patient-oriented and epidemiological research

Gene-environment interactions of *CETP* gene variation in a high cardiovascular risk Mediterranean population[S]

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Abstract Genome-wide association studies show that cholesteryl ester transfer protein (CETP) single nucleotide polymorphisms (SNPs) are more strongly associated with HDL cholesterol (HDL-C) concentrations than any other loci across the genome. However, gene-environment interactions for clinical applications are still largely unknown. We studied gene-environment interactions between CETP SNPs and dietary fat intake, adherence to the Mediterranean diet, alcohol consumption, smoking, obesity, and diabetes on HDL-C in 4,210 high cardiovascular risk subjects from a Mediterranean population. We focused on the −4,502C>T and the TaqIB SNPs in partial linkage disequilibrium (D´= 0.88; *P* < 0.001). They were independently associated with higher HDL-C (*P* < 0.001); this clinically relevant association was greater when their diplotype was considered (14% higher in TT/B2B2 vs. CC/B1B1). No gene-gene interaction was observed. We also analyzed the association of these SNPs with blood pressure, and no clinically relevant associations were detected. No statistically significant interactions of these SNPs with obesity, diabetes, and smoking in determining HDL-C concentrations were found. Likewise, alcohol, dietary fat, and adherence to the Mediterranean diet did not statistically interact with the CETP variants (independently or as diplotype) in determining HDL-C. In conclusion, the strong association of the CETP SNPs and HDL-C was not statistically modified by diet or by the other environmental factors.—Corella, D., P. Carrasco, M. Fitó, M. A. Martínez-González, J. Salas-Salvadó, F. Arós, J. Lapetra, M. Guillén, C. Ortega-Azorín, J. Warnberg, M. Fiol, V. Ruiz-Gutierrez, L. Serra-Majem, J. A. Martínez, E. Ros, and R. Estruch. **Gene-environment interactions of CETP gene variation in a high cardiovascular risk Mediterranean population. *J. Lipid Res.* 2010.** 51: **2798–2807.**

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Results from recent genome-wide association studies carried out in several populations, in which markers are

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Abbreviations: BMI, body mass index; CETP, cholesteryl ester transfer protein; FFQ, food frequency questionnaire; HDL-C, HDL cholesterol; LD, linkage disequilibrium; LDL-C, LDL cholesterol; OR, odds ratio; PREDIMED, Prevención con Dieta Mediterránea; SATFAT, saturated fatty acids; SNP, single nucleotide polymorphism.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one figure and three tables.

studied in all chromosomes, have allowed us to discover that cholesteryl ester transfer protein (CETP) single nucleotide polymorphisms (SNPs) are associated with HDL cholesterol (HDL-C) more strongly than any other loci across the genome (1–4). Hence, e.g., in the work of Kathiresan et al. (3) in which 11 new loci related to lipid metabolism were identified, the locus most associated with HDL-C concentrations was the *CETP* gene ($P = 4 \times 10^{-75}$). The second-most associated locus was the *LPL* gene ($P = 2 \times 10^{-34}$). Still smaller were associations obtained for the newly discovered loci associated with HDL-C (P = between 10^{-8} and 10^{-14}). This highlights the importance of the *CETP* gene variation as a potential genetic marker for future clinical applications related to HDL-C. In addition, the current controversy involving the new drugs (i.e., torcetrapib) designed to inhibit CETP activity and to increase HDL-C concentrations to decrease cardiovascular risk (5–8) have increased interest in this gene for clinical applications.

It is well known that certain variants in the *CETP* gene are associated with decreased plasma CETP protein activity and protein levels, thereby resulting in greater HDL-C concentrations (9–11). Among them, the TaqIB SNP (rs708272) has been the most widely studied. Meta-analyses have shown that carriers of the B2 allele, associated with lower CETP, have higher HDL-C concentrations than B1B1 homozygotes (12, 13). However, given that this SNP is located in an intron, a number of studies (10, 14–16) have been carried out to find the possible functional variant with which this SNP would be in linkage disequilibrium (LD). The study of Thompson et al. (16) has been the most comprehensive, as a dense genotyping of *CETP* and regions up to 15 kb on either side of the gene on >2,000 individuals was undertaken. These authors found that the −4,502C>T promoter SNP (rs183130), which alters two consensus transcription factor binding sites, was the one most associated with HDL-C. Nevertheless, very few studies have analyzed the effect of the promoter SNP on HDL-C concentrations in other populations. Furthermore, even though the associations between *CETP* gene variants and HDL-C are consistent, a controversy still exists over possible gene-environmental interactions (mainly with dietary factors such as fat intake and alcohol consumption). However, the understanding of these gene-environment interactions could be of great interest to potential clinical applications of *CETP* genetic analysis in cardiovascular prevention and treatment. Regarding prior studies that have analyzed gene-environmental interactions, a lack of consistency is observed. Some studies have reported that alcohol consumption statistically interacts with the TaqIB SNP and modifies its effects on HDL-C concentrations (17–19), whereas others have not supported this interaction (20–22). Likewise, although there is one observational study (23) in which a statistically significant interaction between the TaqIB- SNP and fat intake was found, those results have not been replicated (24). Moreover, some intervention studies did not demonstrate TaqIB-fat interactions (25, 26). Therefore, our aims were: *1*) to estimate the impact (additive or interactive effects) between the TaqIB and the −4,502C>T promoter SNP on HDL-C and related parameters, including blood pressure, in a large high cardiovascular risk Mediterranean population; and *2*) to study gene-environment interactions between these SNPs and lifestyle factors (focusing on alcohol consumption, fat intake, adherence to Mediterranean diet, and smoking) as well as with obesity and diabetes in determining HDL-C concentrations.

