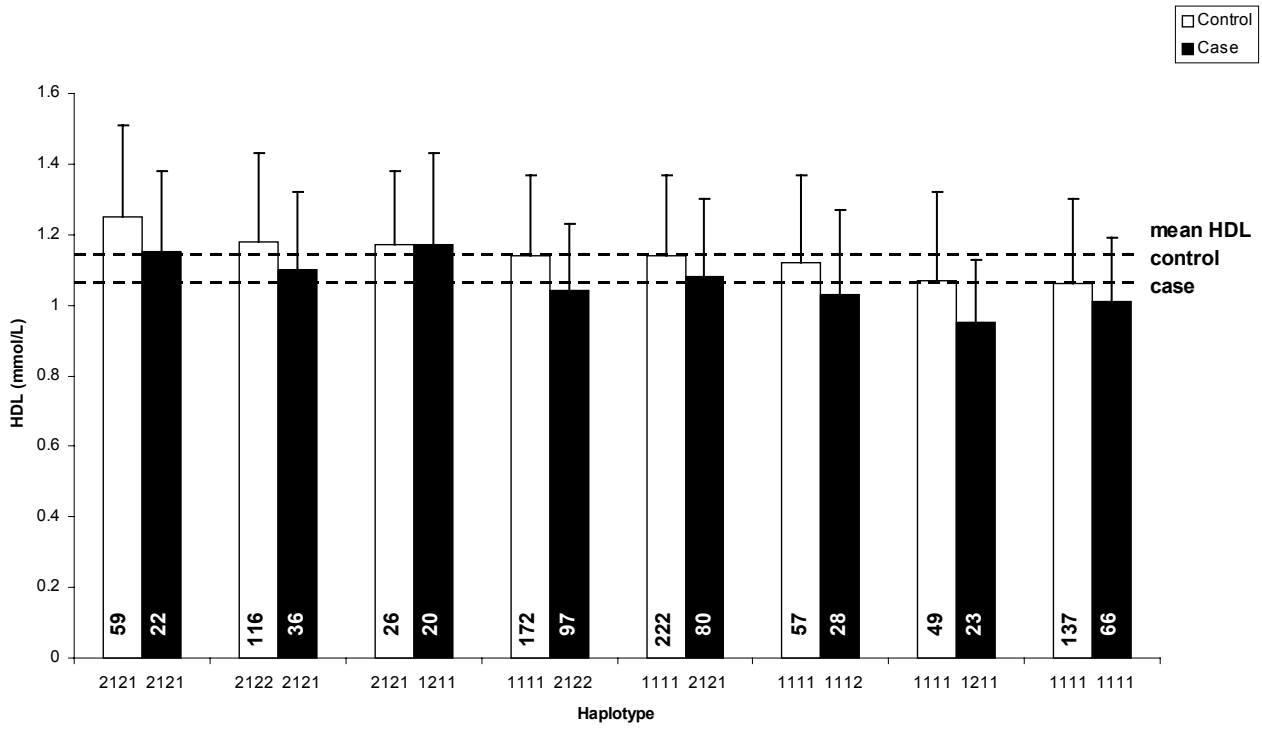

Fig. 1 Histogram of plasma HDL concentration in the common haplotypes. HDL concentrations are plotted for cases and controls separately in decreasing order of control HDL concentration. Data is plotted from haplotypes where the case and control categories contained at least 20 individuals each (number of individuals are shown within the bars). Mean control and case HDL concentrations for the whole sample are indicated by slashed lines.

C(−629)A and I405V polymorphisms did not add any further information. The small differences in haplotype frequency distribution between cases and controls did not identify a particular haplotype that was markedly over- or under-represented in the cases.

Our data are in agreement with the Framingham, REGRESS and VA-HIT studies.[1,15,16] The B1B1 genotype was associated with greater progression of coronary atherosclerosis in REGRESS and more cardiovascular end points in VA-HIT than the B2B2 genotype. This is consistent with our observations on clinical endpoints. In each study B1B1 individuals had lower HDL cholesterol levels than B2B2 individuals. These data are inconsistent with that derived from the Physicians' Health Study[2] where no association between the *Taq*IB polymorphisms and myocardial infarction was found despite a relationship with HDL levels. The fact that the Physicians' Health Study comprised a low risk group with a small number of events might explain these discordant results.

