

Protein-Truncating Variants at the Cholesteryl Ester Transfer Protein Gene and Risk for Coronary Heart Disease

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Rationale: Therapies that inhibit CETP (cholesteryl ester transfer protein) have failed to demonstrate a reduction in risk for coronary heart disease (CHD). Human DNA sequence variants that truncate the *CETP* gene may provide insight into the efficacy of CETP inhibition.

Objective: To test whether protein-truncating variants (PTVs) at the *CETP* gene were associated with plasma lipid levels and CHD.

Methods and Results: We sequenced the exons of the *CETP* gene in 58469 participants from 12 case-control studies (18817 CHD cases, 39652 CHD-free controls). We defined PTV as those that lead to a premature stop, disrupt canonical splice sites, or lead to insertions/deletions that shift frame. We also genotyped 1 Japanese-specific PTV in 27561 participants from 3 case-control studies (14286 CHD cases, 13275 CHD-free controls). We tested association of *CETP* PTV carrier status with both plasma lipids and CHD. Among 58469 participants with *CETP* gene-sequencing data available, average age was 51.5 years and 43% were women; 1 in 975 participants carried a PTV at the *CETP* gene. Compared with noncarriers, carriers of PTV at *CETP* had higher high-density lipoprotein cholesterol (effect size, 22.6 mg/dL; 95% confidence interval, 18–27; $P < 1.0 \times 10^{-4}$), lower low-density lipoprotein cholesterol (–12.2 mg/dL; 95% confidence interval, –23 to –0.98; $P = 0.033$), and lower triglycerides (–6.3%; 95% confidence interval, –12 to –0.22; $P = 0.043$). *CETP* PTV carrier status was associated with reduced risk for CHD (summary odds ratio, 0.70; 95% confidence interval, 0.54–0.90; $P = 5.1 \times 10^{-3}$).

Conclusions: Compared with noncarriers, carriers of PTV at *CETP* displayed higher high-density lipoprotein cholesterol, lower low-density lipoprotein cholesterol, lower triglycerides, and lower risk for CHD. (*Circ Res.* 2017;121:81–88. DOI: 10.1161/CIRCRESAHA.117.311145.)

Key Words: case-control studies ■ cholesteryl ester transfer protein ■ coronary disease ■ lipids

In 3 randomized controlled trials (RCTs), therapies that inhibit CETP (cholesteryl ester transfer protein) have failed to demonstrate a reduction in risk for coronary heart disease (CHD).^{1–3} Possible reasons for this failure include on-target lack of efficacy, off-target adverse effects of the small

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molecule, and RCT design factors, such as insufficient statistical power, concurrent statin therapy, or selection of study

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Novelty and Significance

What Is Known?

- Human DNA sequence variants that truncate a therapeutic target protein may provide insight into the efficacy of pharmacological inhibition.
- It has been uncertain whether carriers of protein-truncating variants (PTVs) at the cholesteryl ester transfer protein (*CETP*) gene have altered plasma lipid levels and lower risk for coronary heart disease (CHD).

What New Information Does This Article Contribute?

- Carriers of a PTV at *CETP* had higher high-density lipoprotein cholesterol, lower low-density lipoprotein cholesterol, and lower triglycerides.
- *CETP* PTV carrier status was also associated with 30% reduced risk for CHD.
- Lifelong reduction in *CETP* function is associated with altered plasma lipids and a lower risk for CHD.

Therapies that inhibit *CETP* have failed to demonstrate a reduction in risk for CHD. Human DNA sequence variants that truncate

a therapeutic target gene may provide insight into the efficacy of pharmacological inhibition. We tested whether humans carrying PTVs at the *CETP* gene were associated with lipid levels and were at reduced risk for CHD. We sequenced the exons of the *CETP* gene in 58 469 participants from 12 case-control and genotyped 1 Japanese-specific PTV in 27 561 participants from 3 case-control studies. PTVs at the *CETP* gene were defined as mutations that lead to a premature stop, disrupt canonical splice sites, or lead to insertions/deletions that shift frame. In an analysis including >80 000 participants, carriers of a PTV at *CETP* had higher high-density lipoprotein cholesterol (+22.6 mg/dL), lower low-density lipoprotein cholesterol (−12.2 mg/dL), and lower triglycerides (−6.3%). *CETP* PTV carrier status was also associated with 30% reduced risk for CHD (summary odds ratio, 0.70). In conclusion, compared with noncarriers, carriers of PTV at the *CETP* gene displayed higher high-density lipoprotein cholesterol, lower low-density lipoprotein cholesterol, lower triglycerides, and lower risk for CHD.

