Cholesteryl Ester Transfer Protein (CETP) Genotype and Reduced CETP Levels Associated With Decreased Prevalence of Hypertension

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OBJECTIVES: To clarify whether reduced cholesteryl ester transfer protein (CETP) activity carries inherent blood pressure risks and to infer whether the increased blood pressure and elevated mortality associated with torcetrapib are idiosyncratic or characteristic of this class of drugs.

PATIENTS AND METHODS: We examined the associations among CETP genotype, phenotype, and blood pressure in a cohort of 521 older adults (who have complete data for the variables required in our primary analysis) enrolled between November 1, 1998, and June 30, 2003, in our ongoing studies of genes associated with longevity, including a cohort with a high prevalence of a genotype coding for a reduced activity variant of CETP and low levels of CETP.

RESULTS: The prevalence of hypertension was actually lower among homozygotes for the variant CETP (48% vs 60% among those with wild-type and 65% among heterozygotes; $P=.03$). Low levels of CETP were associated with reduced prevalence of hypertension (65% in highest tertile, 59% in middle tertile, and 55% in lowest tertile; $P=.04$) and lower systolic blood pressure (140.8, 138.1, 136.2 mm Hg, respectively; $P=.03$).

CONCLUSION: Reduced levels of CETP are associated with lower, not higher, blood pressure. The adverse results with torcetrapib, if mediated through blood pressure, are likely to represent effects of this specific drug, rather than a result of lower CETP levels.

Mayo Clin Proc. 2010;85(6):522-526

CETP = cholesteryl ester transfer protein; CI = confidence interval; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SNP = single nucleotide polymorphism

A decrease in cholesteryl ester transfer protein (CETP) leads to increased high-density lipoprotein (HDL) cholesterol levels and lipoprotein particle size, which may provide significant cardiovascular protection.¹ Earlier work by our group has shown that homozygosity for a single nucleotide polymorphism (SNP), resulting in substitution of valine (V) for isoleucine (I) at codon 405 in the CETP gene, is overrepresented in centenarians.² Therefore, this finding, validated in an independent study,³ suggests that this mutation may contribute to healthy aging by protection from several age-related diseases.⁴ Individuals homozygous for this SNP have decreased CETP levels, increased HDL cholesterol levels, and increased lipoprotein particle sizes. Homozygosity for this SNP is seen significantly more often in the offspring of parents with exceptional longevity than in the offspring of parents with shorter life spans.² Furthermore, a recent review found a consistent association between lower CETP levels, caused by several

different genotypes, and decreased risk of coronary artery disease.⁵ These observations suggest that interventions that reduce CETP levels might promote cardiovascular health and contribute to longevity.

Despite the potential cardiovascular benefits of lower CETP levels, a phase 3 trial of torcetrapib, a CETP inhibitor, was terminated early because of increased risk of death due to cardiovascular disease in the active treatment group.⁶ Torcetrapib has been associated with increased blood pressure,⁷ which is suspected of contributing to the observed increase in mortality. Other investigators have called for investigation of the connection between low CETP levels and blood pressure.⁸

To examine whether naturally occurring CETP polymorphism, decreased CETP levels, increased HDL cholesterol levels, and increased lipoprotein particle sizes are associated with increased blood pressure, we analyzed data from our cohorts of older adults. We reasoned that if blood pressure is independent of spontaneous CETP levels, the problems found in the torcetrapib trial may be specific collateral effects of that drug and not generalizable to its pharmacological class. However, if reduction in CETP levels, due to genetic or other causes, is associated with increased blood pressure, beneficial effects of low CETP levels may apply only to those who have lived to old age or it may be that resulting higher blood pressure levels are tolerated because of other beneficial effects.

PATIENTS AND METHODS

Recruitment for the Searching for Longevity Genes in the Historically Unique Ashkenazi Jewish Population study has been described in detail.² Briefly, Ashkenazi Jews living independently at age 95 years or older (centenarians) were identified by publicity in synagogues and Jewish media. We also recruited the offspring of these probands and

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This work was partially supported by grants AG027734, AG18728, RR12248, DK20541, and M01-RR12248 from the National Institutes of Health.

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a control group consisting of spouses, friends, or neighbors of similar age whose parents died before 85 years of age. The offspring and control groups were typically in their 60s and 70s.

We studied participants (centenarians, their offspring, and controls) recruited between November 1, 1998, and June 30, 2003. From this group of 689, we excluded 122 who lacked information needed to classify hypertension status and an additional 46 who lacked measured CETP levels. Thus, our sample consists of 521 people who have complete data for the variables required in our primary analysis. (Secondary analyses may be based on smaller samples because of incompleteness of other data.) Of these 521 people, 197 (38%) were unrelated to any other member of the sample. The remaining 324 people had 1 ($n=174$), 2 ($n=129$), 3 ($n=19$), or 4 ($n=2$) included relatives. Using a sandwich estimator, we adjusted SEs and P values for clustering of observations within kindreds.

