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TaqIB polymorphism in cholesterol ester transfer protein (*CETP*) gene predicts future cardiovascular death in patients experiencing an acute coronary syndrome

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Abstract

Background: Cholesterol ester transfer protein (CETP) plays a pivotal role in the remodelling of triglyceride (TG)-rich and high-density lipoprotein (HDL) particles. Sequence variations in the *CETP* gene may interfere with coronary atherosclerosis. However, clinical studies of various CETP polymorphisms have shown controversial data in coronary artery outcome. We aimed to investigate whether *TaqIB CETP* gene polymorphism could predict clinical outcome in a prospective cohort of patients hospitalized for an acute coronary syndrome (ACS).

Methods: Two hundred and seventy consecutive Caucasian patients hospitalized for an ACS, and having a significant coronary artery disease in at least one major vessel (stenosis >50%), were prospectively enrolled and followed for 57 months. The mean age was 65.1±12.5 years, and 77% were males. One hundred and thirty-nine patients (51.5%) suffered from unstable angina at inclusion and 131 patients (48.5%) presented with an acute myocardial infarction (MI). The follow-up data were obtained from questionnaires. The major recurrent events recorded were 32 deaths comprising 28 cardiovascular deaths and 49 combined cardiovascular events (28 cardiovascular deaths, 19 non-fatal ACS and 2 non-fatal strokes). *CETP* genotyping was performed using a restriction fragment length polymorphism based method.

Results: A significant relation was found between *B2B2* genotype and combined cardiovascular endpoint ($p<0.02$), mainly driven by a link with cardiovascular death ($p<0.05$). The hazard risk ratio for cardiovascular death associated with *B2B2* genotype was 2.2 [95% confidence interval (CI): 1.01–4.94, $p<0.05$]. In multivariate analyses, no modification except for a significant interaction with statin therapy was observed by inclusion of potential confounders for the association of *B2B2* genotype with cardiovascular death.

Conclusions: These results suggest that patients homozygous for the *B2* allele and not taking statin had a strong increase of recurrent cardiovascular event after an initial acute coronary event. This cardiovascular risk seems to be corrected with statin therapy.

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Keywords: acute coronary syndrome; cardiovascular death; cholesterol ester transfer protein (*CETP*); gene polymorphism; prospective study.

Introduction

The inverse relationship between high-density lipoprotein cholesterol (HDL-C) and the incidence of cardiovascular disease (1, 2) has stimulated interest for pharmacological strategies for raising HDL-C levels. The finding that genetic deficiency of the cholesteryl ester transfer protein (CETP) in humans was associated with increased plasma HDL-C concentrations led to the concept that inhibition of CETP could be an interesting way for preventing coronary artery disease. CETP plays a central role in HDL metabolism, in particular by catalyzing the hetero-exchange of cholesteryl esters (CEs) from HDL particles to apolipoprotein B (apoB)-containing particles, and triglycerides (TGs) from apoB lipoproteins to HDL particles (3). Numerous studies have shown that plasma concentrations of CETP are inversely related to HDL-C concentrations. Administration of the CETP inhibitor torcetrapib was shown to increase HDL-C concentrations by more than 50%. However, the effectiveness of CETP inhibition as a strategy for anti-atherosclerotic therapy is controversial due to the recent unexpected association of torcetrapib with increased cardiovascular mortality (4–6). Today, the question is how to better identify patients who might derive some benefit from such a therapeutic approach. In this way, analysis of the relationship of genetic homozygous or heterozygous CETP deficiency to cardiovascular risk could be very interesting, but remains unclear (7–18). The risk of coronary heart disease might be lower in CETP-deficient heterozygotes, but CETP deficiency in Japanese-American men was associated with an increased prevalence of cardiovascular artery disease (CAD) despite modestly increased

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HDL-C (9, 18). A common polymorphism of the *CETP* gene located in intron 1 (*TaqIB*) has consistently been shown to influence CETP activity and HDL-C concentrations. This polymorphism is non-functional by itself, but is in almost complete linkage disequilibrium with the –629*C/A* promoter polymorphism which directly modulates *CETP* gene transcriptional activity (19–22). After an acute coronary syndrome (ACS), patients with low plasma HDL-C receive clinical benefit from statin therapy. Since genetic variation of the *CETP* gene strongly affects HDL-C and possibly statin therapy, we conducted a prospective study to assess the potential effect of the *TaqIB* polymorphism on the rate of recurrent cardiovascular events in patients hospitalized for an acute coronary event.

