Relationship of HDL and coronary heart disease to a common amino acid polymorphism in the cholesteryl ester transfer protein in men with and without hypertriglyceridemia

Can Bruce,* Dan S. Sharp,† and Alan R. Tall[1,*]

Division of Molecular Medicine,* Columbia University, New York 10032, and Division of Epidemiology and Clinical Applications,† Epidemiology and Biometry Program, National Heart, Lung and Blood Institutes, National Institutes of Health, Bethesda, MD 20892

Abstract **Plasma triglyceride (TG) levels are inversely related to HDL-cholesterol levels and subjects with high TG and low HDL cholesterol have increased coronary heart disease (CHD) risk. Plasma cholesteryl ester transfer protein (CETP) transfers cholesteryl esters from HDL to TG-rich lipoproteins. In this study we determined the relationship between a common CETP amino acid polymorphism (I405V) and CETP and HDL levels and CHD prevalence in 576 men of Japanese ancestry in the Honolulu Heart Program cohort. This conservative substitution was associated with altered plasma CETP concentration (1.95 ± 0.54, 1.91 ± 0.57, and 1.77 ± 0.57 μg/ml for the *II*, *IV* and *VV* genotypes, respectively). The distribution of plasma CETP concentrations among the *VV*, but not *II*, men appeared bimodal ($P < 0.01$), suggesting the presence of a functionally significant CETP gene mutation(s) in a subset of V alleles. HDL-C levels were higher in *VV* than *IV* for *II* men (55.4 ± 17.4, 51.3 ± 16.6, 51.1 ± 17.0 mg/dl, $P < 0.04$). However, the increase in HDL was only significant in *VV* men with plasma TG > 165 mg/dl. Although CHD prevalence was not significantly different among the three genotypes in this population, in the subpopulation with high plasma TG, CHD prevalence appeared higher among *VV* than *IV* or *II* subjects (38% vs. 27% vs. 18%, $P < 0.05$ for an interaction of genotype and plasma TG levels). In fresh plasma from a separate group of normolipidemic subjects, the V/I polymorphism was not associated with any change in plasma CETP specific activity. The data suggest that a widespread and common CETP gene mutation(s) in linkage disequilibrium with 405V causes low CETP. Among hypertriglyceridemic men this is associated with higher HDL and possibly with increased CHD.—**Bruce, C., D. S. Sharp, and A. R. Tall. **Relationship of HDL and coronary heart disease to a common amino acid polymorphism in the cholesteryl ester transfer protein in men with and without hypertriglyceridemia. *J. Lipid Res.* 1998.** 39: **1071–1078.**

Supplementary key words cholesteryl ester transfer protein • lipid transfer protein • high density lipoproteins • hypertryiglyceridemia • coronary heart disease.

Cholesteryl ester transfer protein (CETP) plays an important role in the transport of cholesterol from tissues to the liver by mediating the transfer of cholesteryl esters from plasma high density lipoproteins (HDL) to triglyceride (TG)-rich lipoproteins (1, 2). Because the transfer of the cholesteryl esters occurs as part of an exchange reaction with TG, the transfer rate is dependent on very low density lipoprotein TG, HDL-cholesterol (HDL-C), and CETP concentrations. Mann et al. (3) have suggested that in normolipidemic individuals CETP is in excess, so that cholesteryl ester transfer rates are determined by plasma triglyceride concentrations, while in hypertriglyceridemic patients, because CETP is at limiting concentrations, transfer rates are determined by the concentration of plasma CETP.

Individuals deficient in CETP have increased HDL levels. Although epidemiological studies have shown high HDL levels to be protective against atherosclerosis (4–6), a recent study of Japanese-American men in the Honolulu Heart Program has shown that CETP deficiency is associated with increased prevalence of coronary heart disease (CHD), despite the resulting elevated HDL levels (7). The difference in disease prevalence was found to be most marked in men with intermediate HDL levels (40–60 mg/dl), and men with HDL cholesterol >60 mg/dl with or without CETP deficiency had a low CHD prevalence. These results have suggested the importance of the dynamics of the cholesterol transport in plasma in addition to the lipoprotein profile as contributing to CHD risk.

Several human CETP mutations have been identified that lead to decreased activity and/or expression. The two

Abbreviations: aa405, amino acid 405; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; HDL, high density lipoprotein; HDL-C, HDL-cholesterol; LDL, low density lipoprotein; TG, triglyceride.

[1]To whom correspondence should be addressed.

most common CETP mutations known to date, the D442G (8) and the intron 14 splicing (9) mutations affect about 7 and 2% of the general Japanese population, respectively (10). Recently, other less common mutations have also been described (11–13). No common CETP gene mutations have been described in European populations. The contribution of the CETP locus to HDL-C in Europeans has been assessed by sib-pair analysis, and an association between the CETP gene and HDL levels has been shown in one population having many individuals with heart disease (14), while a larger study on healthy individuals showed no such association (15). Position 405 of CETP is polymorphic, being either an isoleucine or a valine, due to an A-to-G substitution in exon 14 of the CETP gene (16). In a preliminary abstract report, the CETP I405V polymorphism was reported to be significantly associated with HDL-C concentration in two European populations (17). This association suggested to us that this polymorphism may be associated with variations in the plasma CETP concentration, and if so, possibly with CHD status as well. We undertook to examine whether this association was also present in a population of men of Japanese ancestry living in Hawaii (18).

