

# An Increased Coronary Risk Is Paradoxically Associated with Common Cholesteryl Ester Transfer Protein Gene Variations That Relate to Higher High-Density Lipoprotein Cholesterol: A Population-Based Study

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**Background:** Several cholesteryl ester transfer protein (*CETP*) polymorphisms affect high-density lipoprotein (HDL) cholesterol, but the impact of *CETP* gene variants on incident coronary disease in the general population is uncertain after correction for their effect on HDL cholesterol.

**Design:** We determined relationships between the *CETP* -629C→A promoter (n = 8141), the TaqIB (n = 8289), and the I405V (n = 8265) polymorphisms, serum lipids, C-reactive protein, and clinical factors with incident coronary heart disease (defined as death from or hospitalization for myocardial infarction, ischemic heart disease, or coronary intervention) during a median of 4.94 yr follow-up.

**Subjects:** A predominantly Caucasian general population was studied.

**Results:** HDL cholesterol was 0.08 mmol/liter higher in -629A carriers than in -629CC homozygotes ( $P < 0.001$ ). The unadjusted

coronary hazard was 1.26 [95% confidence interval (CI), 0.95–1.68;  $P = 0.11$ ] in A carriers compared with CC homozygotes and increased to 1.46 (95% CI, 1.10–1.95;  $P = 0.01$ ) after adjustment for HDL cholesterol. This effect remained after additional adjustment for apolipoprotein A-I, triglycerides, C-reactive protein, age, and gender. Likewise, the HDL-cholesterol-adjusted hazard ratio was also higher in AA than in CC homozygotes (hazard ratio, 1.72; 95% CI, 1.22–2.42;  $P < 0.01$ ). Similar findings were obtained with the TaqIB polymorphism. The 405V allele was weakly associated with incident coronary heart disease after HDL cholesterol adjustment ( $P = 0.09$ ).

**Conclusions:** A common *CETP* promoter polymorphism, which beneficially contributes to higher HDL cholesterol, is paradoxically associated with increased incidence of coronary disease in the general population. Thus, *CETP* gene variation may affect coronary risk apart from the level of HDL cholesterol. (*J Clin Endocrinol Metab* 91: 3382–3388, 2006)

THE RELATIONSHIP BETWEEN plasma high-density lipoprotein (HDL) cholesterol and the risk of coronary artery disease is well established (1, 2). The cardioprotective role of HDL is commonly explained by its function in the reverse cholesterol transport (RCT) pathway, whereby excess cholesterol is transported from vascular tissue back to the liver for metabolism and excretion in the bile (3–5). The cholesteryl ester transfer protein (CETP) plays a pivotal role in HDL metabolism and in RCT (6, 7). CETP enables the transfer of cholesteryl esters from HDL particles toward very-low-density and low-density lipoproteins (VLDL and LDL) (4, 6, 7). As a result, HDL cholesterol is lowered by

CETP action. Because the cholesteryl ester transfer (CET) process may stimulate RCT by providing a route for delivery of HDL-derived cholesteryl esters to the liver via VLDL and LDL and the hepatic LDL receptor (7, 8), it is currently debated whether circulating CETP may act in an atherogenic or even in an antiatherogenic manner (6, 9, 10).

Rare mutations causing CETP deficiency result in very high HDL cholesterol concentrations (11, 12). Paradoxically, an increased prevalence of coronary disease has been reported in men with genetic CETP deficiency, despite their high HDL cholesterol levels (11, 12). It has been suggested that large HDL particles occurring in genetic CETP deficiency do not have antiatherogenic properties (13), whereas LDL particles may have less affinity for their receptor (14). *CETP* gene variants such as -629C→A, TaqIB, and I405V polymorphisms that affect HDL cholesterol are common in the general population (15). The TaqIB variant is in almost complete linkage disequilibrium with the -629C→A promoter polymorphism (16–18), which directly modulates *CETP* gene transcriptional activity *in vitro* (17, 19). The I405V variant is also but to a lesser extent a determinant of circulating CETP (15). Despite much study, it is still unclear whether, how, and under which circumstances these *CETP*

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Abbreviations: Apo, Apolipoprotein; BMI, body mass index; CABG, coronary artery bypass grafting; CET, cholesteryl ester transfer; CETP, CET protein; CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; RCT, reverse cholesterol transport; SNP, single-nucleotide polymorphism; VLDL, very-low-density lipoprotein.

