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

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Abstract

Rationale—Therapies which inhibit cholesteryl ester transfer protein (CETP) have failed to demonstrate a reduction in risk for coronary heart disease (CHD). Human deoxyribonucleic acid 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 58,469 participants from 12 case-control studies (18,817 CHD cases, 39,652 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 one Japanese-specific PTV in 27,561 participants from three case-control studies (14,286 CHD cases, 13,275 CHD-free controls). We tested association of *CETP* PTV carrier status with both plasma lipids and CHD. Among 58,469 participants with *CETP* gene sequencing data available, average age was 51.5 years and 43% were female; 1 in 975 participants carried a PTV at the *CETP* gene. Compared to non-carriers, carriers of PTV at *CETP* had higher high-density lipoprotein cholesterol (HDL-C; effect size, 22.6 mg/dL; 95% confidence interval [CI], 18 to 27; $P < 1.0\times10^{-4}$), lower low-density lipoprotein cholesterol (LDL-C; −12.2 mg/dL; 95% CI, −23 to −0.98; P = 0.033), and lower triglycerides (−6.3%; 95% CI, −12 to −0.22, P = 0.043). *CETP* PTV carrier status was associated with reduced risk for CHD (summary odds ratio, 0.70; 95% CI, 0.54 to 0.90; $P = 5.1\times10^{-3}$).

Conclusions—Compared with non-carriers, carriers of PTV at *CETP* displayed higher HDL-C, lower LDL-C, lower triglycerides, and lower risk for CHD.

Keywords

Cholesteryl ester transfer protein; exome sequencing; protein truncating variants; coronary heart disease; genetics; human; lipids

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DISCLOSURES

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Subject Terms

Genetics; Lipids and Cholesterol; Coronary Artery Disease

INTRODUCTION

In three randomized controlled clinical trials (RCTs), therapies which inhibit cholesteryl ester transfer protein (CETP) 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 molecule, and/or randomized controlled trial design factors such as insufficient statistical power, concurrent statin therapy, or selection of study participants.[4–6] A randomized trial 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 deoxyribonucleic acid (DNA) sequence variants, rare mutations that truncate a therapeutic target gene may be of particular value because they most closely mirror pharmacologic inhibition.[8, 9, 17] Indeed, protein truncating variants (PTVs; i.e., nonsense, canonical splice-site, and frameshift mutations) at two therapeutic targets – NPC1 Like Intracellular Cholesterol Transporter 1 (NPC1L1)[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 if 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 Myocardial Infarction Genetics (MIGen) Consortium of African, European, and South Asian ancestries (N=25,273), the DiscovEHR project of the Regeneron Genetics Center and the Geisinger Health System (DiscovEHR) of European ancestry (N=24,138),[20] and TAICHI Consortium of East Asian ancestry (N=9,058)[21] (Table 1). The MIGen Consortium consists of the Italian Atherosclerosis Thrombosis and Vascular Biology (ATVB) Study[22], the Deutsches Herzzentrum München Myocardial Infarction Study (DHM)[9], the Exome Sequencing Project Early-Onset Myocardial Infarction Study (ESP-EOMI)[23, 24] of European and African ancestries, the Jackson Heart Study (JHS),[25] the Leicester Acute Myocardial Infarction Peptide Study (Leicester),[26] the Lübeck Myocardial Infarction Study (Lubeck),[27] the Ottawa Heart Study (OHS),[28] the Precocious Coronary Artery Disease Study

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(PROCARDIS),[29] the Pakistan Risk of Myocardial Infarction Study (PROMIS),[30] and the Registre Gironi del COR (REGICOR) Study.[31]

We also genotyped a Japanese-specific PTV at the *CETP* gene (rs5742907; IVS14+1G>A; splice-donor variant[32]) in a total of 27,561 Japanese participants from BioBank Japan (BBJ)[33] and the Cardio-metabolic Genome Epidemiology Network and Coronary Artery Disease (CAGE-CAD) 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 protein truncating variants

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, 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 Supplemental Table I.

Sequencing and genotyping to characterize protein truncating variants

Whole exome sequencing of the MIGen Consortium was performed at the Broad Institute (Cambridge, MA, USA) 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 non-duplicate reads were locally realigned, and base qualities were recalibrated using the Genome Analysis ToolKit (GATK) software.[38] Variants were jointly called using the GATK 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]

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We also genotyped one splice-donor variant (IVS14+1G>A [rs5742907]) at the *CETP* gene using the multiplex PCR-based target sequencing in BBJ,[40] 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, gender, study, and the first five principal components of ancestry (MIGen), or by age and gender (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 (OR) are zero. We removed ESP-EOMI and JHS from this analysis because no participant in these two studies carried a PTV at the *CETP* gene.