MATERIALS AND METHODS

Subjects

The Honolulu Heart Program is a prospective epidemiologic investigation of heart disease and stroke in a population-based cohort of Japanese-American men who were living in the island of Oahu in 1965 (18). The men genotyped in this study are a subgroup of the men reported previously (7) and consist of all men with the low (<30 mg/dl) and high (≥75 mg/dl) HDL-C, and a random 14% of men with intermediate HDL levels. The HDL distribution of the sample was normal. Only those with either definite CHD or no CHD were studied; "possible" CHD cases were excluded. Definite CHD categorization required *a*) prior myocardial infarction detected by hospital surveillance, *b*) silent myocardial infarction from electrocardiogram data, *c*) acute coronary insufficiency, or angina pectoris resulting in surgical intervention, and *d*) temporal changes in electrocardiogram diagnostic of myocardial infarction. Men carrying the intron 14 splicing defect and the D442G mutations of CETP were excluded in the study of the relationship between the aa405 polymorphism and HDL-C levels and CHD (see Table 4, Fig. 1–3). To study the relationship of the intron 14 and D442G mutations to the aa405 polymorphism, a subset of the subjects carrying these mutations were genotyped for I405V (see Tables 1–3).

DNA analysis

Genomic DNA used for genotyping was obtained from buffy coat. The A→G transversion at position 1394 of the CETP cDNA (corresponding to aa405 of the protein) was detected by using a pair of PCR primers (5′-ctcaccatgggcatttgattgcagagcagctccgactcc-3′ and 5′-aatgggaagctctgtcagcctcggccacccag-3′) designed to create a *Tth111* I site in the amplification product when the 405V allele was present. For PCR, a 50 μl reaction mixture contained 1 unit of *Taq* polymerase, about 100 ng of genomic DNA, 50 pmol of each primer, and 10 μm each of dNTPs in a buffer containing 50 mm KCl, 10 mm Tris-HCl, pH 8.3, 1.5 mm $MgCl_2$ and 0.01% gelatin. After 30 cycles (94°C 30 sec, 63°C 1 min, 74°C 1 min) using a Perkin-Elmer DNA Temperature Cycler, 15 μl of the reaction mixture was digested with 1 unit of *Tth111* I at the conditions recommended by the manufacturer (New England Biolabs). The *Msp* I polymorphism was determined as described (19). The M1 and M2 alleles refer to the presence and absence of the *Msp* I cutting site.

Plasma CETP concentration was determined by competitive radioimmunoassay (20). HDL cholesterol was measured as described previously (21). Activity assays were done as described (22), essentially by incubating 1 μl of fresh plasma at 37°C for 4 h with HDL (5 μg protein) containing radiolabeled cholesteryl ester and low density lipoproteins (LDL) (100 μg protein) in a volume of 100 μl, then precipitating the LDL and counting the radioactivity left in the supernatant.

Statistical analyses

Analyses were conducted using the SAS software program. Descriptive statistics included means, standard deviations, and frequencies statistics were done using PROC FREQ. For continuous variables, adjustments were made to 23.5 kg/m^2 body mass index, 50 mg/dl HDL-C, 110 mg/dl calculated LDL-cholesterol, 78 years of age, 80 mm Hg diastolic blood pressure, and 110 mg/dl blood glucose. CHD prevalence was adjusted to reflect no alcohol consumption, no smoking, and no use of medication for hypertension by logistic regression methods (23). Measures of association included differences in adjusted means for continuous measures among allelic groups, and odds ratios for measures of frequency as the outcome variable. Statistical significance was set as two-sided type I error of 0.05. Measures of association included differences in adjusted means for continuous measures among allelic groups and odds ratios for measures of frequency as the outcome variable. Statistical significance was set as a two-sided type I error of 0.05. An adjustment with a weighing factor to account for the 14% sampling frame for the 30–75 mg/dl HDL-C range did not change magnitudes of association or descriptive statistics significantly and was not used in order to allow the calculation of *P* values in statistical tests. The test for bimodality was done using the NOCOM program (24), which estimates the means, variances, and proportions of a mixture of normal distributions for independent observations and calculates their likelihood values. Briefly, this program calculates the best fit to a given sample distribution after it is constrained to assume that this population consists of a mixture of two normal distributed populations or of a single one. The difference in the likelihood values for the two alternatives indicates whether the assumption of a second component in the population fits the distribution significantly better.

RESULTS

Allele frequencies

The CETP aa405 polymorphism reported previously in European populations (17) was found to be similarly prevalent in the Japanese-American population of the Honolulu Heart Program cohort. Of 576 subjects genotyped who had neither the D442G mutation nor the intron 14 splicing mutation in the CETP gene, 126, 301, and 149 had the *II*, *IV* and *VV* genotypes, respectively, at the amino acid 405 (aa405) locus. The I and V alleles have gene frequencies of 0.46 and 0.54, respectively, while the genotype frequencies are consistent with the Hardy-Weinberg equilibrium.

We characterized the CETP aa405 polymorphism also in

TABLE 1. Association of the intron 14 splicing mutation and the aa405 polymorphism

	aa405 Polymorphism			
Int 14 mutation	*II*	*IV*	*VV*	Total
−/−	126	301	149	576
+/−	5	9	0	14
+/+	0	0	0	0
Total	131	310	0	590

Plus (+) indicates presence of the mutation.

men who did carry the D442G and the intron 14 splicing mutation. The relationships between the genotypes are shown in **Tables 1–3**. We also genotyped a subset of our population (96 men) for the *Msp* I restriction fragment polymorphism in intron 8 (23). Individuals who were heterozygous at any of these three loci did not have the *VV* genotype for the aa405 locus (Tables 1–3). The data suggest that the D442G and the intron 14 mutations and the *Msp* I M2 allele are in linkage disequilibrium with the aa405 I allele (Fisher exact test *P*-value (two-sided) <0.001) (Tables 1 and 2). The CETP levels were not significantly different between the M2M1 and M2M2 individuals (Table 3).