METHODS

Subjects

We included the first 4,210 participants (1,840 men and 2,370 women) entering the Prevención con Dieta Mediterránea (PREDIMED) Study from whom DNA was isolated and the TaqIB and −4,502C>T CETP promoter SNP were determined and who had valid data for the main variables analyzed. The PREDIMED study is a multi-center clinical trial aimed at assessing the effects of the Mediterranean diet on the primary prevention of cardiovascular disease. Details of this study have been reported elsewhere (27). The trial is currently taking place and the anticipated completion date is December 2011. In this report, we present data of a cross-sectional analysis at baseline including subjects recruited over 4 years. From October 2003 to October 2007, potential high cardiovascular risk subjects were selected by physicians in primary care centers affiliated to 10 teaching hospitals in Spain participating in the study. Eligible subjects were community-dwelling people (55–80 years of age for men; 60–80 years of age for women) who fulfilled at least one of two criteria: type 2 diabetes; three or more cardiovascular risk factors [current smoking, hypertension (blood pressure >140/90 mm Hg), or treatment with antihypertensive drugs], dyslipidemia [LDL cholesterol (LDL-C) $\geq$ 160 mg/dl or treatment with hypolipidemic drugs; or HDL-C $\leq$ 40 mg/dl], body mass index (BMI) $\geq$25 kg/m^2, or a family history of premature cardiovascular disease. Diagnosis of type 2 diabetes (adult-onset) was based on at least one of the following criteria: *1*) current treatment with insulin or oral hypoglycemic drugs; *2*) fasting blood glucose $\geq$ 126 mg/dl in two measurements; *3*) casual blood glucose $\geq$ 200 mg/dl; or *4*) 2 h glucose $\geq$ 200 mg/dl.

Exclusion criteria included a personal history of cardiovascular disease, any severe chronic illness (terminal cancer, severe hepatic or renal diseases, and severe dementia), and drug or alcohol addiction (27). The Institutional Review Board of each participating center approved the study protocol, and all participants provided written informed consent.

Demographic, clinical, anthropometric, and dietary measurements

The baseline examination included assessment of standard cardiovascular risk factors, medication use, sociodemographic factors, and lifestyle variables, as previously detailed (27). Food consumption was determined by a validated semiquantitative 137-item food frequency questionnaire (FFQ) also including consumption of alcoholic drinks (28). Energy and nutrient intake were calculated from Spanish food composition tables (29). This FFQ was also specifically validated in a sample of this population (30). The results of this validation study showed a very good reproducibility for food groups, energy, and nutrient intake explored by intraclass correlation coefficients that ranged from 0.63 to 0.90, which is comparable to other FFQs used in association studies between diet and cardiovascular diseases (31, 32). We calculated alcohol intake (in g/d) for each individual on

the basis of the type and amount of alcoholic beverages consumed. Three groups were defined according to the reported daily intake of alcohol: no intake (0 g/d), moderate intake (<26.4 g/d for men and <13.2 g/d for women), and high intake (26.4 g/d for men and 13.2 g/d for women). These gram amounts correspond to 1 drink/d for women and 2 drinks/d for men (33). The baseline examination also included the administration of a validated 14-item questionnaire indicating the degree of adherence to the traditional Mediterranean diet (27). Values of 0 or 1 were assigned to each of 14 dietary components. The greater score obtained from the questionnaire, the greater the adherence to the Mediterranean diet. Dichotomic variables of adherence to the Mediterranean diet and of nutrient intake were created by considering population means. Later studies undertaken on this same population have shown a good performance of this scale (34). Physical activity was estimated by the Minnesota Leisure Time Physical Activity, validated for its use in Spanish subjects (35). The reliability of this questionnaire following its repeated administration in the pilot study (27) was calculated using the Spearman correlation coefficient (rho) for total physical activity at baseline and 3 months and was high (rho = 0.83; $P < 0.001$).

Weight and height were measured with calibrated scales and a wall-mounted stadiometer, respectively. BMI was calculated as weight in kilograms divided by the square of height in meters. Trained personnel measured blood pressure in triplicate with a validated semiautomatic sphygmomanometer (Omron HEM-705CP, The Netherlands) in a seated position after a 5 min rest.

Biochemical determinations, DNA extraction, and genotyping

At baseline, blood samples were obtained for each participant after an overnight fast and were frozen at −80°C and shipped to central laboratories for analyses. Fasting glucose, total cholesterol, triglycerides, HDL-C, and LDL-C were determined as previously reported (27). Plasma glucose was analyzed by the glucose-oxidase method, triglycerides and total cholesterol were measured using standard enzymatic procedures, and HDL-C was determined after precipitation with phosphotungstic acid and magnesium chloride. LDL-C concentrations were estimated with the equation of Friedewald et al. whenever triglycerides were <400 mg/dl (36).

Genomic DNA was extracted from buffy-coat with the MagNa-Pure LC DNA Isolation kit (Roche Diagnostics, Mannheim, Germany). We analyzed the TaqIB SNP (rs708272) using the polymerase chain reaction-restriction fragment length polymorphism method described previously (20). The −4,502C>T promoter SNP (rs183130) was genotyped on a 7900HT Sequence Detection system (Applied Biosystems) using a fluorescent allelic discrimination TaqMan™ assay. For quality control purposes, 10% of randomly selected samples were genotyped a second time. There were no discrepancies between the two results.

