The CETP TaqIB Polymorphism is Associated with the Risk of Sudden Death in Type 2 Diabetic Patients.

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Short running title: CETP SNP and Sudden Death in Type 2 Diabetes

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Abstract

OBJECTIVES - Type 2 diabetic patients are at high risk of CHD and sudden death. This cardiovascular risk can be partly attributed to low levels of HDL-cholesterol (HDL-C). The B2 allele of the CETP TaqIB polymorphism has been repeatedly reported to be associated with high HDL-C levels in both healthy and type 2 diabetic subjects, but its association with CHD is unclear. We investigated the association of the CETP TaqIB polymorphism with coronary heart disease (CHD), and sudden death in particular, in a prospective cohort of type 2 diabetic patients.

RESEARCH DESIGN AND METHODS - The CETP TaqIB polymorphism was genotyped in 3124 type 2 diabetic subjects with high cardiovascular risk: the DIABHYCAR study. We used Cox regression analysis to estimate the impact of the TaqIB SNP on the CHD events (myocardial infarction or sudden death) during follow-up.

RESULTS - The incidence of CHD was higher in B1B1 homozygotes than in B2 carriers (P = 0.02). This effect was mainly due to sudden death: hazard ratio (B1B1 vs B2+) = 1.51 [95% CI = 1.05-2.18]. Although the B1 allele was associated in a dose-dependent fashion with lower HDL-C (P < 0.001), the association with sudden death persisted after adjusting for multiple risk factors, including HDL-C levels.

CONCLUSION - In type 2 diabetic patients, the CETP TaqIB polymorphism is a good genetic predictor of cardiac mortality. This association is partly independent of the effect on HDL-C levels.

Abbreviations:
CHD: coronary heart disease
DBP: diastolic blood pressure
HDL-C: high density lipoprotein-cholesterol
CETP: cholesteryl ester transfer protein
CRP: C-reactive protein
MI: myocardial infarction
SBP: systolic blood pressure
SNP: single nucleotide polymorphism
UAE: urinary albumin excretion
Type 2 diabetic patients are at high risk of coronary heart disease (CHD) (1). Sudden death occurs frequently among diabetic patients (2-3). The increased CHD risk is partly due to low high-density lipoprotein cholesterol (HDL-C) levels (4-5), that are a common feature of insulin resistance (6). The cholesterol ester transfer protein (CETP) plays a key role in HDL metabolism and reverse cholesterol transport; it exchanges CE from HDL, for triglycerides from apo-B rich particles (7). The CETP gene is localized on chromosome 16q31 and several CETP gene single nucleotide polymorphisms (SNPs) have been described. The most extensively studied CETP SNP is located in the first intron in the gene and disrupts a TaqI restriction site (TaqIB SNP: rs708272). The B2 allele of this SNP is associated with higher HDL-C concentrations and lower CETP levels in both healthy and type 2 diabetic subjects (8, 9), likely due to a nearly complete linkage disequilibrium with the -629G>A functional promoter polymorphism modifying the transcriptional activity of the CETP gene (10). Some studies report TaqIB SNP to be associated with CHD (11-14). Nevertheless, other studies found no correlation, and some studies support the hypothesis of a CETP-effect independent of HDL levels (15). A recent study of a cohort from the general population reported a higher CHD risk with TaqIB2 and -629A (16). Only a few longitudinal prospective studies have been reported, especially in type 2 diabetes.

We assessed the association of the CETP TaqIB polymorphism with the incidence of CHD, and with sudden death in particular, in a large cohort of more than 3100 French type 2 diabetic patients (the DIABHYCAR study). The mean follow-up was four years.

RESEARCH DESIGN AND METHODS - The design and results of the DIABHYCAR study have been reported (17-19). Briefly, DIABHYCAR was a multicentric, random, double blind, and parallel group trial; it compared, the cardiovascular and renal outcomes of patients taking ramipril (1.25 mg/day) and those taking placebo, in addition to their usual treatment (both groups). The participants were men or women with type 2 diabetes, aged ≥ 50 years, with serum creatinine ≤ 150 µmol/L, and elevated urinary albumin excretion (UAE ≥ 20mg/l, two times consecutively). The investigators examined the participants every six months for at least three years. The mean duration of follow up was 4 years. The low dose of ramipril was found to be ineffective (19).