Nonstandard Abbreviations and Acronyms

BBJ	BioBank Japan
CAGE-CAD	Cardio-metabolic Genome Epidemiology Network and Coronary Artery Disease
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CI	confidence interval
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
MIGen	Myocardial Infarction Genetics
PCSK9	proprotein convertase subtilisin/kexin type 9
PTV	protein-truncating variant
RCT	randomized controlled trial

participants.^{4–6} An RCT of a fourth *CETP* inhibitor—anacetrapib—is ongoing.⁷

Studies of humans with naturally occurring genetic variation in genes encoding drug targets can provide insight into the potential efficacy and safety of therapeutic modulation targeting the gene product.^{8–10} Genetic studies of common, regulatory variants at the *CETP* gene region initially showed mixed results^{11–15} but more recently have converged on a consensus finding: alleles with lower *CETP* expression are associated with reduced CHD risk.¹⁶

Beyond common DNA sequence variants, rare mutations that truncate a therapeutic target gene may be of particular value because they most closely mirror pharmacological inhibition.^{8,9,17} Protein-truncating variants (PTVs; ie, nonsense, canonical splice site, and frameshift mutations) at 2 therapeutic targets—NPC1L1 (NPC1-like intracellular cholesterol transporter 1)⁹ and proprotein convertase subtilisin/kexin type 9 (PCSK9)⁸—are associated with lower low-density lipoprotein cholesterol (LDL-C) and reduced CHD risk. A therapeutic trial testing NPC1L1 inhibition was consistent with the human genetic findings,¹⁸ and a

trial testing PCSK9 inhibition was consistent as well.¹⁹ Here, we tested whether rare PTVs at the *CETP* gene were associated with plasma lipids and reduced odds of CHD.

Methods

Study Participants

First, we sequenced a total of 58 469 participants from the MIGen consortium (Myocardial Infarction Genetics) of African, European, and South Asian ancestries (n=25 273), the DiscovEHR (DiscovEHR project of the Regeneron Genetics Center and the Geisinger Health System) of European ancestry (n=24 138),²⁰ and Taiwanese-Chinese (TAICHI) consortium of East Asian ancestry (n=9058)²¹ (Table 1). The MIGen consortium consists of the Italian ATVB study (Atherosclerosis Thrombosis and Vascular Biology),²² the DHM study (Deutsches Herzzentrum München Myocardial Infarction),⁹ the ESP-EOMI study (Exome Sequencing Project Early-Onset Myocardial Infarction)^{23,24} of European and African ancestries, the JHS (Jackson Heart Study),²⁵ the Leicester (Leicester Acute Myocardial Infarction Peptide Study),²⁶ the Lubeck (Lübeck Myocardial Infarction Study),²⁷ the OHS (Ottawa Heart Study),²⁸ the PROCARDIS (Precocious Coronary Artery Disease Study),²⁹ the PROMIS (Pakistan Risk of Myocardial Infarction Study),³⁰ and the REGICOR (Registre Gironi del COR) study.³¹

We also genotyped a Japanese-specific PTV at the *CETP* gene (rs5742907; IVS14+1G>A; splice-donor variant³²) in a total of 27 561 Japanese participants from BioBank Japan (BBJ)³³ and the CAGE-CAD (Cardio-metabolic Genome Epidemiology Network and Coronary Artery Disease) stage 1 and stage 2 studies (Table 1).³⁴

All participants in the study provided written informed consent for genetic studies. The Institutional Review Boards at the Broad Institute and each participating institution approved the study protocol.

Definition of *CETP* PTVs

PTVs were defined as premature stop (nonsense), canonical splice sites (splice donor or splice acceptor), including IVS14+1G>A (rs5742907), or insertion/deletion variants that shifted frame (frameshift). The positions of these PTVs were based on the GRGh37 human genome reference and the canonical transcript for *CETP* (transcript ID: ENST00000200676).

Clinical Characteristics, Lipid Measurements, and Definition of CHD

A medical history and laboratory data for cardiovascular risk factors were obtained from all the study participants. Plasma total cholesterol,

Table 1. Clinical Characteristics of Each Study by Protein-Truncating Variant Carrier Status

	MIGen		DiscovEHR		TAICHI		BBJ		CAGE-CAD Stage 1		CAGE-CAD Stage 2	
	PTV Carrier	Noncarrier	PTV Carrier	Noncarrier	PTV Carrier	Noncarrier	PTV Carrier	Noncarrier	PTV Carrier	Noncarrier	PTV Carrier	Noncarrier
	n=26	n=25 247	n=16	n=24 122	n=18	n=9040	n=122	n=15 476	n=21	n=2584	n=76	n=9282
Age, y, mean (SD)	53.5 (13)	53.3 (13)	47.2 (12)	46.2 (12)	61.2 (15)	60.9 (15)	65.1 (10)	65.2 (10)	66.4 (8)	65.9 (8)	63.6 (8)	62.5 (7)
Male gender, n (%)	26 (79)	18 387 (73)	9 (56)	5777 (24)	12 (67)	6166 (68)	78 (64)	10 943 (71)	12 (57)	1711 (66)	48 (63)	6041 (65)
BMI, kg/m ² , median (IQR)	25.7 (23–28)	26.2 (24–29)	33.7 (31–38)	31.2 (26–37)	24.8 (23–30)	24.9 (23–28)	23.2 (21–25)	23.4 (21–26)	23.6 (21–24)	23.3 (21–25)	23.7 (22–26)	23.2 (21–24)
Current smoker, n (%)	7 (21)	7389 (29)	3 (19)	5048 (21)	N/A	N/A	78 (64)	10 282 (66)	11 (52)	1336 (51)	36 (47)	4752 (51)
Medical history												
Hypertension, n (%)	12 (36)	9499 (38)	7 (44)	12 933 (54)	6 (33)	4783 (53)	54 (44)	6408 (41)	6 (28)	1182 (45)	39 (51)	4760 (51)
Type 2 diabetes mellitus, n (%)	8 (24)	5069 (20)	5 (31)	4126 (17)	8 (44)	4343 (48)	78 (64)	8568 (55)	9 (42)	917 (35)	16 (21)	2174 (23)
Lipid-lowering medication,* n (%)	1 (3)	3682 (15)	4 (25)	6129 (25)	3 (19)	1781 (22)	43 (35)	5164 (33)	6 (28)	377 (14)	N/A	N/A
Lipid profile												
LDL cholesterol, mean (SD)	121 (57)	130 (48)	114 (39)	124 (38)	126 (42)	120 (50)	118 (35)	125 (38)	117 (32)	130 (38)	N/A	N/A
HDL cholesterol, mean (SD)	61 (24)	41 (14)	58 (14)	51 (15)	58 (23)	45 (14)	67 (25)	50 (15)	78 (24)	58 (16)	N/A	N/A
Triglycerides, median (IQR)	124 (70–163)	150 (102–222)	162 (105–198)	126 (89–177)	138 (89–186)	121 (83–176)	138 (89–156)	145 (86–175)	74 (57–131)	109 (80–154)	N/A	N/A
Total cholesterol, mean (SD)	211 (61)	206 (54)	207 (50)	205 (42)	210 (45)	190 (46)	239 (72)	231 (61)	218 (37)	214 (41)	N/A	N/A