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gene variations affect coronary risk. The associations of the TaqIB polymorphism with coronary heart disease are inconsistent (20–28). A meta-analysis has suggested that cardiovascular risk is decreased in TaqIB B2B2 homozygotes, *i.e.* the genotype that is associated with high HDL cholesterol levels (29). With some exceptions (26, 30), cardiovascular risk was found to be unaffected by the *CETP* –629C→A promoter polymorphism (22, 28, 31, 32). Importantly, it remains uncertain whether an association of the TaqIB polymorphism with cardiovascular risk could be attributable to its effect on HDL cholesterol (29). Moreover, as yet, all clinical end-point studies with the –629C→A and the TaqIB polymorphisms either were cross-sectional (20–23, 31, 32) or were carried out in men (20, 22, 24–26, 31, 33, 34) or in subjects with a cardiac history (23, 24, 27, 28, 30, 34) rather than in the general population.

Therefore, we questioned how the –629C→A promoter polymorphism may affect coronary heart risk in the general population when its effect on HDL cholesterol is taken into account. In the present study, the impact of the *CETP* –629C→A promoter polymorphism on incident coronary disease was determined in a population-based cohort. The effects of the TaqIB and the I405V *CETP* gene variants were also assessed.

## Subjects and Methods

### Study population

Inhabitants of the city of Groningen participating in the PREVENTD (prevention of renal and vascular end-stage disease) study were studied. Details of the protocol have been described elsewhere (35). The PREVENTD study is designed as a prospective longitudinal follow-up study to evaluate the impact of baseline albuminuria level on cardiovascular (36) and renal (37) outcome in a predominantly Caucasian general population. The study was approved by the local medical ethics committee. All participants gave written informed consent.

The present study is focused on the effects of the *CETP* –629C→A promoter polymorphism on coronary disease in a population of 8592 subjects. Therefore, participants who were not genotyped for –629C→A ( $n = 451$ ) were excluded, resulting in data of 8141 subjects. For similar reasons, analyses concerning the effects of the TaqIB and the I405V polymorphisms were carried out in 8289 and in 8265 subjects, respectively.

### Definitions

All data were coded according to the International Classification of Diseases, Ninth Revision (ICD-9-CM), classification of diseases and the classification of interventions. The combined end-point of this study was defined as death from myocardial infarction (MI) (ICD-9 410) and ischemic heart disease (ICD-9 411) and hospitalization for MI (ICD-9 410), ischemic heart disease (ICD-9 411), percutaneous transluminal coronary angioplasty (PTCA), and coronary artery bypass grafting (CABG). Vital status was evaluated through the municipal register. Primary cause of death was obtained from the death certificates coded by the Central Bureau of Statistics (Voorburg/Heerlen, The Netherlands). Morbidity data were registered from the national registry of hospital discharge diagnoses (Prismant, Utrecht, The Netherlands).

The first coronary heart event of each participant was used for analysis. Event-free survival time for participants was defined as the period from the date of the outpatient clinic baseline assessment to the date of death, MI, PTCA, or CABG or death from any cause until December 31, 2003, or December 31, 2002, until which date information regarding specific causes of death was available. If a person had moved to an unknown destination, the date on which the person was dropped from the municipal registry was used as the census date.

