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Association of Genetic Variants Related to CETP Inhibitors and Statins With Lipoprotein Levels and Cardiovascular Risk

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IMPORTANCE Some cholesteryl ester transfer protein (CETP) inhibitors lower low-density lipoprotein cholesterol (LDL-C) levels without reducing cardiovascular events, suggesting that the clinical benefit of lowering LDL-C may depend on how LDL-C is lowered.

OBJECTIVE To estimate the association between changes in levels of LDL-C (and other lipoproteins) and the risk of cardiovascular events related to variants in the *CETP* gene, both alone and in combination with variants in the 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) gene.

DESIGN, SETTING, AND PARTICIPANTS Mendelian randomization analyses evaluating the association between *CETP* and *HMGCR* scores, changes in lipid and lipoprotein levels, and the risk of cardiovascular events involving 102 837 participants from 14 cohort or case-control studies conducted in North America or the United Kingdom between 1948 and 2012. The associations with cardiovascular events were externally validated in 189 539 participants from 48 studies conducted between 2011 and 2015.

EXPOSURES Differences in mean high-density lipoprotein cholesterol (HDL-C), LDL-C, and apolipoprotein B (apoB) levels in participants with *CETP* scores at or above vs below the median.

MAIN OUTCOMES AND MEASURES Odds ratio (OR) for major cardiovascular events.

RESULTS The primary analysis included 102 837 participants (mean age, 59.9 years; 58% women) who experienced 13 821 major cardiovascular events. The validation analyses included 189 539 participants (mean age, 58.5 years; 39% women) with 62 240 cases of coronary heart disease (CHD). Considered alone, the *CETP* score was associated with higher levels of HDL-C, lower LDL-C, concordantly lower apoB, and a corresponding lower risk of major vascular events (OR, 0.946 [95% CI, 0.921-0.972]) that was similar in magnitude to the association between the *HMGCR* score and risk of major cardiovascular events per unit change in levels of LDL-C (and apoB). When combined with the *HMGCR* score, the *CETP* score was associated with the same reduction in LDL-C levels but an attenuated reduction in apoB levels and a corresponding attenuated nonsignificant risk of major cardiovascular events (OR, 0.985 [95% CI, 0.955-1.015]). In external validation analyses, a genetic score consisting of variants with naturally occurring discordance between levels of LDL-C and apoB was associated with a similar risk of CHD per unit change in apoB level (OR, 0.782 [95% CI, 0.720-0.845] vs 0.793 [95% CI, 0.774-0.812]; $P = .79$ for difference), but a significantly attenuated risk of CHD per unit change in LDL-C level (OR, 0.916 [95% CI, 0.890-0.943] vs 0.831 [95% CI, 0.816-0.847]; $P < .001$) compared with a genetic score associated with concordant changes in levels of LDL-C and apoB.

CONCLUSIONS AND RELEVANCE Combined exposure to variants in the genes that encode the targets of CETP inhibitors and statins was associated with discordant reductions in LDL-C and apoB levels and a corresponding risk of cardiovascular events that was proportional to the attenuated reduction in apoB but significantly less than expected per unit change in LDL-C. The clinical benefit of lowering LDL-C levels may therefore depend on the corresponding reduction in apoB-containing lipoprotein particles.

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Mendelian randomization studies and randomized trials of various lipid-lowering therapies have consistently demonstrated that lower levels of low-density lipoprotein cholesterol (LDL-C) may be causally associated with a lower risk of cardiovascular disease.[1-4] Together, these studies suggest that lowering LDL-C levels should reduce the risk of cardiovascular events proportional to the absolute reduction in LDL-C, largely independent of the mechanism by which LDL-C is lowered.[5]

The notable exception to this observation is the class of drugs known as cholesteryl ester transfer protein (CETP) inhibitors.[4] Although CETP inhibitors were originally designed to increase levels of high-density lipoprotein cholesterol (HDL-C),[6] the more potent CETP inhibitors also robustly lower levels of LDL-C.[7-11] However, in the ACCELERATE trial, treatment with the CETP inhibitor evacetrapib reduced LDL-C levels by 29 mg/dL (0.75 mmol/L) but did not reduce the risk of cardiovascular events.[11] This result has created uncertainty about the causal effect of LDL-C on the risk of cardiovascular disease and raises the possibility that the clinical benefit of lowering LDL-C may depend on how LDL-C is lowered.

In this study, a mendelian randomization analysis was conducted to evaluate the association between lower levels of LDL-C (and other measures of lipoprotein concentration) and the risk of cardiovascular events due to variants in the gene that encodes the target of CETP inhibitors and compare it to the association between lower LDL-C levels and the risk of cardiovascular events due to variants in the genes that encode the targets of statins, ezetimibe, and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors to make inferences about whether the clinical benefit of lowering LDL-C might depend on how LDL-C is lowered. Because the cardiovascular outcome trials have evaluated the effect of treatment with a CETP inhibitor on the background of statin therapy, the associations of the *CETP* variants were evaluated both alone and in combination with variants of the 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) gene, which encodes the target of statins.

Key Points

Question Does the clinical benefit of lowering low-density lipoprotein cholesterol (LDL-C) levels depend on how LDL-C is lowered?

Findings In a mendelian randomization analysis of an individual-participant data meta-analysis that included 102 837 participants, combined exposure to variants related to the action of CETP inhibitors and statins was significantly associated with discordant reductions in LDL-C and apolipoprotein B levels; the corresponding association with cardiovascular events was proportional to the attenuated reduction in apolipoprotein B but less than expected per unit change in LDL-C.

Meaning The clinical benefit of lowering LDL-C may be related to the corresponding absolute reduction in apolipoprotein B-containing lipoprotein particles and therefore may depend on how LDL-C is lowered.

