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CLINICAL RESEARCH

Alcohol consumption, TaqIB polymorphism of cholesteryl ester transfer protein, high-density lipoprotein cholesterol, and risk of coronary heart disease in men and women

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Aims To investigate whether a common polymorphism in the cholesteryl ester transfer protein (CETP) gene modifies the relationship of alcohol intake with high-density lipoprotein cholesterol (HDL-C) and risk of coronary heart disease (CHD).

Methods and results Parallel nested case-control studies among women [Nurses' Health Study (NHS)] and men [Health Professionals Follow-up Study (HPFS)] where 246 women and 259 men who developed incident CHD were matched to controls (1:2) on age and smoking. The TaqIB variant and alcohol consumption were associated with higher HDL-C, with the most pronounced effects of alcohol among B2 carriers. In the NHS we did not find an inverse association between alcohol and CHD in B2 non-carriers (*P* trend: 0.5), but did among B2 carriers (*P* trend <0.01). Among non-carriers the odds ratio (OR) for CHD among women with an intake of 5–14 g/day was 1.4 (95% CI: 0.6–3.7) compared with non-drinkers, whereas among B2 carriers the OR was 0.4 (0.2–0.8). Results in men were less suggestive of an interaction; corresponding OR's were 1.9 (0.8–4.5) and 0.9 (0.5–1.6), for B2 non-carriers and carriers, respectively.

Conclusions The association of alcohol with HDL-C levels was modified by CETP TaqIB2 carrier status, and there was also a suggestion of a gene–environment interaction on the risk of CHD.

Keywords Alcohol • Gene–environment interaction • CHD • Cholesterol transport • Lipoproteins

Introduction

In prospective cohort studies, moderate alcohol consumption is associated with a lower risk of coronary heart disease (CHD) than abstention or very light drinking.[1,2] The primary mechanism proposed for this association is the higher levels of high-density lipoprotein cholesterol (HDL-C) found among moderate drinkers.[3] Randomized trials of alcohol administration demonstrate that intake of 30 g of alcohol daily (about 2 drinks) raises HDL-C levels by 0.1 mmol/L (4 mg/dL).[4] In prospective studies with data on alcohol, HDL-C, and incident CHD, about half of the lower risk of CHD among moderate drinkers can be attributed to HDL-C levels.[5–7]

Biological mechanisms underlying the positive association between alcohol and HDL-C are not yet fully understood. One pathway could be through regulation of cholesteryl ester transfer protein (CETP) activity, as CETP mediates transfer of cholesteryl esters from HDL to low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) particles. The concentration of CETP is inversely associated with HDL-C levels.[8,9] Observational studies have reported lower CETP activity among both alcohol abusers and young men with moderate alcohol

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intake.[10,11] Genetic variation in the gene encoding CETP is another important determinant of its activity. Although many single nucleotide polymorphisms (SNPs) in the gene have been identified, the most commonly studied occurs at the TaqIB restriction site in intron 1. The TaqIB2 variant allele is associated with lower CETP levels and activity and higher HDL-C levels,[11–14] most likely via its link to one or more functional promoter SNPs.[14–16]

Given that the association of alcohol with HDL-C and possibly cardiovascular risk may be partly mediated by CETP activity, genetic variation in *CETP* may modulate these relationships. Although such an interrelationship has not been extensively studied, two reports suggest that alcohol consumption could interact with the *CETP* TaqIB SNP to alter risk of myocardial infarction (MI).[11,14]

To investigate the importance of alcohol intake and the *CETP* TaqIB SNP in relation to levels of HDL-C and risk of CHD more definitively, we performed two independent nested case-control studies among men and women enrolled in the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS).

Methods

Study populations

The NHS cohort was established in 1976 at the Channing Laboratory of the Brigham and Women's Hospital. The study population consists of 121 700 married female registered nurses aged 30–55 years residing in one of 11 larger US states. Women have received follow-up questionnaires biennially to update information on exposures and newly diagnosed illnesses. Since 1980, participants have updated information on diet, alcohol, and vitamin supplements through a food frequency questionnaire approximately every 4 years.

The HPFS was established in 1986 at the Harvard School of Public Health, when 51 529 male health professionals 40–75 years of age from throughout the US completed the initial HPFS questionnaire. The population includes 29 683 dentists, 3745 optometrists, 2218 osteopathic physicians, 4185 pharmacists, 1600 podiatrists, and 10 098 veterinarians. Participants update information biennially, in a manner similar to the NHS.