For logistical reasons, only French participants (3124 of the 4912 participants) were included in this study. Incident myocardial infarction (MI) was defined as the first occurrence of a fatal or nonfatal MI after the baseline examination. Sudden death was defined as death occurring instantaneously or within 1 hour after the onset of new cardiac symptoms (arrhythmia, other cardiovascular causes) or non-witnessed death, where the body of the deceased was found, and no cause could be discovered. Fatal stroke and myocardial infarction were not included in this group. Coronary heart disease (CHD) was defined as the combination of MI and sudden death. However, as some people with MI died from sudden death, the number of CHD patients is not the sum of MI and sudden death groups. An independent adjudication committee without access to the genotyping data evaluated the events (18). The study design was approved by the Angers University Ethics Committee. All participants provided written informed consent.

The CETP TaqIB SNP was genotyped using a PCR–molecular Beacon technique (20). The PCR was performed in a 96-well microtitration plate. A total of 200 ng of DNA was
amplified in a total volume of 25 µl containing 20 pmol of 5’ and 3’ primers, 0.2 mmol/L dNTPs, 4 mmol/L MgCl₂, 10 mmol/L Tris-HCl (pH 8.3), 5 pmol of each allele-specific molecular Beacon, and 1 unit of Taq polymerase (Gold Taq; Perkin-Elmer, Paris, France). DNA denaturation and Taq activation was carried out at 95°C for 10 min in a thermocycler (PTC-200; MJ Research, Watertown, MA). This was followed by 40 cycles of 20 s at 55°C, 10 s at 72°C, and 10 s at 95°C. After a final denaturation at 95°C for 2 min, hybridization with the probes was carried out at 60°C for 1 min. Fluorescence emission was recorded with a plate fluorometer (Fluostar; BMG, Offenburg, Germany) using two wavelength systems: 480–520 nm for fluorescein (FAM) and 520–590 for tetramethylrhodamine (TAMRA). The amplifiers and allele-specific probes were synthesised by Eurogentec (Seraing, Belgium). All of the sequences of primers and probes are available from the authors on request.

Quantitative variables were described by mean ± standard deviation (SD) or geometric mean (95 % CI), if the distribution was skewed. The association of CETP TaqIB genotypes with baseline characteristics, not including lipids, was tested by ANOVA (continuous variables) or chi square test (categorical variables) in univariate analysis. The association of CETP TaqIB genotypes with baseline lipid levels was tested by ANCOVA, adjusting for potential confounding variables (age, sex, BMI, HbA1c, smoking, alcohol). We defined survival time as the period from the date of entry into the study to the date of first event (MI or sudden death), or the end of the study. Cox regression was used to estimate the hazard ratio; we adjusted for age, sex, prior myocardial infarction, systolic blood pressure, HbA1c, C-reactive protein, urinary albumin excretion (UAE), serum creatinine, total and HDL cholesterol, smoking, diabetes duration, BMI, triglyceride levels, and for the use of drugs at baseline: different antidiabetic and lipid lowering treatments, treatments for hypertension and platelet anti-aggregants. We adjusted also for insulin and ACE inhibitor treatments which could be only introduced during the follow-up (treatments by insulin and/or ace inhibitor at entry were exclusion criteria). The distribution of the use of the different drugs did not differ among genotypes. There was no interaction effect between genotype and the use of drugs. No interaction effect was found between sex and genotype, either on lipid levels, or on CHD events. Therefore data were not presented separately according to sex. All calculations were performed using SYSTAT 11 for Windows statistical package.

RESULTS - Table 1 provides a summary of features, clinical and lipid profiles at baseline of the DIABHYCAR population as well as the stratified data according to combined CHD incidence (MI or cardiovascular death). Subjects with incident CHD during follow-up were different in age, total, LDL and HDL-cholesterol and TG levels, blood pressure, known duration of diabetes, prevalence of prior coronary antecedents (MI, angina pectoris), serum CRP, serum creatinine, and UAE. The sex ratio was similar in the two groups. Genotype and allele distributions of the TaqIB SNP are shown in Table 2. The CETP TaqIB 1B allele was associated with low levels of HDL-C (P <0.001), with a gene dose effect (Table 2).