Methods

Study Design

The study consisted of 3 sequential parts as summarized in **Figure 1**. First, a mendelian randomization study was conducted to measure the association between lipid changes due to a genetic score consisting of variants in the *CETP* gene and the risk of cardiovascular events. The magnitude of the association between the *CETP* genetic score and the risk of cardiovascular events was then compared with magnitude of the association between the risk of cardiovascular events and genetic scores consisting of variants in the *HMGCR* gene (NCBI Entrez Gene 3156, which encodes for the target of statins), the Niemann-Pick C1-Like 1 intracellular cholesterol transporter 1(*NPC1L1*) gene (NCBI Entrez Gene 29881, which encodes for the target of ezetimibe), and the *PCSK9* gene (NCBI Entrez Gene 255738, which encodes for the target of PCSK9 inhibitors), respectively. The objective of this analysis was to make inferences about whether lower LDL-C levels due to CETP inhibition has the same causal effect on the risk of cardiovascular events as other methods of lowering LDL-C levels.[12]

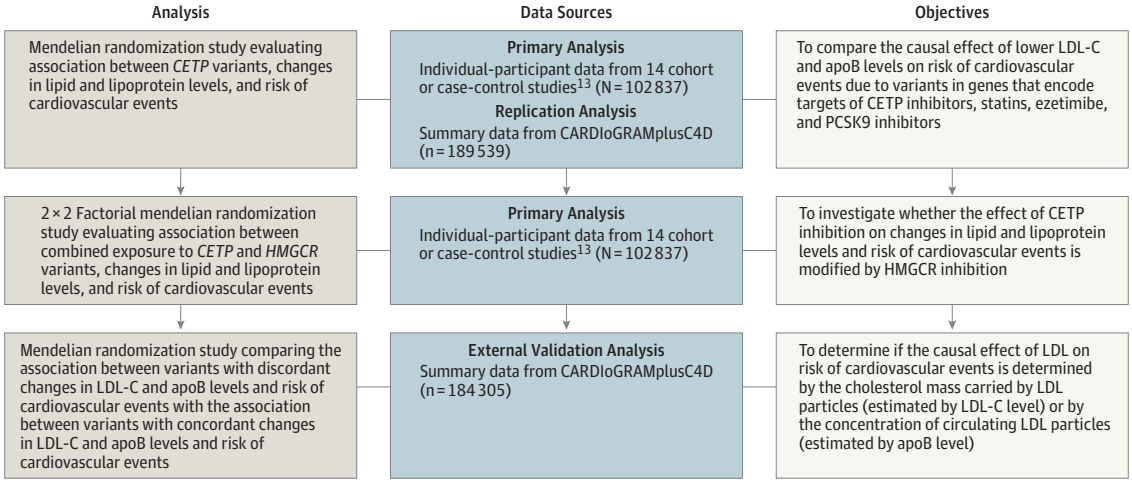
Second, a 2 × 2 factorial mendelian randomization study was conducted to measure the association between lipid changes due to combined exposure to the *CETP* and *HMGCR* genetic scores and the risk of cardiovascular events. The magnitude of these associations were then compared with the magnitude of the associations with the *CETP* score alone. The objective of this analysis was to make inferences about whether the effect of CETP inhibition on lipid changes and the risk of cardiovascular events is modified by inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase.

Third, a mendelian randomization study was conducted to measure the association between the risk of coronary heart disease and a score consisting of genetic variants associated with discordant changes in levels of LDL-C and apolipoprotein B (apoB). The magnitude of the association between this discordant variant genetic score and the risk of cardiovascular events was then compared with the magnitude of the association between a genetic score consisting of variants associated with concordant changes in levels of LDL-C and apoB and the risk of cardiovascular events, measured both per unit change in LDL-C and per unit change in apoB, respectively. The objective of this analysis was to make inferences about whether the causal effect of LDL on the risk of cardiovascular events is determined by the cholesterol mass carried by LDL particles (as measured by LDL-C level) or by the concentration of circulating LDL particles (as estimated by apoB level) and therefore to make further inferences about whether the clinical benefit of lowering LDL-C level may depend on how it is lowered.

Study Population

The primary analyses included individual-participant data from all studies in the National Center for Biotechnology Information database of Genotypes and Phenotypes program that reported data on cardiovascular outcomes.[13] These 14 cohort or case-control studies included a total of 112 772 participants. Of these, 102 837 participants had adequate genetic information for all variants included in the various genetic scores investigated in this study and were included in the analysis without

Figure 1. Study Design



CARDIoGRAMplusC4D indicates Coronary Artery Disease Genome Wide Replication and Meta-analysis plus the Coronary Artery Disease Genetics Consortium; CETP, cholesteryl ester transfer protein; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9.

restrictions or exclusions. All racial/ethnic groups for which data were reported were included in the analysis. In each cohort or case-control study, race/ethnicity was self-identified using a study-specific fixed-category questionnaire. A description of the included studies and the genotyping platforms used in each study is provided in eTable 1 in the Supplement.

External replication and validation analyses included summary-level data from a total of up to 189 539 participants from 48 studies as part of the Coronary Artery Disease Genome-Wide Replication and Meta-analysis plus the Coronary Artery Disease Genetics Consortium (CARDIOGRAMplusC4D).[14,15] Contributing studies received ethical approval from their respective institutional review boards, and written informed consent was obtained from all participants. Analysis of the individual-participant data was approved by the Wayne State University institutional review board.