Nested case-control studies

Between 1989 and 1990, a blood sample was requested from all active participants in NHS and collected from 32 826 women. Similarly, blood samples were obtained from 18 224 men in the HPFS between 1993 and 1995. With the exception of a modestly lower prevalence of smoking, those who returned blood samples did not differ substantially from those who did not in both cohorts, including average alcohol intake of 12.8 vs. 12.2 g/day in the HPFS and 6.5 vs. 6.3 g/day in the NHS. Participants underwent local phlebotomy and returned samples to our laboratory via overnight courier. Upon arrival, whole blood samples were centrifuged and stored in cryotubes as plasma, buffy coat, and red blood cells in the vapour phase of liquid nitrogen freezers.

The outcome for the nested case-control studies was incident CHD, defined as non-fatal MI and fatal CHD. We wrote to participants who reported incident CHD on the follow-up questionnaires to confirm the report and request permission to review medical records. We also sought medical records for deceased participants, whose deaths were identified by families and postal officials and through the National Death Index. Physicians blinded to the participant's questionnaire reports reviewed all medical records. Cases of MI and fatal CHD were identified primarily through review of medical records, as previously described.[17,18] Among participants who provided blood samples and who were without cardiovascular disease or cancer at blood draw, 212 women sustained an incident MI and 37 died from fatal CHD between blood draw and June 30, 1998. The corresponding numbers in HPFS were 196 non-fatal MI and 70 fatal CHD cases prior to January 31, 2000. As a secondary endpoint, we additionally identified 564 men who had coronary artery bypass graft surgery (CABG) or percutaneous transluminal coronary angioplasty (PTCA) during follow-up. Confirmation of CABG/PTCA was based on self-report only; hospital records obtained for a sample of 102 men confirmed the procedure for 96% of these.[18]

Using risk-set sampling,[19] controls were selected randomly and matched in a 2:1 ratio on age, smoking, and month of blood return, among participants who were free of cardiovascular disease at the time CHD was diagnosed in the case patient. In the NHS, we also matched on fasting status.

Information on genetic variants and plasma lipids

DNA was extracted from the buffy coat fraction of centrifuged blood with the QLAmp Blood Kit (Qiagen, Chatsworth, CA, USA). The primary genotyping technique was Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA, USA), using rs708272.[20] Genotype data were available for 732 women from the NHS (246 cases and 486 controls), 772 men (259 MI cases and 513 controls), and an additional 531 men with CABG/PTCA and their 1075 controls.

Plasma lipids assessed using standard methods with reagents from Roche Diagnostics (Indianapolis, IN, USA) and Genzyme (Cambridge, MA, USA) included triglycerides, total cholesterol, HDL-C, and directly obtained LDL cholesterol (LDL-C). Study samples were sent to the laboratory for analysis in batches where cases were paired with their two controls in random order. The intra-assay coefficient of variation was (CV%) $<2.5\%$ for the lipid parameters. Plasma lipids were measured among for nonfatal MI and fatal CHD cases and their controls (due to limited funding the set of CABG/PTCA cases and controls did not have plasma biomarkers measured).

Assessment of alcohol consumption

We assessed diet with a 131-item semi quantitative food frequency questionnaire that includes separate items for beer, white wine, red wine, and liquor, as described elsewhere.[17] We previously validated estimated alcohol consumption against 3-week dietary records collected approximately 6 months apart from 136 HPFS participants and 173 NHS participants residing in Eastern Massachusetts,[21] with Spearman correlation coefficients between these two measures of 0.90 in women and 0.86 in men.

In these analyses, we used average alcohol consumption assessed in 1990 among women and 1994 among men. Previous assessments were used for 37 women and 25 men with missing information for alcohol intake at the time of the blood draw.