In an exploratory analysis, we evaluated if 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 four 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 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 protein truncating variants

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 three 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 (ten premature stop, nine frameshifts, three splice-donor, and one splice-accepter variants). A total of 60 individuals carried one of the *CETP* PTVs, including 18 CHD cases (0.096%; 95% confidence interval (CI), 0.051 to 0.14%) and 42 CHD-free controls (0.11%; 95% CI, 0.074 to 0.14%). Baseline characteristics by variant carrier status are shown in Supplemental Table II. We genotyped a Japanese-specific splice-donor variant (IVS14+1G>A [rs5742907]) in three studies from Japan and found the carrier frequency to be: BBJ, 0.78%; CAGE-CAD Stage1, 0.81%; and CAGE-CAD Stage2, 0.92%.

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Association of CETP protein truncating variants with plasma lipids

We assessed whether *CETP* PTV carrier status was associated with lipid levels (Table 2 and Supplemental Figure). We obtained plasma lipid profiles in 11,205 control participants from the MIGen Consortium and 6,955 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 to 27; $P < 1\times10^{-4}$), 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 protein truncating variants 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 Supplemental Table III and Supplemental 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 OR, 0.70; 95% CI, 0.54 to 0.90; $P = 5.1\times10^{-3}$) (Figure 1).

DNA sequence variants, LDL-C, and CHD risk across four 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 three 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 more than 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 non-carriers, *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 the *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 three small molecule inhibitors of CETP function all failed to show lower CHD outcomes in randomized clinical trials? Several possibilities emerge. First, this could be due to off-target adverse effects of small molecule inhibitors. Torcetrapib,

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dalcetrapib, and evacetrapib treatment all led to higher blood pressure in randomized controlled trials[1–3]; torcetrapib also led to hyperaldosteronism.[1]

Second, RCT design factors such as limited statistical power could play a role.[4, 5] 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 due to the lowering of apolipoprotein B-containing lipoproteins. On background statin therapy, the LDL cholesterol and apolipoprotein B lowering effect is smaller, as shown in the ACCELERATE trial. [3] As such, it is unclear if 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 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 (e.g. atherosclerotic plaque) and excreted from the body. Indeed, overexpression of CETP leads to enhanced reverse cholesterol transport via a 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 less than 80 mg/dl.[47] Under this framework, pharmacologic 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 cholesterols or bile acids in both on- and off-statin individuals.[49]

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

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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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
PTV	Protein truncating variant
RCT	Randomized controlled clinical trial

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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 pharmacologic 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 HDL cholesterol, lower LDL 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 which 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 pharmacologic 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 one Japanese-specific PTV in 27561 participants from three 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 more than 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 non-carriers, carriers of PTV at the *CETP* gene displayed higher HDL-C, lower LDL-C, lower triglycerides, and lower risk for CHD.

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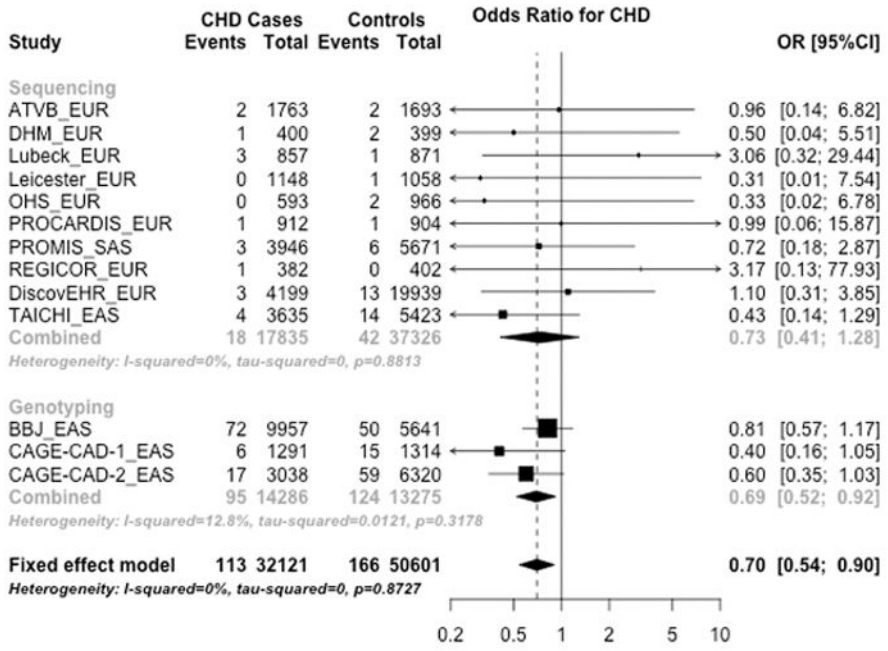

Figure 1. Association of *CETP* protein truncating variant carrier status with risk for coronary heart disease

CETP protein truncating variant carrier status was associated with reduced risk for CHD. Each study column indicates [Study name] _[Ancestry].

Abbreviations: CHD, coronary heart disease; EAS, East Asian ancestry; EUR, European ancestry; OR, odds ratio; SAS, South Asian ancestry.