Study Outcomes

The primary outcome for the individual participant data analyses was major cardiovascular events, defined as a composite of the first occurrence of myocardial infarction, coronary revascularization, stroke, or coronary death. The primary outcome for analyses using summary-level data was coronary heart disease (CHD), as defined by the CARDIoGRAMplusC4D Consortium.[14,15]

Genetic Instruments

The *CETP* genetic score was constructed by combining all variants within 100kb on either side of the *CETP* gene that were conditionally associated with HDL-C levels (the major lipid effect of CETP inhibition) at a genome-wide level of significance ($P < 5 \times 10^{-8}$) and that were in low linkage disequilibrium ($r^2 < 0.4$) with all other variants included in the score, using a forward stepwise conditional regression procedure among participants free from cardiovascular disease at baseline in each study (eFigure 1 in the Supplement). The exposure allele for each variant was defined as the allele associated with higher HDL-C levels.[16]

For each participant with individual data, a weighted *CETP* genetic score was calculated by summing the number of HDL-C–raising alleles that each participant inherited at each variant included in the score, weighted by each variant's conditional effect on HDL-C levels measured in mg/dL. For analyses involving summary-level data, genetic scores were calculated using the usual ratio of effect estimates method. Genetic scores for *HMGCR*, *NPC1L1*, and *PCSK9* were constructed using a similar procedure, as previously described.[17,18]

Allocation of Exposures

In individual-participant analyses, the *CETP* genetic score was dichotomized and used as an instrument to allocate participants into 2 approximately equal-sized groups based on whether their *CETP* score was either equal to or above the median or below the median (eFigure 2 in the Supplement). To evaluate dose response, participants were divided into 4 groups based on the quartile value of their *CETP* score.

To conduct the 2 × 2 factorial mendelian randomization analysis, participants were first allocated into 2 groups based on whether their *HMGCR* genetic score was equal to or above the median or below the median; participants in either of these 2 groups were then allocated into 2 further groups based on whether their *CETP* genetic score was equal to or above the median or below the median (eFigure 2 in the Supplement).[17,18] To conduct the stratified analysis, participants were first divided into 2 groups based on whether their *HMGCR* score was equal to or above the median or below the median, and the associations of the *CETP* score with cardiovascular events was then evaluated as a continuous variable (without dichotomization to increase statistical power to detect effect modification) in each of these 2 groups separately.

Table 1. Baseline Characteristics of 102 837 Participants From 14 Cohort or Case-Control Studies by *CETP* Genetic Score[a]

Characteristic	Mean (95% CI)		*P* Value
	CETP Score <Median (n = 49 435)	CETP Score ≥Median (n = 53 402)	
Age, y	59.8 (59.7-59.9)	60.0 (59.9-60.1)	.13
Women, %	58.3	58.2	.87
Blood pressure, mm Hg			
Systolic	127.3 (127.1-127.5)	127.5 (127.3-127.7)	.11
Diastolic	75.0 (74.9-75.1)	74.9 (74.8-75.0)	.16
Weight, kg	76.9 (76.7-77.1)	76.8 (76.6-77.0)	.48
Body mass index[c]	27.7 (27.6-27.8)	27.6 (27.5-27.7)	.19
Prevalent diabetes at baseline, No. (%)	4.2	4.1	.57
Ever smoker, No. (%)	54.4	54.3	.65
Lipid levels, mg/dL			
LDL-C	130.8 (130.3-131.3)	128.7 (128.2-129.2)	<.001
apoB	102.1 (101.5-102.7)	100.7 (100.1-101.3)	.004
HDL-C	49.6 (49.4-49.8)	54.4 (54.2-54.6)	<.001
Triglycerides	119.7 (81-159)	115.2 (77-156)	<.001
Total cholesterol	205.7 (205.3-206.1)	207.5 (207.1-207.9)	<.001
Non–HDL-C[b]	155.1 (154.7-155.5)	151.9 (151.5-152.3)	<.001

Abbreviations: apoB, apolipoprotein B; CETP, cholesteryl ester transfer protein; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol.

SI conversion factors: To convert HDL-C, LDL-C, and non–HDL-C values to mmol/L, multiply by 0.0259.

[a] Values in the table represent weighted mean values of the baseline characteristics for the entire study sample (age and sex) or from the cohort studies (for all other variables) in either group, after combining study-specific estimates in an inverse variance–weighted meta-analysis of the individual-participant data from up to 102 837 participants enrolled in one of 14 cohort or case-control studies. Among all participants, median *CETP* score was 34.8 (IQR, 28.3-41.1; range, 0-54.3). For participants in the group with *CETP* scores below the median, median *CETP* score was 28.2 (IQR, 23.3-32.0; range, 0-34.7). For participants in the group with *CETP* scores equal to or above the median, median *CETP* score was 41.1 (IQR, 37.9-44.8; range, 34.8-54.3). Higher scores indicate a greater number of HDL-C–raising alleles (weighted by the effect of each allele on HDL-C level).

[b] Calculated as the difference between total cholesterol and high-density lipoprotein cholesterol levels.

[c] Calculated as weight in kilograms divided by height in meters squared.

Statistical Analysis

For analyses involving individual-participant data, the absolute difference in mean levels of various biomarkers between groups was measured using linear regression, and the association with the risk of cardiovascular events was measured using logistic regression. All analyses were adjusted for age, sex, and the first 5 principal components of ancestry. Analyses were performed separately in each included study. Mendelian randomization estimates were then obtained by combining these summarized associations using a previously reported method that accounts for correlation between variants.[19]

For analyses involving summary-level data, the association between a genetic score and the risk of CHD was calculated by looking up the effect estimate of each variant included in that score on the risk of CHD as reported by the CARDIoGRAMplusC4D Consortium, dividing that effect size (and corresponding standard error) by the effect estimate of that variant on levels of HDL-C, LDL-C, or apoB (depending on the analysis) and then combining the adjusted effect estimates for all variants included in that score in an inverse-variance-weighted fixed-effects meta-analysis. Pleiotropy was assessed using MR-Egger regression.[20]

To identify variants associated with discordant changes in levels of LDL-C and apoB, a genome-wide association study was conducted among 65 829 participants from 15 studies in Europe and the United Kingdom, all of whom had LDL-C and apoB measurements performed on the same nuclear magnetic resonance metabolomic platform (eTable 2 in the Supplement).[21,22] Discordant variants were defined as being associated with LDL-C at $P < 5 \times 10^{-4}$ and with at least a 2-fold greater change in LDL-C level as compared with apoB, measured in mg/dL.