The CHD incidence (myocardial infarction and/or sudden death) according to the CETP genotype is shown in Table 3. There were a higher percentage of B1B1 subjects in the CHD group than in
the group without CHD. Cox survival analysis indicated that B1B1 homozygotes had more risk than carriers of the B2 allele; this was independent of other risk factors, including many variables listed in Table 1 (age, HbA1c, systolic blood pressure, diabetes duration, prior MI, total plasma cholesterol, triglycerides, serum CRP, serum creatinine, urinary albumin). There was no interaction between genotype and presence of prior coronary events on the incidence of CHD. Nevertheless, the association was still significant when we excluded subjects with prior myocardial infarction.

After further adjustment for HDL-C levels, the association remained significant, and only slightly lower than without the adjustment. Sudden death was a major factor in this association (Table 3). The 4-year overall and sudden death-free survival rates were 91 % and 95 % for B1B1 and B2 carriers, respectively. The B1B1 genotype was also more frequent in the “incident MI” group (Table 3), but the association was not significant. This could be due to a lack of power of the study, because there were few cases of incident myocardial infarction.

**CONCLUSIONS** - We report a prospective study based on a large cohort of French type 2 diabetic patients with micro or macroalbuminuria, and found, for the first time, a strong negative association between the B2 allele and CHD incidence, and particularly sudden death. Although the *CETP TaqIB* polymorphism was associated with HDL-C in these patients, the association with the occurrence of sudden death remained highly significant after adjustment for most potential confounders, including age and HDL-C.

The association of *CETP TaqIB* polymorphism and HDL-C levels is widely recognized in many different populations, including type 2 diabetic patients. The B1 allele has also been associated with a higher prevalence of coronary heart disease (11-13). Nevertheless, there is a lack of consistent correlation between the *CETP* genotype and the clinical outcome. This association may depend on gene-gene or gene-environment interactions (21). A recent meta-analysis of large studies (11) showed that *CETP TaqIB* was associated with cardiovascular risk, and that this relationship was mediated by lower HDL cholesterol levels. In contrast, we show that HDL only slightly modified the risk associated with TaqIB genotype. One of the reasons could be the interaction with the diabetic phenotype. Studies of type 2 diabetic populations mostly find an association between CETP and cardiovascular disease (9, 12, 22-23). HDL-C levels do not greatly modify the relationship between the CETP genotype and the clinical outcome. This could be specific to type 2 diabetic subjects in which HDL-C levels are already lower than in non-diabetic subjects.

The most striking result from our study is the association with sudden death. There have been only a few prospective studies of *CETP TaqIB* polymorphisms. Blankenberg et al. (24) investigated the association between the risk of fatal cardiovascular events in 1211 CAD patients and -629C>A, an SNP in near complete positive linkage disequilibrium with the *TaqIB* polymorphism (10). Mortality was lower for carriers of the minor A allele (4-4.6%) (the one linked to *TaqIB*2 allele) than for CC homozygotes (10.8%); this was not the case for other cardiovascular outcomes. This allele was also associated with higher HDL-C levels and lower CETP activity, but, similarly to our results, the strong protective effect on future cardiovascular mortality was independent of its effect on HDL-cholesterol levels. The *TaqIB* polymorphism in cases of micro-macroalbuminuria has been associated with atrial fibrillation (25). This is very relevant to our findings for T2D patients with micro/macroalbuminuria since atrial
fibrillation is associated with an increase in cardiovascular mortality (26, 27).

The B2 allele has been associated with lower levels and activity of CETP, which leads to higher HDL-C levels. However, there is evidence that CETP inhibition goes beyond raising HDL-C levels alone for purpose of cardiovascular prevention (28, 29) and our study supports this hypothesis. Nevertheless, the ILLUMINATE trial with a CETP inhibitor, torcetrapib, has been interrupted because of a higher mortality rate in the torcetrapib and atorvastatin group than in the atorvastatin group (30). In another trial (ILLUSTRATE), there was no significant difference in the atherosclerotic plaque burden in the 2 study groups (atorvastatin with / without torcetrapib) (31). In both of these trials, the use of torcetrapib was associated with an increased blood pressure. In our study, the TaqIB polymorphism was not associated with blood pressure (data not shown). Therefore, our results indirectly suggest that the problem may have been due to an adverse effect of the drug, unrelated to the CETP inhibition, as already suggested (32).

