

A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly

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Abstract High density lipoprotein cholesterol (HDL-C) levels are inversely associated with the incidence of coronary heart disease (CHD) in middle-aged individuals; in the elderly, the association is less clear. Genetic factors, including variations in the cholesteryl ester transfer protein (CETP) gene, play a role in determining HDL-C levels. Controversy remains about whether CETP deficiency and the resultant rise in HDL-C are antiatherogenic, or whether CETP has the opposite effect due to its role in reverse cholesterol transport. In a seven-year follow-up of 2,340 men aged 71–93 in the Honolulu Heart Program, the age-adjusted CHD incidence rates were significantly lower in men with high versus low HDL-C levels. After adjustment for age, hypertension, smoking, and total cholesterol, the relative risk of CHD for those with HDL-C levels ≥ 60 mg/dl, compared with those with HDL-C levels < 40 mg/dl, was 0.6. Men with a CETP mutation had the lowest rates of CHD, although this was not statistically significant. These data indicate that HDL-C remains an important risk factor for CHD in the elderly. Whether a CETP mutation offers additional protection against CHD warrants further investigation.—Curb, J. D., R. D. Abbott, B. L. Rodriguez, K. Masaki, R. Chen, D. S. Sharp, and A. R. Tall. A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly. *J. Lipid Res.* 2004. 45: 948–953.

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High density lipoprotein cholesterol (HDL-C) blood levels have been inversely associated with the incidence of coronary heart disease (CHD) in middle-aged and, to a

lesser extent, in elderly individuals (1–9). Reports from the Honolulu Heart Program (HHP) were among the first to describe the inverse relationship of “ α ” cholesterol (HDL-C) with CHD (10). This inverse HDL-C to CHD relationship in the Honolulu Japanese-American sample has also appeared in four other cohorts (Albany, Framingham, Evans County, and San Francisco) in the Cooperative Lipoprotein Phenotyping Study (1).

The relationships of HDL-C to cardiovascular events in older individuals have been far less consistent than the strong relationships seen in middle-aged individuals. There have been few studies in elderly minority populations. Most studies in older individuals have been case control studies. In addition, few have been able to reliably exclude prevalent atherosclerotic disease.

The mechanistic relationships of the association between HDL-C and CHD remain poorly understood. A number of antiatherogenic properties of HDL-C have been suggested as possible explanations for the association between HDL-C and CHD (11, 12). Genetic factors, including variations in the cholesteryl ester transfer protein (CETP) gene, are thought to play a major role in the determination HDL-C levels. CETP mediates the transfer of cholesteryl esters from HDL-C and low density lipoprotein cholesterol (LDL-C) into triglyceride (TG)-rich lipoproteins (11). Controversy remains about whether CETP deficiency and the resultant rise in HDL-C is antiatherogenic, or whether CETP has the opposite effect due to its role in reverse cho-

Abbreviations: BMI, body mass index; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; HHP, Honolulu Heart Program; LDL-C, low density lipoprotein cholesterol; MI, myocardial infarction.

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lesterol transport. This determination has become important because of the development of pharmaceutical compounds that can alter CETP levels in the body.

We have previously shown in elderly Japanese-American men of the HHP that the prevalence of CETP mutations is high and often associated with elevated concentrations of HDL-C (13). The continued follow-up of the now elderly HHP cohort, and the careful classification of cardiovascular events in this cohort, provide an opportunity to further examine the relationship of HDL-C and CETP mutations to incident CHD.

METHODS

The HHP is a long-term, prospective study of heart disease, stroke, and other diseases in a sample of 8,006 Japanese-American men, aged 45 to 68 years at the time of study enrollment in 1965–1968. Kuakini Medical Center’s Institutional Review Board approved the project. Follow-up in this report began at physical examinations given from 1991–1993 to 3,741 surviving HHP subjects. Men with a history of CHD at this examination, and those who reported being on lipid lowering medications, were excluded from these analyses. Prevalent cases of stroke and cancer were also excluded.