Based on the 96 people who were genotyped for both the aa405 and the *Msp* I polymorphism, we determined the haplotype frequencies of the M2-I, M1-I, M2-V, and M1-V alleles to be 0.30, 0.07, 0.63, and 0.00. These numbers are comparable to those reported for the Dutch population (in its middle HDL-C decile): 0.48, 0.13, 0.37, 0.02 (19), suggesting that the *Msp* I mutation in the two populations has the same origin.

Effect on CETP levels

Measurement of plasma CETP concentration of 576 men in the Honolulu Heart Program cohort showed that the aa405 polymorphism is associated with differences in CETP levels. Men with the *VV* genotype had a lower CETP concentration (1.77 ± 0.57 mg/ml) than *II* men (1.95 ± 0.54 mg/ml; $P < 0.01$) while *IV* heterozygotes were intermediate (1.91 ± 0.57; $P < 0.02$ for *IV* vs. *VV*, *P* not significant for *IV* vs. *II*) (**Table 4**). As a recent study reported an *Msp* I polymorphism in the CETP gene to be a better predictor of plasma CETP levels than the aa405 polymorphism (19), we examined whether this was the case for the Japanese-American population of Hawaii. The CETP levels were not significantly different between the M2M1 and M2M2 individuals (Table 3c).

The I405V substitution of CETP is a conservative substitution that is unlikely to alter function. Moreover, the specific activity of plasma CETP is identical in *II* and *VV* subjects (see below). This suggests that the V allele may be linkage disequilibrium with one or more mutations that determine plasma CETP levels.

TABLE 2. Association of the D442G mutation and the aa405 polymorphism

	aa405 Polymorphism			
aa442	*II*	*IV*	*VV*	Total
DD	126	301	149	576
DG	18	24	0	42
GG	4	0	0	0
Total	148	325	149	618

TABLE 3. Relationship of the MspI and aa405 polymorphisms on plasma CETP concentrations

	aa405 Polymorphism[b]			
MspI Polymorphism[a]	*II*	*IV*	*VV*	Total
M2M2	13 (2.03 ± 0.52)	32 (1.71 ± 0.57)	38 (1.57 ± 0.49)	83
M2M1	2 (2.10, 2.10)	11 (1.73 ± 0.41)	0	13
M1M1	0	0	0	0
Total	15	43	38	96

[a]M1 and M2 indicate the presence and absence of the *Msp* I cutting site, respectively.
[b]Number of subjects and their mean ± SD of plasma CETP (μg/ml).

To evaluate this possibility, the frequency distribution of CETP levels among the three genotypes (*II*, *IV*, *VV*) was determined. Despite their different mean CETP values, their modes were essentially identical. The distribution of CETP values in subjects below the 50th percentile appeared strikingly different for the three genotypes. In the *VV*, *IV*, and *II* groups about 34%, 20%, and 15% of the subjects had a CETP concentration less than 1.5 μg/ml, respectively (**Fig. 1**). A bimodal distribution is suggested by a shoulder in the VV curve, while the II curve has the typical shape for a normal distribution. A test for bimodality of the distribution curve using the NOCOM program (24), indicated that for the *VV* population the distribution is more consistent with a 2-component mixture (1.03 ± 0.05 μg/ml, 8% proportion, and 1.83 ± 0.55 μg/ml, 92% proportion) than a one-component mixture ($P < 0.01$). Two subpopulations with 22% and 78% proportions, having means of 1.26 ± 0.29 and 1.91 ± 0.54 μg/ml, respectively, also provide a good fit to the observed distribution of the *VV* subjects, but their likelihood value does not quite reach significance. For the *II* population, the likelihood value for a two component mixture was not significantly better than a one component mixture with a mean CETP concentration of 1.95 ± 0.54 μg/ml.

HDL levels

Similar to previous observations on European populations (17), there was an association between the polymor-

TABLE 4. Relationship of the CETP aa405 polymorphism to CETP and HDL-cholesterol

	CETP aa405 Genotype		
	II	*IV*	*VV*
CETP (μg/ml)	1.95 ± 0.54	1.91 ± 0.57	1.77 ± 0.57[a]
HDL-C (mg/dl)	51.15 ± 17.0	51.27 ± 16.6	55.38 ± 17.4[b]
n	126	301	149

[a]Less than *II* and *IV* values with $P < 0.01$ and < 0.02, respectively.
[b]More than *II* and *IV* values with $P < 0.04$ and < 0.02, respectively.