Statistical analyses

Chi-square tests were used to test differences between observed and expected genotype frequencies, assuming Hardy-Weinberg equilibrium, and to test differences in percentages. The LD between the CETP SNPs was assessed with the Haploview software package and Lewontin's disequilibrium coefficient D´ was estimated. A combined diplotype variable was created taking three categories into consideration for the −4,502C>T and the TaqIB SNP: *1*) pure homozygotes for the C-B1 (C-B1/C-B1) diplotype; *2*) pure homozygotes for the T-B2 (T-B2/T-B2) diplotype; and *3*) a mixed category including any other combination of diplotypes (as we first carried out an exploratory analysis and no relevant differences among them were found). Triglycerides and alcohol intake were log-transformed for the statistical analyses. T and ANOVA tests were applied to compare crude means. Multivariate adjustments for continuous variables were carried out by ANCOVA. Models were adjusted for age, gender, BMI, diabetes, tobacco smoking, alcohol consumption, physical activity, education, and medications (lipid-lowering and antihypertensive drugs). The homogeneity of the effects by gender was also statistically tested. To test the interaction between the *CETP* gene variants and type 2 diabetes, obesity (BMI $\geq$ 30 kg/m^2), tobacco smoking, alcohol consumption, fat intake variables, or adherence to Mediterranean diet, separated multivariate regression models including the corresponding main effects and interaction terms in addition to the above variables and total energy intake to control for potential confounders were fitted. Stratified analyses were also carried out. Total fat intake, saturated fatty acids (SATFAT), and MUFA were considered as continuous as well as dichotomic. Two cutoff points were considered, one based on cutoff points previously published for the American population (23) and the other on the Mediterranean population mean intake. Adherence to Mediterranean diet was also analyzed as continuous and as dichotomic based in the mean score. In addition, stratified analyses of CETP genotype associations and interactions with clinical and lifestyle variables, depending on whether lipid-lowering drugs are taken or not, have been undertaken.

Logistic regression methods were also used to estimate the contribution of the genetic polymorphisms and their combination to predict HDL-C. A dichotomous trait, denominated low HDL (HDL-C < 40 mg/dl in men and <50 mg/dl in women), which was based on the National Cholesterol Education Program Adult Treatment Panel III (37), was created. Multivariate logistic regression models including the control for the different environmental factors were fitted. Statistical analyses were performed with the SPSS package, version 15.0 (SPSS, Chicago, IL). All tests were two-tailed and *P*-values < 0.05 were first considered statistically significant. However, taking into account that multiple comparisons have been carried out, a corrected *P*-value of <0.01 was also considered to minimize the Type I error that could derive from this.

RESULTS

Participant's characteristics

Table 1 shows demographic, biochemical, clinical, and lifestyle characteristics of the 4,210 high risk participants in the PREDIMED study at baseline. The analysis of macronutrient contribution revealed a high MUFA intake, which is a typical profile of the Mediterranean population. There were no statistically significant differences in the genotype distribution between men and women for either the TaqIB SNP or the −4,502C>T promoter SNP ($P = 0.36$ and $P = 0.55$, respectively). Genotype frequencies did not deviate from Hardy-Weinberg equilibrium expectations ($P = 0.62$ and $P = 0.06$ for the -4,502C>T SNP and the TaqIB SNP, respectively). TaqIB and the −4,502C>T SNP were found to be in LD ($P < 0.001$), although this disequilibrium was not complete (D´= 0.88; $r^2 = 0.49$). From the genotypes, a diplotypic variable was created as indicated in "Methods." There were no significant differences in diplotype distribution between the recruitment field-centers of participants ($P = 0.24$). We did not observe differences in

TABLE 1. Demographic, clinical, biochemical, lifestyle, and genetic characteristics of the study participants

	Men (n = 1,840)	Women (n = 2,370)
	Mean SD	Mean SD
Age (years)	66.0 (6.6)	67.9 (5.9)
BMI (kg/m^2)	29.2 (3.4)	30.5 (4.3)
Total cholesterol (mg/dl)	204.2 (41.2)	217.4 (39.0)
LDL-C (mg/dl)	127.8 (36.8)	134.0 (34.4)
HDL-C (mg/dl)	49.6 (12.1)	56.8 (13.0)
Triglycerides (mg/dl)	136.2 (75.3)	133.6 (74.0)
Fasting glucose (mg/dl)	133.7 (49.0)	125.8 (47.3)
Systolic blood pressure (mmHg)	151.7 (21.5)	149.8 (21.3)
Diastolic blood pressure (mmHg)	84.6 (11.2)	83.6 (11.7)
Physical activity (kcal/d)	314.5 (290.3)	176.4 (164.6)
Energy intake (kcal/d)	2514.1 (676.0)	2183.8 (618.2)
Total fat (g/d)	108.0 (32.7)	96.6 (31.1)
SATFAT (g/d)	27.8 (9.9)	24.7 (9.5)
MUFA (g/d)	53.7 (17.0)	48.1 (16.1)
PUFA (g/d)	17.5 (7.7)	15.2 (7.0)
Proteins (g/d)	97.6 (24.5)	91.8 (24.4)
Carbohydrates (g/d)	258.3 (93.0)	230.7 (82.7)
% Energy carbohydrates	40.7 (7.5)	42.0 (6.7)
% Energy total fat	38.9 (6.9)	39.9 (6.8)
% Energy saturated fat	10.0 (2.3)	10.1 (2.3)
% Energy MUFA	19.4 (4.5)	20.0 (4.6)
Alcohol (in drinkers) (g/d)	20.2 (20.0)	7.3 (8.3)
Nondrinkers (n, %)	294 (16.0)	1242 (52.4)
Drinkers (moderate intake) (n, %)[a]	1047 (56.9)	986 (41.6)
Drinkers (high intake) (n, %)[a]	497 (27.0)	142 (6.0)
Current smokers (n, %)	497 (27.0)	128 (5.4)
Former smokers (n, %)	857 (46.6)	173 (7.3)
Education (n, %)		
Primary	1238 (67.3)	2005 (84.6)
Secondary	372 (20.2)	258 (10.9)
University	230 (12.5)	100 (4.2)
Type 2 diabetes (n, %)	973 (52.9)	1107 (46.7)
Obesity (n, %)	736 (40.0)	1209 (51.0)
Hypertension (n, %)	1378 (74.9)	1991 (84.0)
Dyslipemia (n, %)	1121 (60.9)	1678 (70.8)
Subjects on lipid-lowering drugs (n, %)	760 (41.3)	1130 (47.6)
CETP TaqIB, rs708272 (n, %)		
B1B1	740 (40.2)	951 (40.1)
B1B2	848 (46.1)	1060 (45.5)
B2B2	252 (13.7)	359 (14.9)
CETP −4,502C>T, rs183130 (n, %)		
CC	1005 (54.6)	1262 (53.2)
CT	706 (38.4)	930 (39.3)
TT	130 (7.0)	177 (7.5)
CETP diplotype (n, %)		
CC/B1B1	686 (37.3)	905 (38.1)
Others	1038 (56.4)	1307 (55.2)
TT/B2B2	116 (6.3)	158 (6.7)