The threshold of statistical significance for the association between each genetic score and all outcomes or biomarker differences was $P < .05$ (2-sided). All analyses were performed using STATA 14, R version 3.2.5 (R Project for Statistical Computing), or Golden Helix SNP & Variation Suite (version 8.1.4). A detailed description of the methods is provided in the Supplement.

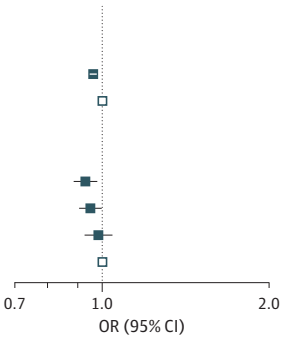
Results

The weighted mean age of the 102 837 study participants was 59.9 years. The weighted mean level of high-density lipoprotein cholesterol (HDL-C) was 52.0 mg/dL; of LDL-C, 129.7 mg/dL (to convert lipid values to mmol/L, multiply by 0.0259); and of apoB, 101.4 mg/dL (eTable 3 in the Supplement). Study participants experienced a total of 13 821 first major cardiovascular events. The stepwise selection procedure identified 8 genetic variants conditionally associated with HDL-C level that were included in the *CETP* genetic score; 6 variants were included in the *HMGCR* genetic score as previously reported (eTables 4-7 in the Supplement).[17,18] There were no differences in any nonlipid baseline characteristics between the groups being compared, thus demonstrating that allocation to each group appeared to be random (**Table 1**). The distribution of genetic scores for each group being compared in all analyses is presented in eTable 8 in the Supplement.

Figure 2. Association of *CETP* Score With Risk of Major Cardiovascular Events Among 102 837 Participants From 14 Cohort or Case-Control Studies

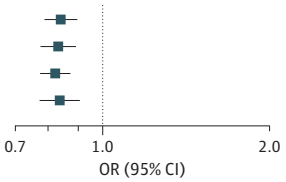
A Main effects and dose response

Group	Major Cardiovascular Events, No. of Cases/ No. of Participants (%)	Change, Mean (95% CI), mg/dL HDL–C	LDL–C	apoB	OR (95% CI)
Primary analysis					
CETP score					
≥Median	7048/53 402 (13.2)	4.62 (3.84-5.40)	-2.15 (-3.33 to -0.97)	-1.47 (-2.54 to -0.40)	0.964 (0.955-0.983)
<Median	6773/49 435 (13.7)				Reference
Dose-response analysis					
CETP score quartile					
4	3371/25 925 (13.0)	7.12 (5.47- 8.77)	-3.29 (-5.52 to -1.06)	-2.14 (-4.20 to -0.08)	0.934 (0.889-0.979)
3	3459/26 048 (13.3)	4.98 (3.39-6.57)	-2.32 (-4.27 to -0.37)	-1.38 (-3.52 to 0.75)	0.953 (0.910-0.998)
2	3415/25 011 (13.7)	2.37 (1.14-3.60)	-0.95 (-3.09 to -1.19)	-0.69 (-2.65 to 1.27)	0.985 (0.929-1.041)
1	3576/25 853 (13.8)				Reference



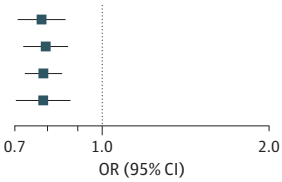
B Association of genetic scores with major cardiovascular events

Genetic Score	OR (95% CI) per 10-mg/dL Lower LDL–C
CETP	0.843 (0.788-0.901)
HMGCR	0.834 (0.775-0.896)
PCSK9	0.824 (0.774-0.876)
NPC1L1	0.839 (0.773-0.911)



C Association of genetic scores with major cardiovascular events

Genetic Score	OR (95% CI) per 10-mg/dL Lower apoB
CETP	0.781 (0.708-0.861)
HMGCR	0.794 (0.725-0.871)
PCSK9	0.787 (0.729-0.849)
NPC1L1	0.786 (0.703-0.879)



All information derived from the individual-participant data. A total of 102 837 participants who experienced a total of 13 821 first major cardiovascular events were included in the analysis. Among all participants, median cholesteryl ester transfer protein (*CETP*) genetic score was 34.8 (interquartile range [IQR], 28.3-41.1; range, 0-54.3). For participants in the group with *CETP* scores below the median, median *CETP* score was 28.2 (IQR, 23.3-32.0; range, 0-34.7). For participants in the group with *CETP* scores equal to or above the median, median *CETP* score was 41.1 (IQR, 37.9-44.8; range, 34.8-54.3). Higher scores indicate a greater number of high-density lipoprotein cholesterol (HDL-C)–raising alleles (weighted by the effect of each allele on HDL-C level) and is analogous to treatment with increasingly potent CETP inhibitors. Lipid and lipoprotein values are presented in mg/dL (to convert HDL-C and low-density lipoprotein cholesterol [LDL-C] values to mmol/L, multiply by 0.0259) as the difference in mean value for each group compared with the reference group, with 95% confidence intervals. Associations with major cardiovascular events were calculated using an inverse variance–weighted fixed-effects meta-analysis of the study-specific estimates of effect. In panels B and C, the association between the *CETP* score and risk of major cardiovascular events is compared with the association between the risk of major cardiovascular events and genetic scores consisting of variants in the 3-hydroxy-3-methyl-glutaryl-CoA reductase (*HMGCR*) gene (encodes the target of statins), proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene (encodes target of PCSK9 inhibitors), and Niemann-Pick C1-Like 1 intracellular cholesterol transporter 1 (*NPC1L1*) gene (encodes target of ezetimibe). All associations between the genetic scores and risk of major cardiovascular events are standardized per 10-mg/dL lower level of LDL-C (panel B) or 10-mg/dL lower level of apolipoprotein B (apoB) (panel C) and measured in the overall sample of studies that contributed individual-participant data. Data markers indicate point estimates of effect and are of equal size because the analysis compared approximately equal-sized groups divided by the median *CETP* score value or quartiles of the *CETP* score (panel A). OR indicates odds ratio.