We have previously reported on the association between alcohol consumption and risk of CHD in these nested case-control studies,[7] which was similar to the relationship found in the full NHS and HPFS cohorts.[17,22]

Statistical analysis

Multiple regression analysis was used to address the associations between *CETP* TaqIB genotype and plasma lipids among NHS and HPFS controls. All plasma lipids were log-transformed due to positive

skewness, and geometric means with 95% confidence intervals (CI) are presented. Participants who were homozygous B1 served as the natural reference, and comparisons of means with this group were corrected for mass significance using Dunnett adjustment.[23] Fully adjusted models included age (5-year intervals), smoking (never smoker, current smoker, past smoker), body mass index (BMI) (<20, 20–24.9, 25–29.9, 30–34.9, ≥35 kg/m^2), alcohol intake (0, 0.1–4.9, 5–14.9, 15+ g/day for women, and 15–29.9 and 30+ g/day for men), history of hypertension, diabetes, and parental history of CHD before the age of 60. Analyses among NHS controls were further adjusted for menopause status and postmenopausal hormones (PMH) at blood draw (premenopausal, postmenopausal no PMH, postmenopausal currently taking PMH, postmenopausal past PMH). As expected from the random distribution of alleles, these variables had little impact on the odds ratios (OR), but were kept in the model because of their recognition as risk factors for CHD and because they may account for some of the heterogeneity between study participants. We used covariate information from the time of blood draw, defined as 1994 in the HPFS and 1990 in the NHS. Information from previous questionnaires was used when covariate data from the time of blood draw were missing. In analyses of the combined study populations of NHS and HPFS we further adjusted for study origin and used 15+ g/day as the top category for alcohol consumption among both women and men.

Both conditional and unconditional analyses (adjusted for matching factors) provided essentially the same results for the association between *CETP* and CHD. We present the results from unconditional logistic regression models for all analyses because this parallels the stratified analyses. Due to small numbers among the light-drinkers, we used non-drinkers as the reference group in all analyses. Previous analyses in the entire HPFS cohort have shown that abstainers and very light drinking men have similar CHD risks.[18] We conducted tests of linear trend across increasing categories of alcohol consumption by treating the midpoints of consumption in categories as a continuous variable. Statistical interactions between the *CETP* TaqIB variant and categorical alcohol intake were tested comparing likelihood ratios in models with and without all interaction terms. All statistical tests were two-tailed and *P*-values below 0.05 were considered statistically significant. Analyses were performed using SAS 9 (SAS Institute Inc., Cary, NC, USA).

Results

Table 1 shows baseline characteristics of cases and controls in both studies. As expected, physical activity and HDL-C were lower, and BMI, diabetes, hypertension, LDL-C and triglycerides were higher, among cases than controls (*Table 1*). We found no departures from Hardy–Weinberg equilibrium for CETP in neither the NHS ($P = 0.96$) nor the HPFS ($P = 0.46$). Frequency of the TaqIB2 allele was 0.4. Genotype and allele frequencies did not differ between cases and controls of both studies.

Cholesteryl ester transfer protein TaqIB, alcohol, and plasma lipids

In analyses restricted to the controls, carriers of the B2 allele had higher HDL-C concentrations compared with non-carriers (*Table 2*). There was also a suggestion of higher triglyceride concentrations among B2 homozygotes, however, this was not statistically significant. Alcohol consumption was associated with HDL-C

Table 1 Characteristics of covariates among cases of myocardial infarction and controls in the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS)[a]

Variable	NHS		HPFS	
	Cases ($n = 246$)	Controls ($n = 486$)	Cases ($n = 259$)	Controls ($n = 513$)
Alcohol (g/day)	0.9 (0; 24)	1.8 (0; 29)	5.8 (0; 46)	6.8 (0; 77)
Drinking frequency (days/week)	1 (0; 7)	1 (0; 7)	2 (0; 7)	2 (0; 7)
CETP B1B2 genotype	120 (49%)	235 (48%)	126 (49%)	244 (48%)
CETP B2B2 genotype	42 (17%)	85 (17%)	44 (17%)	89 (17%)
Age (years)	62 (47; 69)	62 (48; 68)	66 (50; 78)	66 (51; 78)
Physical activity (METS/week)	11 (0; 47)	12 (1; 53)	24 (1; 125)	27 (1–124)
Body mass index (BMI) (kg/m^2)	24.8 (18.5; 34.2)	23.4 (18.8; 32.4)	25.7 (20.9; 31.9)	25.1 (19.8; 31.8)
Diabetes	49 (20%)	32 (7%)	25 (9%)	24 (5%)
Hypertension	142 (58%)	144 (30%)	111 (42%)	158 (31%)
Hypercholesterolemia	132 (54%)	194 (40%)	128 (49%)	209 (41%)
Postmenopausal hormone (PMH) use	76 (31%)	178 (37%)	N/A	N/A
Plasma lipids				
HDL-C (mmol/L)	1.3 (1.1; 1.6)	1.5 (1.2; 1.8)	1.1 (0.7; 1.7)	1.1 (0.7; 1.8)
LDL-C (mmol/L)	3.7 (3.1; 4.3)	3.4 (2.7; 4.0)	3.5 (2.0; 4.9)	3.2 (2.8; 3.8)
Cholesterol (mmol/L)	6.1 (5.5; 6.7)	5.8 (5.2; 6.6)	5.6 (4.0; 7.1)	5.2 (3.9; 7.0)
Triglycerides (mmol/L)[b]	1.5 (1.1; 2.3)	1.2 (0.8; 1.7)	1.7 (0.6; 5.3)	1.2 (0.5; 3.5)