[a] Moderate intake (<26.4 g alcohol/d for men and <13.2 g/d for women) and high intake (26.4 g alcohol/d for men and 13.2 g alcohol/d for women).

the consumption of lipid-lowering drugs depending on the CETP diplotype ($P = 0.297$).

Relationships between CETP variants and continuous biochemical and clinical parameters

Table 2 details BMI, plasma lipid concentrations, fasting glucose, and blood pressure according to the *CETP* TaqIB SNP, the −4,502C>T SNP, and their diplotypic variable at baseline. There were no differences in the mean age of each genetic group, all being 67 ± 6 years. No association between BMI, fasting glucose, total cholesterol, or LDL-C concentrations and the genetic variables was observed. However, a highly significant and clinically relevant association between the two *CETP* SNPs and HDL-C concentrations was found. In the regression analysis even after adjustment for potential confounders, both SNPs were independent predictors of HDL-C ($P < 0.01$ for each of them) with additive effects (a mean estimated adjusted increase of 1.26 mg/dl; 95% CI: 0.37–2.15 per B2 allele, and 1.51 mg/dl; 95% CI: 0.05–2.96 per T allele), without their interaction term being statistically significant ($P = 0.774$). Accordingly, in the diplotype analysis, the B2B2 homozygotes presented an increase of 9% in their HDL-C concentrations compared with B1B1, whereas in the combined genotype analysis, B2B2, who in turn had the TT genotype for the −4,502C>T SNP, had a greater clinically relevant HDL-C increase (14%; $P < 0.001$) than those individuals carrying two copies of the C-B1 diplotype. These results remained statistically significant following adjustments for sex, age, BMI, diabetes, drug treatment (lipid-lowering and antihypertensive drugs), tobacco smoking, alcohol consumption, education, physical activity, and energy intake. Having undertaken these analyses on both the group of individuals taking lipid-lowering drugs (mainly statins) and the group who did not take them, the associations of the CETP genotypes and diplotypes with HDL-C concentrations did not vary in their statistical significance. Supplementary Table I shows the results for the CETP diplotypes.

Regarding the controversial association between CETP activity inhibition and greater blood pressure (7), no association of the SNPs was found with systolic blood pressure. Nevertheless, a low, statistically significant ($P < 0.01$), but not clinically relevant, association was found for the diplotype with diastolic blood pressure. However, this association did not present any linear trend (the lowest values were detected in the heterozygote group). If, instead of these continuous variables, the association of *CETP* SNPs with hypertension is studied, no significant results are found in any of the three genetic variables. Thus, for the diplotype variable, the prevalence of hypertension was 80.4% in the C-B1/C-B1, 79.8% in the intermediate diplotypes, and 80.3% in the T-B2/T-B2 subjects ($P = 0.906$).

Gene-environment interactions between the CETP SNPs and lifestyle variables

Given that HDL-C was the phenotype showing the highest significant association with the genetic variants studied, we then analyzed whether that association was modulated by environmental factors and clinical conditions. Supplementary Fig. I shows multivariate adjusted means of HDL-C according to the TaqIB SNP stratified by obesity (A), type 2 diabetes (B), alcohol consumption (C), and tobacco smoking (D). Those estimations for the *CETP* diplotypic variable are presented in **Table 3**. No statistical significant interactions were found, and a great homogeneity in the genetic effects of the *CETP* variants was observed in the different strata considered.

