**CETP** genotype predicts increased mortality in statin-treated men with proven cardiovascular disease: an adverse pharmacogenetic interaction

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**Aims**

Inhibition of cholesteryl ester transfer protein (CETP) increases HDL-cholesterol. However, its combination with statins may increase mortality by factors incompletely understood. We previously observed that patients with intrinsically low CETP levels (carriers of the TaqIB-B2 allele) may have less benefit from statin therapy, and here tested this pharmacogenetic hypothesis on long-term outcomes.

**Methods and results**

We performed a 10-year follow-up analysis in 812 coronary artery disease (CAD) patients (REGRESS cohort), treated with statins after an initial 2-year study period. Carriers of TaqIB-B2 showed reduced CETP levels and higher HDL-cholesterol ($P_{0.001}$ for both). Despite these lower CETP and higher HDL-cholesterol levels, hazard ratios per B2 copy were 1.59 ($P_{0.01}$) for atherosclerotic disease death, 1.53 ($P_{0.03}$) for ischaemic heart disease death, and 1.30 ($P_{0.04}$) for all-cause mortality. Haplotype-effects analysis provided even stronger basis for the genetics involved: one risk-haplotype was identified that was highly significantly associated with these endpoints.

**Conclusion**

In statin-treated male CAD patients, genetic variation conferring low CETP levels is associated with increased 10-year mortality. This suggests that efficacy of statin therapy to reduce cardiovascular risk depends on CETP genotype and associated CETP plasma levels. This effect may need consideration when administering CETP inhibition to CAD patients.

**Keywords**

Cholesteryl ester transfer protein • Lipoprotein metabolism • Pharmacogenetics • Risk factors • Prognosis

**Introduction**

Lowering plasma low-density lipoprotein (LDL)-cholesterol is the cornerstone of secondary prevention to reduce cardiovascular events. The formation of atherosclerotic plaques in the arterial wall is promoted by the accumulation of atherogenic LDL particles, as well as by their oxidation and subsequent interactions with cellular and molecular components of the inflammatory response. Several lines of evidence suggest that each of these atherogenic processes can be counteracted by high-density lipoprotein (HDL).2,3 Low plasma HDL cholesterol (HDL-C) is consistently related to excess cardiovascular risk,4 and raising HDL-C levels thus seems a promising strategy to further reduce cardiovascular risk. Cholesteryl ester transfer protein (CETP)5 is considered a promising target. The main action of CETP is to transfer cholesteryl esters from HDL to apolipoprotein-B-containing particles in exchange for triglycerides, thereby reducing the concentration of HDL-C. Furthermore, recent insight suggests that CETP also

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plays a role in macrophage cholesterol homeostasis, which is considered atheroprotective, especially in normolipidemic states. The net effect of CETP activity in humans is still debated and conflicting results from studies in mice, human CETP deficiency, and CETP gene polymorphisms constitute a prominent part of that discussion. Interactions with concomitant statin therapy appear to lead to further complications since the efficacy of these drugs may vary due to patients’ CETP genotype and CETP concentration. Moreover, the safety of pharmacological CETP inhibition on top of statin therapy was recently questioned in view of the results of the Investigation of Lipid Level Management to Understand its impact in Atherosclerotic Events (ILLUMINATE) trial. In this trial, in 15,067 symptomatic statin-treated coronary artery disease (CAD) patients randomized to the CETP inhibitor torcetrapib or placebo, a 58% increase in total mortality was observed in the torcetrapib arm, leading to early termination of the trial.

The Regression Growth Evaluation Statin Study (REGRESS) was a prospective placebo-controlled double-blinded angiographic trial evaluating the effects of 2-year pravastatin 40 mg therapy vs. placebo, on the evolution of established atherosclerotic lesions in male patients with proven CAD. In this study, a pharmacogenetic interaction between statin therapy and CETP genotype was observed: the frequent TaqIB-B1 allele (higher CETP level, lower HDL-C) was associated with a better response to statin-treatment compared with the rare TaqIB-B2 allele (lower CETP level, higher HDL-C). Since statins reduce CETP activity up to 30%, this led to our hypothesis that such CETP activity reduction by statins, in patients with intrinsically low CETP levels may have adverse effects. The unexpected outcome of ILLUMINATE prompted us to investigate CETP genotype and mortality in the REGRESS patients who had been using statins for 8 years after the initial 2-year angiographic study.