[a]Medians (5th and 95th percentiles) of continuous covariates. Counts and percentages of categorical covariates.
[b]Fasting participants only (NHS, $n = 302$; HPFS, $n = 298$).

Table 2 Mean [95% confidence intervals (CI)] plasma lipid concentrations according to cholesteryl ester transfer protein (*CETP*) genotype among controls in the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS)[a]

	NHS				HPFS			
	B1B1	B1B2	B2B2	P^b	B1B1	B1B2	B2B2	P^b
n	166	235	85		180	244	89	
HDL-C (mmol/L)	1.41 (1.36–1.47)	1.54 (1.49–1.59)	1.57 (1.49–1.66)	<0.01	1.10 (1.07–1.14)	1.14 (1.11–1.18)	1.26 (1.20–1.32)	<0.01
LDL-C (mmol/L)	3.32 (3.18–3.47)	3.24 (3.13–3.36)	3.37 (3.17–3.58)	0.95	3.18 (3.05–3.30)	3.25 (3.14–3.36)	2.97 (2.82–3.16)	0.10
Cholesterol (mmol/L)	5.70 (5.58–5.87)	5.75 (5.61–5.86)	5.98 (5.76–6.20)	0.13	5.15 (5.02–5.29)	5.30 (5.18–5.42)	5.10 (4.93–5.32)	0.91
Triglycerides (mmol/L)[c]	1.20 (1.09–1.32)	1.16 (1.07–1.26)	1.42 (1.22–1.63)	0.13	1.30 (1.16–1.46)	1.32 (1.21–1.45)	1.39 (1.20–1.62)	0.72

[a]Geometric means obtained from regression analyses adjusted for age, smoking, time of blood draw, BMI, alcohol intake, family history of MI before age 60, diabetes and hypertension at baseline. Not all lipid parameters available on all participants.
[b]*P* for test of differences between means of B2 homozygotes and B1 homozygotes (Dunnett adjustment for mass significance applied).
[c]Fasting participants only (NHS, $n = 302$; HPFS, $n = 298$).

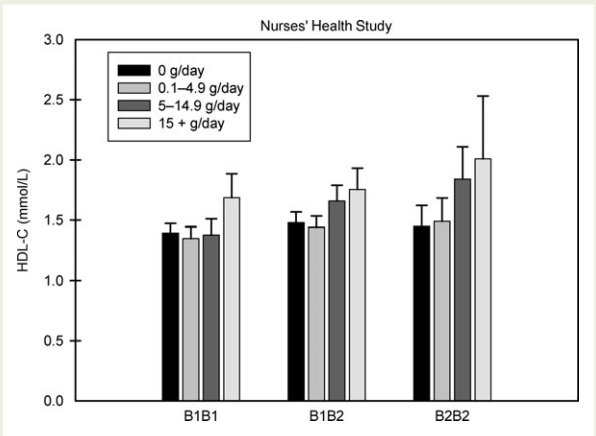

Figure 1 Geometric means of high-density lipoprotein cholesterol (HDL-C) concentrations (error bar represents upper limit of 95% confidence interval) among female controls obtained from regression analyses adjusted for age, smoking, time of blood draw, body mass index (BMI), family history of myocardial infarction (MI) before age 60, diabetes, hypertension at baseline, post-menopausal status and hormone use. All *P* for trend <0.01. *P* for interaction: Wald test for the inclusion of a separate interaction term between cholesteryl ester transfer protein (CETP) (modelled dominantly) and medians of alcohol categories treated linearly, $P < 0.01$