Materials and methods

Study design and patients

Two hundred and eighty-four consecutive patients of Caucasian origin, hospitalized from October 1998 to January 2001 at the Cardiology Unit in Bordeaux University Hospital for an acute coronary event associated with a coronary artery disease confirmed by coronary angiography on the basis of a luminal diameter stenosis $\geq$50% in at least one coronary artery, were recruited for this study. Patients of any age and gender were recruited. Each gave written informed consent for blood collection for use in a confidential ''deoxyribonucleic (DNA) bank'', approved by the Local Research Ethics Committee. Key demographic characteristics at baseline were recorded using computerized angiographic data forms, including age, gender, risk factors, clinical presentation and biological data. Only patients presenting with an acute coronary event defined as unstable angina or acute myocardial infarction (MI) were recruited. The diagnosis of unstable angina included the presence of typical angina at rest associated with acute or transient ST segment or T wave changes without enzyme increases. Acute MI was diagnosed according to the occurrence of chest pain, increased myocardial enzymes (total and creatine kinase-MB and troponin I $\geq$twice the upper normal limit) and electrographic changes (ST elevation or depression). Hypercholesterolemia was defined as plasma low-density lipoprotein cholesterol (LDL-C) concentrations $>$2.58 mmol/L; hypoalphalipoproteinemia was defined as plasma HDL-C concentrations $<$1.032 mmol/L for men and 1.29 mmol/L for women; hypertriglyceridemia was defined as plasma TG concentrations $>$1.7 mmol/L (23, 24). Hypertension was defined 1) in non-diabetic patients as systolic pressure $>$140 mm Hg or diastolic pressure $>$90 mm Hg; 2) in diabetic patients as systolic pressure $>$130 mm Hg or diastolic pressure $>$80 mm Hg; 3) as a history of hypertension requiring treatment (25). Body mass index was calculated as body weight in kg divided by the square of height in meters. Medications at discharge were noted. As indicated in Table 1, the mean age of the study population was 65.1$\pm$12.5 years, with 77% male. At inclusion, 139 patients (51.5%) suffered from unstable angina and 131 patients (48.5%) presented with an acute MI. A previous MI was found in 51 patients (18.9%). Sixty-nine percent of the patients presented with multiple coronary stenosis, and 8% presented with a low ejection fraction (EF) of $<$40%.

Table 1 Baseline characteristics of the study population (n=270).

Age, years (mean$\pm$SD)	65.1$\pm$12.5
Gender (male), n (%)	208 (77)
Diabetes, n (%)	51 (19)
BMI, kg/m^2 mean$\pm$SD	25.8$\pm$3.9
Hypercholesterolemia, n (%)	207 (77)
Hypertriglyceridemia, n (%)	93 (34)
HypoHDLemia, n (%)	115 (43)
Hypertension, n (%)	187 (69)
Current smoker, n (%)	82 (30)
EF $<$ 40%, n (%)	21 (8)
MVD, n (%)	186 (69)
Unstable angina, n (%)	139 (51)
MI, n (%)	131 (49)
Previous MI, n (%)	51 (19)
Glycemia, mmol/L	5.95$\pm$1.42
Total cholesterol, mmol/L	5.32$\pm$1.14
TGs, mmol/L	1.72$\pm$1.13
HDL-C, mmol/L	1.19$\pm$0.48
LDL-C, mmol/L	3.41$\pm$1.00
Fibrinogen, g/L	4.35$\pm$1.22
ESR, mm/h	27.38$\pm$23.64
Aspirin, n (%)	260 (96)
β-Blocker, n (%)	178 (66)
ACE inhibitor or ARAII, n (%)	140 (52)
Statin, n (%)	188 (70)
Overall mortality, n (%)	32 (12)
Cardiovascular death, n (%)	28 (10)
Combined cardiovascular event, n (%)	49 (18)

Medications are treatment during follow-up. BMI, body mass index; EF, ejection fraction; MVD, multiple vessels diseased; MI, myocardial infarction; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ESR, erythrocyte sedimentation rate; ACE, angiotensin converting enzyme; ARAII, angiotensin II receptor antagonists.