BBJ indicates BioBank Japan; BMI, body mass index; CAGE-CAD, the Cardio-metabolic Genome Epidemiology Network and Coronary Artery Disease study; DiscovEHR, the DiscovEHR project of the Regeneron Genetics Center and the Geisinger Health System; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; MIGen, Myocardial Infarction Genetics Consortium; N/A, not applicable; PTV, protein-truncating variant; and TAICHI, Taiwanese-Chinese consortium.
*At the time of lipid measurement.

triglycerides, and high-density lipoprotein cholesterol (HDL-C) levels were determined enzymatically. LDL-C level was calculated using the Friedewald equation^{35,36} for those with triglycerides <400 mg/dL. If triglycerides ≥400 mg/dL, LDL-C level was directly measured or set to missing. The effect of lipid-lowering therapy at the time of lipid measurement was taken into account by dividing the measured total cholesterol and LDL-C levels by 0.8 and 0.7, respectively.³⁷ HDL-C and triglyceride levels were not adjusted by lipid-altering medication use, and triglyceride levels were natural logarithm transformed for statistical analysis. CHD case and CHD-free control definitions of each study are in Online Table I.

Sequencing and Genotyping to Characterize PTVs

Whole-exome sequencing of the MIGen consortium was performed at the Broad Institute (Cambridge, MA) as previously described.²³ Sequencing reads were aligned to a human reference genome (build 37) using the Burrows–Wheeler Aligner-maximal exact match algorithm. Aligned nonduplicate reads were locally realigned, and base qualities were recalibrated using the Genome Analysis ToolKit software.³⁸ Variants were jointly called using the Genome Analysis ToolKit HaplotypeCaller program. The sensitivity of the Variant Quality Score Recalibration threshold was 99.6% for single nucleotide variants and 95% for insertion/deletion variants. All identified variants were annotated with the use of the Variant Effect Predictor software (version 82).³⁹ The DiscovEHR project and TAICHI consortium participants were exome sequenced as previously described.²⁰

We also genotyped 1 splice-donor variant (IVS14+1G>A [rs5742907]) at the *CETP* gene using the multiplex polymerase chain reaction–based target sequencing in BBJ⁴⁰ or the TaqMan assay in CAGE-CAD stage 1 and stage 2.

Statistical Analysis

We tested the association of *CETP* PTV carrier status with lipid levels using linear regression adjusted by age, sex, study, and the first 5 principal components of ancestry (MIGen), or by age and sex (BBJ and CAGE-CAD stage 1). Only CHD-free controls in each study were included in this assessment to minimize the effect of ascertainment bias. These data were meta-analyzed to calculate overall summary effect sizes with an inverse-variance weighted fixed-effects model.

We tested the association of *CETP* PTV carrier status with CHD risk using a Cochran–Mantel–Haenszel method without continuous correction. This method combines score statistics instead of Wald statistics and is useful for rare exposures when some observed odds ratios are zero. We removed ESP-EOMI and JHS from this analysis because no participant in these 2 studies carried a PTV at the *CETP* gene.

In an exploratory analysis, we evaluated whether the effect of *CETP* PTVs on LDL-C could explain the reduction in CHD risk. We used an inverse-variance weighted model to draw a regression line with a 95% confidence interval (CI). Across 4 genes (*APOB*, *NPC1L1*, *PCSK9*, and *CETP*), we plotted the effect of DNA sequence variants in these genes on both LDL-C and CHD risk. The results for *APOB*, *NPC1L1*, and *PCSK9* are derived from samples of the MIGen

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consortium to draw a dose-response reference line. The results for *CETP* are summary estimate from all studies.

Statistical analyses were performed using R software version 3.2.3 (The R Project for Statistical Computing, Vienna, Austria).