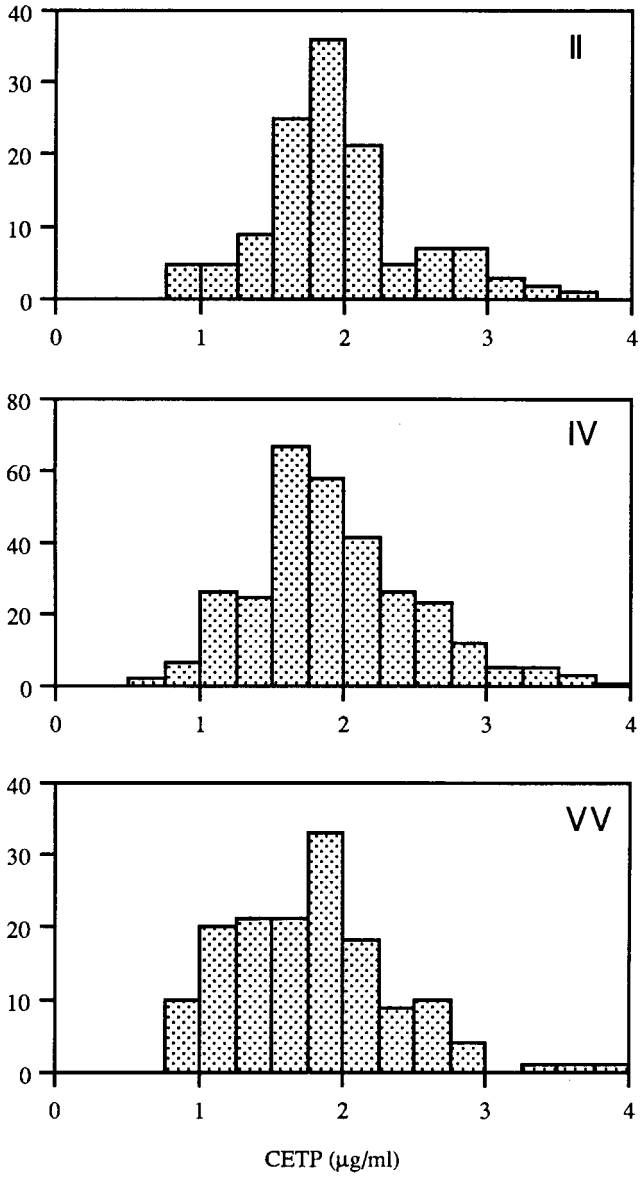

Fig. 1. Effect of aa405 polymorphism on the cumulative distribution of CETP concentrations. The *VV* and *II* curves are consistent with bimodal and unimodal distributions, respectively. See text for details.

phism at aa405 and HDL levels (Table 4). When HDL-C levels were analyzed among the three aa405 polymorphism categories, the adjusted mean value of 55.4 mg/dl is about 10% higher in the *VV* group compared to the *II* and *IV* groups ($P < 0.04$ and < 0.02, respectively), while there appears to be no significant difference between the *II* and *IV* groups in HDL-C (Table 4). There was no correlation between the CETP concentration in the subjects analyzed and their HDL levels.

Because the activity of plasma CETP is driven by hypertriglyceridemia (3, 25), we next segregated the population by plasma total triglyceride values to see whether the relationships between the aa405 polymorphism and CETP were enhanced among men with high TG values. A cut-off of 165 mg/dl TG placed about one-third of our population in the "high TG" group. With this cut-off, the high-TG group had lower HDL levels than the low-TG group (**Fig. 2**). The TG ≥ 165 mg/dl group accounted for nearly all the effect of the aa405 polymorphism on HDL levels ($P < 0.05$), while the low-TG group's HDL values did not differ significantly among the *II*, *IV*, and *VV* men (Fig.2). Cut-off points at higher TG levels produced similar results, i.e. the slope of the relationship between CETP genotype and HDL levels was steeper in more hypertriglyceridemic subjects (**Fig. 3**).

There was no significant difference in CETP levels between the low- and high-TG groups: the mean (±SD) of plasma CETP concentration for the *II*, *IV*, and *VV* groups were, respectively, 1.94 (±0.56), 1.88 (±0.56), and 1.80 (±0.59) for the low-TG group, and 1.99 (±0.50), 1.98 (±0.59), and 1.68 (±0.51) for the high-TG group. *VV* men had significantly lower CETP levels than *II* men ($P < 0.05$) in both the low and high triglyceride groups.

Relationship to CHD prevalence

A logistic regression model predicting CHD status from HDL cholesterol was used to confirm that in the present population, the odds ratio for definite CHD for a 1 mg/dl increase in HDL cholesterol is 0.978, which is highly significant ($P < 0.001$) and consistent with previous data showing an overall inverse relationship between HDL levels and CHD (4). However, several statistical tests failed to show any overall relationship between the aa405 polymorphism and CHD status. Also, there was no significant difference in CHD prevalence among the three aa405 genotypes when tertiles of HDL-C values were analyzed individually.

However, when the effect of the aa405 polymorphism on disease prevalence was examined for the low- and high-TG groups, the patterns of CHD prevalence showed different relationships with the aa405 genotype, i.e., amongst high TG subjects, disease prevalence tended to increase going from the II to IV to VV genotypes, while amongst low TG subjects it tended to decrease (chi-square = 6.010, df = 2, $P = 0.05$). In the hypertriglyceridemic group, CHD prevalence among *VV* men was found to be about twice that of *II* men (37% vs. 18%) (**Fig. 4**). In the group with TG < 165 mg/dl, there was an insignificant trend for lower CHD prevalence among *VV* men relative to *IV* and *II* men (20% vs. 30% and 29%, respectively). These findings suggest that hypertriglyceridemic *VV* subjects have increased CHD, while normotriglyceridemic *VV* subjects have a trend toward lower CHD prevalence.

Relationship to specific activity

Although the aa405 polymorphism of CETP is a conservative amino acid substitution, the lower CETP levels in *VV* subjects could directly reflect this change. We reasoned that a functionally significant missense mutation causing low CETP levels would probably cause changes in specific activity. To assess this possibility, we collected fresh plasma from 22 healthy volunteers (reliable activity measurements necessitate fresh plasma), measured their plasma CETP concentration, performed an activity assay on their diluted plasma, and also genotyped them. The specific activity of CETP was not significantly different among the three aa405 genotypes (**Table 5**).