Follow-up

Follow-up data were obtained from questionnaires sent to the patient or family, and the patient's general physician and cardiologist. Questionnaires were sent 2 years following examination and then every other year. The following variables were recorded: death, cardiac death, stroke and ACS. Relevant clinical data were also analyzed for the patients who were readmitted or died in the hospital. Cardiac death was defined as that from any cardiac cause (sudden death, fatal MI, heart failure). Treatment during follow-up, although not standardized, was recorded. The mean follow-up period was 57 months. Among the 284 patients included in this study, 14 patients were lost to follow-up and excluded from the study. The final analysis was performed using the 270 patients with follow-up information. As indicated in Table 1, the major events recorded were 32 deaths, consisting of 28 deaths from cardiovascular causes, two from cancer, one from lymphoma and one from articular gangrene; and 49 combined cardiovascular events comprised of 28 deaths from cardiovascular causes, 19 non-fatal ACS and two non-fatal strokes. Median delay for combined end points was 12 months.

Lipids and lipoproteins

Total serum cholesterol (TC), TG, and HDL-C concentrations were determined using enzymatic assay kits (Roche modular, Roche Diagnostics SA, Meylan, France). LDL-C was calculated using the Friedewald equation (26). Lipid profiles

Table 2 Distribution of follow-up events according to *CETP TaqIB* genotypes.

	Number of subjects	Overall mortality (n=32)	Cardiovascular death (n=28)	Combined CV event (n=49)
CETP TaqIB genotypes				
B1B1, n (%)	83	7 (8.4)	6 (7.2)	11 (13.3)
B1B2, n (%)	137	16 (11.7)	13 (9.5)	23 (16.8)
B2B2, n (%)	50	9 (17.6)	9 (17.6)	15 (29.4)
Overall p-value		0.253	0.127	**0.044**
p-Value for *B2B2*		0.136	**0.049**	**0.017**

Differences in genotypes frequencies were evaluated by the χ^2-test. CV, cardiovascular. Bold: $p<0.05$.

obtained the day of ACS diagnosis were the only ones considered for analysis.

Blood sampling for DNA analysis

All blood samples for DNA analysis were collected at discharge. DNA was extracted from 200 μL of the buffy coat of a centrifuged EDTA anticoagulated blood sample, with a QiaAmp®DNA minikit (QIAGEN S.A., Courtaboeuf, France) according to the manufacturer's protocol.

CETP genotyping

CETP genotyping was performed using a restriction fragment length polymorphism based method. Briefly, polymerase chain reaction (PCR) amplification was performed using primers surrounding the *TaqIB* polymorphism in intron 1 of the *CETP* gene: 5′-CAC ACC ACT GCC TGA TAA CC-3′ (forward) and 5′-GTG ACC CCC AAC ACC AAA TA-3′ (reverse). The amplification mixture included 25 pmol of each primer, 100 ng genomic DNA, 1x GoTaq® reaction buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, and 0.5 U of GoTaq® DNA polymerase in a total volume of 25 μL (Promega Corporation, Madison, WI, USA). Amplification was performed for 35 cycles of 30 s at 95°C, 15 s at 60°C, and 15 s at 72°C with an initial denaturation period of 5 min at 95°C. Approximately 20 μL of PCR products were digested with the restriction enzyme TaqI according to the recommendations of the supplier (Fermentas, Life Sciences, St. Rémy les chevreuse, France). Fragments were separated using 2% MP agarose gel (Boehringer Mannheim, Mannheim, Germany) and stained with ethidium bromide. One fragment of 505 bp indicated the absence of the *TaqI* restriction site (*B2B2* genotype), two fragments of 415 and 90 bp indicated the presence of the restriction site (*B1B1*), and three fragments of 505, 415, and 90 bp indicated heterozygosity for the restriction site (*B1B2*).