Results

Prevalence of *CETP* PTVs

Sequencing of the 16 exons at the *CETP* gene was performed in 58 469 participants (18 817 CHD cases and 39 652 CHD-free controls) from 3 projects: the MIGen consortium, the DiscovEHR project, and TAICHI consortium. Baseline characteristics of each study are shown in Table 1. A total of 23 PTVs were identified (10 premature stop, 9 frameshifts, 3 splice-donor variants, and 1 splice-accepter variant). A total of 60 individuals carried one of the *CETP* PTVs, including 18 CHD cases (0.096%; 95% CI, 0.051%–0.14%) and 42 CHD-free controls (0.11%; 95% CI, 0.074%–0.14%). Baseline characteristics by variant carrier status are shown in Online Table II. We genotyped a Japanese-specific splice-donor variant (IVS14+1G>A [rs5742907]) in 3 studies from Japan and found the carrier frequency to be BBJ, 0.78%; CAGE-CAD stage 1, 0.81%; and CAGE-CAD stage 2, 0.92%.

Association of *CETP* PTVs With Plasma Lipids

We assessed whether *CETP* PTV carrier status was associated with lipid levels (Table 2; Online Figure). We obtained plasma lipid profiles in 11 205 control participants from the MIGen consortium and 6955 control participants from BBJ and CAGE-CAD stage 1. *CETP* PTV carrier status was associated with increased HDL-C (effect size, 22.6 mg/dL; 95% CI, 18–27; $P < 1 \times 10^{-4}$), decreased LDL-C (–12.2 mg/dL; 95% CI, –23 to –0.98; $P = 0.033$), and decreased triglycerides (–6.3%; 95% CI, –12 to –0.22; $P = 0.043$).

Association of *CETP* PTVs With CHD

We evaluated the association of *CETP* PTV carrier status with CHD. Baseline characteristics and lists of *CETP* PTVs by case–control status in each study are shown in Online Table III and Online Table IV. In an analysis including a total of 82 722 participants, *CETP* PTV carrier status was significantly associated with lower risk for CHD (summary odds ratio, 0.70; 95% CI, 0.54–0.90; $P = 5.1 \times 10^{-3}$; Figure 1).

DNA Sequence Variants, LDL-C, and CHD Risk Across 4 Genes

We explored whether the effect size of *CETP* PTV on CHD risk was consistent with its effect on LDL-C. We drew a

dose-response reference line for CHD risk as a function of LDL-C change conferred by DNA sequence variants in 3 genes other than *CETP*. DNA sequence variants in *APOB*, *NPC1L1*, and *PCSK9* associated with lower LDL-C also correlated with lower CHD risk. The effect of *CETP* PTV on CHD risk (30% reduction in risk) was consistent with the estimate based on the change in LDL-C (–12.2 mg/dL; Figure 2).

Discussion

Across >80 000 participants, we evaluated whether *CETP* PTVs were associated with lipid levels and risk for CHD. About 1 in 975 participants carried a PTV at the *CETP* gene in sequencing studies, and compared with noncarriers, *CETP* PTV carriers exhibited significantly higher plasma HDL-C levels and lower LDL-C and triglyceride levels. The presence of a *CETP* PTV was also associated with decreased risk for CHD.

This evidence from rare human mutations that disrupt the *CETP* gene is consistent with earlier data on common, regulatory variants at the *CETP* locus. Common variants in *CETP* have been associated with increased HDL-C, decreased LDL-C, decreased triglyceride levels,⁴¹ and reduced risk for CHD.^{13,42–44} And recently, the statistical evidence for association of common *CETP* variants with CHD has exceeded a stringent genome-wide threshold.¹⁶ Exploratory analyses suggest that the effect of *CETP* PTV on lower CHD risk is consistent with lower LDL-C change conferred by these variants.

If human genetics shows loss of *CETP* function mutations to be associated with reduced CHD risk, why have 3 small molecule inhibitors of *CETP* function all failed to show lower CHD outcomes in randomized clinical trials? Several possibilities emerge. First, this could be because of off-target adverse effects of small molecule inhibitors. Torcetrapib, dalcetrapib, and evacetrapib treatment all led to higher blood pressure in RCTs^{1–3}; torcetrapib also led to hyperaldosteronism.¹

Second, RCT design factors, such as limited statistical power, could play a role.⁴⁵ Human genetic evidence is supportive for apolipoprotein B–containing lipoproteins (low-density lipoprotein, triglyceride-rich lipoproteins, lipoprotein[a]) as causal factors for CHD, whereas this is not the case for HDL-C.⁶ As such, any benefit from *CETP* inhibition may be solely because of the lowering of apolipoprotein B–containing lipoproteins. On background statin therapy, the LDL cholesterol and apolipoprotein B–lowering effect are smaller, as shown in the ACCELERATE trial (Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition With Evacetrapib in Patients at a High Risk for Vascular Outcomes).³

Table 2. Associations of *CETP* Protein-Truncating Variant Carrier Status With HDL Cholesterol, LDL Cholesterol, Triglycerides, and Total Cholesterol

	MIGen			BBJ and CAGE-CAD			Overall		
	Effect Size	95% CI	P Value	Effect Size	95% CI	P Value	Effect Size	95% CI	P Value
HDL cholesterol, mg/dL	19.2	12 to 27	2.5×10^{-7}	24.5	19 to 30	<0.0001	22.6	18 to 27	<0.0001
LDL cholesterol, mg/dL	–20.2	–45 to 4.8	0.11	–10.2	–23 to 2.4	0.11	–12.2	–23 to –0.98	0.033
Triglycerides, %	2.8%	–27 to 44	0.16	–6.6%	–12 to –0.43	0.036	–6.3%	–12 to –0.22	0.043
Total cholesterol, mg/dL	5.2	–23 to 33	0.72	8.6	–8.7 to 26	0.97	7.6	–7.1 to 22	0.31

BBJ indicates BioBank Japan; CAGE-CAD, the Cardio-metabolic Genome Epidemiology Network and Coronary Artery Disease study; *CETP*, cholesteryl ester transfer protein; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and MIGen, Myocardial Infarction Genetics consortium.