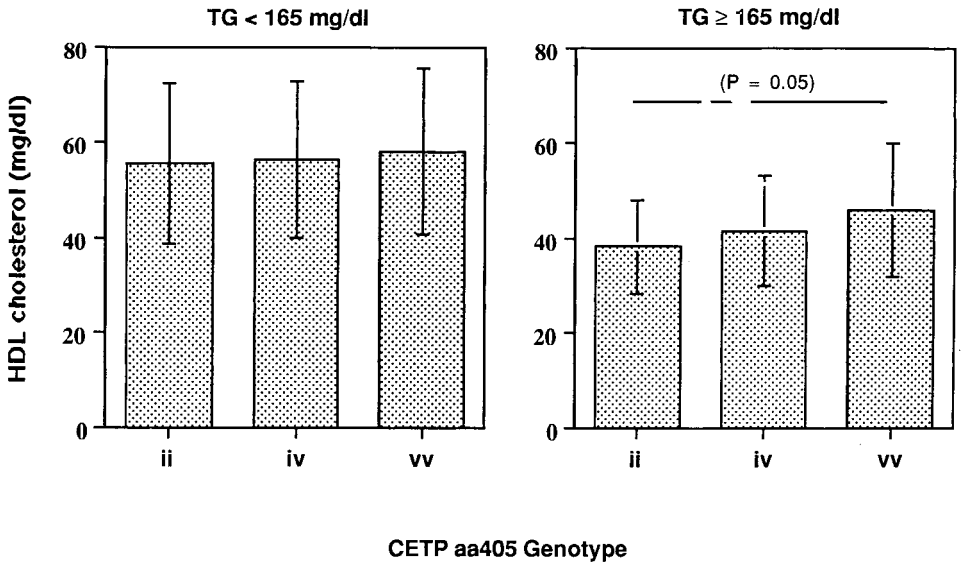

Fig. 2. The effect of the aa405 polymorphism on adjusted HDL-C levels is modulated by plasma TG levels. Subjects were segregated into the TG < 165 (n = 374) and TG ≥ 165 (n = 102) mg/dl groups. HDL-C levels between the *II* and *VV* subjects in the high-TG group were significantly different ($P = 0.05$ by unpaired *t* test).

DISCUSSION

The population effects of a polymorphic missense mutation at amino acid 405 of the CETP gene (16) were originally described in a preliminary report in German and Sardinian populations with V allele frequencies of 0.3 and 0.4, respectively (17). More recently the aa405 and the *Msp* I polymorphisms have been described in a Dutch population, along with the *Taq* I-B polymorphism (19). We find here that these two polymorphisms are also present among the Japanese (V allele frequency = 0.54, M2 frequency = 0.6). Similar to the findings in the German

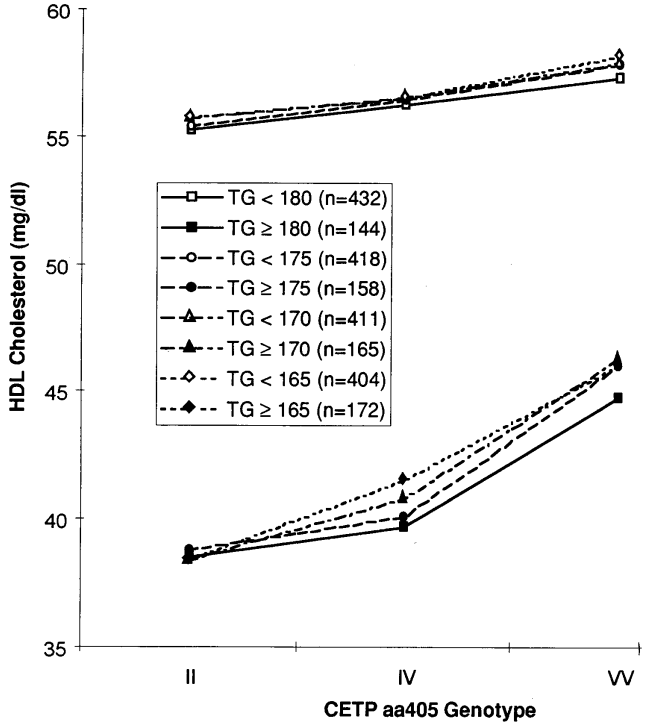

Fig. 3. The effect of diffferent TG cutoff points on the interaction between HDL levels and aa405 genotype.

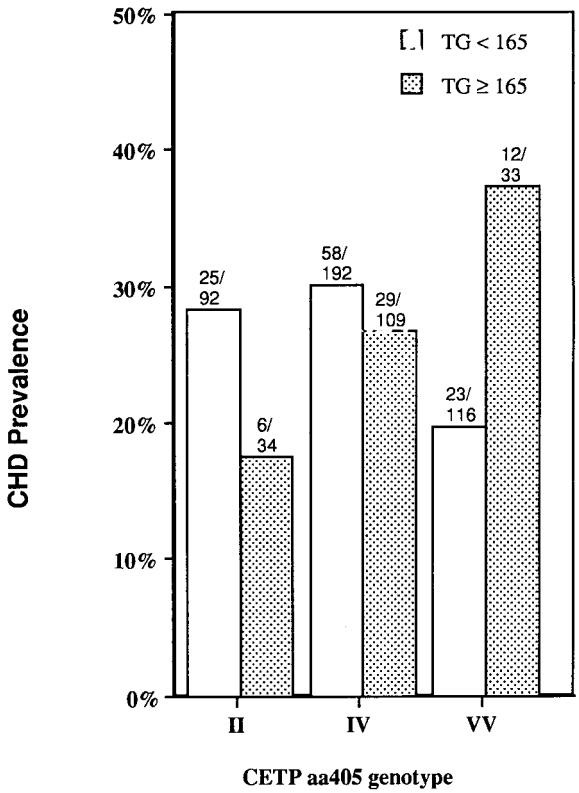

Fig. 4. The effect of the aa405 polymorphism on adjusted CHD prevalence. Adjusted prevalence reflects adjustment to alcohol consumption, smoking, and use of medication for hypertension. A chi-test for multiplicative interaction gives $P = 0.05$ (chi-square test). Fractions on each bar indicate the actual number of subjects with CHD, over the total number of subjects in that group.

TABLE 5. Relationship of the CETP aa405 polymorphism to CETP specific activity

	CETP aa405 Genotype		
	II	*IV*	*VV*
Specific activity	100 ± 25[a]	92 ± 18	104 ± 12
n	7	10	5

The specific activity was determined by dividing the cholesteryl ester transfer activity by the CETP concentration of each fresh plasma sample.