The study was approved by the Institutional Review Board of the Albert Einstein College of Medicine. All participants provided written informed consent.

STUDY MEASURES

The principal outcome variable is hypertension, defined as any of the following: (1) systolic blood pressure greater than 140 mm Hg, (2) diastolic blood pressure greater than 90 mm Hg, or (3) current treatment with antihypertensive medication. Because a more subtle effect might be recognized as a slight upward shift in blood pressure with only minor effect on the prevalence of hypertension, we also analyzed systolic and diastolic blood pressure measurements as secondary outcome variables. (Participants taking blood pressure medications were included in these analyses as well.) Blood pressure was measured with a mercury sphygmomanometer while the participant was sitting after a 5-minute period of rest. The average of 2 measurements was recorded. Medication history was obtained by participant self-report.

Levels of CETP were measured by enzyme-linked immunosorbent assay (Wako Chemicals, Richmond, VA). Genotyping of CETP was performed as described previously.⁴ Lipoprotein particle sizes were measured by nuclear magnetic resonance spectroscopy (LipoScience, Raleigh, NC).^{9,10}

STATISTICAL ANALYSES

Means and SDs of continuous study variables are presented, along with tabulations of categorical variables to describe the study sample. The principal independent variable is the level of CETP. (Additional analyses categorizing CETP levels led to identical conclusions and are not shown.)

Whether CETP may affect blood pressure directly or by mechanisms involving its effects on lipoproteins is un-

known. Therefore, we examined the associations of CETP to blood pressure or hypertension, both with and without adjustment for lipoprotein levels and lipoprotein particle sizes.

The primary hypothesis test was performed using logistic regression analysis and examining the t statistic of the coefficient for CETP level. We controlled for the following variables: age, sex, body mass index, serum creatinine, and blood glucose. Goodness of fit of the logistic regression model was tested with the Hosmer-Lemeshow statistic, and discrimination was assessed using the area under the receiver operating characteristic curve. Secondary outcomes (systolic and diastolic blood pressures) were analyzed using ordinary least squares linear regression, again examining the t statistic of the coefficient for CETP level.

To adjust for these other effects, we also tested models including HDL cholesterol levels and average sizes of low-density lipoprotein (LDL) and HDL particles, testing for the effect of each while controlling for all the others.

To verify that our findings are not distorted by misspecification of associations to continuous covariates as linear when in fact they are not, we repeated the analyses, representing each continuous variable by categorical variables corresponding to tertiles, or natural clinical cutoffs. The conclusions of those analyses did not differ from the conclusions of the original analyses, so we did not report them in this article.

Our sample size was determined by the availability of participants with sufficient data in a study originally undertaken for other purposes. It provides 90% power to test the association of hypertension with a 1-SD increase in CETP level and to detect an adjusted odds ratio of 1.42 with $\alpha=.05$.

RESULTS

Table 1 shows the basic demographic and clinical characteristics of the sample included in the primary analysis and compares them with the characteristics of persons who were excluded because of incomplete information on hypertension status or lack of a measured CETP level.

ASSOCIATION OF CETP WITH HYPERTENSION

Primary Analysis. Figure 1 presents the prevalence of hypertension in each tertile of CETP level and by CETP genotype. The crude prevalence of hypertension increases with the CETP level. Among those in the lowest tertile of CETP (0.4-1.5 $\mu\text{g/mL}$; $n=170$), the prevalence is 51.2%. It increases to 59.9% in the middle tertile (1.5-2.2 $\mu\text{g/mL}$; $n=177$) and to 63.2% in the highest tertile (2.2-4.8 $\mu\text{g/mL}$; $n=174$) ($P=.02$ by χ^2 for trend). Note that the prevalence

TABLE 1. Characteristics of Study Sample and Persons Excluded Because of Missing CETP or Blood Pressure Data^a

Characteristic	Sample (N=521)		Excluded (N=168)	
	Mean	SD	Mean	SD
Age (y)	77.8	15.1	77.7	16.6
Body mass index (kg/m ²)	25.0	4.3	24.2	4.1
Cholesterol (mg/dL)	204.3	41.2	206.0	40.2
Serum creatinine (mg/dL)	1.0	0.5	1.0	0.3
Fasting glucose (mg/dL)	98.8	35.0	99.8	36.7
HDL cholesterol (mg/dL)	60.4	18.5	58.5	18.0
HDL particle size (nm)	9.3	0.5	9.3	0.5
LDL cholesterol (mg/dL)	114.2	34.7	119.4	36.0
LDL particle size (nm)	21.2	0.7	21.3	0.6
Triglycerides (mg/dL)	152.6	111.9	146.5	80.8
Sex, No. (%) female	313 (60.1)		111 (66.1)	
CETP genotype, No. (%)				
II	148 (29.8)		42 (29.6)	
IV	235 (47.3)		21 (50.0)	
VV	114 (22.9)		79 (20.4)	