Overall, as compared with participants with *CETP* scores below the median, participants with *CETP* scores equal to or above the median had lower mean CETP activity resulting in 4.62 mg/dL higher mean HDL-C level, 2.15 mg/dL lower mean LDL-C, 1.39 mg/dL lower mean apoB, and a corresponding lower risk of major cardiovascular events (odds ratio [OR], 0.964 [95% CI, 0.955-0.983]; $P < .001$). In dose response analyses, increasing quartiles of the *CETP* score were associated with a step-wise increase in mean HDL-C, step-wise decreases in mean LDL-C and apoB, and a corresponding step-wise decrease in the risk of major cardiovascular events (**Figure 2**A).

In standardized analyses, the *CETP* score was associated with a very similar risk of major cardiovascular events per 10-mg/dL lower LDL-C level (and per 10-mg/dL lower apoB) as compared with the *HMGCR*, *NPC1L1*, and *PCSK9* genetic scores (Figure 2B and C).

In external replication analyses involving up to 62 240 participants with CHD and 127 299 control participants from the CARDIoGRAMplusC4D Consortium, the *CETP* score was associated with a lower risk of CHD (OR, 0.968 [95% CI, 0.956-0.981]; $P < .001$). This association was very similar in magnitude compared with the association between the *HMGCR*, *NPC1L1*, and *PCSK9* genetic scores and the risk of CHD per unit change in LDL-C level (and per unit change in apoB) (eFigure 3 in the Supplement).

In the 2 × 2 factorial analysis, compared with participants with both *CETP* and *HMGCR* scores below the median, participants in the group with both *CETP* and *HMGCR* scores equal to or above the median had additively higher mean HDL-C levels and additively lower mean LDL-C (**Table 2**). However, participants in this group had an attenuated less than additively lower change in mean apoB, with no further significant reduction in

Table 2. Association Between *CETP* and *HMGCR* Genetic Scores With Biomarkers and Cardiovascular Events Among 102 837 Participants From 14 Cohort or Case-Control Studies[a]

	Overall Study Sample (N = 102 837)	Mean (95% CI)			
		Both Scores <Median (n = 25 693)	*CETP* Score ≥Median (n = 27 031)	*HMGCR* Score ≥Median (n = 23 854)	Both Scores ≥Median (n = 26 259)
No. of cases (%)	13 821 (13.4)	3622 (14.1)	3631 (13.4)	3145 (13.2)	3423 (13.0)
OR (95% CI) for major cardiovascular events		Reference	0.952 (0.910 to 0.995)	0.929 (0.880 to 0.981)	0.920 (0.869 to 0.976)
Lipid levels, mg/dL					
HDL-C (n = 72 411)[b,c]		49.3 (49.0 to 49.6)	53.9 (53.6 to 54.2)	50.2 (49.8 to 50.6)	54.7 (54.4 to 55.0)
LDL-C (n = 56 754)[b,c]		132.4 (131.7 to 133.2)	130.3 (129.5 to 131.0)	129.2 (128.4 to 129.9)	127.1 (126.3 to 127.9)
apoB (n = 18 312)[b,c]		103.4 (102.5 to 104.3)	101.5 (100.6 to 102.3)	100.7 (99.8 to 101.6)	100.1 (99.2 to 100.9)
CETP activity, SMD (n = 6436)[b,d]		Reference	−0.319 (0.462 to −0.176)	−0.008 (−0.026 to 010)	−0.323 (−0.451 to −0.196)
Genetic scores, median (IQR) [range]					
CETP	34.8 (28.3 to 41.1) [0 to 54.3]	28.4 (23.3 to 31.8) [0 to 34.7]	41.4 (37.9 to 45.1) [34.8 to 54.3]	28.1 (23.1 to 31.8) [0 to 34.7]	40.9 (37.8 to 44.7) [34.8 to 54.3]
HMGCR	16.8 (14.6 to 20.8) [4.1 to 22.4]	14.6 (12.2 to 16.2) [4.1 to 16.7]	14.6 (11.8 to 16.2) [4.1 to 16.7]	20.8 (19.1 to 22.3) [16.8 to 22.4]	20.8 (19.1 to 22.4) [16.8 to 22.4]

Abbreviations: apoB, apolipoprotein B; CETP, cholesteryl ester transfer protein; HMGCR, HMG-CoA reductase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; SMD, standardized mean difference.

SI conversion factors: To convert HDL-C and LDL-C values to mmol/L, multiply by 0.0259.

[a] Values are derived from individual-participant data.

[b] No. in parentheses indicates participants with biomarker data.

[c] Means and 95% confidence intervals defined by different combinations of genetic scores were derived from a fixed-effects inverse variance–weighted meta-analysis of studies that reported these values.

[d] Because CETP activity was measured on different scales in the studies that measured CETP activity, values were transformed to unit-less standardized mean differences.

the risk of major cardiovascular events compared with participants in the group with higher *HMGCR* scores alone (Table 2 and **Figure 3**A). A synthesis of the evidence from all published CETP inhibitor randomized trials demonstrated a similar attenuation in apoB reduction resulting in a discordance between the observed reduction in LDL-C and apoB levels when any CETP inhibitor was added to treatment with a statin, regardless of whether LDL-C was measured using the Friedewald equation or the β-quantification method (eTable 9 in the Supplement).[7-11,23,24] The observed attenuation in apoB reduction with combination therapy in these randomized trials recapitulates the genetic association with combined exposure to *CETP* and *HMGCR* variants observed in this study.