[a] Mean and standard deviation, expressed as a percentage of the mean value for the *II* subjects.

population where the V allele is associated with increased HDL cholesterol (by 6.5 mg/dl) (17) and the Dutch population where *VV* subjects have about 20% higher HDL-C than *II* subjects, in men of Japanese ancestry of the Honolulu Heart Project cohort, those with the *VV* genotype have a 4.2 mg/dl (~10%) higher HDL-C value than those with the *II* genotype. Because an effect of the aa405 polymorphism on HDL levels is seen in several divergent populations, it is likely that this effect is mediated by the CETP locus rather than being the result of genetic admixture. The fact that the aa405 polymorphism is associated with low CETP and increased HDL in both European and Japanese populations suggests that a CETP gene mutation(s) of clinical importance is present among a significant segment of human populations.

The conservative modification of CETP at residue 405 from an isoleucine to a valine may not be the direct cause of reduced plasma CETP levels and increased HDL. Consistent with this suggestion, the specific activity values of CETP from a group of normolipidemic individuals of the *II*, *IV*, and *VV* genotypes did not differ significantly from each other. In contrast, the common D442G missense mutation of CETP affects both the secretion and specific activity of CETP (8). We cannot rule out the possibility that this polymorphism somehow affects the secretion or catabolism of the protein without changing the specific activity, but we believe it is more likely to be a marker for a mutation(s) in strong linkage disequilibrium that reduces expression levels of the gene. In other studies, the modeling of the three-dimensional structure of CETP based on that of the evolutionarily related bactericidal/permeability inducing protein (26) indicates that aa405 is too distant from the lipid binding pocket to affect lipid binding directly (L. Beamer and D. Eisenberg, unpublished results).

The skewed distribution of CETP levels in the *VV* population of the Honolulu Heart Program population is consistent with a bimodal distribution and also suggests that a subpopulation of the V alleles carry one or more linked mutations that alter the expression of CETP. Other associations reported between plasma CETP levels and the intronic *Taq* I-B and *Msp* I polymorphisms support the existence of common genetic variants that influence CETP and HDL levels (19, 27). The aa405 polymorphism has been shown to be in linkage disequilibrium to both the *Taq* IB and *Msp* I polymorphisms (19). Recently sib-pair studies have shown a common genetic effect on CETP levels in a white Caucasian US population (28). The nature of the underlying mutation(s) is unknown. However, sequencing and single-strand conformation polymorphism analysis of Japanese subjects have revealed no other common mutations apart from the D442G and intron 14 splicing mutations (10), and sequencing and single-stranded conformation polymorphism analysis of the proximal promoter, intron 1, and intron 9 have not revealed a causative mutation (C. Bruce and A. Tall, unpublished results). However, as there are known regulatory elements extending out to 3 kb upstream of the gene (29), the putative mutation(s) could be in a distant regulatory region or intron.

An interesting finding in this study was the apparent modification of the relationship between CETP and HDL levels by hypertriglyceridemia. Although based on a post-hoc analysis of the data, this finding is biologically plausible (30–33). Our findings are consistent with those of Tato, Vega, and Grundy (32) and Foger et al. (31) who showed that among hypertriglyceridemic men, variation in plasma CETP levels was the major factor determining HDL cholesterol concentrations, while the contribution of CETP was less in normotriglyceridemic subjects. The sensitivity of HDL-C levels to variations in CETP concentration is explainable by the finding that in hypertriglyceridemia, the rate-limiting factor in cholesteryl ester transfer is CETP (3). This is further confirmed experimentally in human-CETP transgenic mice, in which the presence of the human apo-CIII transgene leads to hypertriglyceridemia accompanied by profound reductions in HDL-C (34). Thus, although CETP levels of the men studied here were in the normal range, the sensitivity that is brought out by hypertriglyceridemia causes a relatively small variation in CETP levels to have a detectable effect on HDL levels as well as CHD prevalence. Apparent contradictory results in linking CETP as a genetic determinant of HDL levels in sib-pair studies (14, 15) could be related to an effect of hypertriglyceridemia. The study by Cohen et al. (14) excluded individuals with plasma TG >200 mg/dl, while the study population of Bu et al. (15) was enriched for CHD and therefore was likely to harbor more individuals with hypertriglyceridemia. However, the former study had much larger numbers of informative sib-pairs (14, 15) and the modifying effect of plasma triglyceride levels suggested in the present study will need to be replicated in a separate population.

The observation that the effect of the aa405 polymorphism on CHD prevalence, like HDL levels, is limited to hypertriglyceridemic subjects could indicate a causal relationship between the increase in HDL associated with low CETP and disease risk. The result of the chi-square test suggests that TG levels and the aa405 genotype interact differently with respect to their effect on disease prevalence. A possible interpretation of this is that there is an excess in disease prevalence seen in VV men with high TG relative to either VV men with low TG, or II men with high TG, due to low CETP predisposing to CHD at high TG levels. The bimodality analysis suggests that 8% or possibly 22% of the VV men represent a subpopulation with a lower mode in the CETP distribution. If 8% of the VV men carry

an underlying mutation that lowers their CETP level, then the excess disease prevalence among this subgroup of VV men with high TG relative to II men with high TG corresponds to 4-fold increase in disease prevalence. For the 22% figure, the calculated odds ratio is about 2, which is of the same order as found for the D442G mutation (7). Although the 22% proportion estimated by the bimodality analysis is not as likely as an 8% proportion, it may be biologically more plausible.