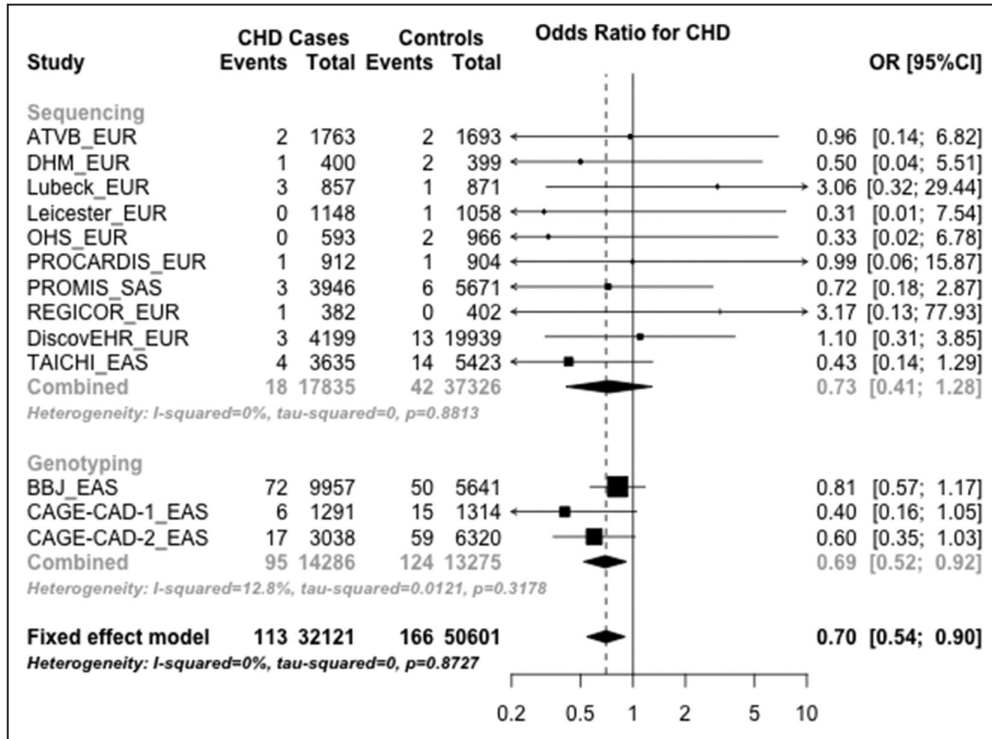


Figure 1. Association of CETP (cholesteryl ester transfer protein) protein-truncating variant carrier status with risk for coronary heart disease (CHD). CETP protein-truncating variant carrier status was associated with reduced risk for CHD. Each study column indicates [Study name]_[Ancestry]. ATVB indicates Atherosclerosis Thrombosis and Vascular Biology; BBJ, BioBank Japan; CAGE-CAD, Cardio-metabolic Genome Epidemiology Network and Coronary Artery Disease; CI, confidence interval; DHM, Deutsches Herzzentrum München Myocardial Infarction Study; DiscovEHR, DiscovEHR project of the Regeneron Genetics Center and the Geisinger Health System; EAS, East Asian ancestry; EUR, European ancestry; OHS, Ottawa Heart Study; OR, odds ratio; PROMIS, Pakistan Risk of Myocardial Infarction Study; REGICOR, Registre Gironi del COR; and SAS, South Asian ancestry.

As such, it is unclear whether RCTs were adequately powered to detect this benefit.

Third, statin therapy may modify the relationship of CETP activity and coronary disease. CETP promotes the transfer of cholesteryl

esters from HDL to atherogenic apolipoprotein B-containing lipoproteins, including LDL.⁴ If not cleared from the circulation, accumulation of such particles in the bloodstream promotes atherosclerotic progression. However, statins lead to substantial