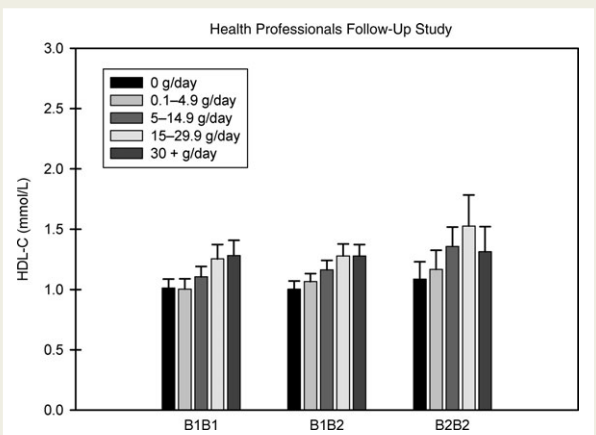

Figure 2 Geometric means of high-density lipoprotein cholesterol (HDL-C) concentrations (error bar represents upper limit of 95% confidence interval) among male controls obtained from regression analyses adjusted for age, smoking, time of blood draw, body mass index (BMI), family history of myocardial infarction (MI) before age 60, diabetes and hypertension at baseline. All *P* for trend: <0.01. *P* for interaction: Wald test for the inclusion of a separate interaction term between cholesteryl ester transfer protein (CETP) (modelled dominantly) and medians of alcohol categories treated linearly, $P < 0.01$

levels in a dose-dependent manner, with the most pronounced effects among both men and women who were homozygous for the B2 allele (*Figures 1* and *2*). B2 homozygous women with an alcohol intake of 15 g/day or more had 0.6 mmol/L (43%) higher HDL-C levels than

Table 3 Odds ratios (OR) and 95% confidence intervals (CI) of coronary heart disease (CHD) [and coronary artery bypass graft surgery (CABG)/CHD] according to cholesteryl ester transfer protein (*CETP*) genotype in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)

	NHS			HPFS		
	B1B1	B1B2	B2B2	B1B1	B1B2	B2B2
CHD cases/controls	84/166	120/235	42/85	89/180	126/244	44/89
OR (95% CI)[a]	1	1.0 (0.7–1.5)	1.0 (0.7–1.6)	1	1.0 (0.7–1.5)	1.0 (0.6–1.6)
OR (95% CI)[b]	1	1.2 (0.8–1.7)	1.2 (0.8–2.1)	1	1.1 (0.8–1.6)	1.1 (0.7–1.7)
CABG and CHD cases/controls	–	–	–	275/530	373/771	142/287
OR (95% CI)[a]				1	0.9 (0.8–1.1)	1.0 (0.7–1.2)
OR (95% CI)[b]				1	0.9 (0.8–1.1)	1.0 (0.7–1.2)

[a]Adjusted for age, smoking, and time of blood draw.
[b]Additional adjustment for BMI, alcohol intake, parental history of CHD before age 60, diabetes, and high blood pressure at baseline. Analyses among women also included postmenopausal status and hormone use.

Table 4 Odds ratios (OR) and 95% confidence intervals (CI) of coronary heart disease (CHD) according to alcohol intake within strata of cholesteryl ester transfer protein (*CETP*) genotype in the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS)[a]