We did not observe associations between the CETP TaqIB SNP and prior MI. We think that the prospective design of the DIABHYCAR study allowed us to avoid the biases of cross sectional/case-control studies which can lower the power to detect associations but such biases could explain why we observed only an effect on the CHD incidence but not on the prevalence at entry.

In summary, we have demonstrated that the TaqIB polymorphism of the CETP gene is a good genetic predictor of CHD complications of type 2 diabetes, and especially cardiac mortality. This association is not fully explained by the effect of TaqIB SNP on HDL-C levels.

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APPENDIX
The DIABHYCAR study group included: Principal Investigator: Prof. M. Marre (Bichat Hospital, Paris, France); Steering Committee: F. Alhenc-Gelas, J.P. Boissel, F. Cambien, S. Etienne, A. Girault-Louvel, P. Gueret, M. Lièvre, J. Mann (Vice-Chairman), M. Marre, J. Ménard, P. Passa (Chairman), P.F. Plouin, D. Vasmant, L. Vaur (Secretary), G.C. Viberti, and C. Weisselberg; Central Co-ordinating Center: J.P. Boissel and M. Lièvre; Executive Committee: J.P. Boissel, V. Bost, M. Cambien, Y. Gallois, N. Genes, J. Gillet, M. Hervé, M. Lièvre, M. Marre, L. Martin, A. Perret-Hantzperg, P.F. Plouin, and L. Vaur; Biological Committee: F. Alhenc-Gelas (Chairman), F. Cambien, A. Girault-Louvel (Vice-Chairman), M. Lièvre, M. Marre, and J. Ménard; Central End Point Committee: E. Bonnefoy, G. Chatellier (Chairman), T. Moreau, and L. Pinède; Independent Data and Safety Committee: E. Eschwege, C.E. Mogensen, N. Victor, and S. Weber.
References


CETP SNP and Sudden Death in Type 2 Diabetes


Table 1: Baseline characteristics of the DIABHYCAR study

<table>
<thead>
<tr>
<th>Incident CHD</th>
<th>Overall</th>
<th>No</th>
<th>Yes</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3,124</td>
<td>2,901</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.5 ± 8.4</td>
<td>65.3 ± 8.2</td>
<td>69.1 ± 9.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.4 ± 4.6</td>
<td>29.4 ± 4.6</td>
<td>28.9 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Male (%)</td>
<td>73.1</td>
<td>73.1</td>
<td>74.0</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>14.3</td>
<td>14.5</td>
<td>12.6</td>
<td>NS</td>
</tr>
<tr>
<td>Prior MI (%)</td>
<td>6.1</td>
<td>4.9</td>
<td>11.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes duration (years)*</td>
<td>7.7 (7.6-7.8)</td>
<td>7.6 (7.5-7.7)</td>
<td>8.8 (8.5-9.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>9.51 ± 3.04</td>
<td>9.51 ± 3.05</td>
<td>9.48 ± 3.01</td>
<td>NS</td>
</tr>
<tr>
<td>HBA1C (%)</td>
<td>7.86 ± 1.76</td>
<td>7.84 ± 1.75</td>
<td>8.09 ± 1.87</td>
<td>0.03</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>145.0 ± 14.1</td>
<td>144.8 ± 14.1</td>
<td>147.4 ± 13.6</td>
<td>0.010</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>82.1 ± 8.5</td>
<td>82.1 ± 8.5</td>
<td>82.6 ± 8.0</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.79 ± 1.07</td>
<td>5.77 ± 1.06</td>
<td>5.97 ± 1.16</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.31 ± 0.35</td>
<td>1.32 ± 0.36</td>
<td>1.25 ± 0.30</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.52 ± 0.88</td>
<td>3.51± 0.88</td>
<td>3.65 ± 0.93</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)*</td>
<td>1.90 (1.85-1.97)</td>
<td>1.89 (1.84-1.96)</td>
<td>2.04 (1.84-2.24)</td>
<td>0.03</td>
</tr>
<tr>
<td>CRP (mg/L)*</td>
<td>3.15 (3.04-3.26)</td>
<td>3.09 (2.99-3.21)</td>
<td>3.81 (3.45-4.19)</td>
<td>0.004</td>
</tr>
<tr>
<td>Urinary albumin (mg/L) *</td>
<td>98.2 (98.1-98.3)</td>
<td>95.2 (95.1-95.3)</td>
<td>139.5 (139.0-139.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)*</td>
<td>87.0 (87.0-87.1)</td>
<td>86.6 (86.5-86.6)</td>
<td>92.4 (92.3-92.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ramipril group (%)*</td>
<td>49.5</td>
<td>49.5</td>
<td>49.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± SD except where indicated *.
* Geometric mean (95 %CI) when appropriate.
† Comparison between CHD = “yes” and CHD = “no” by ANOVA or chi² as appropriate
### Table 2: Association of Taq1B SNP with plasma lipid levels (mmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Triglycerides*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1B1 (n = 1107)</td>
<td>5.73 ± 1.01</td>
<td>3.50 ± 0.89</td>
<td>1.25 ± 0.35</td>
<td>1.93 (1.83-2.03)</td>
</tr>
<tr>
<td>B1B2 (n = 1526)</td>
<td>5.82 ± 1.05</td>
<td>3.55 ± 0.87</td>
<td>1.33 ± 0.35</td>
<td>1.89 (1.80-1.98)</td>
</tr>
<tr>
<td>B2B2 (n = 491)</td>
<td>5.84 ± 1.12</td>
<td>3.49 ± 0.89</td>
<td>1.39 ± 0.37</td>
<td>1.91 (1.76-2.06)</td>
</tr>
</tbody>
</table>