In this report, up to 7 years of follow-up data are available to examine the relationship between HDL-C and CHD. Based on a comprehensive system of follow-up, cases of CHD were identified by continuous surveillance of hospital discharge records, obituary notices, and death certificates. Using a standardized protocol and case definition criteria, a physician committee classified suspected endpoints. For this report, subjects were followed for the first occurrence of CHD. Here, CHD is defined to include unequivocal findings of a nonfatal myocardial infarction (MI), coronary death, and sudden death within an hour that could not be attributed to another cause. Risk factor measurements included body mass index (BMI) (kg/m²), physical activity index, alcohol consumption, hypertension, serum cholesterol, smoking habits, serum glucose levels, and TG levels. A diagnosis of hypertension was made when either a systolic or diastolic blood pressure was ≥ 160 and 95 mm Hg, respectively, or when a subject was receiving medication for high blood pressure. Assessment of physical activity was based on the use of the physical activity index, a common measure used to quantify overall metabolic output in a typical 24-h period and known to be inversely associated with the risk of cardiovascular disease (CVD) (14). The methods and procedures utilized in the HHP are documented in detail elsewhere (15, 16).

Laboratory analyses

Blood was centrifuged within 30 min of collection at 3,000 *g* for 10 min at 4°C, and the plasma was frozen at –70°C for up to 2 months. Samples were then shipped on dry ice to the University of Vermont, where HDL-C was separated by precipitation with dextran sulfate and magnesium chloride (17). DNA was obtained from white blood cells separated from plasma samples procured at the examination, and genotyping was carried out for intron 14 and exon 15 mutations in the CETP gene. For this report, a CETP mutation is defined as the presence of either an intron 14 or an exon 15 mutation.

Statistical methods

The percent of men who fell within a range of HDL-C and who had a CETP mutation, were calculated within age-specific

strata for ages that were observed at the time of study enrollment. Age-adjusted risk factors across the ranges of HDL-C levels and according to CETP status were estimated from analysis of covariance models (18). Similar procedures were used to assess changing levels of HDL-C and the percent of men with a CETP mutation as they might occur with age (a test for trend). Age-adjusted tests further examined trends in risk factor changes across levels of HDL-C.

Crude and age-adjusted incidence rates of CHD in person-years were estimated according to ranges of HDL-C concentrations and by CETP mutation status based on the 7 years of follow-up data that were available for the 2,340 men without prevalent disease, who were examined from 1991 to 1993 (18). To test for an independent effect of HDL-C level and CETP mutation on the risk of CHD after adjusting for age and the other covariates, proportional hazard regression models were used (19). While HDL-C was modeled as a continuous risk factor, relative risks of CHD (and associated confidence intervals) were also estimated comparing the risk of CHD between the ranges of HDL-C levels. When treated as a continuous variable, a test for trend was provided for whether there was a change in the risk of CHD with changes in levels of HDL-C. Similar models were used to compare the incidence of CHD between men with and without a CETP mutation. All reported *P* values were based on two-sided tests of significance. Clinically significant cut points based on the National Cholesterol Education Program guidelines were used to examine the relationship of HDL-C to age and age-adjusted risk factors (20).

RESULTS

Shown in **Table 1** are the percent of HHP men whose HDL-C levels fell in three clinically important categories (<40, 40–59, and ≥ 60 mg/dl), and who were characterized with either of the two CETP mutations, by 5-year age group. As can be seen, 23.6% of the men had HDL-C levels of ≥ 60 mg/dl. Prevalence of HDL-C values of ≥ 60 mg/dl increased significantly with age. There was no indication of a relationship between CETP mutations and age.

Shown in **Table 2** are the age-adjusted baseline characteristics of the study participants by HDL-C category. Significant positive associations between HDL-C levels and physical activity index, alcohol intake, and serum cholesterol were seen. In contrast, BMI, the prevalence of hypertension, serum glucose, and TGs declined with increasing

TABLE 1. Percent of men within ranges of HDL-C levels and the percent prevalence of CETP mutation according to age

Age Group	HDL-C Range			CETP Mutation	Sample Size
	<40	40–59	60+		
	<i>mg/dl</i>			<i>%</i>	
71–74	17.9	61.0	12.1	5.0	720
75–79	18.7	58.2	23.1	4.6	961
80–84	16.4	55.0	28.6	7.1	409
85+	18.0	58.0	24.0	3.6	250
Overall	18.0	58.5	23.6	5.0	2,340
Test for trend ^a	0.73	0.13	0.04	0.88	

CETP, cholesteryl ester transfer protein; HDL-C, high density lipoprotein cholesterol.