The *Taq*IB polymorphism was in strong linkage disequilibrium with the promoter polymorphisms in agreement with previous observations[12,13] and may explain why a non-functional *Taq*IB polymorphism is associated with CETP mass.[6] However there is evidence that the C(-629)A polymorphism has independent associations with HDL and CETP concentration.[12] The C(−629)A rare A allele is associated with reduced promoter activity and lower CETP mass[11] and in the present study is associated with a trend towards a reduced risk of a cardiovascular event. The *Taq*IB and C(−629)A polymorphisms have a similar magnitude of association with CVD risk reflected in similar odds ratios and confidence intervals. The C(−631)A rare homozygote is associated with a greater CVD risk but the relationship does not reach significance perhaps due to the lack of power associated with a five-fold reduced rare allele frequency compared to the former two polymorphisms. It is possible that other functional polymorphisms in the CETP gene may explain the association between genetic variation at the CETP gene and plasma HDL, CETP levels or both. Two studies published after completion of our analysis have identified a number of new polymorphisms in the promoter of the CETP gene.[12,25] Klerkx et al.[12] suggested that the linkage disequilibrium of the C(−629)A polymorphism with the *Taq*IB polymorphism explains its association with HDL cholesterol and CETP concentrations in the REGRESS study. However, Lu et al[25] found that neither the C(−629)A nor the *Taq*IB polymorphisms were strongly associated with HDL levels and CETP concentrations in a population of 357 elderly Japanese men. Instead, a novel C(−2505)A polymorphism seemed to best explain CETP concentrations while a [gaaa](n) repeat and/or the C(−2505)A polymorphism independently determined HDL cholesterol levels in this population.

The exact mechanism whereby HDL protects against CVD is not known but its role in reverse cholesterol transport is of paramount importance. Thus a likely mechanism for the association of CETP genotype with cardiovascular risk is the influence on plasma HDL. Variation in the CETP gene is associated with plasma CETP mass and HDL cholesterol as evidenced in the REGRESS study. In the presence of triglyceride-rich

Table 6 Univariate and multivariate odds ratios with 95% confidence intervals and *P* values for baseline predictors of a cardiovascular event

Univariate analysis	Odds ratio	95% Confidence interval	*P*-value
Body mass index (kg/m^2)	1.04	1.00–1.07	0.04
Pravastatin treatment	0.66	0.54–0.82	<0.001
Alcohol consumption (units/week)			
Level 1=0	Referent		
Level 2 1–10	0.92	0.69–1.23	0.59
Level 3 11–40	0.87	0.64–1.18	0.36
Level 4 >40	0.89	0.44–1.78	0.74
Ln Triglycerides (mmol/l)	1.42	1.12–1.90	0.005
LDL cholesterol (mmol/l)	1.42	1.12–1.78	0.004
HDL cholesterol (mmol/l)	0.26	0.16–0.43	<0.001
LDL diameter (nm)	0.92	0.81–1.04	0.17
Multivariate analysis adjusting for all confounding variables[a]			
*Taq*IB genotype			
B1B2	1.08	0.84–1.38	0.55
B2B2	0.78	0.56–1.09	0.14
C(−631)A genotype			
CA	1.07	0.78–1.47	0.65
AA	2.17	0.71–6.65	0.17
C(−629)A genotype			
CA	1.09	0.84–1.41	0.53
AA	0.87	0.63–1.19	0.37
I405V genotype			
CT	1.06	0.85–1.33	0.61
TT	1.32	0.90–1.95	0.16
Multivariate analysis adjusting for confounding variables excepting HDL			
*Taq*IB genotype			
B1B2	1.00	0.79–1.28	0.99
B2B2	0.69	0.50–0.95	0.02
C(−631)A genotype			
CA	1.08	0.79–1.47	0.65
AA	2.48	0.81–7.55	0.11
C(−629)A genotype			
CA	1.01	0.78–1.30	0.94
AA	0.76	0.56–1.04	0.08
I405V genotype			
CC	1.02	0.81–1.28	0.88
CT	1.22	0.83–1.79	0.31
Multivariate analysis adjusting for confounding variables including LDL diameter			
*Taq*IB genotype			
B1B2	1.07	0.82–1.39	0.62
B2B2	0.75	0.53–1.07	0.12
C(−631)A genotype			
CA	1.07	0.77–1.50	0.68
AA	2.34	0.69–7.90	0.17
C(−629)A genotype			
CA	1.15	0.87–1.52	0.31
AA	0.88	0.63–1.24	0.47
I405V genotype			
CC	0.99	0.77–1.26	0.91
CT	1.27	0.84–1.92	0.26