At baseline, information regarding the use of antihypertensive, an-

ti-diabetic, and lipid-lowering drugs and smoking and alcohol consumption (categorized as  $<1$  and  $\geq 1$  U/d, *i.e.*  $<10$  and  $\geq 10$  g/d) was obtained using a check-list as described (35). Body mass index (BMI) was calculated as the ratio between weight and height squared (in kilograms per meter<sup>2</sup>). Waist circumference was measured on bare skin between the 10th rib and the iliac crest. Hypertension was characterized as systolic blood pressure of at least 140 mm Hg or diastolic blood pressure of at least 90 mm Hg or the use of antihypertensive drugs. Microalbuminuria was defined as urinary albumin excretion of 30–300 mg/24 h (35). Diabetes mellitus was diagnosed by fasting plasma glucose of at least 7.0 mmol/liter or use of antidiabetic drugs. MI was documented if the participant had a history of hospital admission for MI.

### Laboratory methods

Blood samples were taken after 15 min rest. Plasma glucose was measured shortly after blood sampling. Serum samples for lipid and apolipoprotein (Apo) measurements as well as for C-reactive protein (CRP) assay were stored at –20 C until analysis. EDTA-anticoagulated plasma samples for *CETP* and *CET* measurement were frozen at –80 C until assay in a subset of subjects. HDL cholesterol was measured with a homogeneous method (direct HDL, no. 7D67, AEROSSET System; Abbott Laboratories, Abbott Park, IL). Serum triglycerides were measured enzymatically. Serum total cholesterol and plasma glucose were assessed using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY). Serum Apo A-I was determined by nephelometry applying commercially available reagents for Dade Behring nephelometer systems (BN II; Dade Behring, Marburg, Germany; Apo A-I test kit, code no. OUED) (38, 39). CRP was also determined by nephelometry with a threshold of 0.175 mg/liter (BNII; Dade Behring). Urinary albumin concentration was determined by nephelometry (Dade Behring Diagnostic). Albuminuria is given as the mean of two 24-h urine excretions. Plasma *CETP* concentration was analyzed using a double-antibody sandwich ELISA as described (40). *CET* was assayed using an isotope method (41).

### Genotyping

The –629C→A promoter single-nucleotide polymorphism (SNP) and the TaqIB polymorphism were genotyped exactly as described (42). The *CETP* I405V SNP was analyzed using TaqMan-MGB probes and primers, designed through the Assay-by-Design service of Applied Biosystems (Applied Biosystems, Applied Biosystems, Applera Nederland, Nieuwerkerk aan de IJssel, The Netherlands). For the I405V SNP, the forward primer sequence was 5'-CTCACCATGGGCATTTGATTGG, the reversed primer sequence 5'-CGGTGATCATTGACTGCAGGAA, and the TaqMan-MGB probes were FAM-TCCGAGTCCGTCAGAGA and VIC-CTCCGAGTC-CATCCAGA. Assays were carried out according to the manufacturer's recommendations on an ABI 7900HT apparatus.

### Statistical analyses

Stata SE 8, SPSS 12, and Excel were used for data analysis. Hardy-Weinberg equilibria and linkage equilibrium were calculated using Excel (43). Data are expressed as mean  $\pm$  SD or median (interquartile range). Between-group differences of means were compared with Student's *t* test, medians with Mann-Whitney *U* test, and frequencies with  $\chi^2$  analysis.  $\chi^2$  analysis was used to compare frequencies between groups. HDL cholesterol is lower in –629CC than in –629CA and –629AA subjects (17, 18, 42). For modeling, the association of the –629A allele (–629CA + –629AA subjects combined and –629AA homozygotes alone) with event-free coronary heart disease survival was investigated using the *CETP* –629CC homozygotes as reference group. Likewise, the effects of the TaqIB and the I405V polymorphisms on coronary risk were evaluated using the B1B1 and the II homozygotes, *i.e.* the genotype groups with the lowest HDL cholesterol levels, as reference group. The effects of the *CETP* –629C→A promoter, *CETP* TaqIB, and *CETP* I405V polymorphisms on coronary events were studied with Cox regression analysis, and hazard models were fit to test differences in survival. Proportional hazards assumptions were assessed by graphing the log-log(survival) and by testing the Schoenfeld residuals against time. Event-free survival data are plotted graphically. Our cohort consisted of a random sample of control subjects with less than 10 mg/liter of urinary

albumin and a selected sample of subjects with more than 10 mg/liter of urinary albumin. Therefore, risk estimates were calculated by adding the selection parameter as a confounder interacting with the *CETP* -629C→A promoter, the TaqIB, or the I405V polymorphism in a secondary analysis. A two-sided *P* value < 0.05 was considered significant.