Similar findings were made in studying hyperlipidemic transgenic mice overexpressing human apoC-III with and without human CETP; on a high cholesterol diet, the area and number of lesions in the proximal aorta of the animals was significantly lower when the CETP transgene was present (34). More recently, markedly decreased HDL levels resulting from CETP expression in a mouse model of combined hyperlipidemia did not lead to an increase in atherosclerosis (35). In the presence of hypertriglyceridemia, CETP expression markedly increased the formation of small, preβ HDL particles which may be the preferred initial acceptor of cellular cholesterol efflux (35). On the other hand, CETP expression in mice without hypertriglyceridemia caused increased atherosclerosis (25, 36). This may be analogous to the apparently decreased CHD prevalence in *VV* men who have normal plasma TG levels (Fig. 4). Together the data could suggest that reverse cholesterol transport via the CETP pathway is more important in hypertriglyceridemia, whereas in normotriglyceridemia reverse cholesterol transport by a non-CETP pathway (such as the HDL cholesteryl ester selective uptake pathway (37)) may be more important. In the future it will be important *a*) to confirm to the bimodal distribution and find the underlying mutation and *b*) to relate significant mutations directly to increased TG, HDL, and CHD.

This work was supported by National Institutes of Health grants HL22682 and HL56984 (SCOR in Molecular Medicine and Atherosclerosis) and Contract No. NO1-HC-02901 from the National Heart, Lung, and Blood Institute.

Manuscript received 30 September 1997, in revised form 18 December 1997, and in re-revised form 20 January 1998.

REFERENCES

1. Tall, A. 1995. Plasma lipid transfer proteins. *Annu. Rev. Biochem.* **64:** 235–257.
2. Bruce, C., and A. R. Tall. 1995. Cholesteryl ester transfer protein, reverse cholesterol transport and atherosclerosis. *Curr. Opin. Lipidol.* **6:** 306–311.
3. Mann, C. J., F. T. Yen, A. M. Grant, and B. E. Bihain. 1991. Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. *J. Clin. Invest.* **88:** 2059–2066.
4. Rhoads, G. G., C. L. Gulbrandsen, and A. Kagan. 1976. Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. *N. Engl. J. Med.* **294:** 293–298.
5. Miller, N. E., D. S. Thelle, O. H. Forde, and O. D. Mjos. 1977. The Tromso Heart Study: high density lipoprotein and coronary heart disease: a prospective case-control study. *Lancet.* **1:** 965–968.
6. Gordon T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease: the Framingham Heart Study. *Am. J. Med.* **62:** 707–714.
7. Zhong, S., D. S. Sharp, J. S. Grove, C. Bruce, K. Yano, J. D. Curb, and A. R. Tall. 1996. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J. Clin. Invest.* **97:** 2917–2923.
8. Takahashi, K., X-C. Jiang, N. Sakai, S. Yamashita, K. Hirano, H. Bujo, H. Yamazaki, J. Kusunoki, T. Miura, P. Kussie, Y. Matsuzawa, Y. Saito, and A. Tall. 1993. A missense mutation in the cholesteryl ester transfer protein gene with possible dominant effects on plasma high density lipoproteins. *J. Clin Invest.* **92:** 2060–2064.
9. Brown, M. L., A. Inazu, C. B. Hesler, L. B. Abellon, C. Mann, M. E. Whitlock, Y. L. Marcel, R. W. Milne, J. Koizumi, H. Mabuchi, R. Takeda, and A. R. Tall. 1989. Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature (London)* **342:** 448–451.
10. Inazu, A., X-C. Jiang, T. Haraki, K. Yagi, N. Kamon, J. Koizumi, H. Mabuchi, R. Takeda, K. Takata, Y. Moriyama, M. Doi, and A. R. Tall. 1994. Genetic cholesteryl ester transfer protein deficiency caused by two prevalent mutations as a major determinant of increased levels of high density lipoprotein cholesterol. *J. Clin. Invest.* **94:** 1872–1882.
11. Arai, T., S. Yamashita, N. Sakai, K. Hirano, S. Okada, M. Ishigami, T. Maruyama, M. Yamane, H. Kobayashi, S. Nozaki, T. Funahashi, K. Kameda-Takemura, N. Nakajima, and Y. Matsuzawa. 1996. A novel nonsense mutation (G181X) in the human cholesteryl ester transfer protein gene in Japanese hyperalphalipoproteinemic subjects. *J Lipid Res.* **37:** 2145–2154.
12. Sakai, N., S. Santamarina-Fojo, S. Yamashita, Y. Matsuzawa, and H. B. Brewer, Jr. 1996. Exon 10 skipping caused by intron 10 splice donor site mutation in cholesteryl ester transfer protein gene results in abnormal downstream splice site selection. *J Lipid Res.* **37:** 2065–2073.
13. Gotoda T., M. Kinoshita, H. Shimano, K. Harada, M. Shimada, J. Ohsuga, T. Teramoto, Y. Yazaki, and N. Yamada. 1993. Cholesteryl ester transfer protein deficiency caused by a nonsense mutation detected in the patient's macrophage mRNA. *Biochem. Biophys. Res. Commun.* **194:** 519-524
14. Cohen, J. C., Z. Wang, S. M. Grundy, M. R. Stoesz, and R. Guerra. 1994. Variation at the hepatic lipase and apolipoprotein AI/CII/AIV loci is a major cause of genetically determined variation in plasma HDL cholesterol levels. *J. Clin. Invest.* **94:**2377–2384.
15. Bu, X., C. H. Warden, Y. R. Xia, C. De Meester, D. L. Puppione, S. Teruya, B. Lokensgard, S. Daneshmand, J. Brown, R. J. Gray, J. I. Rotter, and A. J. Lusis. 1994. Linkage analysis of the genetic determinants of high density lipoprotein concentrations and composition: evidence for involvement of the apolipoprotein A-II and cholesteryl ester transfer protein loci. *Hum. Genet.* **93:** 639–648.
16. Agellon, L. B., E. M. Quinet, T. G. Gillette, D. T. Drayna, M. L. Brown, and A. R. Tall. 1990. Organization of the human cholesteryl ester transfer protein gene. *Biochemistry.* **29:** 1372–1376.
17. Funke H., H. Wiebusch, L. Fuer, S. Muntoni, H. Schulte, and G. Assmann. 1994. Identification of mutations in the cholesterol ester transfer protein in Europeans with elevated high density lipoprotein cholesterol. *Circulation.* **90 (pt 2):** I–241.(Abstr.)
18. Trombold J. C., R. C. Moellering, Jr., and A. Kagan. 1966. Epidemiological aspects of coronary heart disease and cerebrovascular disease: the Honolulu Heart Program. *Hawaii Med J.* **25:** 231–234.
19. Kuivenhoven, J. A., P. de Knijff, J. M. A. Boer, H. A. Smalheer, G. Botma, J. C. Seidell, J. J. P. Kastelein, and P. H. Pritchard. 1997. Heterogeneity at the CETP gene locus. Influence on plasma CETP concentrations and HDL cholesterol levels. *Arterioscler. Thromb. Vasc. Biol.* **17:** 560–568.
20. Marcel, Y. L., R. McPherson, M. Hogue, H. Czarneka, Z. Zawadski, P. Weech, M. E. Whitlock, A. R. Tall, and R. W. Milne. 1990. Distribution and concentration of cholesteryl ester transfer protein in plasma of normolipidemic subjects. *J. Clin. Invest.* **85:** 10–17.
21. Fried, L. N., N. O. Borhani, and P. Enright, for the CHS Investigators. 1991. The Cardiovascular Health Study: design and rationale. *Ann. Epidemiol.* **1:** 263–276.
22. Tall, A. R., E. Granot, R. Brocia, I. Tabas, C. Hesler, K. Williams, and M. Denke. 1987. Accelerated transfer of cholesteryl esters in dyslipidemic plasma. Role of cholesteryl ester transfer protein. *J. Clin. Invest.* **79:** 1217–1225.
23. Wilcosky, T. C. and L. E. Chanbless. 1985. A comparison of direct adjustment and regression adjustment of epidemiologic measures. *J. Chronic Dis.* **38:** 849–856.
24. Ott, J. 1979. Detection of rare major genes in lipid levels. *Hum. Genet.* **51:** 79–91.