Statistical analysis

Hardy-Weinberg equilibrium was tested using the χ^2-test with two degrees of freedom. Differences between the groups of patients were evaluated with the unpaired t-test for continuous variables, and with the χ^2-test for categorical variables. The cumulative survival plot in relation to *CETP* genotypes was estimated by the Kaplan-Meier method with use of the log-rank test. Hazard risk ratios according to *CETP* genotypes were estimated using Cox regression analysis. The interaction between the *CETP TaqIB B2B2* genotype and statin treatment was tested by introducing a corresponding interaction term into the Cox model. In survival analyses, the p-value associated with the genotype was obtained by assuming a co-dominant allele effect (genotype entered as an ordinal variable 0, 1, or 2). A $p<0.05$ was considered statistically significant. All computations were carried out with NCSS 2000 (NCSS Inc, Kaysville, UT, USA).

Results

CETP genotypes and clinical outcomes

A significant association was found between the *CETP TaqIB* polymorphism and combined cardiovascular end point (Table 2). Homozygousity for the mutant

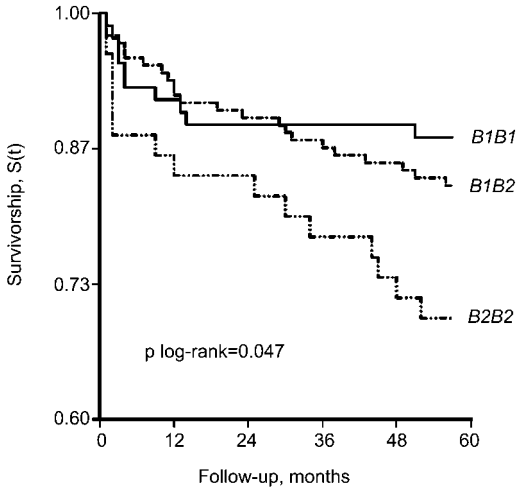

Figure 1 Combined cardiovascular event: survival analysis (Kaplan-Meier) for 270 coronary patients with ACS according to *CETP TaqIB* genotypes.

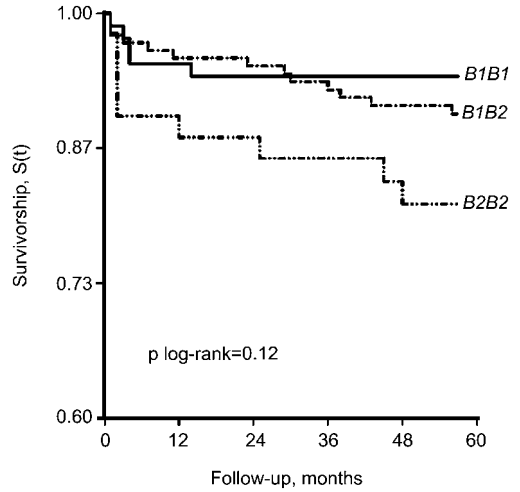

Figure 2 Death from a cardiovascular cause: survival analysis (Kaplan-Meier) for 270 coronary patients with ACS according to *CETP TaqIB* genotypes.

Table 3 Baseline characteristics of patients according to cardiovascular death.