[a]Confounding variables are alcohol consumption, body mass index, treatment, triglycerides (natural logarithm), LDL cholesterol, HDL cholesterol.

lipoproteins, CETP removes cholesteryl ester from HDL and replaces it with triglyceride. In combination with hepatic lipase (HL) activity this results in smaller HDL particles[26] that are more rapidly cleared from the circulation.[27] Hence high CETP levels are associated with low HDL levels. Because plasma samples that had not been freeze-thawed were unavailable for the present study, CETP levels were not measured. The observation that the

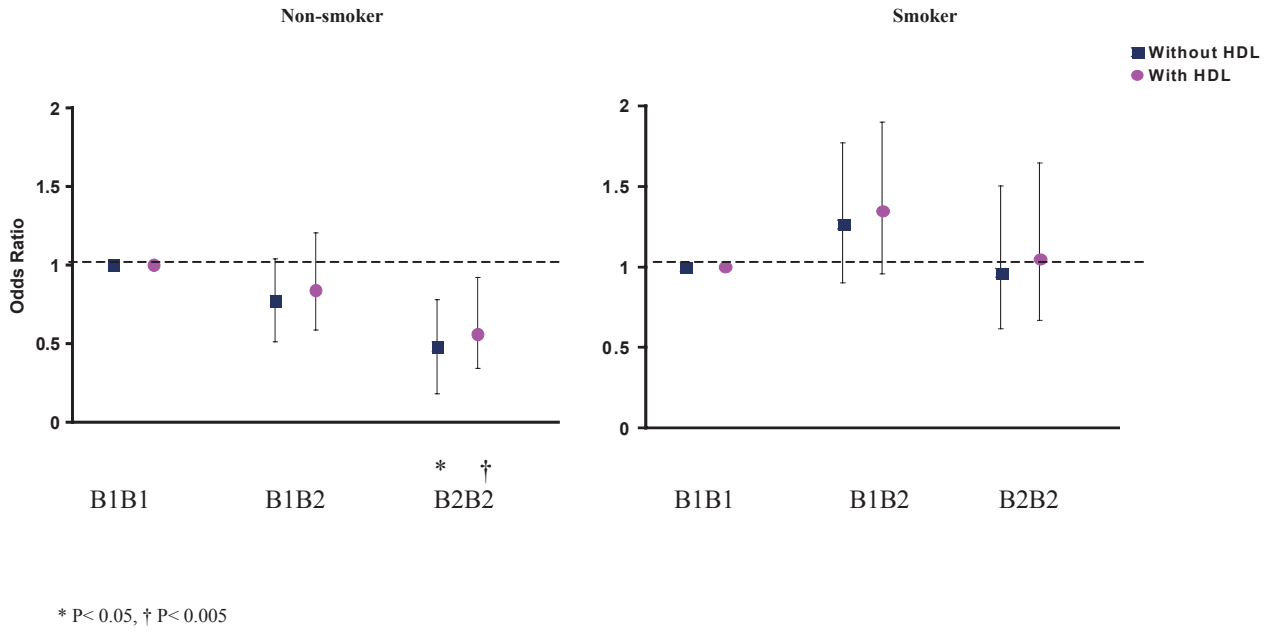

Fig. 2 Multivariate analysis of the association between CETP genotype and risk of a cardiovascular event in smokers and non-smokers. Alcohol consumption, body mass index, treatment, triglycerides (natural logarithm) and LDL cholesterol were included in the model and the effect of inclusion or exclusion of HDL cholesterol is demonstrated. Odds ratios and 95% confidence intervals are shown.

relationship between CETP genotype and cardiovascular risk was independent of lifestyle factors but not of HDL might be consistent with a mechanism involving HDL. However, it should be noted that the association between *Taq*IB genotype and risk of an event is only partially confounded by HDL (OR changed from 0.70 to 0.79). In addition, there was no difference in risk for a cardiovascular event between B1B2 heterozygotes (OR 1.01) and B1B1 homozygotes (Table 3) despite heterozygotes having intermediate HDL levels (Table 4). This suggests that other as yet unknown mechanisms may also be involved in the association of CETP genotype with cardiovascular risk.