^a Percentages may not total 100% because of rounding. Numbers for some characteristics may be smaller because of incomplete data. CETP = cholesteryl ester transfer protein; HDL = high-density lipoprotein; I = isoleucine; LDL = low-density lipoprotein; V = valine.

^b SI conversion factors: To convert cholesterol, HDL cholesterol, and LDL cholesterol values to mmol/L, multiply by 0.0259; to convert serum creatinine values to $\mu\text{mol/L}$, multiply by 88.4; to convert fasting glucose values to mmol/L, multiply by 0.0555; to convert triglyceride values to mmol/L, multiply by 0.0113.

of hypertension increases with increasing levels of CETP within each genotype and is lower in the VV genotype at all levels of CETP.

In the logistic regression model, adjusting for other factors associated with hypertension, we found that hypertension appears to be associated with *increased* levels of CETP; the adjusted odds ratio of hypertension associated with a 1- $\mu\text{g/mL}$ increase in CETP level is 1.27 (95% confidence interval [CI], 0.97-1.69; $P=.09$). Although this finding is not statistically significant, it is in the *opposite* direction from what would be expected if CETP inhibition were inherently associated with increased risk of hypertension. The fit of the logistic regression model was satisfactory (Hosmer-Lemeshow $\chi^2 = 7.8$; $P=.45$), as was calibration (area under the receiver operating characteristic curve, 0.66).

Secondary Analyses. In linear regression analyses, a 1- $\mu\text{g/mL}$ increase in CETP level was associated with an increase of 2.9 mm Hg in systolic blood pressure (95% CI, 0.3-5.5; $P=.03$). The mean increase in diastolic blood pressure with the same increase in CETP level was 1.0 mm Hg (95% CI, -0.4 to +2.3; $P=.16$). The regression models with CETP levels as the independent variable explained 7% and 18% of the variance in systolic and diastolic blood pressure levels, respectively. Adjusted mean systolic and diastolic blood pressures by genotype and tertile of CETP level are shown in Table 2.

FIGURE. Crude prevalence of hypertension by cholesteryl ester transfer protein (CETP) level and genotype. I = isoleucine; V = valine.

Controlling for all recognized effects of CETP genotype by incorporating indicator variables for the nonwild genotypes, quintiles of HDL cholesterol, and classes of HDL and LDL particle size further reinforces the conclusions of the analysis. As can be seen in Table 3, lower levels of CETP are associated with lower prevalence of hypertension, as is the homozygous VV genotype associated with reduced CETP. The large LDL particle size also associated with this genotype is, itself, associated with a reduced prevalence of hypertension. However, hypertension prevalence does not differ according to HDL cholesterol level or particle size.

If the 158 centenarians in these analyses are excluded to obtain a study population more representative of the potential recipients of lipid therapy, the effects found are essentially unchanged, although statistical significance is lost because of the decreased sample size (data not shown).

TABLE 2. Adjusted Mean Systolic and Diastolic Blood Pressure by Genotype and Level of CETP

CETP genotype	Tertiles of CETP level ($\mu\text{g/mL}$)		
	0.4-1.5	1.5-2.2	2.2-4.8
II/IV			
Systolic/diastolic (mm Hg)	136.4/77.3	138.2/77.9	140.9/78.9
Adjusted mean (SE)	(1.6/0.8) ^a	(1.2/0.6) ^b	(1.5/0.7) ^c
VV			
Systolic/diastolic (mm Hg)	135.8/76.2	137.7/76.8	140.4/77.7
Adjusted mean (SE)	(1.9/1.1) ^d	(1.8/1.0) ^e	(2.2/1.2) ^f

CETP = cholesteryl ester transfer protein. I = isoleucine; V = valine.

^a n=106.

^b n=140.

^c n=142.

^d n=39.

^e n=39.

^f n=34.