	With cardiovascular death (n = 28)	Without cardiovascular death (n = 242)	p-Value
Age, years (mean ± SD)	71.0 ± 10.2	64.5 ± 12.6	**0.003**
Gender (male), n (%)	19 (68)	189 (78)	0.22
Diabetes, n (%)	13 (46)	38 (16)	**< 0.001**
BMI, kg/m^2 mean ± SD	27.2 ± 4.9	25.6 ± 3.8	0.12
Hypercholesterolemia, n (%)	18 (64)	189 (78)	0.10
Hypertriglyceridemia, n (%)	8 (29)	85 (36)	0.46
HypoHDLemia, n (%)	12 (43)	103 (43)	ns
Hypertension, n (%)	23 (82)	164 (68)	0.12
Current smoker, n (%)	7 (25)	75 (31)	ns
EF < 40%, n (%)	6 (21)	15 (6)	**0.004**
MVD, n (%)	23 (82)	163 (69)	0.15
Unstable angina, n (%)	18 (64)	121 (50)	0.15
MI, n (%)	10 (36)	121 (50)	0.15
Previous MI, n (%)	8 (29)	43 (18)	0.17
Glycemia, mmol/L	6.83 ± 1.83	5.85 ± 1.33	**0.01**
Total cholesterol, mmol/L	5.54 ± 1.67	5.30 ± 1.06	0.48
TGs, mmol/L	1.98 ± 1.56	1.70 ± 1.08	0.38
HDL-C, mmol/L	1.11 ± 0.33	1.20 ± 0.49	0.24
LDL-C, mmol/L	3.74 ± 1.63	3.37 ± 0.92	0.29
Fibrinogen, g/L	4.88 ± 0.94	4.29 ± 1.23	**0.008**
ESR, mm/h	41.95 ± 31.66	26.00 ± 22.35	**0.044**
Aspirin, n (%)	26 (93)	234 (97)	0.31
β-Blocker, n (%)	11 (39)	167 (69)	**0.001**
ACE inhibitor or ARAII, n (%)	19 (68)	121 (50)	0.07
Statin, n (%)	18 (64)	170 (70)	ns

Medications are those prescribed during follow-up. Continuous variables were analyzed with the unpaired t-test and categorical variables using the χ^2-test. BMI, body mass index; EF, ejection fraction; MVD, multiple vessels diseased; MI, myocardial infarction; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ESR, erythrocyte sedimentation rate; ACE, angiotensin converting enzyme; ARA, angiotensin II receptor antagonists. Bold: $p < 0.05$.

allele was more frequent in the patient group with a future combined cardiovascular event compared with non-carriers ($p < 0.02$). This association was primarily driven by cardiovascular mortality ($p < 0.05$), with no effect observed for non-fatal ACS or stroke. The relationship between genotypes and combined cardiovascular event or cardiovascular mortality are illustrated in the Cox survival plots (Figures 1 and 2).

Characteristics of study population according to cardiovascular death

Major traits significantly associated with cardiovascular death were age, diabetes mellitus, alteration of left ventricular function, and use of β-blockers (Table 3). It is also interesting to note a more severe inflammatory response to the initial coronary event.

Table 4 Hazard risk ratios of future cardiovascular death.

Categories	Variables	β	SE	Z-Value	p-Value	HR	CI (95%)	
Model 1	*B2B2*	0.803	0.405	1.98	0.047	**2.23**	1.01	4.93
Model 2	Age, years	0.049	0.019	2.45	0.014	**1.05**	1.01	1.09
	Gender (male)	–0.161	0.425	–0.38	0.706	0.85	0.37	1.96
	B2B2	0.778	0.405	1.92	0.055	2.18	0.98	4.82
Model 3	Age, years	0.042	0.021	1.95	0.051	1.04	1.00	1.09
	Gender (male)	–0.010	0.446	–0.02	0.982	0.99	0.41	2.37
	Diabetes	0.949	0.412	2.30	0.021	**2.58**	1.15	5.79
	BMI > 25 kg/m^2	1.086	0.502	2.16	0.030	**2.96**	1.11	7.92
	Hypertension	0.086	0.520	0.17	0.868	1.09	0.39	3.02
	β-Blocker	–0.720	0.424	–1.70	0.090	0.49	0.21	1.12
	ACE inhibitor or ARAII	0.190	0.447	0.43	0.671	1.21	0.50	2.91
	EF < 40%	1.348	0.547	2.46	0.014	**3.85**	1.32	11.24
	B2B2	0.892	0.422	2.11	0.035	**2.44**	1.07	5.58

Multiple variate Cox proportional hazards analyses were performed with cardiovascular death as the dependent variable and various independent variables as mentioned in the Table. HR, hazard risk ratio; *B2B2*, patients homozygous for *B2* allele; BMI, body mass index; ACE, angiotensin converting enzyme; ARA, angiotensin II receptor antagonists; EF, ejection fraction. Bold: $p < 0.05$.