To further clarify the association of combined exposure to *CETP* and *HMGCR* variants with both lipoprotein changes and the risk of cardiovascular events, a stratified analysis was performed. Among participants with *HMGCR* scores below the median (analogous to CETP inhibitor monotherapy), the *CETP* score was associated with 4.81-mg/dL higher mean HDL-C level, 2.21-mg/dL lower mean LDL-C, a concordant 2.06-mg/dL lower mean apoB, and a corresponding lower risk of cardiovascular events (OR, 0.946 [95% CI, 0.921-0.972]; $P < .001$). By contrast, among participants with *HMGCR* scores equal to or above the median (analogous to combination therapy with a CETP inhibitor added to a statin), the *CETP* score was associated with a similar 4.42-mg/dL higher mean HDL-C level and 2.08-mg/dL lower mean LDL-C but with an attenuated 0.59-mg/dL lower mean apoB and no significant reduction in cardiovascular events (OR, 0.985 [95% CI, 0.959-1.012]; $P = .26$) (Figure 3B). The association with major cardiovascular events in this group was less than expected for the observed change in LDL-C level ($P = .04$) and instead was proportional to the attenuated change in apoB.

The genome-wide association study identified 21 independently inherited variants associated with discordant changes between levels of LDL-C and apoB similar in magnitude to that seen when *CETP* variants are combined with *HMGCR* variants (eTable 10 in the Supplement). In analyses involving up to 60 801 participants with coronary artery disease and 123 504 control participants from the CARDIoGRAMplusC4D Consortium, a genetic score consisting of these 21 variants was associated with a significantly less than expected risk of CHD per 10-mg/dL lower LDL-C level (OR, 0.916 [95% CI, 0.890-0.943] vs 0.831 [95% CI, 0.816-0.847]; $P = 2.9 \times 10^{-8}$ for difference), but a very similar risk of CHD per 10-mg/dL lower apoB (OR, 0.772 [95% CI, 0.701-0.844] vs 0.788 [95% CI, 0.769-0.807]; $P = .79$ for difference), as compared with an LDL-C genetic score consisting of 36 genetic variants associated with LDL-C at genome-wide level of significance and concordant changes in apoB level (**Figure 4**; eTables 11 and 12 in the Supplement).

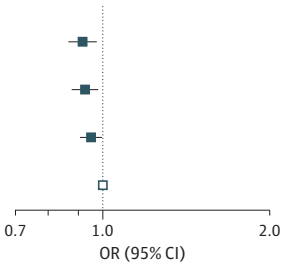
Discussion

Genetic variants in the target of CETP inhibitors were associated with higher HDL-C levels and concordant reductions in levels of LDL-C and apoB and a corresponding lower risk of cardiovascular events that was similar in magnitude to the association between genetic variants in the target of other LDL-lowering therapies and the risk of cardiovascular events per

Figure 3. Separate and Combined Effects of the *CETP* and *HMGCR* Scores on Risk of Major Cardiovascular Events Among 102 837 Participants From 14 Cohort or Case-Control Studies

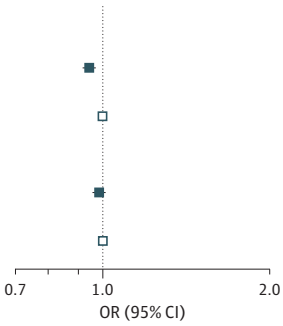
A 2 × 2 Factorial analysis of *CETP* and *HMGCR* genetic scores

Group	Major Cardiovascular Events, No. of Cases/ No. of Participants (%)	Change, Mean (95% CI), mg/dL HDL–C	LDL–C	apoB	OR (95% CI)
Both scores ≥median	3423/26 259 (13.0)	5.40 (4.16-6.64)	-5.29 (-7.29 to -3.29)	-3.33 (-5.19 to -1.46)	0.920 (0.869-0.976)
HMGCR score ≥median	3145/23 854 (13.2)	0.83 (0.11-1.56)	-3.27 (-5.08 to -1.46)	-2.74 (-4.31 to -1.07)	0.929 (0.880-0.981)
CETP score ≥median	3631/27 031 (13.4)	4.64 (3.44-5.83)	-2.16 (-3.69 to -0.63)	-1.93 (-3.27 to -0.57)	0.952 (0.910-0.995)
Both scores <median	3622/25 693 (14.1)				Reference



B Effect of *CETP* genetic score, stratified by *HMGCR* score

Group	Major Cardiovascular Events, No. of Cases/ No. of Participants (%)	Change, Mean (95% CI), mg/dL HDL–C	LDL–C	apoB	OR (95% CI)
HMGCR score < median					
CETP score ≥median	3631/27 031 (13.4)	4.64 (3.44-5.83)	-2.16 (-3.69 to -0.63)	-1.93 (-3.27 to -0.57)	0.946 (0.921-0.972)
CETP score <median	3622/25 693 (14.1)				Reference
HMGCR score ≥ median					
CETP score ≥median	3423/26 259 (13.0)	4.57 (3.26-5.88)	-2.02 (-3.76 to -0.28)	-0.59 (-1.81 to 0.64)	0.985 (0.959-1.012)
CETP score <median	3145/23 854 (13.2)				Reference