## Results

### Baseline population characteristics

Table 1 shows baseline characteristics of the population according to the *CETP* -629C→A promoter polymorphism. The population consisted of 95.6% Caucasians. The promoter polymorphism was distributed in Hardy-Weinberg equilibrium (*P* > 0.99), and the -629A allele frequency was 48%. No differences were observed in gender distribution, age, BMI, waist, prevalence of hypertension, systolic and diastolic blood pressure, smoking, use of alcohol and lipid-lowering drugs, prevalence of diabetes, previous MI, prevalence of microalbuminuria, and urinary albumin excretion between the -629CC and the -629A carriers as well as between the -629CC and -629AA homozygotes. HDL cholesterol and Apo A-I were higher in -629A carriers and in -629AA homozygotes as compared with -629CC homozygotes. Triglycerides were slightly lower in -629A carriers and in -629AA homozygotes than in -629CC subjects.

### Incident coronary disease and hazard models on -629C→A promoter polymorphism

During a median follow-up of 4.94 yr (range, 1 d to 6.3 yr) and a total observation of 43,440 person-years, 276 subjects (3.4%) suffered a coronary event. Table 2 summarizes the first coronary events according to the *CETP* -629C→A genotypes. As shown in model 1 (Table 3), the hazard ratio (HR) for coronary disease was 26% higher in -629A allele carriers than in -629CC homozygotes (*P* = 0.11). When the Cox survival function was adjusted for HDL cholesterol levels,

-629A carriers had a significantly 46% higher HR than -629CC subjects (model 2, Table 3, and Fig. 1). These increased HRs for the -629A allele remained significant when confounders, Apo A-I and triglycerides (model 3), and subsequently age, gender, and CRP (model 4) were included. Additional adjustment for use of alcohol, smoking, use of lipid-lowering drugs, presence of diabetes mellitus, or history of MI did not change the model. These parameters did not interact with the *CETP* genotype on coronary heart disease. When the models were repeated to obtain proportional hazards of AA vs. CC homozygotes, similar results were obtained for model 2 [HR, 1.72; 95% confidence interval (CI), 1.22–2.42; *P* = 0.01] and model 4 (HR, 1.53; 95% CI, 1.06–2.20; *P* = 0.02). The design of the study concerning enrichment of subjects with microalbuminuria did not alter the results. Moreover, no interaction of the sampling of the study population with the genotype on coronary risk was found (data not shown).

*CETP* TaqIB polymorphism was also in Hardy-Weinberg equilibrium (*P* > 0.99) and in almost complete linkage disequilibrium with the -629C→A polymorphism (*D'* = 0.942; *P* < 0.001). The univariate HR of *CETP* TaqIB B2 allele on incident coronary disease was 1.31 (95% CI, 1.01–1.72; *P* = 0.04), as compared with B1B1 homozygotes. The HDL-cholesterol-adjusted HR of the B2 allele was 1.51 (95% CI, 1.16–1.98; *P* < 0.01). The HR was 1.62 (95% CI, 1.22–2.15; *P* < 0.01) after additional adjustment for Apo A-I and triglycerides and 1.48 (95% CI, 1.12–1.97; *P* < 0.01) after subsequent age, gender, and CRP adjustment. Results were similar when the models were repeated to obtain proportional hazards of B2B2 vs. B1B1 homozygotes (model 2: HR, 1.75; 95% CI, 1.24–2.47; *P* < 0.001) (model 4: HR, 1.58; 95% CI, 1.10–2.27; *P* = 0.01). The sampling of the database did not alter the analyses with respect to the TaqIB polymorphism (data not shown).