Table 5 Hazard risk ratios of future cardiovascular death and interaction analyses with lipid profile parameters.

Categories	Variables	β	SE	Z-Value	p-Value	HR	CI (95%)	
Model 1	*B2B2*	0.803	0.405	1.98	0.047	**2.23**	1.01	4.93
Model 2	Decreased HDL	0.621	0.516	1.2	0.229	1.86	0.68	5.12
	B2B2	1.13	0.605	1.87	0.062	3.10	0.95	10.16
	I	−0.923	0.983	−0.94	0.348			
Model 3	Hypertriglyceridemia	−0.335	0.532	−0.63	0.528	0.72	0.25	2.03
	B2B2	0.809	0.500	1.62	0.105	2.25	0.84	5.98
	I	0.222	0.885	0.25	0.801			
Model 4	Hypercholesterolemia	0.511	0.756	0.68	0.499	1.67	0.38	7.34
	B2B2	1.96	0.913	2.15	0.031	**7.13**	1.19	42.70
	I	−1.71	1.074	−1.59	0.112			
Model 5	Statin therapy	0.209	0.521	0.4	0.687	1.23	0.44	3.42
	B2B2	1.82	0.633	2.87	0.004	**6.15**	1.78	21.31
	I	−1.624	0.820	−1.99	**0.047**			

Multiple variate Cox proportional hazards analyses were performed with cardiovascular death as dependent variable and various independent variables as mentioned in the Table. HR, hazard risk ratio; HDL, high-density lipoprotein; *B2B2*, patients homozygous for *B2* allele; I, interaction term which correspond to the co-presence of the first two variables. Bold: $p < 0.05$.

Patient characteristics and genotype frequencies

Overall allele frequencies in the study population were 0.56 for *B1* and 0.44 for *B2*. The distribution of *CETP TaqIB* genotypes was *B1B1* 30.74%; *B1B2* 50.74%; and *B2B2* 18.52%. These distributions conformed to the Hardy-Weinberg equilibrium. Overall, demographic and key clinical characteristics of patients were generally similar by genotype (data not shown). Importantly, distribution by genotype of clinical presentation at inclusion was similar (i.e., 44% of patients homozygous for *B2* allele presented with an acute MI vs. 50% in *B1* carriers, $n = 22$ and 111, respectively, $p = 0.48$).

Multivariate analyses

On the basis of a recessive allele effect on risk for cardiovascular death, the hazard risk ratio associated with the *TaqIB B2* allele was 2.23 [95% confidence interval (CI): 1.01–4.94; $p = 0.047$] (Table 4). To determine whether the *CETP TaqIB B2B2* genotype predicted future fatal cardiovascular events independently of most significant clinical and therapeutic confounders, several models were fitted (Table 4). No modification was observed. Similarly, no modification of the association with *B2B2* genotype was observed when hypoalphalipoproteinemia or hypertriglyceridemia status were included in the analysis. Hypercholesterolemia or statin therapy strongly modified this association (Table 5).

Benefit of statin therapy according to *CETP TaqIB B2B2* genotype

A significant interaction between the *CETP TaqIB B2B2* genotype and statin therapy on cardiovascular mortality was observed (Table 5; p for interaction $= 0.047$). As indicated in Figure 3, patients homozygous for the *CETP TaqIB B2* allele and not treated with statin had a higher prevalence of cardiovascular events during the follow-up period in comparison with patients carrying the *B1* allele. This apparent deleterious effect of the *B2B2* genotype disappears in patients treated with statins.

Patient characteristics according to genotype and treatment status

Patient characteristics differed somewhat by genotype and treatment (e.g., age, hyperlipidemia and concomitant therapy), but major baseline traits associated with the *B2B2* genotype and absence of statin medication were hypercholesterolemia and hypertension (Table 6).