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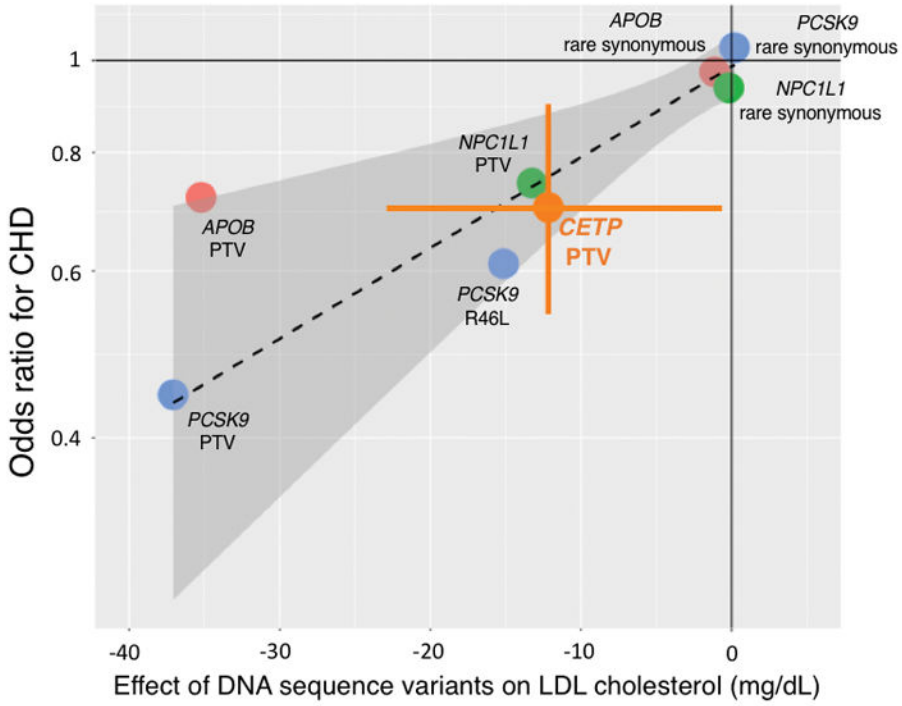

Figure 2. Effects of DNA sequence variants in four genes on LDL-C and CHD risk

Dashed line denotes a dose-response reference line, with the 95% CI indicated by shadow. Error bar indicates *CETP* PTV 95% CIs of an effect size on LDL-C and odds ratio for CHD. Abbreviations: CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; LDL-C, low-density lipoprotein cholesterol; PTV, protein truncating variant.

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Table 1

Clinical characteristics of each study by protein truncating variant carrier status.

	MIGen		DiscovEHR		TAICHI		BBJ		CAGE-CAD Stage1		CAGE-CAD Stage2	
	PTV carrier	Non-carrier	PTV carrier	Non-carrier	PTV carrier	Non-carrier	PTV carrier	Non-carrier	PTV carrier	Non-carrier	PTV carrier	Non-carrier
	N = 26	N = 25,247	N = 16	N = 24,122	N = 18	N = 9,040	N = 122	N = 15,476	N = 21	N = 2,584	N = 76	N = 9,282
Age, years, 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)	5,777 (24)	12 (67)	6,166 (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)	7,389 (29)	3 (19)	5,048 (21)	N/A	N/A	78 (64)	10,282 (66)	11 (52)	1336 (51)	36 (47)	4752 (51)
Medical history												
Hypertension, n (%)	12 (36)	9,499 (38)	7 (44)	12,933 (54)	6 (33)	4,783 (53)	54 (44)	6,408 (41)	6 (28)	1182 (45)	39 (51)	4760 (51)
Type 2 Diabetes, n (%)	8 (24)	5,069 (20)	5 (31)	4,126 (17)	8 (44)	4,343 (48)	78 (64)	8,568 (55)	9 (42)	917 (35)	16 (21)	2174 (23)
Lipid-lowering medication *, n (%)	1 (3)	3,682 (15)	4 (25)	6,129 (25)	3 (19)	1,781 (22)	43 (35)	5,164 (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

*At the time of lipid measurement.

Abbreviations: BBJ, BioBank Japan; 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; IQR, interquartile range; MIGen, Myocardial Infarction Genetics Consortium; N/A, not applicable; PTV, protein truncating variant; TAICHI, TAICHI consortium; SD, standard deviation; LDL, low-density lipoprotein; HDL, high-density lipoprotein

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Table 2

Associations of *CETP* protein truncating variant carrier status with HDL cholesterol, LDL cholesterol, triglycerides, and total cholesterol.

	MIGen			BBJ + 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

Abbreviations: BBJ, BioBank Japan; 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; IQR, interquartile range; MIGen, Myocardial Infarction Genetics Consortium; N/A, not applicable; PTV, protein truncating variant; TAICHI, TAICHI consortium; SD, standard deviation; LDL, low-density lipoprotein; HDL, high-density lipoprotein