**TABLE 1.** Baseline population characteristics (n = 8141)

	<i>CETP</i> -629C→A promoter polymorphism			
	CC	CA	AA	CA + AA
n (%)	2148 (26.4)	4122 (50.6)	1871 (23.0)	5993 (73.6)
Male gender (%)	49.7	51.3	49.9	50.9
Age (yr)	49.1 ± 12.5	49.4 ± 12.7	49.6 ± 12.8	49.5 ± 12.8
BMI (kg/m <sup>2</sup> )	26.1 ± 4.2	26.1 ± 4.2	26.1 ± 4.4	26.1 ± 4.3
Waist (cm)	88.2 ± 12.8	88.8 ± 12.9	88.7 ± 13.4	88.8 ± 13.1
Hypertension (%)	31.3	32.7	31.8	32.4
Systolic blood pressure (mm Hg)	129 ± 20	129 ± 20	129 ± 20	129 ± 20
Diastolic blood pressure (mm Hg)	74 ± 10	74 ± 10	74 ± 10	74 ± 10
Cigarette smokers (%)	36.5	38.4	37.5	38.1
Alcohol users, >1 U/d (%)	59.3	59.0	59.3	59.1
Use of lipid-lowering drugs (%)	5.3 <sup>a</sup>	4.8	4.0	4.5
Diabetes (%)	3.4	4.0	4.0	4.0
History of MI (%)	3.0	3.8	2.7	3.4
Microalbuminuria (%)	14.0	13.4	14.4	13.7
Urinary albumin excretion (μg/min)	9.4 (6.4–18.4)	9.4 (6.3–16.9)	9.4 (6.2–18.4)	9.4 (6.3–17.3)
CRP (mg/liter)	1.31 (0.55–2.98)	1.26 (0.55–3.06)	1.31 (0.57–2.90)	1.27 (0.56–2.98)
HDL cholesterol (mmol/liter)	1.26 ± 0.37	1.32 ± 0.39	1.39 ± 0.42 <sup>c</sup>	1.34 ± 0.40 <sup>e</sup>
Non-HDL cholesterol (mmol/liter)	4.34 ± 1.21	4.36 ± 1.21	4.26 ± 1.20 <sup>a</sup>	4.33 ± 1.21
Triglycerides (mmol/liter)	1.21 (0.86–1.75)	1.16 (0.85–1.67)	1.12 <sup>b</sup> (0.83–1.64)	1.15 <sup>d</sup> (0.85–1.66)
Apo A-I (g/liter)	1.36 ± 0.29	1.38 ± 0.29	1.41 ± 0.31 <sup>c</sup>	1.39 ± 0.30 <sup>e</sup>

To convert cholesterol from mmol/liter to mg/dl, multiply by 38.7. To convert triglycerides from mmol/liter to mg/dl, multiply by 88.6. One unit of alcohol per day = 10 g alcohol/d.

<sup>a</sup> *P* < 0.05; <sup>b</sup> *P* < 0.01; <sup>c</sup> *P* < 0.001 for difference between AA and CC.

<sup>d</sup> *P* < 0.05; <sup>e</sup> *P* < 0.001 for difference between CA + AA and CC.

**TABLE 2.** Incidence of coronary heart disease per 1000 person-years, according to deaths or hospital admission for MI, ischemic heart disease, coronary PTCA, and CABG

	<i>CETP</i> -629C→A promoter polymorphism			
	CC	CA	AA	CA + AA
n (%)	2148 (26.4)	4122 (50.6)	1871 (23.0)	5993 (73.6)
Total of person-years of follow-up	11,451	22,011	9978	31,988
Coronary disease (n)	61	142	73	215
Coronary disease (n/1000 yr)	5.3 (4.1–6.8)	6.5 (5.5–7.6)	7.3 (5.8–9.2)	6.7 (5.9–7.7)
Subgroups of events				
Death from MI or ischemic heart disease (n/1000 yr)	0.3 (0.1–0.9)	0.3 (0.1–0.6)	0.6 (0.3–1.3)	0.4 (0.2–0.7)
MI (n/1000 yr)	1.6 (1.0–2.5)	2.9 (2.2–3.7)	2.8 (1.9–4.1)	2.8 (2.3–3.5)
Ischemic heart disease (n/1000 yr)	1.8 (1.2–2.8)	1.7 (1.3–2.4)	2.6 (1.8–3.8)	2.0 (1.6–2.6)
CABG (n/1000 yr)	0.4 (0.2–1.0)	0.5 (0.3–0.9)	0.7 (0.3–1.5)	0.6 (0.4–0.9)
PTCA (n/1000 yr)	1.1 (0.7–2.0)	1.1 (0.7–1.6)	0.6 (0.3–1.3)	0.9 (0.7–1.3)