Discussion

The present prospective study reveals a significant higher frequency of *B2* homozygotes than carriers for the *B1* allele in patients experiencing a cardiovascular death or a combined cardiovascular event. On the basis of a recessive model, risk value (Hazard Ratio) for cardiovascular death in *B2* homozygous patients is estimated to be 2.23 with a 95% CI of 1.01–4.94 ($p = 0.047$). This association remained significant even after the addition of potential confounding variables in the Cox regression. No modification can be seen when variables, such as age, gender, diabetes melli-

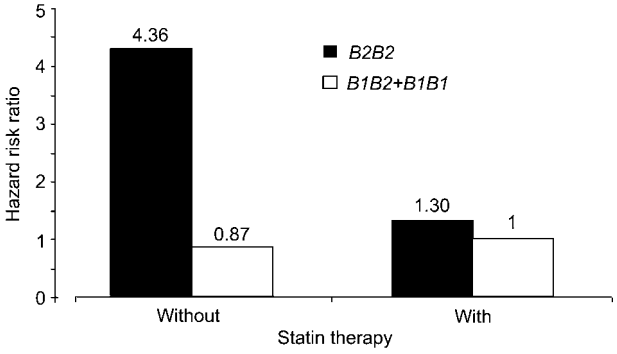

Figure 3 Hazard risk ratios of future cardiovascular death according to *CETP TaqIB* genotypes and statin therapy.

Table 6 Baseline characteristics of patients according to statin treatment and *CETP TaqIB* genotypes.

Statin treatment	Untreated	Treated		Untreated
CETP genotypes	*B2B2* (n = 14)	*B2B2* (n = 36)	*B1*[a] (n = 152)	*B1*[a] (n = 68)
Age, years (mean ± SD)	72.9 ± 10.1[b]	63.9 ± 13.4	63.5 ± 12.3	68.0 ± 12.0[g]
Gender (male), n (%)	10 (71)	30 (83)	117 (77)	51 (75)
Diabetes n (%)	4 (29)	5 (14)	31 (20)	11 (16)
BMI, kg/m^2 mean ± SD	25.2 ± 3.9	25.9 ± 2.9	26.1 ± 4.3	25.1 ± 3.6
Hypercholesterolemia, n (%)	7 (50)[c,d]	31 (86)	115 (76)	54 (79)
Hypertriglyceridemia, n (%)	3 (21)	14 (39)	59 (39)	17 (25)[g]
HypoHDLemia, n (%)	4 (29)	12 (33)	69 (45)	30 (44)
Hypertension, n (%)	13 (93)[d]	27 (75)	104 (68)	43 (63)
Current smoker, n (%)	3 (21)	6 (17)[f]	55 (36)	18 (26)
EF < 40%, n (%)	2 (14)	1 (3)	11 (7)	7 (10)
MVD, n (%)	11 (78)	21 (58)	106 (71)	48 (73)
Aspirin, n (%)	14 (100)	36 (100)	146 (96)	64 (94)
β-Blocker, n (%)	6 (43)	22 (61)	146 (72)	40 (59)[g]
ACE inhibitor, n (%)	9 (64)	16 (44)	75 (49)	40 (59)
Statin, n (%)	0 (0)	36 (100)	152 (100)	0 (0)
Overall mortality, n (%)	5 (36)[b,e]	4 (11)	17 (11)	6 (9)
Cardiovascular death, n (%)	5 (36)[b,e]	4 (11)	14 (9)	5 (7)
Combined cardiovascular event, n (%)	7 (50)[b,e]	8 (22)	24 (16)	10 (15)

[a]*B1*, carriers for *B1* allele; [b]*B2B2* homozygous patients without vs. with statin therapy, $p < 0.05$; [c]*B2B2* homozygous patients without vs. with statin therapy, $p < 0.01$; [d]*B2B2* homozygous vs. carriers for *B1* allele in patients without statin therapy, $p < 0.05$; [e]*B2B2* homozygous vs. carriers for *B1* allele in patients without statin therapy, $p < 0.01$; [f]*B2B2* homozygous vs. carriers for *B1* allele in patients with statin therapy, $p < 0.05$; [g]*B1* carriers without vs. with statin therapy, $p < 0.05$. Medications are treatment during follow-up. *B2B2*, patients homozygous for *B2* allele; BMI, body mass index; EF, ejection fraction; MVD, multiple vessels diseased; ACE, angiotensin converting enzyme.

tus, arterial hypertension or heart failure (defined as an EF < 40%) were added in the model. Similarly, no modification was observed when variables, such as low concentrations of HDL-C or hypertriglyceridemia were added. One variable that significantly modified this association was the presence or absence of statin therapy. This suggests that patients homozygous for the *B2* allele and not taking statin had a strong likelihood of recurrent cardiovascular events after an initial acute coronary event, unstable angina or MI. This risk seems to be corrected with statin treatment.