Delving deeper into the study of the interaction between the two polymorphisms and diet, **Table 4** shows HDL-C concentrations according to *CETP* gene variation (the TaqIB SNP and the diplotype) and the different levels of

TABLE 2. Association between the CETP polymorphisms and BMI, plasma lipids, glucose, and blood pressure in the high cardiovascular risk Mediterranean population (n = 4,210)

	Genotype	Mean SD	Genotype	Mean SD	Diplotype	Mean SD
BMI (kg/m^2)	TaqIB		−4,502C>T		C>T/TaqIB	
	B1B1	29.9 (4.1)	CC	29.9 (4.0)	CC/B1B1	29.9 (4.1)
	B1B2	30.0 (4.0)	CT	30.0 (4.0)	Others	30.0 (4.0)
	B2B2	30.0 (3.9)	TT	30.0 (3.9)	TT/B2B2	30.0 (4.0)
		P1: 0.838		P1: 0.657		P1: 0.867
		P2: 0.695		P2: 0.435		P2: 0.390
HDL-C (mg/dl)	TaqIB		−4.502C>T		C>T/TaqIB	
	B1B1	52.2 (12.8)	CC	52.5 (12.9)	CC/B1B1	51.9 (12.6)
	B1B2	54.0 (13.0)	CT	54.3 (12.8)	Others	54.2 (12.9)
	B2B2	57.0 (13.5)	TT	59.2 (14.6)	TT/B2B2	59.1 (14.8)
		P1: <0.001		P1: <0.001		P1: <0.001
		P2: <0.001		P2: <0.001		P2: <0.001
LDL-C (mg/dl)	TaqIB		−4,502C>T		C>T/TaqIB	
	B1B1	132.0 (38.3)	CC	131.7 (37.0)	CC/B1B1	132.5 (38.6)
	B1B2	130.4 (34.3)	CT	130.6 (34.2)	Others	130.4 (33.9)
	B2B2	131.9 (33.0)	TT	130.8 (33.8)	TT/B2B2	131.7 (34.3)
		P1: 0.406		P1: 0.632		P1: 0.200
		P2: 0.353		P2: 0.524		P2: 0.116
Triglycerides (mg/dl)	TaqIB		−4,502C>T		C>T/TaqIB	
	B1B1	137.5 (77.4)	CC	135.3 (74.8)	CC/B1B1	137.8 (78.5)
	B1B2	133.1 (69.5)	CT	134.8 (75.0)	Others	132.7 (70.6)
	B2B2	129.6 (76.3)	TT	128.8 (72.4)	TT/B2B2	130.6 (74.6)
		P1: 0.007		P1: 0.108		P1: 0.018
		P2: 0.145		P2: 0.538		P2: 0.304
Total cholesterol (mg/dl)	TaqIB		−4,502C>T		C>T/TaqIB	
	B1B1	212.7 (42.3)	CC	212.2 (41.2)	CC/B1B1	213.1 (42.5)
	B1B2	211.7 (39.1)	CT	212.3 (39.0)	Others	211.6 (38.8)
	B2B2	215.5 (37.5)	TT	217.9 (38.5)	TT/B2B2	218.7 (38.3)
		P1: 0.141		P1: 0.058		P1: 0.054
		P2: 0.138		P2: 0.106		P2: 0.097
Glucose (mg/dl)	TaqIB		−4,502C>T		C>T/TaqIB	
	B1B1	129.4 (49.2)	CC	128.5 (48.2)	CC/B1B1	129.5 (49.2)
	B1B2	128.4 (47.2)	CT	129.5 (47.7)	Others	128.6 (47.0)
	B2B2	129.0 (48.0)	TT	129.1 (49.8)	TT/B2B2	129.2 (51.1)
		P1: 0.870		P1: 0.854		P1: 0.889
		P2: 0.932		P2: 0.926		P2: 0.957
Systolic BP (mm Hg)	TaqIB		−4,502C>T		C>T/TaqIB	
	B1B1	151.0 (20.9)	CC	150.9 (20.9)	CC/B1B1	151.0 (20.9)
	B1B2	150.4 (21.6)	CT	149.8 (22.1)	Others	150.1 (21.8)
	B2B2	150.3 (22.4)	TT	151.9 (21.8)	TT/B2B2	151.8 (21.6)
		P1: 0.598		P1: 0.126		P1: 0.279
		P2: 0.532		P2: 0.137		P2: 0.236
Diastolic BP (mm Hg)	TaqIB		−4,502C>T		C>T/TaqIB	
	B1B1	84.5 (11.6)	CC	84.2 (11.6)	CC/B1B1	84.5 (11.5)
	B1B2	83.3 (11.2)	CT	83.5 (11.2)	Others	83.5 (11.4)
	B2B2	84.8 (12.1)	TT	85.3 (12.9)	TT/B2B2	85.8 (13.0)
		P1: 0.002		P1: 0.013		P1: 0.000
		P2: 0.010		P2: 0.011		P2: 0.001

N: B1B1: 1691, B1B2: 1908, B2B2: 611; CC: 2267, CT: 1636, TT: 307; CC-B1B1: 1591, Others: 2345 and TT-B2B2: 274. BP: Blood pressure. P1: *P*-value obtained in the ANOVA test for crude comparison of means among CETP genotypes. P2: *P*-value obtained in the multivariate adjusted model after control for potential confounders (sex, age, BMI, diabetes, alcohol consumption, tobacco smoking, physical activity, education, and medications).

fat intake (total fat, SATFAT, and MUFA). For that stratification, two cutoff points were taken into consideration: one based on the Mediterranean population mean intake and the other on cutoff points previously published for the American population (23). We found no statistically significant interaction between the CETP SNPs and dietary fat. Given that a significant interaction was reported in diabetic men (23), we carried out a new analysis including only diabetic men. This analysis also found no statistically significant interaction between the *CETP* SNPs and dietary fat for any of the fatty acids studied or for any of the cutoff points considered (results not shown).