		NHS				HPFS			
CETP genotype	Alcohol consumption	Cases (n = 247)	Controls (n = 486)	OR (95% CI) Crude	OR (95% CI) Adjusted	Cases (n = 259)	Controls (n = 513)	OR (95% CI) Crude	OR (95% CI) Adjusted
B1B1	0 g/day	39	65	1	1	17	47	1	1
	0.1–4.9 g/day	22	51	0.8 (0.4–1.6)	1.1 (0.5–2.3)	24	37	1.9 (0.9–4.1)	1.7 (0.7–4.1)
	5–14.9 g/day	14	29	1.0 (0.4–2.2)	1.4 (0.6–3.7)	28	41	1.7 (0.8–3.8)	1.9 (0.8–4.5)
	15–29.9 g/day	8	21	0.9 (0.3–2.2)	1.3 (0.5–3.8)	13	27	1.3 (0.5–3.3)	1.6 (0.6–4.4)
	30+ g/day	–	–	–	–	7	28	0.6 (0.2–1.8)	0.6 (0.2–2.0)
	P trend[b]			0.9	0.5			0.1	0.2
X/B2	0 g/day	79	118	1	1	46	72	1	1
	0.1–4.9 g/day	55	97	0.8 (0.5–1.2)	0.8 (0.5–1.4)	39	78	0.9 (0.5–1.5)	0.9 (0.5–1.6)
	5–14.9 g/day	17	67	0.3 (0.2–0.6)	0.4 (0.2–0.8)	38	75	0.8 (0.5–1.5)	0.9 (0.5–1.6)
	15–29.9 g/day	12	38	0.4 (0.2–0.8)	0.4 (0.2–0.9)	23	53	0.7 (0.4–1.4)	0.8 (0.4–1.5)
	30+ g/day	–	–	–	–	24	55	0.8 (0.4–1.4)	0.8 (0.4–1.6)
	P trend[b]			<0.01	<0.01			0.4	0.5
	P interaction[c]			0.4	0.4			0.2	0.2

[a]Crude model adjusted for age, smoking and time of blood draw. Adjusted models included age, smoking, time of blood draw, BMI, family history of MI before age 60, diabetes and hypertension at baseline. Analyses among women also included postmenopausal status and hormone use.
[b]P trend: median of alcohol categories modelled continuously.
[c]P interaction: Likelihood ratio test of nested models with and without all interaction terms between CETP (dominant effects) and the alcohol categories.

non-drinking, B1 homozygous women. Tests of interaction between *CETP* (modelled with dominant effects of the B2 allele) and alcohol intake on HDL-C were statistically significant ($P < 0.01$).

Cholesteryl ester transfer protein TaqIB, alcohol, and risk of coronary heart disease

The TaqIB polymorphism was not associated with risk of CHD in the NHS and neither with CHD nor the combined endpoint of CHD and CABG in the HPFS (*Table 3*).

An inverse association between average alcohol consumption and risk of CHD was observed among both women and men, as has previously been reported.[7] For power reasons, we modelled the *CETP* genotype according to dominant effects of the B2 allele in the interaction analyses. In the NHS, a strong inverse association between alcohol and CHD was only observed in B2 carriers (P trend <0.01), whereas a light to moderate alcohol intake was not associated with a lower risk of CHD among the homozygous B1 women (P trend = 0.5) (*Table 4*). Among the HPFS men,