Data of first events are shown. The 95% CI are shown in parentheses.

### Hazard models on I405V polymorphism

*CETP* I405V polymorphism was also in Hardy-Weinberg equilibrium ( $P > 0.99$ ). The 405V allele frequency was 31.7%. Unlike the TaqIB, the I405V polymorphism was relatively weakly linked with the -629C→A polymorphism ( $D' = 0.554$ ;  $P < 0.001$ ). HDL cholesterol levels in 405VV homozygotes ( $1.39 \pm 0.42$  mmol/liter) and in 405V allele carriers ( $1.34 \pm 0.41$  mmol/liter) were higher than in 405II homozygotes ( $1.30 \pm 0.39$  mmol/liter;  $P < 0.001$  for both). The univariate HR of *CETP* 405V allele on incident coronary disease was 1.16 (95% CI, 0.91–1.47;  $P = 0.24$ ), whereas the HDL-adjusted HR was 1.23 (95% CI, 0.97–1.57;  $P = 0.09$ ). After additional adjustment for Apo A-I and triglycerides and after subsequently adjusting for age, gender, and CRP as well, the HR of the 405V allele was 1.24 (95% CI, 0.97–1.60;  $P = 0.09$ ). The procedure of enrichment of the population with microalbuminuria again did not alter the analyses.

### *CETP* mass and *CET*

In 226 men, plasma *CETP* mass and *CET* was measured. As expected, *CETP* mass decreased over the -629CC ( $2.95 \pm 0.95$  mg/liter), -CA ( $2.27 \pm 0.67$  mg/liter), and -AA ( $2.15 \pm 0.71$  mg/liter) as well as over the TaqIB B1B1 ( $2.72 \pm 0.98$  mg/liter), -B1B2 ( $2.28 \pm 0.62$  mg/liter), and -B2B2 ( $2.09 \pm 0.74$  mg/liter) genotypes ( $P < 0.001$  for both). In I405V V allele carriers, *CETP* mass was modestly lower than in II homozygotes ( $2.52 \pm 0.90$  mg/liter vs.  $2.27 \pm 0.73$  mg/liter;  $P = 0.02$ ). In parallel, plasma *CET* was higher in -629CC homozygotes [12.00 (8.71–17.35) nmol/liter·h] than in -629AA homozygotes [9.07 (6.13–12.56) nmol/liter·h] with intermediate values in -629CA subjects [9.72 (6.58–13.72) nmol/liter·h] ( $P < 0.01$ ).