We also tested whether *CETP* polymorphisms interacted with adherence to the Mediterranean diet in determining HDL-C. Two strata of adherence based on the 14-point scale were considered (low adherence, ⩽8 points and high adherence, >8 points). No statistically significant modification of the effect of the *CETP* polymorphisms was found (P for interaction adherence to Mediterranean diet-TaqIB SNP = 0.627; P for interaction adherence to Mediterranean diet × *CETP* diplotype = 0.695). To prevent confusion about the cutoff points, we also studied the interaction of the diplotype with continuous variables of diet, but for neither of them did we find statistically significant interactions ($P = 0.804$, $P = 0.378$, $P = 0.922$, and $P = 0.215$ for the interaction terms between the *CETP* diplotype and total fat intake, SATFAT intake, MUFA intake, and adherence to the Mediterranean diet, respectively).

TABLE 3. Interaction between the CETP diplotype and obesity, diabetes, tobacco smoking, and alcohol consumption in determining HDL-C concentrations in a Mediterranean population

	-4,502C>T/TaqIB CETP diplotype				
	CC/B1B1	Mixed haplotypes	TT/B2B2		
Variable	(n = 1,591) Mean SE	(n = 2,345) Mean SE	(n = 274) Mean SE	P^b	P for interaction[c]
Obesity					0.211
Nonobese (n = 2,265)	51.8 (0.6)	55.1 (0.5)	59.3 (1.1)	<0.001	
Obese (n = 1,945)	50.4 (0.6)	52.1 (0.6)	56.2 (1.2)	<0.001	
Type 2 diabetes					0.375
Nondiabetic (n = 2,130)	52.6 (0.6)	55.6 (0.5)	60.2 (1.1)	<0.001	
Diabetic (n = 2,080)	49.6 (0.6)	51.7 (0.5)	55.4 (1.2)	<0.001	
Tobacco smoking					0.089
Nonsmoker (n = 2,555)	52.4 (0.6)	55.3 (0.5)	60.1 (1.1)	<0.001	
Former smoker (n = 1,030)	51.7 (0.8)	53.2 (0.7)	55.1 (1.5)	0.048	
Current smoker (n = 625)	49.6 (0.9)	52.2 (0.8)	60.2 (2.4)	<0.001	
Alcohol consumption[a]					0.536
Nondrinker (n = 1,536)	48.5 (0.7)	51.2 (0.6)	54.3 (1.3)	<0.001	
Moderate intake (n = 2,033)	50.9 (0.6)	53.4 (0.5)	57.2 (1.2)	<0.001	
High intake (n = 639)	53.9 (0.9)	56.1 (0.8)	62.9 (1.8)	<0.001	

Values are multivariate adjusted means and SE.

[a] Moderate intake (<26.4 g alcohol/d for men and <13.2 g/d for women) and high intake (26.4 g alcohol/d for men and 13.2 g alcohol/d for women).

[b] *P*-value for the CETP diplotype in each strata after multivariate adjustment for sex, age, physical activity, hypertension, medications, education, obesity, diabetes, tobacco smoking and alcohol consumption.

[c] *P*-value for the interaction term between the CETP diplotype and the corresponding variable by considering each one singly.

Having undertaken the analysis of these interactions on both the group of individuals taking lipid-lowering drugs and those who did not take them, no statistically significant interactions were observed in either of the subgroups, and the relationships were similar (point estimates differing by <10% and the confidence intervals overlapping). Supplementary Table II shows the results of the interaction analysis with the main environmental variables and clinical conditions determining HDL-C depending on CETP diplotype in subjects not taking lipid-lowering medications.

Association between the CETP variants and risk of having low HDL-C

Given the homogeneity of the effect of the variations in the *CETP* gene on HDL-C concentrations, we assessed their impact in determining defined low HDL-C concentrations (34) **Table 5** shows the odds ratio (OR) of having low HDL-C, depending on the CETP SNP, and their comparison with other well-known cardiovascular risk factors. For the diplotypic variable, these estimates were: OR = 0.44; 95% CI: 0.31–0.65 for theT-B2/T-B2 diplotype, and OR = 0.64; 95% CI: 0.55–0.76 for the mixed category, both in comparison with the B1-C/B1-C diplotype (reference category). These associations are clinically very relevant, as they were higher or of similar magnitude than those observed for smoking, alcohol consumption, diabetes, and obesity in this population. No statistically significant gene-environment interactions with lifestyle variables and clinical conditions were observed (supplementary Table III).

DISCUSSION

In this study, carried out in a large sample of high cardiovascular risk subjects from a Mediterranean population, we found that variations in the *CETP* gene (TaqIB or −4,502C>T) are strong genetic determinants of HDL-C concentrations despite the concurrence of multiple risk factors and drug treatments. There is little insight into which other genetic variant in the *CETP* gene could explain the effects of the intronic TaqIB (10, 11, 14, 38). The −4,502C>T promoter variant, proposed as being the most important determinant of HDL-C by Thompson et al. (16), does not completely explain the effects of the TaqIB SNP in this population, despite being found in LD. Both SNPs have additive effects in discordant subjects and the diplotype defined by them can be considered clinically relevant, because increases of more than 10% (approximately 7 mg/dl) of HDL-C in subjects carrying two copies of the variant diplotype versus wild type were detected. Considering that a 1% increase in HDL-C has been associated with a 2–3% reduction in cardiovascular morbidity and mortality (39, 40), and that our study involves a high-risk population, these *CETP* SNPs could have a relevant role in atherogenesis. Comparing the effects with other populations, we found that the TaqIB SNP was similar to that described (13), whereas the effect of the −4,502C>T SNP in our study appears to be slightly higher than that reported by Thompson et al. (16). The other study that analyzed the association of the −4,502C>T SNP with HDL-C (41) did not provide mean values, but this SNP exhibited the most significant association.