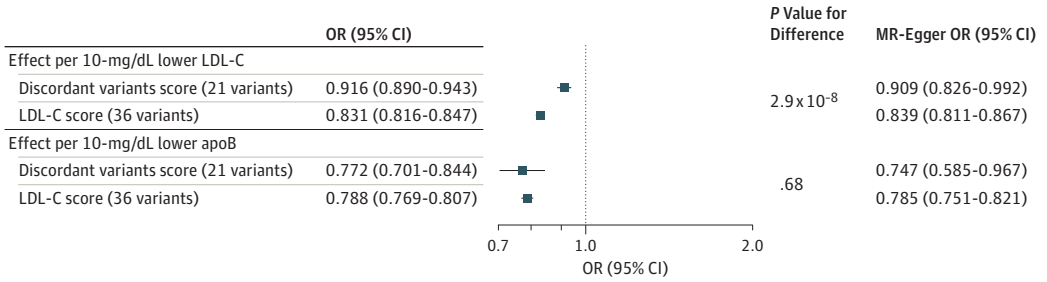
All information derived from the individual-participant data. A total of 102 837 participants who experienced a total of 13 821 first major cardiovascular events were included in the analysis. Among all participants, the median cholesteryl ester transfer protein (*CETP*) genetic score was 34.8 (interquartile range [IQR], 28.3-41.1; range, 0-54.3). The median *CETP* and 3-hydroxy-3-methyl-glutaryl-CoA reductase (*HMGCR*) score, IQR, and range of values for each group is presented in Table 2. Lipid and lipoprotein values are presented in mg/dL (to convert high-density lipoprotein cholesterol [HDL-C] and low-density lipoprotein cholesterol [LDL-C] values to mmol/L, multiply by 0.0259) as the difference in mean value for each group compared with the reference group, with 95% confidence intervals. Associations with major cardiovascular events were calculated using an inverse variance–weighted fixed-effects meta-analysis of the study-specific estimates of effect. In panel B, the study population was first divided into 2 groups based on whether the *HMGCR* score was below or equal to or greater than the median value. The association between the *CETP* score and the risk of major cardiovascular events was then estimated modeling the *CETP* score as a continuous variable scaled to the lipid effects in the dichotomous score analysis. There was evidence for effect modification of the *HMGCR* score on the association between the *CETP* genetic score and the risk of major cardiovascular events ($P = .04$). Data markers indicate point estimates of effect and are of equal size because the analysis compared approximately equal-sized groups divided into a factorial analysis (panel A) or the median *HMGCR* score value (panel B). Error bars represent 95% confidence intervals. apoB indicates apolipoprotein B; OR, odds ratio.

unit change in level of LDL-C (and apoB). However, when combined with variants in the target of statins, variants in the target of CETP inhibitors were associated with discordant reductions in LDL-C and apoB level and a corresponding reduction in cardiovascular events that was proportional to the attenuated reduction in apoB but significantly less than expected per unit change in LDL-C.

These mendelian randomization analyses suggest that the causal effect of CETP inhibition on the risk of cardiovascular events appears to be determined by changes in the concentration of apoB-containing lipoproteins rather than changes in LDL-C or HDL-C level. In external validation analyses, a genetic score consisting of 21 variants with similar naturally occurring discordance between changes in levels of LDL-C and apoB also was associated with the risk of cardiovascular disease proportional to change in level of apoB rather than LDL-C. Together, these findings suggest more generally that the causal effect of LDL on the risk of cardiovascular disease appears to be determined by the concentration of circulating apoB-containing lipoprotein particles rather than by the total cholesterol mass carried by those particles (as estimated by the plasma LDL-C level).

These mendelian randomization results are consistent with the results of several previous discordant effects analyses of LDL-C and apoB, suggesting that the risk of cardiovascular disease is more closely related to apoB level than to LDL-C level. Under most circumstances, LDL-C and apoB levels are highly correlated and therefore provide similar information about cardiovascular risk. It is only when they become discordant that the differential effects of LDL-C and apoB on the risk of

Figure 4. Association of Genetic Variants With Naturally Occurring Discordance Between Changes in Concentrations of LDL-C and apoB and the Risk of CHD Among CARDIoGRAMplusC4D Consortium Participants



Analyses are based on summary data from up to 62 240 participants with coronary heart disease (CHD) and 127 299 control participants from the Coronary Artery DIsease Genome Wide Replication and Meta-analysis plus the Coronary Artery Disease Genetics (CARDIoGRAMplusC4D) Consortium. Effect sizes are standardized per 10-mg/dL lower level of low-density lipoprotein cholesterol (LDL-C) or 10-mg/dL lower level of apolipoprotein B (apoB). MR-Egger regression estimates are presented for sensitivity analyses. Data markers indicate point estimates of effect; error bars, 95% confidence intervals.

atherosclerosis can be evaluated. The results of these mendelian randomization analyses provide naturally randomized genetic evidence to support the previous discordant analysis findings that the likelihood of an apoB-containing lipoprotein particle entering into and being trapped within the subintimal space of the arterial wall is more closely related to the concentration of circulating apoB-containing lipoproteins than to the variable mass of cholesterol within them.[25-27]

The results of the current mendelian randomization analyses suggest that the clinical benefit of lowering LDL-C level may be determined by the corresponding absolute reduction in concentration of apoB-containing particles. Therefore, the clinical benefit of lowering LDL-C may depend on how LDL-C is lowered. Therapies such as statins, ezetimibe, and PCSK9 inhibitors that lower LDL-C level by reducing circulating LDL particle concentration through up-regulation of LDL receptors should reduce the risk of cardiovascular events proportionally to the absolute reduction in either LDL-C level or the concordant absolute reduction in apoB. By contrast, therapies that lower LDL-C level without proportionally reducing apoB level, for example by altering the lipid content of apoB-containing lipoproteins without necessarily decreasing the concentration of those particles, may have an attenuated benefit on cardiovascular disease risk reduction that is proportional to the change in apoB level but less than expected per unit change in LDL-C.