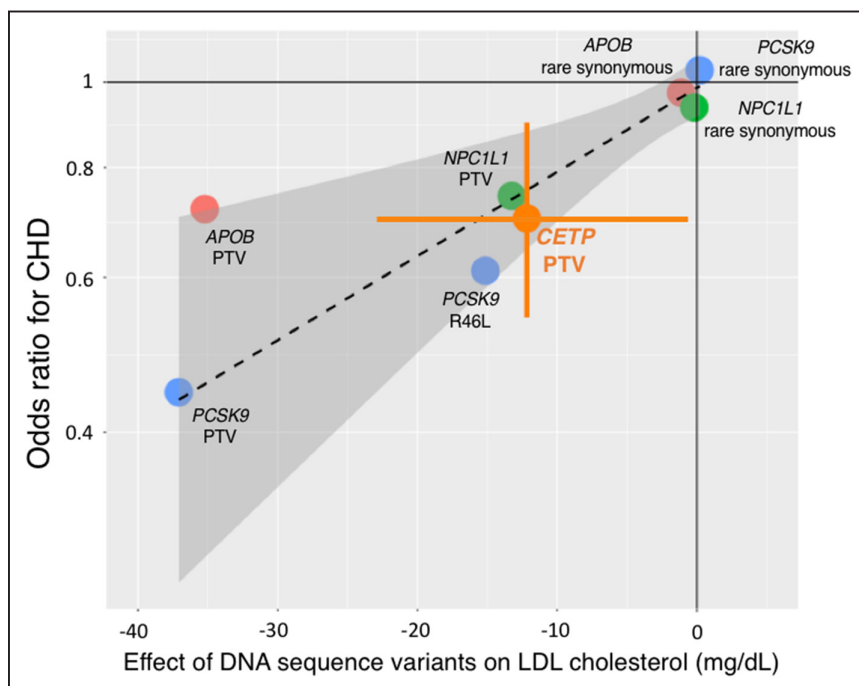


Figure 2. Effects of DNA sequence variants in 4 genes on low-density lipoprotein cholesterol (LDL-C) and coronary heart disease (CHD) risk. Dashed line denotes a dose-response reference line, with the 95% confidence interval (CI) indicated by shadow. Error bar indicates cholesteryl ester transfer protein (CETP) protein-truncating variant (PTV) 95% CIs of an effect size on LDL-C and odds ratio for CHD.

upregulation of hepatic LDL receptor density.⁴⁵ In this context, apolipoprotein B-containing lipoproteins may be rapidly cleared from the circulation and excreted into the feces. CETP may, therefore, play a role in promoting reverse cholesterol transport, the process by which cholesterol is extracted from peripheral tissues (eg, atherosclerotic plaque) and excreted from the body. Overexpression of CETP leads to enhanced reverse cholesterol transport via an LDL receptor-dependent pathway in mouse models.⁴⁶ Furthermore, individuals with increased on-statin CETP mass were protected from recurrent coronary events, particularly when the achieved LDL cholesterol was <80 mg/dL.⁴⁷ Under this framework, pharmacological CETP inhibition might prove less effective or potentially harmful among those in whom statin therapy leads to efficient clearance of apolipoprotein B-containing lipoproteins. However, the impact of CETP inhibition on reverse cholesterol transport has been questioned because the mouse studies might have been confounded by cholesterol pool size changes.⁴⁸ Also, torcetrapib did not elevate fecal cholesterol or bile acids in both on- and off-statin individuals.⁴⁹

Fourth, a major difference in individuals with heterozygous PTV mutations in CETP and individuals taking the CETP inhibitors is the accumulation of a cholesteryl ester-rich HDL in the latter, which may be a toxic lipoprotein particle like cholesteryl ester-rich LDL.

Finally, phenotypic consequences of human PTVs reflect lifelong perturbation of a gene in every human tissue. In contrast, the results of RCTs reflect pharmacological inhibition initiated later in life. As such, there are intrinsic limitations in using human mutations to anticipate efficacy and safety of pharmacological manipulation.

These results should be interpreted in the context of study limitations. Definitions of CHD were different among studies. Cases in the MiGen consortium and the DiscovEHR project were limited to only early-onset CHD, whereas those in East Asian studies were not. Loss of *CETP* function alters the distribution of cholesterol and triglycerides in lipoproteins, and as such, LDL-C levels estimated by the Friedewald equation might overestimate the reduction in participants harboring *CETP* PTVs. We only assessed 4 major lipid levels to evaluate effects of *CETP* PTV carrier status, and other traits such as lipoprotein (a) or function of reverse cholesterol transport were unavailable. Results were somewhat stronger in participants from the Japanese genotyping studies, but the point estimates of the odds ratios for CHD were consistent between populations of Japanese and non-Japanese ancestries (0.69 and 0.73, respectively).

Conclusions

In this meta analyses of data from 15 case-control studies, rare PTVs at the *CETP* gene were associated with higher HDL-C, lower LDL-C, lower triglycerides, and reduced risk for CHD.

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APPENDIX

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APPENDIX

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Supplemental Table I. Definitions of coronary heart disease across studies.