Since its initial description, the relationship between the *TaqIB* genotype and the risk of CAD has been investigated in numerous population-based studies. Meta-analysis performed individual patient data from seven large, population-based studies and three randomized, placebo-controlled, pravastatin trials has shown that the *TaqI* genotype was significantly associated with the risk of coronary artery disease, with a lower risk for *B2* carriers. Usually the significant association between *TaqIB* genotype and CAD risk is largely mediated through HDL-C concentrations. Our results did not find a lower risk for *B2B2* individuals or mediation of the risk through HDL-C concentrations. These conflicting results could be due to the fact that our study analyzed recurrent cardiovascular events in patients initially recruited for an initial coronary event. The higher prevalence of cardiovascular events in *B2B2* could suggest a cardiovascular death risk independent of HDL-C concentrations. In our study, it did not appear to be dependant of potential interactions with TG concentrations, known to affect CETP activity and previously reported as potentially mediating the CAD risk of CETP (27). It is difficult to assume that the worst evolution of *B2* carriers could be due to more rapid progression of coronary atherosclerosis which is related to CETP mass and the *B1* allele (14). Moreover, in patients with coronary artery disease, a previous study demonstrated that the *CETP*/–629A allele, in nearly complete concordance with the *B2* allele, had a strong protective effect on future mortality from cardiovascular causes (7). All of these results suggest that *B2* carriers should be protective against future or recurrent coronary event.

In order to explain our results, we could not exclude a selection effect. *B2B2* patients, theoretically protected against coronary artery disease and presenting with an acute coronary event, could be more sensitive to undetected or unknown risk factors leading to a more severe prognosis. In the same way, the apparent deleterious action of the *B2* allele could be linked to an associated genotype, such as hepatic lipase lowering gene variants that have been previously reported (27). The impact of the *CETP* genotype on risk mediated by a prothrombotic or proarrhythmic effects of CETP within the vessel wall or within ischemic myocardium is always possible. Nevertheless, our finding of a less favorable outcome in *B2B2* subjects is in agreement with results reported by Mohrschladt et al. (16) in statin-treated patients with familial hypercholesterolemia.

Based on these results, we hypothesize that in patients with an ACS, a low CETP level and activity as

observed in *B2B2* subjects could be deleterious. This can lead to future fatal cardiovascular events by decreasing the reverse cholesterol transport from HDL and peripheral tissues to the liver under conditions of efficient hepatic apoB lipoprotein clearance. The strong amplification of this mechanism by statin therapy with the significant decrease in LDL-C concentrations could explain the disappearance of the deleterious action of CETP deficiency.

Limitation

The present study has limitations. First, the small population and small number of events preclude an exhaustive statistical analysis. Second, the sole inclusion of patients experiencing an ACS could result in selection bias. Moreover, we did not have the ability to evaluate lipid parameters before and after statin therapy. The results on the genotype-statin interaction should thus be considered as a hypothesis. Because we tested several primary and secondary hypotheses that were not independent, it was not possible to correct for multiple testing; hence, the p-values provided are nominal and require further confirmation. Finally, serum CETP concentrations and activity were not measured. Therefore, no association between them could be observed.

Conclusions

This study demonstrates an association between the *B2B2* genotype in the *CETP* gene and risk of future cardiovascular death in patients experiencing an ACS. Specifically, patients at increased risk are those with an ACS who are not receiving statin medication and homozygous for the *B2* allele. We speculate that these patients express an increased sensitivity to less effective peripheral cholesterol removal. As the action of CETP may have different effects depending on the metabolic and/or clinical context, the question is whether CETP is a good marker and/or therapeutic target for patients at increased risk of coronary events.

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