This finding may help to reconcile the causal effect of LDL on cardiovascular disease with the results of the ACCELERATE trial by providing a possible explanation for how treatment with evacetrapib can lower LDL-C level without reducing the risk of cardiovascular events. Adding a CETP inhibitor to a statin leads to an attenuated reduction in apoB-containing lipoproteins compared with treatment with a CETP inhibitor alone, thus resulting in a discordance between the observed changes in levels of LDL-C and apoB. In the ACCELERATE trial, treatment with evacetrapib plus a statin reduced LDL-C level by 37% compared with treatment with a statin plus placebo, but only reduced plasma apoB level by 15%, less than half the expected apoB reduction with evacetrapib monotherapy.[11] After 2 years of treatment, the corresponding 29-mg/dL absolute reduction in LDL-C would be expected to reduce the risk of major cardiovascular events by approximately 13% to 19%, which is much greater than the 5% to 8% reduction in risk that would be expected from the attenuated 12-mg/dL absolute reduction in apoB.[4,28] This attenuated expected effect size based on the attenuated change in apoB falls well within the confidence bounds of the effect reported in the ACCELERATE trial (hazard ratio, 0.97 [95% CI, 0.85-1.10] for the composite outcome of cardiovascular death, myocardial infarction, or stroke).[11]

Similarly, the recently completed and much larger 30 000 person REVEAL (Randomized Evaluation of the Effects of Anacetrapib Through Lipid Modification) trial reported that treatment with anacetrapib plus a statin significantly reduced the risk of cardiovascular events, but further details have not yet been reported.[29] This trial had a median follow-up of 4 years, which should be long enough to observe the full effect of the CETP inhibitor–induced lipoprotein changes on the risk of cardiovascular events.[28] The results of the current mendelian randomization analyses would suggest that treatment with anacetrapib plus a statin should reduce the risk of cardiovascular events proportional to the absolute change in apoB level, which may be less than the expected risk reduction per unit lower LDL-C level depending on the level of discordance between the change in LDL-C and apoB that occurs when anacetrapib is added to treatment with atorvastatin.

Furthermore, the results of these mendelian randomization analyses also suggest that treatment with a CETP inhibitor as monotherapy may have the potential to effectively reduce the risk of cardiovascular events. Both genetic and therapeutic inhibition of CETP leads to quantitatively concordant changes in LDL-C and apoB levels when considered in the absence of HMG-CoA reductase inhibition. Therefore, treatment with a potent CETP inhibitor without a statin could lead to large concordant absolute reductions in both LDL-C and apoB, which could in turn lead to large relative reductions in cardiovascular events. Future cardiovascular outcomes trials evaluating CETP inhibitor therapy in statin-intolerant patients could test this hypothesis directly.

The current mendelian randomization analyses reconcile the conflicting results between previous mendelian randomization studies and the CETP inhibitor randomized trials. Prior mendelian randomization studies have reported that some *CETP* variants are weakly associated with lower CETP activity, higher HDL-C level, slightly lower LDL-C, and a marginally lower risk of cardiovascular disease.[30,31] These studies helped to establish CETP as a "genetically validated" drug target. The failure of CETP inhibitors to reduce the risk of cardiovascular events in 3 large randomized outcome trials therefore challenges the utility of using mendelian randomization studies to inform drug discovery and development programs. However, the prior mendelian randomization studies did not evaluate the combined effect of *CETP* and *HMGCR* variants and therefore could not have detected the attenuation in apoB reduction that occurs when CETP inhibition is combined with HMG-CoA inhibition. To provide relevant information to inform drug discovery and development, future mendelian randomization studies should be designed to explicitly evaluate the effect of variants in therapeutic targets the way therapies directed against those targets are likely to be used in clinical practice, including in combination with relevant required background therapy.[17,18]

This study has several limitations. First, the results of a mendelian randomization study do not establish causality. Second, mendelian randomization studies cannot evaluate the impact of acutely raising HDL-C to very high, supraphysiologic levels with a CETP inhibitor. It is possible that very high levels of HDL-C, reflected by a predominance of large, cholesterol-rich particles, may be deleterious and therefore may offset the potential clinical benefit of lowering levels of apoB-containing lipoprotein particles with a CETP inhibitor.

Third, treatment with the combination of a CETP inhibitor and a statin has resulted in a small increase in systolic blood pressure in each of the 3 large cardiovascular outcome trials completed to date.[7,11,23] This increased blood pressure may have offset some of the potential benefit of the already attenuated apoB-lowering effect of combination therapy on the risk of cardiovascular events. Fourth, CETP inhibition might increase cardiovascular risk through some other as-yet unknown mechanism that counterbalances any benefit from LDL lowering. Fifth, the mechanism by which changes in LDL-C and apoB levels become discordant when CETP inhibition is combined with HMG-CoA reductase inhibition is unclear, but could be related to the redistribution of cholesterol between HDL and LDL particles.[32] Regardless of the mechanism, this effect has been observed in all randomized trials evaluating combined treatment with a CETP inhibitor and a statin and recapitulates the genetic effect observed in this study.

Conclusions

Combined exposure to variants in the genes that encode the targets of CETP inhibitors and statins was associated with discordant reductions in LDL-C and apoB levels and a corresponding risk of cardiovascular events that was proportional to the attenuated reduction in apoB but significantly less than expected per unit change in LDL-C. The clinical benefit of lowering LDL-C levels may therefore depend on the corresponding absolute reduction in apoB-containing lipoprotein particles.

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Acquisition, analysis, or interpretation of data: Ference, Kastelein, Chapman, Nicholls, Ray, Packard, Brook, Oliver-Williams, Butterworth, Danesh, Catapano, Sabatine.
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Administrative, technical, or material support: Ference, Kastelein, Nicholls.
Study Supervision: Ference, Kastelein, Ray, Butterworth, Danesh, Catapano.

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