**P †** 0.009 0.30 <0.001 0.71

Mean ± SD

* Geometric mean (95 % CI)

† by ANCOVA, adjusting for age, sex, BMI, HbA1c, smoking, alcohol
Table 3: Frequencies of CETP genotypes according to the incidence of coronary events. Data are given as numbers (%)

<table>
<thead>
<tr>
<th>TaqIB CETP</th>
<th>Combined CHD</th>
<th></th>
<th>Sudden Death</th>
<th></th>
<th>Incident MI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>B1B1</td>
<td>1,012 (34.9)</td>
<td>95 (42.6)</td>
<td>1,046 (35.0)</td>
<td>61 (44.5)</td>
<td>1,068 (35.2)</td>
<td>39 (41.2)</td>
</tr>
<tr>
<td>B1B2</td>
<td>1,431 (49.3)</td>
<td>95 (42.6)</td>
<td>1,469 (49.2)</td>
<td>57 (41.6)</td>
<td>1,484 (49.1)</td>
<td>42 (44.2)</td>
</tr>
<tr>
<td>B2B2</td>
<td>458 (15.8)</td>
<td>33 (14.8)</td>
<td>472 (15.8)</td>
<td>19 (13.9)</td>
<td>477 (15.8)</td>
<td>14 (14.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B1B1 vs B2+</th>
<th>Hazard Ratio (95% CI)</th>
<th>B1B1 vs B2+</th>
<th>Hazard Ratio (95% CI)</th>
<th>B1B1 vs B2+</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.37 (1.05-1.79) P = 0.020*</td>
<td>1.47 (1.05-2.08) P = 0.023*</td>
<td>1.30 (0.85-1.96) P = 0.22*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.41 (1.06-1.87) P = 0.018†</td>
<td>1.51 (1.05-2.18) P = 0.027†</td>
<td>1.42 (0.93-2.17) P = 0.10†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.35 (1.01-1.79) P = 0.043‡</td>
<td>1.46 (1.01-2.12) P = 0.043‡</td>
<td>1.33 (0.87-2.05) P = 0.19‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* unadjusted
† model 1: adjusted for age, systolic blood pressure, sex, prior MI, urinary albumin, serum CRP, serum creatinine, total cholesterol and HBA1C, smoking, diabetes duration, BMI and triglycerides levels, drug treatments
‡ model 2 = model 1 + adjustment for HDL-C.