### Methods

#### Participants and the 2-year follow-up angiographic study

The study participants were derived from the REGRESS angiographic trial cohort, which enrolled 884 male patients with symptomatic CAD between 1989 and 1993. The trial design and main findings have been reported. In brief, the primary objectives of this randomized, placebo-controlled double-blinded angiographic trial were to evaluate the effects of 24 months of 40 mg pravastatin vs. placebo therapy on the evolution of atherosclerotic lesions in male patients with proven CAD. Within the framework of the trial, clinical and angiographic follow-up was documented after the initial 2-year trial. The clinical outcomes assessed (by an independent clinical event committee) were fatal or non-fatal myocardial infarction (MI), death due to ischaemic heart disease (IHD), repeated coronary revascularization (coronary artery bypass grafting or percutaneous transluminal coronary angioplasty), stroke, and death due to non-coronary causes. All participants gave written informed consent. The 2-year REGRESS results demonstrated a distinct positive effect of pravastatin treatment on both the risk of adverse cardiac events as well as on the angiographic progression of CAD. After termination and presentation of the study (along with the publication of the 45 clinical and the Multicentre Anti-Atheroma Study (MAAS) angiographic stain studies), all participants and treating physicians were informed of the major trial outcomes and were explicitly recommended to start (placebo group) or continue (pravastatin group) statin therapy. A survey at 5 years after completion of the trial provided data that 91% of patients were using statin therapy, according to national and international guidelines.

#### Morbidity and mortality 10-year follow-up study

In order to obtain 10-year follow-up data of the REGRESS participants, cause-specific mortality and hospitalization until 1 January 2001 were extracted from nation-wide registries. All diagnoses in these registries are coded according to the International Classification of Diseases, 9th and 10th Revisions (ICD9 and ICD10) for mortality, and ICD9 Clinical Modification (ICD9-CM) for morbidity. The research protocol was approved by the institutional review board and ethics committee of the coordinating center (UMC Utrecht).

#### Linkage process method

The study database, comprising all 884 REGRESS participants, was linked to the national inhabitant registers on the basis of birth date, sex, and postal address code. As is customary due to privacy legislation, patient names were omitted in the linkage process. On a per-patient basis, historical registers of the Dutch inhabitants were searched for this unique combination of characteristics, and once found, this automatically merged migration history over the follow-up time. The vital status of the participants was then obtained through linking municipal administration registries using a six-character postal code. Out of the 884 participants in the original trial, 861 (97%) could be uniquely traced using the above method. The 23 patients who could not be uniquely traced were right-censored at the end of the 24-month follow-up in the mortality analyses. Information on the occurrence of non-fatal MI was obtained through linkage with the register of hospital discharge diagnoses which uses the four-digit part of the postal code. The register files admissions of all general and university hospitals in the Netherlands. Out of the 884 participants, 740 (84%) could be uniquely traced. The 144 patients who could not be uniquely traced were right-censored at the end of the 24-month follow-up for morbidity analyses.

#### Outcome events

In the outcome events analyses, we considered the primary causes of mortality and the primary clinical diagnosis recorded during hospitalization. The composite endpoint ‘death due to IHD’ consisted of all primary causes of mortality within the ICD9 codes 410–414 and ICD10 codes I20–125, ‘non-fatal MI or death due to IHD’, additionally comprised the clinical occurrence of ICD9CM codes 4100–4109. The composite endpoint ‘death due to atherosclerotic disease’ consisted of all primary causes of mortality within the ICD9 codes 410–414, 430–438, 440–448 and ICD10 codes I20–I25, I60–I69, I70–I79 and F01.

#### DNA analyses

Genomic DNA was extracted from blood collected at baseline according to standard procedures. Genotyping of available samples for the TaqIB variant (dbSNP number rs708272), the G-2708A (rs12149545), the C-629A (rs1800775), and the CCC-784A (no dbSNP rs number designated) polymorphisms was performed using restriction fragment length polymorphism methods as described earlier.
Data analyses

Differences in baseline clinical characteristics between the TaqIB variant genotype groups were assessed by one-way analysis of variance and Pearson’s $\chi^2$ test. The latter was also used to assess Hardy–Weinberg equilibrium. Since triglyceride concentrations had a skewed distribution, the statistical analyses were based on log-transformed data. However, the triglyceride concentrations in the table are given as means [± standard error (SE)]. The time-to-death due to IHD, time-to-death due to atherosclerotic disease, time to all-cause mortality, and time-to-non-fatal MI or death due to IHD, was calculated for survival analyses. The absolute risk in each TaqIB genotype group was estimated using the Kaplan–Meier method and temporal pattern displayed in survival curves. Next, a competing risks analysis was performed according to the method of Fine and Gray, in order to account for potential bias from competing events in the case of cause-specific mortality endpoints (death due to IHD, death due to atherosclerotic disease, non-fatal MI or death due to IHD).