25. Hayek, T., N. Azrolan, R. B. Verdery, A. Walsh, T. Chajek-Shaul, L. B. Agellon, A. R. Tall, and J. L. Breslow. 1993. Hypertriglyceridemia and cholesteryl ester transfer protein interact to dramatically alter high density lipoprotein levels, particle sizes and metabolism. Studies in transgenic mice. *J. Clin. Invest.* **92:** 1143–1152.
26. Beamer, L. J., S. F. Carroll, and D. Eisenberg. 1997. Crystal structure of human BPI and two bound phospholipids at 2.4 Ångstrom resolution. *Science.* **276:** 1861–1864
27. Freeman, D. J., C. J. Packard, J. Shepherd, and D. Gaffney. 1990. Polymorphisms in the gene coding for cholesteryl ester transfer protein are related to plasma high-density lipoprotein cholesterol and transfer protein activity. *Clin. Sci. (Colch).* **79:** 575–581.
28. McPherson, R., S. M. Grundy, R. Guerra, and J. C. Cohen. 1996. Allelic variation in the gene encoding the cholesteryl ester transfer protein is associated with variation in the plasma concentrations of cholesteryl ester transfer protein. *J. Lipid Res.* **37:** 1743–1748.
29. Oliveira, H. C. F., R. A. Chouinard, L. B. Agellon, C. Bruce, L. Ma, A. Walsh, J. L. Breslow, and A. R. Tall. 1996. Human cholesteryl ester transfer protein gene proximal promoter contains dietary cholesterol positive responsive elements and mediates expression in small intestine and periphery while predominant liver and spleen expression is controlled by 5′-distal sequences. *Cis*-acting sequences mapped in transgenic mice. *J. Biol. Chem.* **271:** 31831–31838.
30. Tatò, F., G. L. Vega, A. R. Tall, and S. M. Grundy. 1995. Relation between cholesterol ester transfer protein activities and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **15:** 112–120.
31. Föger, B., A. Ritsch, A. Doblinger, H. Wessels, and J. R. Patsch. 1996. Relationship of plasma cholesteryl ester transfer protein to HDL cholesterol. Studies in normotriglyceridemia and moderate hypertriglyceridemia. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1430–1436.
32. Tatò, F., G. L. Vega, and S. M. Grundy. 1997. Determinants of Plasma HDL-cholesterol in hypertriglyceridemic patients: role of cholesterol ester transfer protein and lecithin cholesteryl acyl transferase. *Arterioscler. Thromb. Vasc. Biol.* **17:** 56–63.
33. Murakami, T., S. Michelagnoli, R. Longhi, G. Gianfranceschi, F. Pazzuconi, L. Calabresi, C. R. Sirtori, and G. Franceschini. 1995. Triglycerides are major determinants of cholesterol esterification / transfer and HDL remodeling in human plasma. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1819–1828.
34. Hayek, T., L. Masucci-Magoulas, X. Jiang, A. Walsh, E. Rubin, J. L. Breslow, and A. R. Tall. 1995. Decreased early atherosclerotic lesions in hypertriglyceridemic mice expressing cholesteryl ester transfer protein transgene *J. Clin. Invest.* **96:** 2071–2074.
35. Masucci-Magoulas, L., I. J. Goldberg, C. L. Bisgaier, H. Serajuddin, O. L. Francone, J. L. Breslow, and A. R. Tall. 1997. A mouse model with features of familial combined hyperlipidemia. *Science.* **275:** 391–394.
36. Marotti, K. R., C. K. Castle, T. P. Boyle, A. H. Lin, R. W. Murray, and G. W. Melchior. 1993. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. *Nature.* **364:** 73–75.
37. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.