The association of *CETP* SNPs with blood pressure has not been routinely examined in prior studies, and it was not included as a phenotype in the prior meta-analyses (12, 13). However, a recent meta-analysis (39) has been focused on it given the controversy arising from clinical trials with torcetrapib, in which an increase in blood pressure was observed (7, 42). In our study, no association of

TABLE 4. Interaction between the CETP genotypes and fat intake (total fat, SATFAT, and MUFA) in determining HDL-C concentrations based on Mediterranean population mean intakes and on American cutoff points

	CETP-TaqIB Genotype					-4,502C>T/TaqIB CETP Diplotype				
	B1B1 (n = 1,691) Mean SE	B1B2 (n = 1,908) Mean SE	B2B2 (n = 611) Mean SE			CC/B1B1 (n = 1,591) Mean SE	Mixed (n = 2,345) Mean SE	TT/B2B2 (n = 274) Mean SE		
Fat intake	Mediterranean population means			P^a	P interaction^b	Mediterranean population means			P^a	P interaction^b
Total fat					0.251					0.311
≤39.5% (n = 2,092)	51.2 (0.6)	53.2 (0.6)	56.5 (0.8)	<0.001		50.7 (0.6)	53.6 (0.5)	58.7 (1.1)	<0.001	
>39.5% (n = 2,118)	51.9 (0.6)	54.0 (0.6)	55.5 (0.8)	<0.001		51.7 (0.6)	54.1 (0.6)	57.4 (1.2)	<0.001	
Saturated fat					0.543					0.677
≤10% (n = 2,164)	51.4 (0.6)	53.0 (0.6)	55.4 (0.8)	<0.001		50.9 (0.6)	53.3 (0.5)	56.9 (1.1)	<0.001	
>10% (n = 2,046)	51.7 (0.6)	54.2 (0.6)	56.6 (0.8)	<0.001		51.5 (0.6)	54.3 (0.5)	58.9 (1.1)	<0.001	
Monounsaturated fat					0.253					0.239
≤19.5% (n = 2,088)	50.9 (0.6)	53.4 (0.6)	56.3 (0.8)	<0.001		50.4 (0.6)	53.7 (0.5)	57.7 (1.1)	<0.001	
>19.5% (n = 2,121)	52.1 (0.6)	53.8 (0.6)	55.7 (0.8)	<0.001		51.9 (0.6)	53.8 (0.5)	58.3 (1.2)	<0.001	
	North-American population cutoff points^c					North-American population cutoff points^c				
Total fat					0.157					0.129
≤33.5% (n = 838)	50.7 (0.8)	53.5 (0.8)	57.5 (1.2)	<0.001		50.1 (0.8)	53.9 (0.7)	59.8 (1.7)	<0.001	
>33.5% (n = 3,372)	51.7 (0.5)	53.6 (0.5)	55.6 (0.7)	<0.001		51.4 (0.5)	53.7 (0.5)	57.5 (0.9)	<0.001	
Saturated fat					0.889					0.803
≤11.47% (n = 3,157)	51.3 (0.5)	53.3 (0.5)	55.7 (0.7)	<0.001		50.9 (0.5)	53.5 (0.5)	57.4 (0.9)	<0.001	
>11.47% (n = 1,053)	52.1 (0.7)	54.5 (0.7)	56.8 (1.1)	<0.001		51.9 (0.6)	54.5 (0.7)	59.6 (1.6)	<0.001	
Monounsaturated fat					0.832					0.831
≤12.75% (n = 295)	52.7 (1.3)	53.7 (1.2)	56.2 (1.9)	0.174		52.1 (1.4)	54.1 (1.1)	59.9 (2.9)	0.048	
>12.75% (n = 3,915)	51.4 (0.5)	53.5 (0.5)	56.0 (0.7)	<0.001		51.1 (0.5)	53.6 (0.5)	57.7 (0.9)	<0.001	

Values are multivariate adjusted means and SE.

[a] *P*-value for the CETP genotype in each strata after multivariate adjustment for sex, age, physical activity, hypertension, medications, education, BMI, diabetes, tobacco smoking, alcohol consumption, and total energy intake.

[b] *P*-value for the interaction term between the CETP genotype and the corresponding fat intake variable.

[c] Cutoff points for the North American population are based on those reported by Li et al. (23) corresponding to the median of intake.

TABLE 5. Association between the CETP diplotype and the risk of having low HDL-C[1]

Variable	CETP-4,502C>T/TaqIB* OR 95% CI	*P*
CETP haplotype		
CC-B1B1	1.00	
Mixed	0.64 (0.55–0.76)	<0.001
TT-B2B2	0.44 (0.31–0.66)	<0.001
Type 2 diabetes		
Yes	1.00	
No	0.53 (0.45–0.62)	<0.001
Obesity		
No	1.00	
Yes	1.32 (1.12–1.55)	0.001
Hypertension		
Yes	1.00	
No	0.94 (0.77–1.16)	0.577
Tobacco smoking		
Nonsmoker	1	
Past-smoker	1.18 (0.931–50)	0.178
Current-smoker	1.51 (1.15–1.99)	0.003
Alcohol consumption		
Nondrinker	1.00	
Moderate-drinker	0.74 (0.62–0.88)	0.001
High-drinker	0.44 (0.33–0.60))	<0.001
Total fat intake		
≤39.5%	1.00	
>39.5%	0.98 (0.83–1.14)	0.768

Low HDL-C (<40 mg/dl in men and <50 mg/dl in women). N = 1095 subjects with low HDL-C and 3115, nonlow. Multivariate logistic regression models including the CETP diplotype, diabetes, obesity, hypertension, smoking, alcohol, and total fat were additionally adjusted for sex, age, physical activity, education, and medications.

the *CETP* variants with systolic blood pressure was found. Nevertheless, some statistically significant associations were observed with diastolic blood pressure. However, this association was small, not clinically relevant, and did not follow a gene-dosage effect. In addition, bearing in mind the results of no association of the *CETP* SNPs with systolic or diastolic blood pressure in a recent meta-analysis (42), the association observed in our study with diastolic blood pressure may be produced at random. In this regard, functional studies pointed out that torcetrapib induces aldosterone and cortisol synthesis by an intracellular calcium-mediated mechanism that is independent of CETP inhibition (43); thus, other CETP-inhibiting interventions could still increase HDL-C without having off-target effects on blood pressure (44).