The effect of genotype on outcome was analysed using proportional hazards (Cox’ regression); the number of rare allele copies was entered as a linear covariate into the model. The proportional hazards assumption was satisfied. Furthermore, in order to take into account and time, and verified visually by a log–log plot; in all instances the regression coefficient of the interaction between the covariate(s) and time was similar to those reported in other Caucasian populations. Base-line characteristics according to genotypes are shown in Table 1 with means and SE between brackets for continuous variables, and frequencies with percentages between brackets for dichotomous variables.

Moreover, possible heterogeneity of these effects between the randomized treatment groups was explored by modelling interaction terms of genotype and randomization group.

## Results

### Distribution of TaqIB allele frequencies

Genotype data regarding the TaqIB variant were available from 812 REGRESS participants and comprised 292 (36%) B1B1, 392 (48%) B1B2, and 128 (16%) B2B2 subjects, respectively. These frequencies are similar to those reported in other Caucasian populations. Base-line characteristics according to genotypes are shown in Table 1 with means and SE between brackets for continuous variables, and frequencies with percentages between brackets for dichotomous variables.
Mean age of the patients was around 56 years, with controlled blood pressures (RR around 135/82 mmHg) and preserved left ventricular ejection fraction (LVEF). At baseline, plasma CETP concentration (µg/mL) was 2.06 (SE 0.033) for B1B1, 1.93 (SE 0.028) for B1B2, and 1.70 (SE 0.066) for B2B2 patients and HDL-C (mmol/L) was 0.89 (SE 0.012) for B1B1, 0.92 (SE 0.011) for B1B2, and 1.02 (SE 0.023) for B2B2 patients (both P < 0.001). CETP concentration was 17% lower and HDL-C concentrations were 15% higher in B2B2 homozygotes relative to the B1B1 homozygotes (P < 0.001), consistent with literature. Otherwise, no significant differences were observed between the genotypes in baseline CAD risk factors including lipoprotein profile, angiographic, or lifestyle parameters as depicted in Table 1.

Figure 1: Survival curves for time-to-death from the four defined endpoints. Time in years is displayed horizontally and cumulative risk vertically.

Table 2: Effect of CETP-TaqIB genotype on outcome

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Absolute risk per genotype</th>
<th>Crude HR</th>
<th>HR adjusted for randomization group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHD death</td>
<td>0.05 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td>IHD death or MI</td>
<td>0.09 (0.02)</td>
<td>0.18 (0.03)</td>
<td>0.18 (0.04)</td>
</tr>
<tr>
<td>Atherosclerotic disease death</td>
<td>0.05 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.15 (0.04)</td>
</tr>
<tr>
<td>All-cause death</td>
<td>0.18 (0.05)</td>
<td>0.17 (0.02)</td>
<td>0.22 (0.01)</td>
</tr>
</tbody>
</table>

Ten-year absolute risk (and SE) of composite outcome events are displayed per genotype on the left. Hazard ratios from the competing risks analysis (HR, with corresponding 95% confidence interval). P-value per each additional B2 allele copy are displayed on the right. IHD, ischaemic heart disease; MI, myocardial infarction.
The effects of the separate haplotypes on outcomes, relative to a threshold of 5% were entered into a haplotype effects analysis.

The REGRESS 10-year follow-up study of statin-treated male CAD patients provides evidence that genetic variation associated with low CETP levels is associated with increased 10-year mortality. Specifically, the TaqIB-B2 genotype (low CETP level and high HDL-C), or rather the (ACB2A) haplotype can be considered a risk factor when statins are used. This is counterintuitive as the B2 allele has been shown to be cardioprotective in the original REGRESS placebo group (2 years) and in a recent meta-analysis. In the light of the meta-analysis on the effect of TaqIB variant (most widely studied worldwide), we revisited the current body of evidence in relation to the fraction of patients who received statin therapy in the different studies (Figure 4). In agreement with our initial analysis,24 we again find evidence for a pharmacogenetic interaction between the TaqIB and statin therapy: in the studies in which only 9% of the patients received statin therapy [Physicians’ Health Study (PHS), Northwick Park Heart Study (NPHS), Etude Cas-Témoins de l’Infarctus du Myocarde (ECTIM), Oulu Project Elucidating Risk of Atherosclerosis (OPERA), Reykjavik and Arca studies], the B2 allele was indeed associated with better outcome (OR for CV outcome 0.77). In contrast, in the cohorts in which 50% of the patients received statins [REGRESS-2 year follow-up, West of Scotland Coronary Prevention Study (WOSCOPS), Cholesterol And Recurrent Events (CARE)], the OR for the B2 allele was 1.06 while in the current REGRESS 10 years study, we now show that the HR for the B2 allele is in fact 1.59. These insights also complement our initial report,24 which suggested worse angiographic outcome in B2 carriers when treated with statins, which in turn is in accordance with other reports describing detrimental effects of statins in the context of endogenous low CETP concentration.25,26