^a *P* value.

TABLE 2. Age-adjusted percents and mean risk factor levels by ranges of HDL-C levels

Risk Factors	HDL-C Range			Test for Trend ^a
	<40 (N = 421)	40–59 (N = 1,368)	60+ (N = 551)	
	<i>mg/dl</i>			
BMI	24.7	23.7	21.9	<0.001
Physical activity index	30.4	31.2	31.3	0.001
Alcohol (oz./month)	17.6	16.4	25.8	0.002
Hypertension (%)	77	75	67	<0.001
Serum cholesterol	179.9	191.6	196.6	<0.001
Current smoking (%)	8.2	6.7	8.5	0.889
Glucose	118.5	111.4	106.4	<0.001
TG	218.6	139.9	103.7	<0.001
CETP (%)	2.8	4.7	7.6	<0.001

BMI, body mass index; TG, triglyceride.

^a P value.

levels of HDL-C. Rising HDL-C was also associated with a significant increase in the prevalence of CETP mutations. Prevalence of CETP mutations was more than doubled in men with HDL-C levels ≥ 60 mg/dl versus men with levels < 40 mg/dl.

Table 3 shows age-adjusted characteristics for those with and without CETP mutations. There were 118 individuals with either mutation. Eleven were heterozygous for intron 14, 106 were heterozygotes for exon 15, and 1 was homozygote for exon 15. As expected from Table 2, HDL-C levels were significantly higher in those with a CETP mutation. In contrast, TG and glucose levels were lower in those with a mutation. Relationships with the other risk factors were not apparent.

Figure 1 shows the relative risks of CHD (adjusted for age, hypertension, smoking, and total cholesterol) for those with HDL-C levels between 40 mg/dl to 59 mg/dl and ≥ 60 mg/dl, as compared with those with HDL-C levels that were lower (< 40 mg/dl). As can be seen, the risk of CHD in men with HDL-C concentrations ≥ 60 mg/dl was nearly halved when compared with men with HDL-C levels < 40 mg/dl. Risk of CHD declined significantly with

TABLE 3. Age-adjusted percents of mean risk factor levels by the presence and absence of a CETP mutation

Risk Factors	CETP Mutation	
	Absent (N = 2,222)	Present (N = 118)
BMI	23.44	23.89
Physical activity index	31.08	31.46
Alcohol (oz./month)	18.86	18.57
Hypertension (%)	73.5	74.6
Serum cholesterol	190.65	191.28
Current smoking (%)	7.28	8.89
Glucose	111.81	105.76 ^a
HDL-C	51.02	56.27 ^b
TG	146.44	127.92 ^b

^a Significant difference between those with versus without a CETP mutation ($P = 0.02$).

^b Significant difference between those with versus without a CETP mutation ($P < 0.001$).

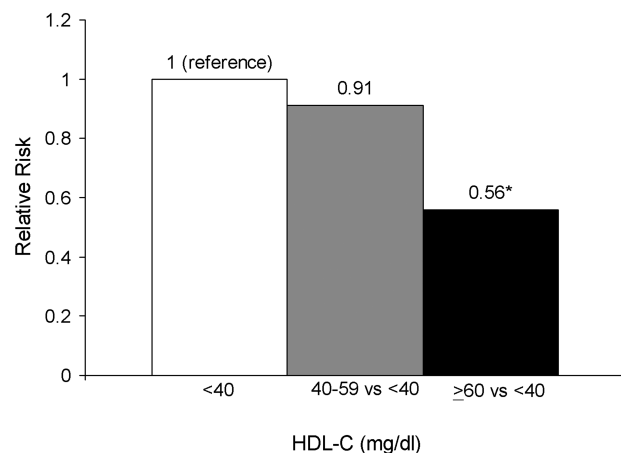


Fig. 1. Age factor- and risk factor-adjusted relative risks of coronary heart disease (CHD) for men with high density lipoprotein cholesterol (HDL-C) levels ≥ 60 mg/dl and between 40–59 mg/dl compared with levels below 40 mg/dl. Adjusted risk factors included hypertension, smoking, and cholesterol. *Significantly lower risk as compared to men with an HDL-C level < 40 mg/dl ($P < 0.05$).