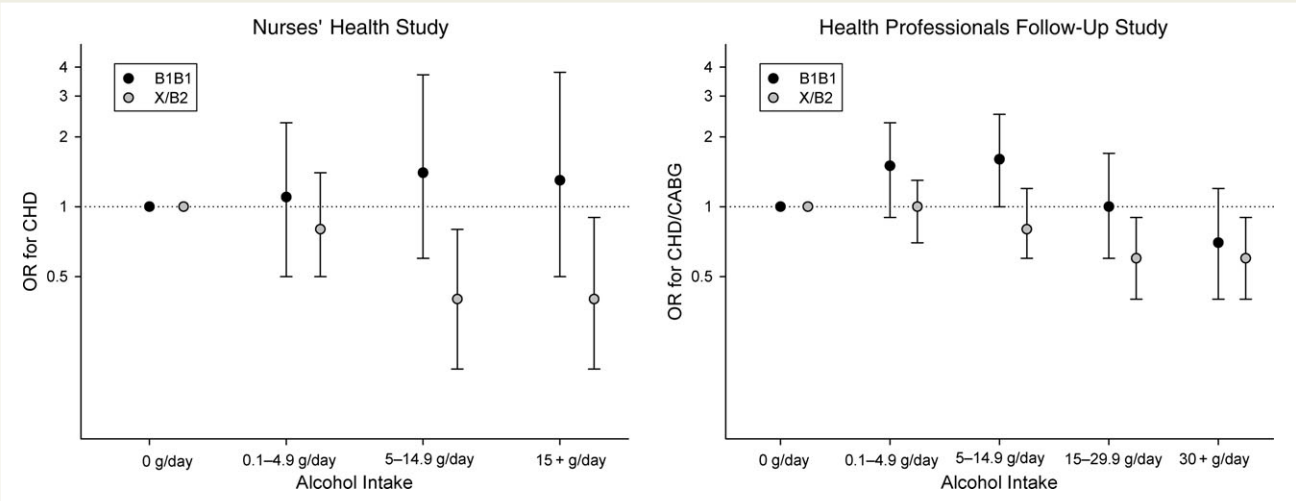

Figure 3 Odds ratio (OR) estimates from analysis in the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS), adjusted for age, smoking, time of blood draw, body mass index (BMI), family history of myocardial infarction (MI) before age 60, diabetes and hypertension at baseline. Analyses among women also included postmenopausal status and hormone use. Black dots: B1B1. Grey dots: X/B2. *P* for trend: NHS – B1B1 individuals: 0.5; X/B2 individuals: <0.01, HPFS – B1B1 individuals: 0.03; X/B2 individuals: <0.01. *P* interaction: likelihood ratio test of nested models with and without all interaction terms between cholesteryl ester transfer protein (CETP) (dominant effects) and the alcohol categories. NHS: $P = 0.4$. HPFS: $P = 0.7$

there was a modest difference in the association between alcohol and CHD by genotype, but the CIs were very broad. Moderate alcohol consumption appeared to be associated with a higher risk of CHD among B2 non-carriers while the association between alcohol and CHD was null in B2 carriers. In additional analyses in the HPFS where the CABG/PTCA endpoints were included (531 additional cases and 1075 controls) differences in the association according to B2 carrier status remained modest (*Figure 3*). There was a strong inverse association between alcohol and risk of CHD/CABG among the B2 carriers (*P* trend <0.01), whereas alcohol intake <15 g/day was associated with a higher risk compared with non-drinking in the B1 homozygotes. However, alcohol intake above 30 g/day was associated with the lowest risk of CHD regardless of genotype in the HPFS. Tests of the interaction terms were, however, not significant (NHS, $P = 0.4$; HPFS, CHD only, $P = 0.2$; including CABG, $P = 0.7$).

Given the similar trends in the two study populations, we combined them to achieve greater statistical power. In analyses stratified by genotype, compared with non-drinkers, the OR for CHD among individuals who drank 5–14.9 g/day was 1.6 (95% CI: 1.1–2.3) for B1B1 and 0.7 (95% CI: 0.6–1.0) for B2 carriers (*P* interaction = 0.02) (data not shown).

Discussion

In this prospective study, the association of alcohol with HDL-C levels was modified according to *CETP* TaqIB genotype. Even though the genotype did not have a main effect on CHD risk our results suggest that the inverse association between alcohol and CHD risk is also modified by the CETP TaqIB genotype.

Alcohol, cholesteryl ester transfer protein, and high-density lipoprotein cholesterol

The association between alcohol intake and higher HDL-C levels is well established.[3] However, the metabolic mechanisms for the increase in HDL-C following alcohol consumption are poorly understood. CETP is a key protein in HDL-C metabolism. Congenital CETP deficiency and pharmacological inhibition of CETP both lead to markedly elevated HDL-C levels.[24,25] Limited evidence suggest that alcohol intake is associated with lower CETP activity[11] and lower concentrations among drinkers.[10] Recently it has been suggested that alcohol or a metabolite may inhibit the glycosylation of CETP, which could affect the binding of CETP to lipoproteins in alcohol drinkers.[26] Other metabolic pathways that may also play a role in the association between alcohol and HDL-C concentration include increased transport rate of apolipoproteins, reduced hepatic lipase activity, and greater lecithin cholesterol acyl transferase activity[27,28] and a combination of all may be the most likely scenario.[29–31]

Consistent with our findings, other observational studies have found that genetic variation in *CETP* influences the association between alcohol consumption and HDL-C, such that the highest HDL-C concentrations are found among B2 carriers who drink alcohol.[32,33] Boekholdt *et al.*[11] also found an interaction of borderline statistical significance between alcohol use of any amount and the TaqIB SNP on HDL-C levels, whereas no interaction on HDL-C levels was found in the Northwick Part Heart Study,[34] or in healthy populations from Spain[35] and rural Japan.[36] As higher levels of HDL-C are related to several lifestyle features, including exercise,[37] smoking cessation,[38] PMH,[39] and lower carbohydrate intake,[40] it is possible that other lifestyle characteristics may partly account for these inconsistencies.