In the current study, we assessed for the first time gene-environment interactions of the −4,502C>T SNP on HDL-C concentrations. Initial studies of interactions were mainly focused on the TaqIB SNP. A classic example is the study of Fumeron et al. (17) in French males in which a gene-alcohol interaction was described where the effect of B2 on HDL-C concentrations was absent in subjects drinking <25 g/d of alcohol but increased at higher levels. Following this observation, many studies have been published with inconsistent results (9, 18–22, 38). In a prior study (20), we found no significant TaqIB-alcohol interaction in determining HDL-C in a healthy Mediterranean population. Also, the Framingham Study reported no statistically significant alcohol-TaqIB interaction (9).

In addition to the potential interaction with alcohol, the presence of obesity, diabetes, and smoking blunted the effect of *TaqIB* SNP on HDL-C concentrations in some studies (45, 46) but not in others (9, 20). In their meta-analysis, Boekholdt et al. (12) concluded that there was not sufficient evidence of a significant interaction between the TaqIB SNP and obesity or diabetes in determining HDL-C. However, they observed a significant interaction with tobacco smoking. In the current study, we did not find any significant interaction between these factors and the CETP SNPs with HDL-C.

The potential interaction of CETP SNPs with fat intake has not been as widely studied and was not included in the published meta-analyses (12, 13). The two prior observational studies that analyzed the interaction between the TaqIB SNP and dietary fat with HDL-C obtained contradictory results (23, 24). Li et al. (23), evaluated 780 diabetic men participating in the Health Professionals' Follow-Up Study and found a statistically significant interaction between the TaqIB SNP and total fat, as well as with different fatty acids, so that the effect of the B2 allele increasing HDL-C was not present with lower intakes of total fat, animal fat, SATFAT, and MUFA. These findings were not replicated in the large biracial community from the Atherosclerosis Risk in Communities study (24) regardless of the manner in which dietary fat intake was modeled. Our results in the Mediterranean population support the Atherosclerosis Risk in Communities findings, as we did not find any statistical interaction of the TaqIB SNP and fat intake, assessed in various ways, with HDL-C. Moreover, we also reported for the first time the results of no significant gene-diet interaction involving the −4,502C>T SNP and the diplotypic variable. Therefore, to increase HDL-C concentrations in subjects with the nonfavorable CETP genotypes, it is necessary to consider biological (47) gene-diet interactions instead of statistical gene-diet interactions, because no genetic determinism was found. HDL-C concentrations were different in subjects with the same genotype that differed in environmental factors or clinical conditions. For example, HDL-C concentrations were higher in B1B1 nondiabetic subjects than in B2B2 diabetic subjects.

One of the strengths of our study is that it was initially designed to analyze gene-environment interactions, and we have measured genetic, anthropometric, clinical, biochemical, and lifestyle variables, including diet, in a large sample to compare the magnitude of the effects and the potential interactions. Despite the fact that some other studies have described statistically significant interactions, we found no statistically significant interaction between any of the genetic variants studied (the TaqIB SNP, the −4,502C>T SNP, or their diplotype) and these factors in determining HDL-C. One of the reasons, and also a strength of our study, that may have contributed to this is our large sample size. This apparent paradox can be easily explained by bearing in mind that if modifications of the effects in the different strata considered really do not exist, a study of greater sample size provides greater stability in the measurements, minimizing the probability of wide

differences being found randomly in means between strata. On the other hand, in a study of smaller sample size, although its statistical power for detecting true modifications of the effect is less, the instability of the measurements is greater, increasing the probability of wrongly detecting statistically significant interactions. Furthermore, having carried out the stratified analyses on individuals who either took lipid-lowering drugs or did not, we found similar associations of CETP genotypes with HDL-C concentrations, and did not find, in any of the strata, statistically significant interactions of CETP genotypes with the environmental variables and clinical conditions analyzed, so increasing internal validity. In our study, most patients on lipid-lowering drugs were taking statins to reduce LDL-C concentrations. Numerous clinical studies have revealed that statins have not only LDL-C-lowering effects but also HDL-C-elevating effects, depending on the type of statin taken (48). Nevertheless, according to our results, taking lipid-lowering drugs does not modify the association between CETP genotypes and HDL-C concentrations in this high cardiovascular risk Mediterranean population and does not have any influence on the interaction between CETP genotypes and lifestyle variables. However, there is a study that has found an adverse pharmacogenetic interaction between the CETP genotype and statins with cardiovascular mortality (49), but this particular issue does not form part of our analysis.

In conclusion, we have found that the −4,502C>T and TaqIB SNPs at the CETP gene locus show additive effects and are very strong determinants of HDL-C in a high-risk population, with diplotypic effects clinically relevant and comparable to those of obesity, tobacco smoking, or alcohol consumption. This genetic effect does not present statistically significant interactions with obesity, type 2 diabetes, smoking, alcohol consumption, dietary fat intake, or even adherence to the Mediterranean diet.

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