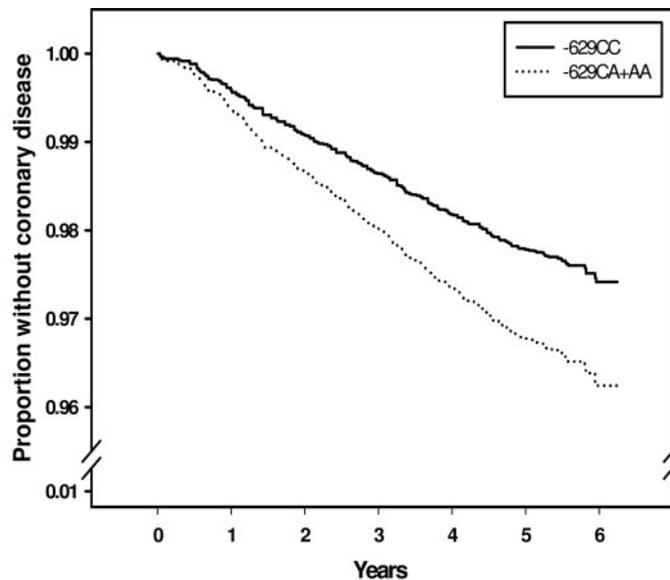
### Discussion

This prospective population-based study in 8141 mostly Caucasians demonstrates that the -629A as well as the TaqIB B2 and the I405VV alleles of the *CETP* gene are not associated with a decreased risk for coronary disease, despite the HDL-cholesterol-raising effect of these common *CETP* polymorphisms. On the contrary, the unadjusted hazard for coronary events tended to be higher in -629A carriers than in -629CC homozygotes and was significantly higher in B2 carriers than in B1B1 homozygotes. As expected (1, 2), incident coronary disease was inversely related to baseline HDL cholesterol. Nevertheless, the association of the -629A allele with higher coronary risk became significant and the hazard associated with the B2 allele was greater after HDL cholesterol adjustment. Moreover, HDL-cholesterol-adjusted coronary hazards were also significantly higher in -629AA than in -629CC and in B2B2 than in B1B1 homozygotes. We consider our findings robust, because this relation remained after additional adjustment for Apo A-I and triglycerides, as well as for age, gender, CRP, smoking, alcohol use, use of lipid-lowering drugs, presence of diabetes mellitus, and history of MI, and because comparable results were obtained with respect to the *CETP* I405V genotype, which is rather weakly linked with the promoter polymorphism. Thus, our study supports the notion that there are other mechanisms than an effect on the HDL cholesterol level through which coronary risk is associated with these *CETP* polymorphisms in the general population.

A paradoxically higher coronary risk associated with the -629A and the B2 allele, which results in lower *CETP* concentration and higher HDL cholesterol (16–18, 20, 21, 30, 31, 33, 34) (present study), is in agreement with subgroup anal-

**TABLE 3.** Variation in *CETP* -629C→A promoter polymorphism (-629CA+AA vs. -629CC) and other determinants of coronary heart disease evaluated by Cox proportional hazards analyses

Model variable	Model 1			Model 2			Model 3			Model 4		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
-629CA+AA vs. -629CC	1.26	0.95–1.68	0.11	1.46	1.10–1.95	0.01	1.53	1.13–2.08	<0.01	1.40	1.03–1.90	0.03
HDL cholesterol (mmol/liter)				0.18	0.12–0.26	<0.001	0.40	0.23–0.71	<0.01	0.79	0.44–1.40	0.42
Apo A-I (g/liter)							0.59	0.33–1.10	0.09	0.45	0.25–0.81	0.01
Triglycerides (ln, mmol/liter)							1.77	1.37–2.27	<0.001	1.58	1.21–2.07	0.001
Age (yr)										1.07	1.06–1.08	<0.001
Male gender										1.91	1.42–2.58	<0.001
CRP (ln, mg/liter)										1.33	1.19–1.50	<0.001



-629 CC	2148	2118	2090	2042	1985	1709	290
-629 CA + AA	5993	5922	5829	5699	5535	4783	896

FIG. 1. Association of HDL cholesterol-adjusted incident coronary disease with *CETP*  $-629C \rightarrow A$  promoter polymorphism by Cox proportional hazards regression analysis (cf. Table 3, model 2). Numbers of subjects are given next to genotypes.