Study	CHD Cases	Controls	CHD Definition	Control Definition
ATVB	1763	1693	EOMI in male or female $\leq 45y$	No history of thromboembolic disease
BBJ	9957	5641	History of CHD validated by an evidence of left ventricular wall motion abnormalities on echocardiography and a coronary angiography.	Free of CHD individuals with 5 diseases (cerebral aneurysm, esophageal cancer, endometrial cancer, chronic obstructive pulmonary disease or glaucoma).
CAGE-CAD Stage1	1291	1314	The CHD criteria include (i) a validated history of either MI or coronary revascularization (coronary artery bypass grafting or percutaneous coronary intervention), or (ii) subjective symptoms of angina pectoris with 1 or more major coronary vessels showing $\geq 75\%$ stenosis documented by coronary angiography.	Free of CHD
CAGE-CAD Stage2	3038	6320		
DiscovEHR	4199	19939	EOCAD in male $\leq 55y$ or female $\leq 65y$	Free of CAD
DHM	400	399	EOMI in male $\leq 40y$ or female $\leq 55y$	Controls without CAD, men $\geq 65y$ and women $\geq 75y$
ESP-EOMI	962	1426	EOMI (male $\leq 50y$ or female $\leq 60y$)	Hospital-based, no report of MI by history
JHS	20	900	Combination of prevalent CHD (self-reported or electrocardiographic evidence of MI) and incident CHD (MI or coronary revascularization) in $\leq 50y$ or female $\leq 60y$.	Free of CHD during follow-up
Leicester	1148	1058	EOMI (male $\leq 50y$ or female $\leq 60y$)	
Lubeck	857	871	EOMI (male $\leq 50y$ or female $\leq 60y$)	Controls without CAD; male and female \leq age 65
OHS	593	966	EOMI (male $\leq 50y$ female $\leq 60y$)	Asymptomatic
PROCARDIS	912	904	EOMI (male $\leq 50y$ or female $\leq 60y$)	No history of CAD
PROMIS	3946	5671	EOMI (male $\leq 50y$ or female $\leq 60y$)	Age and gender frequency-matched. No history of MI/CVD
REGICOR	382	402	EOMI (male $\leq 50y$ or female $\leq 60y$)	Controls from a population-based study; free of MI, coronary revascularization; ≥ 55 and $<80y$
TAICHI	3635	5423	CAD (history of MI/CABG/PCI, or at least 1 major vessel with stenosis $\geq 50\%$)	Free of CAD

Abbreviations: CAD, coronary artery disease; CHD, coronary heart disease; CABG, Coronary artery bypass grafting; CVD, cardiovascular disease; EOCAD, early-onset coronary artery disease; EOMI, early-onset myocardial infarction; MI, myocardial infarction.

Supplemental Table II. Baseline characteristics of MIGen, DiscovEHR, BBJ, CAGE-CAD, and TAICHI.

	MIGen	DiscovEHR	TAICHI	BBJ	CAGE-CAD Stage 1	CAGE-CAD Stage 2
	N = 25,273	N = 24,138	N = 9,058	N = 15,598	N = 2,605	N = 9,358
Age, years, mean (SD)	53.3 (13)	46.2 (12)	60.9 (15)	65.2 (10)	65.9 (8.4)	62.5 (8.0)
Male gender, n (%)	18,413 (73)	5,786 (24)	6,128 (68)	11,021 (71)	1,723 (66)	6,089 (65)
BMI, kg/m ² , median (IQR)	26.2 (24–29)	31.2 (26–37)	24.9 (23–28)	23.4 (21–26)	23.3 (21–25)	23.2 (21–25)
Current smoker, n (%)	7,396 (29)	5051	N/A	10,360 (66)	1,347 (51)	4,788 (51)
Ancestry						
African	1,548 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
European	14,108 (56)	24,138 (100)	0 (0)	0 (0)	0 (0)	0 (0)
South Asian	9,617 (38)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
East Asian	0 (0)	0 (0)	9,058 (100)	15,598 (100)	2,605 (100)	9,358 (100)
Medical history						
Coronary heart disease, n (%)	10,983 (43)	4,199 (17)	3,635 (40)	9,957 (64)	1,291 (49)	3038 (32)
Hypertension, n (%)	9,511 (38)	12,940 (54)	4,789 (53)	6,462 (41)	1,188 (45)	4799 (51)
Type 2 diabetes, n (%)	5,077 (20)	4,131 (17)	4,351 (48)	8,646 (55)	926 (35)	2190 (23)
Lipid-lowering medication*, n (%)	3,683 (14)	6,133 (25)	1,784 (22)	5,207 (33)	383 (14)	N/A
Lipid profile						
LDL cholesterol, mean (SD)	130 (48)	124 (38)	120 (50)	125 (38)	130 (38)	N/A
HDL cholesterol, mean (SD)	41 (14)	51 (15)	45 (14)	50 (15)	58 (16)	N/A
Triglycerides, median (IQR)	150 (102–222)	126 (89–177)	121 (83–176)	145 (86–175)	109 (79–154)	N/A
Total cholesterol, mean (SD)	206 (54)	205 (42)	190 (53)	232 (61)	214 (41)	N/A

*At the time of lipid measurement.

Abbreviations: Same as Table 1.

Supplemental Table III. Clinical characteristics of each study by case-control status.