increasing HDL concentration ($P < 0.05$). The age-adjusted CHD incidence rates declined from 16.1 per 1,000 person years for men with HDL-C levels < 40 mg/dl to 9.8 per 1,000 person years in men with HDL-C levels ≥ 60 mg/dl.

Shown in Fig. 2 is the age-adjusted CHD incidence rate per 1,000 person years by level of HDL-C and according to the presence and the absence of a CETP mutation. The two lower HDL-C subgroups were combined due to the small number of events in those with a CETP mutation. Among the four groups, the only significant difference in the rates of CHD occurred between the two HDL-C strata in men without a CETP mutation ($P < 0.05$). Although

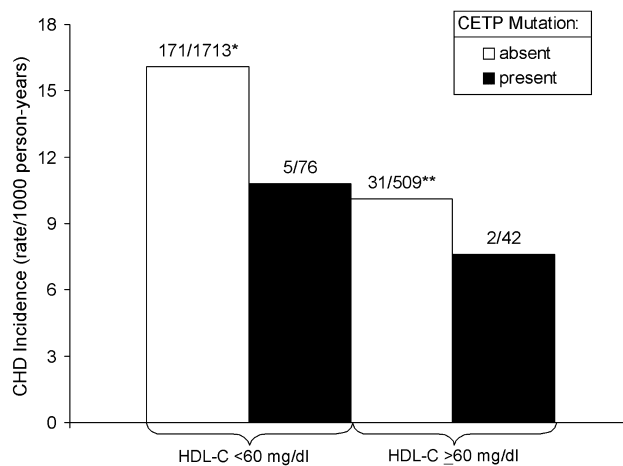


Fig. 2. Age-adjusted incidence of CHD according to ranges of HDL-C and in the absence and the presence of a cholesteryl ester transfer protein (CETP) mutation. *Number of CHD events/men at risk. **Significantly lower risk compared to men with HDL-C < 60 mg/dl and without a CETP mutation ($P < 0.05$).

the presence of a CETP mutation appeared protective, even when HDL-C strata were pooled, the effect of CETP on the risk of CHD failed to reach statistical significance. As noted earlier, however, the number of individuals with a mutation is small (118), and, among this group, there were only seven events. Thus, the power to detect an association between CETP and the future risk of CHD is limited. It may be that with a larger sample size, a significant relationship between CETP and CHD would have emerged, including a possible interaction effect between CETP and HDL-C.

DISCUSSION

An important finding of this study is that HDL-C is a significant risk factor for CHD in this elderly sample. The importance of HDL-C as a risk factor for CHD in those over 70 years-of-age has not been consistently demonstrated (8, 9, 21–23). In contrast, low levels of HDL-C have been independently and strongly associated with an increased risk of coronary artery disease in numerous studies involving middle-aged cohorts (1–7). For example, the Framingham Heart Study found that men aged 50 years to 79 years with HDL-C levels in the bottom quartile had a 60% to 70% excess of MI compared to men with higher HDL-C levels. Effects were even greater in women (2). Combined results from four large observational studies indicated that a 1 mg/dl increment in HDL-C was associated with approximately a 2% to 3% decrease in the risk of coronary artery disease in middle-aged individuals (3). The relationship between HDL-C and CHD is not confined to Caucasians. In middle-aged men in Japan, the incidence of CHD and MI, adjusted for other risk factors, was 3–4× higher in the lowest quartile of HDL-C than in the highest quartile (7).