yses in hypertriglyceridemia men of Japanese ancestry and in Danish women who did not receive hormonal replacement therapy (44, 45). In these reports, the *CETP* I405V genotype leading to a higher HDL cholesterol was associated with an increased cardiovascular risk (44, 45). In apparent contradiction with our study, a meta-analysis that included three case-control studies and four prospective reports has documented a lower cardiovascular risk in B2B2 compared with B1B1 homozygotes (29). The magnitude of the effects of the *CETP* gene variations on circulating CETP and on HDL cholesterol in the currently studied cohort (42) is similar compared with that reported previously (16). This makes it unlikely that the discrepancy can be explained by differences in *CETP* gene effects on CETP and on HDL cholesterol across various populations. Furthermore, the  $-629A$  allele frequency was 48% in our study, being very similar to a frequency of 49% in other European populations (17, 22, 26, 32). As opposed to other prospective studies (24–28, 30, 33, 34), the present findings are based on a single cohort retrieved from the general population. Consequently, coronary risk was much lower than that of the studies included in the meta-analysis (29). Of note, high circulating CETP levels may increase cardiovascular risk in hypertriglyceridemic subjects (46). In agreement, intima media thickness is positively correlated with the rate of plasma CET, which is determined by both plasma triglycerides and CETP (47). The median triglyceride concentration was clearly lower in our cohort (1.16 mmol/liter) compared with the previous survey (1.7 mmol/liter) (46) and to several other studies included in the meta-analysis (29). These differences in triglyceride levels may contribute to the apparent discrepancy between effects of variation in the *CETP* gene and of the circulating CETP level *per se* on cardiovascular risk. Finally, of potential clinical

relevance, amelioration of the lipid profile and cardiovascular risk by statin treatment may be diminished in TaqIB B2 and in  $-629A$  carriers (18, 30). In view of the present findings, a pharmacogenomic approach with assessment of *CETP* gene variation could, therefore, be helpful to identify subjects requiring more stringent lipid-lowering treatment.

Because we performed a genetic association study, mechanisms responsible for the increased HDL-cholesterol-adjusted incidence of coronary heart disease associated with *CETP* genotypes that result in lower CETP and higher HDL cholesterol remain putative. A decreased CETP could contribute to a diminished transfer of cholesteryl esters from HDL toward VLDL and LDL (3, 7–9), which are subsequently metabolized by the liver. Hence, genetically determined lower CETP levels may impede RCT (6, 9, 11). Indeed, our study demonstrates for the first time that plasma CET from HDL toward Apo-B-containing lipoproteins is lower, in parallel with lower CETP mass levels, in  $-629A$  allele and  $-B2$  allele carriers. Furthermore, it is tempting to hypothesize that *CETP* gene variability may also affect atherosclerosis development via other processes involved in RCT. First, CETP contributes to the generation of small lipid-poor pre- $\beta$ -HDL particles (48) that stimulate cellular cholesterol efflux via ATP-binding cassette transporter A-1 expressed on macrophages and fibroblasts (49). Thus, lower circulating CETP levels may diminish the ability of plasma to promote cellular cholesterol removal (our unpublished data). Second, it is likely that CETP affects cholesterol trafficking at the cellular level, thereby stimulating RCT. Macrophages present in human atherosclerotic lesions produce CETP, where it is able to stimulate cellular cholesterol efflux (50). Third, CETP expressed in hepatocytes may promote selective hepatic uptake of HDL-derived cholesteryl esters (51). Hence, it is possible that if cellular CETP production is subject to regulation by *CETP* gene variation, *CETP* polymorphisms that lower CETP may impede peripheral cell cholesterol efflux and/or hepatic cholesterol removal.

A potential limitation of our study is that the participants were recruited from a restricted geographical area, *i.e.* the city of Groningen in the northern part of The Netherlands. Moreover, even though the study cohort was enriched with subjects with microalbuminuria, cardiovascular risk was relatively low. However, in our opinion, the present findings can be extrapolated to the general population. First, statistical evaluation showed that the enrichment procedure had no effect on the models. Second, there was no association of *CETP* gene variations with the degree of urinary albumin excretion or with the prevalence of microalbuminuria. Third, the  $-629A$  allele frequency was similar compared with other European populations, making it very unlikely that microalbuminuria enrichment affected  $-629C \rightarrow A$  allele distribution.

In conclusion, the *CETP*  $-629C \rightarrow A$  and the TaqIB genotypes, which beneficially contribute to higher HDL cholesterol levels, are paradoxically associated with higher incidence of coronary disease in the general population when their effects on HDL cholesterol are taken into account. Thus, *CETP* gene variation may also affect coronary risk by other mechanisms than the HDL cholesterol level *per se*.

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