	MIGen		DiscovEHR		TAICHI		BBJ		CAGE-CAD Stage1		CAGE-CAD Stage2	
	CHD	CHD-free	CHD	CHD-free	CHD	CHD-free	CHD	CHD-free	CHD	CHD-free	CHD	CHD-free
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
	N = 10,983	N = 14,290	N = 4,199	N = 19,939	N = 3,635	N = 5,423	N = 9,957	N = 5,641	N = 1,291	N = 1,314	N = 3,038	N = 6,320
Age, years, mean (SD)	46.6 (8.5)	58.3 (13)	51.8 (7.3)	45.0 (12)	67.2 (11)	56.7 (16)	66.6 (10.4)	62.8 (9.8)	66.4 (9)	65.5 (7)	62.6 (9)	62.4 (6)
Male gender, n (%)	8,263 (75)	10,150 (71)	1,938 (46)	3,848 (19)	2,886 (79)	3,242 (60)	8,133 (82)	2,888 (51)	998 (77)	725 (55)	2364 (77)	3725 (58)
BMI, kg/m ² , median (IQR)	26.7 (24–30)	25.9 (24–29)	32.3 (28–38)	31.0 (26–37)	25.5 (23–28)	24.5 (22–27)	24.0 (22–26)	22.5 (20–25)	23.5 (21–25)	23.1 (21–25)	23.6 (21–25)	23.0 (21–24)
Current smoker, n (%)	4,509 (41)	2,887 (20)	986 (23)	4,065 (20)	N/A	N/A	7,164 (72)	3,196 (57)	809 (62)	538 (40)	1999 (65)	2789 (44)
Medical history												
Hypertension, n (%)	4,331 (39)	5,180 (36)	3,373 (80)	12444 (34)	2,184 (60)	2,605 (48)	3,941 (40)	2,521 (45)	774 (59)	414 (31)	1635 (53)	3164 (50)
Type 2 Diabetes, n (%)	2,679 (24)	2,398 (17)	1,520 (36)	2611 (13)	1,702 (47)	2,648 (49)	4,338 (44)	4,308 (76)	603 (46)	323 (24)	1153 (37)	1037 (16)
Lipid-lowering medication*, n (%)	3,176 (29)	507 (4)	2,494 (59)	3639 (18)	1,226 (34)	738 (14)	4,712 (47)	495 (9)	171 (13)	212 (16)	N/A	N/A
Lipid profile												
LDL cholesterol, mean (SD)	146 (54)	120 (41)	130 (40)	122 (37)	124 (53)	117 (48)	126 (37)	124 (38)	124 (37)	132 (37)	N/A	N/A
HDL cholesterol, mean (SD)	38 (13)	43 (15)	46 (13)	52 (15)	43 (12)	46 (15)	49 (14)	58 (18)	52 (15)	61 (16)	N/A	N/A
Triglycerides, median (IQR)	168 (117–248)	138 (94–206)	154 (112–215)	119 (85–167)	124 (87–178)	118 (81–174)	150 (91–181)	132 (77–158)	133 (97–181)	103 (76–140)	N/A	N/A
Total cholesterol, mean (SD)	222 (57)	196 (48)	214 (43)	203 (42)	192 (52)	189 (53)	234 (62)	223 (58)	207 (41)	216 (41)	N/A	N/A

*: At the time of lipid measurement.

Abbreviations: Same as Table 1.

Supplemental Table IV. List of protein truncating variants in the *CETP* gene and coronary heart disease.

Chr	BP (b37)	REF	ALT	rsID	Consequence	AA change	MIGen		DiscovEHR		TAICHI		BBJ		CAGE-CAD-1		CAGE-CAD-2	
							CHD N = 10,983	Controls N = 14,290	CHD N = 4,199	Controls N = 19,939	CHD N = 3,635	Controls N = 5,423	CHD N = 9,957	Controls N = 5,641	CHD N = 1,291	Controls N = 1,314	CHD N = 3,038	Controls N = 6,320
16	56995902	CCA	C	rs766825140	Frameshift	T5SfsX95	0	1	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	56996963	C	T	rs780627434	Premature stop	D54X	1	0	0	0	0	1	N/A	N/A	N/A	N/A	N/A	N/A
16	56996966	GC	G	rs753876598	Frameshift	A55fsX	0	0	1	1	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	56997025	T	G	rs201790757	Premature stop	Y74X	0	2	1	1	0	1	N/A	N/A	N/A	N/A	N/A	N/A
16	57003374	C	T	rs759621654	Premature stop	Q104X	0	1	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57003536	C	T	rs781363517	Premature stop	Q128X	1	0	0	2	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57003581	AAC	A	.	Frameshift	Q145AfsX4	1	0	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57003587	C	T	.	Premature stop	Q145X	0	1	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57003859	CT	C	rs781445411	Frameshift	D159TfsX34	1	3	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57003914	G	A	.	Splice donor	INT5+1G>A	0	1	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57004961	C	T	rs142750310	Premature stop	Q182X	1	1	0	2	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57005015	G	T	rs777194816	Splice donor	INT6+1G>T	0	1	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57005910	TC	T	rs759929211	Frameshift	L223FfsX12	1	0	0	2	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57007274	ACCTC	A	rs767971569	Frameshift	D261fsX	0	0	0	0	2	5	N/A	N/A	N/A	N/A	N/A	N/A
16	57007345	C	T	rs748488549	Premature stop	R285X	0	3	0	2	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57012122	TC	T	rs769850349	Frameshift	P367fsX	0	0	0	0	2	7	N/A	N/A	N/A	N/A	N/A	N/A
16	57012151	C	CT	.	Frameshift	A377fsX	0	0	1	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57015573	CTG	C	rs760384575	Frameshift	T410fsX	0	0	0	1	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57016086	G	T	.	Premature stop	E420X	0	0	0	1	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57016107	C	T	rs780936328	Premature stop	Q427X	1	0	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57016150	G	A	rs5742907	Splice donor	IVS14+1G>A	1	0	0	1	0	0	72	50	6	15	17	59
16	57017236	A	G	.	Splice acceptor	IVS14-2A>G	1	0	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
16	57017318	C	T	rs577301331	Premature stop	R468X	2	1	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A

Abbreviations: AA, amino acid; ALT, alternative allele; BP, base-pair position; CHD, coronary heart disease; Chr, chromosome; REF, reference allele.

Supplemental Figure. Meta-analysis evaluating effects of *CETP* protein truncating variant carrier status on lipid traits.