It is tempting to speculate that this insight may hold value with regard to the recently observed increased mortality rate, experienced with the CETP inhibitor torcetrapib on top of (high-dose) statin therapy in the ILLUMINATE study, apart from the fact that this compound may also have an off-target drug toxicity via an altered aldosterone mechanism.27 High-dose statin therapy is known to yield potent cholesteryl-ester transferase inhibition30 and this is further blocked by torcetrapib. Therefore, it could be understood that further suppression of CETP by statins may be related with negative outcomes in patients with already intrinsically lower CETP. Recent insight furthermore suggests that CETP also plays a role in macrophage cholesterol homeostasis,4 which is considered atheroprotective,7–9 especially, in normolipidemic states. At present, a balanced level of CETP activity may be crucial in vascular physiology.

Limitations

Some aspects and possible limitations of our study merit consideration. First, the longitudinal REGRESS study constitutes a cohort of CAD patients with 10 years of follow-up. The follow-up data set was not complete for all patients: 3% and 16% of patients could not be uniquely identified in the mortality and hospital registries, respectively. However, as these patients were right-censored at last-to-follow-up time, it seems unlikely that this would have affected the primary outcome of the current study. We elected...
to calculate survival times across genotypes, in contrast to the case–control design. Survival analysis enabled us to efficiently study all the available information, including that of censored participants. Another point is that after the initial 2 years, the patients and treating physicians were only advised to start (placebo group) or continue statin use (active group) during the follow-up period and the data are likely suffering from suboptimal compliance. However, we have no reason to assume that these factors would have differentially affected the three subgroups. A survey at 5 years after completion of this trial provided crucial insight that 91% of patients were indeed on statin therapy according to the guidelines. The original placebo group patients moreover used statins for 8 years and not 10 years as did the pravastatin group, but this fact will have if anything only diluted the primary outcome of the current study. Finally, the results in this study were obtained in a cohort of exclusively male patients with...
established CAD. Confirmation of the present result in males in other trials is needed and the effects in women may need special attention.

**Conclusion**

The results of this 10-year follow-up of a cohort of male patients with symptomatic CAD provide new and clear evidence for a pharmacogenetic interaction between the CETP genotypes, statin therapy, and clinical outcome. The results show that a CETP genotype (TaqIB2 allele—part of a distinct haplotype) associated with lower CETP level and higher HDL-C, adversely affects clinical therapy, and clinical outcome. The results show that a CETP transfer protein gene strongly predicts future cardiovascular death in patients with coronary artery disease. J Am Coll Cardiol 2003;41:1990–1993.

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**Conflict of interest:** none declared.

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**References**


A strangled heart by calcified pericardial band: a rare case of localized pericardial constriction detected by chance

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A 49-year-old gentleman was referred to our emergency department with atypical chest discomfort after traffic accident. Paradoxical pulse could be demonstrated without definite pericardial knock on chest auscultation. A chest X-ray showed mild cardiomegaly and the presence of calcified pericardial band (Panel A). On transthoracic echocardiography, we could recognize the localized cardiac constriction by the thickened and calcified pericardial band located at the atrioventricular (AV) groove of the right side (Panel B). Volume-rendering cardiac computed tomographic (CT) image that was obtained from the left-side view showed calcified pericardial band encircling the left ventricular cavity at the level of the AV groove and crossing the left anterior descending coronary artery (Panel C). Another image constructed from the right-side view revealed the thickened and calcified pericardial ring along the AV groove, leading to stranding of the heart and associated severe right atrial enlargement (Panel D). The presence of constriction was confirmed by cardiac catheterization. As a definitive treatment, we strongly recommended pericardiectomy, which, however, was rejected by the patient. Localized pericardial constriction was reported to be a rare form of constrictive pericarditis, but in most cases constricts the AV groove. Previous pericardiectomy, congenital heart disease, and tuberculous complications could be the leading causes. Depending on the location of pericardial constriction, clinical presentation of localized constriction may be variable including obstruction of right ventricular outflow tract, pulmonary stenosis. It is no doubt that the curative treatment option is pericardiectomy as in generalized pericardial constriction, although it could not be performed in our case.

Panel A. A chest left lateral roentgenogram clearly depicting the presence of the calcified pericardial band (arrow).
Panel B. Transthoracic echocardiographic findings showing constriction of both left and right ventricular cavity by pericardial calcified ring (arrow).
Panel C. Three-dimensional cardiac computed tomographic image from the left-side view demonstrated calcified pericardial band besieging the left ventricular cavity and the left anterior descending coronary artery (arrow) at the level of the atrioventricular groove.
Panel D. Three-dimensional volume-rendering cardiac computed tomographic image from right-side view illustrated pericardial ring (arrow) that was thickened and calcified along the atrioventricular groove. Severe right atrial enlargement was also clearly represented.

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