Alcohol, cholesteryl ester transfer protein, and risk of coronary heart disease

Although the relationship of CHD risk and the *CETP* TaqIB polymorphism is still debated,[13,41] one meta-analysis found the B2 allele associated with a 22% lower risk of CHD.[11] We did not confirm a statistically significant lower risk associated with the B2 allele among participants in this study, but the CI around these estimates were broad and we cannot exclude a lower risk of the magnitude previously identified. Our results suggest we might have seen a stronger effect of the B2 allele if the prevalence of moderate drinking had been higher. This is consistent with the results of the meta-analysis, which was performed in predominantly European populations where the underlying prevalence of alcohol consumption may be somewhat higher. Another hypothesis that has been put forward is effect modification of the association between CETP activity and CHD risk by plasma triglyceride concentration,[9] suggesting that the B2 allele may be associated with lower CHD risk particularly in hypertriglycedemic populations.[13]

In the present study, a light-to-moderate alcohol intake was associated with a lower risk of CHD among B2 carriers, whereas this was not observed among B1B1 individuals who were not genetically predisposed for higher HDL-C levels. Although our results were not entirely consistent among men and women, this could be related to both lack of statistical power or differences in underlying alcohol consumption pattern between genders. Relatively few studies have addressed the possibility of an interaction between alcohol use and *CETP* genotype on CHD risk (rather than just lipids). In the ECTIM study, Fumeron *et al.*[32] found lower risk of MI associated with the B2 variant allele among heavy drinkers, and in a meta-analysis of more than 13 000 individuals, Boekholdt *et al.*[11] reported a borderline statistically significant interaction ($P = 0.07$) between the SNP and alcohol on cardiovascular events.

Some limitations pertinent to the present study should be noted. We conducted these investigations in two groups of predominately white health professionals in the US, hence we cannot necessarily generalize our results to other populations with different distributions of race and ethnicity. However, the relationships of alcohol intake and the TaqIB SNP with HDL-C levels tend to be consistent in a wide variety of populations.[42,43] Although we documented over 500 incident cases of CHD among over 50 000 individuals in this study, the size of the nested case-control studies limited our power to detect interaction effects.

We measured only a single *CETP* SNP although numerous other, potentially functional, SNPs have been identified in the CETP gene.[14] Other SNPs could interact with alcohol in a manner similar to that of the TaqIB variant, in a different direction, or not at all. Furthermore, our study samples of 250 cases in both men and women were not adequately powered for such exploratory analyses. Thus, in the present study we aimed particularly to investigate the previously hypothesized interrelationship of the widely investigated TaqIB variant and alcohol. Of note, the TaqIB SNP is not itself likely to be functional and may be associated with CETP activity via its link to at least two other SNPs.[14–16] Indeed high linkage disequilibrium between SNPs located in the promoter region of the CETP gene has been demonstrated, suggesting that they all capture the same underlying functional variance.[14] As technological advances make sequencing and genotyping increasingly cost effective, further analyses and in-depth exploration of other CETP variants and alcohol should be performed with appropriate statistical methodology and in larger population samples.

We did not have measures of CETP concentration or activity thus we were not able to directly assess the effect of the TaqIB gene polymorphism on CETP activity. However, we did observe an association between the TaqIB variant and HDL metabolism even using HDL-C as a less sensitive parameter.

The self-reported measures of alcohol consumption used in this study have previously been validated in these populations.[21,44] As in most prospective cohort studies, the NHS and HPFS contain few drinkers who consume alcohol heavily on a regular or even episodic basis. Therefore, we were unable to separately assess heavy drinking or binge drinking, although other studies suggest it may be associated with an increased risk of CHD,[45] and Fumeron *et al.*[32] found that only alcohol consumed in very large quantities modulated the association between the TaqIB SNP and CHD among men.

In summary, we found that the TaqIB SNP in the *CETP* gene modified the association of alcohol intake with HDL-C. Furthermore, our analyses in two independent prospective studies lend support for the suggested interaction between CETP and alcohol on risk of CHD. Further studies with greater information on genetic variation in CETP and wider variation in alcohol consumption among men and women are still warranted to investigate this question further.

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