TABLE 3. **Adjusted Prevalence of Hypertension in Sample Subgroups^{a,b}**

	No. in subgroup	Hypertension (%)	P value of trend
<i>CETP</i> genotype			
II	140	60	.04
IV	209	65	
VV	107	48	
<i>CETP</i> level (μg/mL)			
0.4-1.5	132	55	.07
1.5-2.2	165	59	
2.2-4.8	159	65	
HDL cholesterol (mg/dL)			
20-50	155	57	.45
51-66	140	60	
67-144	161	63	
HDL particle size (nm)			
8.1- 8.2	4	65	.59
8.3- 8.7	49	63	
8.8-11.3	403	60	
LDL particle size (nm)			
19.0-19.6	19	81	.01
19.7-21.1	144	68	
21.2-22.0	293	54	

^a Prevalence is adjusted for age, sex, body mass index, serum creatinine, and blood glucose. In addition, each of these analyses is adjusted for all the others among genotype, cholesteryl ester transfer protein (CETP) level, high-density lipoprotein (HDL) cholesterol level, HDL particle size, and low-density lipoprotein (LDL) particle size.

^b SI conversion factors: to convert HDL cholesterol values to mmol/L, multiply by 0.0259.

DISCUSSION

Our findings suggest that low CETP level, its genetic antecedent, or resulting lipoprotein levels and particle sizes are themselves associated with an increased risk of hypertension or higher blood pressure. Indeed, our analyses strongly suggest that the opposite is true. We have consistently found that the *CETP* VV genotype and lower CETP levels are associated with a *lower* prevalence of hypertension, *reduced* systolic blood pressure, and a trend toward lower diastolic blood pressure.

In animal models, torcetrapib-associated blood pressure increases have been found to be independent of the CETP-lowering effect and to be associated with increased levels of aldosterone.¹¹ These findings suggest that the adverse effects observed with torcetrapib may be due to actions outside the sphere of lipoprotein metabolism. Furthermore, a manufacturer-sponsored phase 1 randomized controlled trial of a different CETP inhibitor, anacetrapib, found no effect on blood pressure despite a large reduction in CETP levels.¹² In that double-blind, placebo-controlled crossover

study of healthy normotensive adults, effects on 24-hour average ambulatory systolic and diastolic blood pressures were tested on the last day of a 10-day course of treatment. The study was adequately powered to detect a 6-mm Hg difference in systolic or diastolic blood pressure. The observed mean differences were 0.60 and 0.47 mm Hg, respectively. These findings, like ours, suggest that low levels of CETP do not inherently predispose to hypertension, whether sustained for the brief duration of a clinical trial or for a lifetime.

Inhibition of CETP leads to increasing levels of HDL cholesterol and increased sizes of HDL and LDL particles. We observed no association between HDL cholesterol levels and blood pressure, or between HDL particle size and blood pressure. With regard to LDL particle size, we observed a substantial and statistically significant decline in adjusted prevalence of hypertension as LDL particle size increases. The mechanisms whereby low CETP levels would be associated with reductions in blood pressure are unknown, but we speculate on several possibilities. First, low CETP levels may exert the protective effects on blood pressure that we have observed because they prevent atherosclerosis by changing lipoprotein profiles and conserving arterial wall distensibility. Second, HDL cholesterol may have independent protective effects on endothelial function. Finally, because CETP is also expressed in endothelial cells, it may have a direct modulating effect on endothelial function.

The current study has several limitations. First, as a cross-sectional study, it does not estimate the effects of changing CETP levels on blood pressure: it merely demonstrates the associations between spontaneous CETP levels and concurrently measured blood pressure. Because persons with *CETP* genotype have been exposed to its effects from early development and throughout life, this study may not necessarily reflect what would happen when CETP inhibition begins later in life. Second, although our study enrolled only Ashkenazi Jews (because of its primary genetic goals), our findings about *CETP* genotype and levels have been replicated in Japanese and Italian centenarians.³

Finally, our operational definition of hypertension may induce some misclassification bias because of blood pressure measurement error and erroneous self-reports of blood pressure medications. Nevertheless, we used the standard methods of reducing blood pressure measurement error in our study. Analyses of hypertension as a dichotomous variable may fail to detect small shifts in the blood pressure distribution: directly analyzing the effect on measured blood pressure may have greater statistical power. However, in community-based studies, increases in blood pressure may be masked by the effect of medication. A strength of our approach is that we explored both dichotomous hyper-

tension and blood pressure measurements and found that the same conclusions are reached either way. Furthermore, our conclusions are robust to different specifications of the covariate–blood pressure associations.

CONCLUSION

Inhibition of CETP and its consequences for the lipoprotein phenotype are unlikely to directly increase blood pressure. On the contrary, CETP inhibition may lead to reduced blood pressure. Therefore, future trials of drugs in this pharmacologic class other than torcetrapib should proceed, with appropriate safeguards and monitoring. Other CETP inhibitors may achieve the benefits that were expected with torcetrapib without adversely affecting blood pressure.

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