In elderly samples, the relationship between HDL-C and CHD appears equivocal. In a population-based sample of 997 men and women over 70 years of age from Connecticut, HDL-C was not associated with CHD mortality or hospitalizations from CVD (22). In contrast, a follow-up of a larger population from three centers (3,904 women and men) found that low HDL-C predicted CHD mortality and the occurrence of new CHD events (8). Both studies also adhered to the same protocol and laboratory techniques with case ascertainment based on death certificates. Unlike the HHP, however, access to and utilization of clinically rigorous methods for detecting and classifying events were not available.

Another principal goal of the present study was to provide prospective information on the relationship between heterozygous CETP gene mutations and CHD. Although not statistically significant, the risk of CHD is lower in the presence versus the absence of a CETP mutation, especially for those individuals with HDL-C levels ≥ 60 mg/dl. This study's findings are similar to an earlier prevalence study reported by Moriyama et al. (24), in which subjects with CETP mutations and HDL-C levels >80 mg/dl were found to have very little CHD.


Our current findings did not confirm an earlier observation of a cross-sectional excess of CHD in subjects with a CETP gene mutation and HDL-C levels ranging from 40 mg/dl to 60 mg/dl (13). The differences in the longitudinal and cross-sectional findings may be partly explained by the exclusion of prevalent cases of CHD, stroke, and cancer in the current longitudinal analysis. Although CETP mutations should not change with age, it may be that the gene mutations have different effects in older individuals. The prospective study design also includes other advantages, which may have contributed to differences from the cross-sectional study. A shortcoming of both studies is the relatively low number of men with a CETP mutation and, therefore, limited statistical power.

Earlier studies in subjects with CETP mutations have indicated a possible susceptibility to CHD. A small number of patients with a CETP mutation, hepatic lipase deficiency, high HDL-C, and multiple risk factors were reported to have an excess of coronary artery disease (25). Moreover, a study in a Japanese community with a high prevalence of the intron 14 mutation found a high rate of ECG changes in subjects with very high HDL-C levels (26). These changes, however, were not specifically related to CETP mutations, and the data could be potentially confounded by other factors such as alcohol intake (27). Studies on CETP gene polymorphisms in European samples have shown associations with HDL-C levels and conflicting data concerning relationships with CHD (27, 28). These polymorphisms appear to have modest and indirect effects on plasma CETP levels.

Animal studies have also provided data on the relationship between CETP and atherogenesis. Studies on the inhibition of CETP in rabbits by several different strategies have indicated an antiatherogenic effect of CETP (29, 30). In particular, a CETP inhibitor drug was highly efficacious in reducing atherosclerosis (29). Other studies in CETP transgenic mice have shown that effects on atherogenesis depend on the metabolic context of CETP expression (31–33).

Therapeutic inhibition of CETP has been suggested as a treatment for elevating HDL-C levels in humans (33, 34), and recent studies indicate that CETP inhibitors can raise HDL-C and lower LDL-C (35). While it is clear that high-level inhibition is effective at raising HDL-C, one cannot predict with certainty the ultimate effects on atherosclerosis. Although this might represent a disruption of reverse cholesterol transport, it is likely that HDL-C and cholesteryl ester clearance in the liver can occur by other mechanisms, including via scavenger receptor BI. The large, apoE-rich HDL-C particles that accumulate in the absence of CETP may have other beneficial properties, such as antioxidant or antiinflammatory effects (36) and may compete with atherogenic lipoproteins for retention on the arterial matrix (37). Potential adverse properties of these particles have been suggested by others (38, 39).

In summary, these data indicate that HDL-C remains an important risk factor for CHD in the elderly. This important relationship is seen here in a prospective cohort study with rigid quality control and detailed and clinically rigor-

ous endpoint ascertainment and classification. Inasmuch as many of the classic risk factors have altered relationships with cardiovascular endpoints in the elderly, HDL-C may prove to be an increasingly useful clinical tool for identifying high-risk individuals in this age group (40). While definitive conclusions are not possible, the results are also suggestive of a lower rate of coronary events in those with a CETP mutation and a high HDL-C. Though the present study is consistent with the idea that therapeutic inhibition could be beneficial for CHD, more data are needed to determine whether CETP mutation significantly reduces the risk of CHD. 

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