Increased small, dense LDL concentrations are associated with increased risk of cardiovascular disease. A linkage of the CETP gene to LDL particle size has been reported and this may also be a potential mechanistic link between variation at the CETP gene and cardiovascular risk.[14] When LDL diameter was included in a multivariate model there was no significant attenuation of risk. It is noteworthy that LDL diameter did not differ between cases and controls and therefore did not represent a significant risk factor in WOSCOPS. Similarly in VA-HIT, LDL size did not differ between *Taq*IB genotype groups.[16] It was reported that the *Taq*IB polymorphism is not associated with LDL size while the I405V polymorphism is,[28] suggesting independent relationships between specific CETP gene variants and HDL cholesterol and LDL size.

The mechanism for the interaction of the CETP genotype with smoking is unclear. Smoking interferes with the activity of plasma enzymes involved in HDL metabolism, namely CETP, lecithin cholesterol acyl transferase (LCAT), lipoprotein lipase (LPL), HL and phospholipid transfer protein (PLTP). The circulating levels of HDL may be dependent in part on the relative activity of these enzymes. Smoking appears to impede the HDL-raising effect of the *Taq*IB2 allele. This could potentially be due to smoking directly counteracting the low CETP levels normally associated with the *Taq*IB2 allele. However there is only one report of increased CETP concentrations in smokers[29] and several reports of either neutral or CETP reducing effects of smoking.[30–32] Alternatively smoking may interfere with other enzymes in such a manner as to neutralize the potential benefits of a reduced CETP activity e.g. inhibition of LCAT[30,33,34] or post-prandial PLTP activity in smokers.[32] The complex interaction between HDL, smoking and plasma enzyme activities has been discussed elsewhere.[31,32]

In WOSCOPS 50% of the subjects received pravastatin therapy, a treatment associated with reduced CHD events and mortality.[18] In the subset of patients comprising the present study pravastatin was associated with a 34% reduction in cardiovascular events (P<0.001). There was no evidence for a differential benefit with therapy in B2B2 compared to B1B1 homozygotes. Similarly there was no difference in benefit between the genotype groups of the other polymorphisms studied. These data are at odds with the REGRESS study where investigators found that pravastatin therapy slowed progression of coronary atherosclerosis in B1B1 but not B2B2 individuals. On the basis of this observation the authors argued that B2B2 individuals did not benefit from treatment which prompted some debate in the literature.[35] This contradiction might be explained by the different end-points used: cardiovascular events in WOSCOPS and mean luminal diameter in REGRESS which may not correlate strongly to clinical outcome.[36] In our population the

benefit of pravastatin did not differ between genotype groups despite there being differences in HDL levels suggesting that the beneficial effects of pravastatin may be independent of its effects on HDL.

We conclude that variation at the CETP gene, in this male West of Scotland population, has significant impact on cardiovascular risk, which may be independent of its influence on HDL levels and LDL size and is restricted to non-smokers. We found no evidence that any of the CETP genotypes failed to benefit from pravastatin therapy.

Acknowledgements

We acknowledge the generous support of a British Hyperlipidaemia Association Research Award and the British Heart Foundation (97/160). The West of Scotland Coronary Prevention Study and its follow up were supported by an educational grant from Bristol-Myers Squibb Co. Research.

Appendix A

West of Scotland Coronary Prevention Study Group

Executive Committee

James Shepherd (chairman), Stuart M. Cobbe, A. Ross Lorimer, James H. McKillop, Ian Ford, Christopher J. Packard, Peter W. Macfarlane, Christopher Isles.

Data and Safety Monitoring Committee

Michael F. Oliver (chairman), Anthony F. Lever, Byron W. Brown, John G. G. Ledingham, Stuart J. Pocock, Basil M. Rifkind.

Cardiovascular End-points Committee

Stuart M. Cobbe (chairman), Barry D. Vallance, Peter W. Macfarlane.

Adverse Events Review Board

A. Ross Lorimer, James H. McKillop, David Ballantyne.

Data centre staff

Liz Anderson, David Duncan, Sharon Kean, Audrey Lawrence, June McGrath, Vivette Montgomery, John Norrie.

Population screening

Melvyn Percy.

Clinical coordination, monitoring, and administration

Elspeth Pomphrey, Andrew Whitehouse, Patricia Cameron, Pamela Parker, Fiona Porteous, Leslie Fletcher, Christine Kilday.

Computerized ECG analysis

David Shoat (deceased), Shahid Latif, Julie Kennedy

Laboratory operations

M. Anne Bell, Robert Birrell

Company liaison and general support

Margot Mellies, Joseph Meyer, Wendy Campbell.

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