Journal of the American Heart Association

ORIGINAL RESEARCH

Genome-Wide Association Study of Cardiovascular Resilience Identifies Protective Variation in the *CETP* Gene

Chenglong Yu, PhD; Andrew Bakshi, PhD; Gerald F. Watts, MD, DSc; Alan E. Renton, PhD; Brian Fulton-Howard, PhD; Alison M. Goate, DPhil; Pradeep Natarajan, MD; Daniel I. Chasman, PhD; Liubov Robman, MBBS, PhD; Robyn L. Woods, PhD; Robyn Guymer, MBBS, PhD; Rory Wolfe, PhD; Le Thi Phuong Thao, PhD; John J. McNeil, MD, PhD; Andrew M. Tonkin, MD; Stephen J. Nicholls, MBBS, PhD; Paul Lacaze, PhD

BACKGROUND: The risk of atherosclerotic cardiovascular disease (ASCVD) increases sharply with age. Some older individuals, however, remain unaffected despite high predicted risk. These individuals may carry cardioprotective genetic variants that contribute to resilience. Our aim was to assess whether asymptomatic older individuals without prevalent ASCVD carry cardioprotective genetic variants that contribute to ASCVD resilience.

METHODS AND RESULTS: We performed a genome-wide association study using a 10-year predicted ASCVD risk score as a quantitative trait, calculated only in asymptomatic older individuals aged ≥70 years without prevalent ASCVD. Our discovery genome-wide association study of N=12 031 ASCVD event-free individuals from the ASPREE (Aspirin in Reducing Events in the Elderly) trial identified 2 independent variants, rs9939224 ($P<5\times10^{-8}$) and rs56156922 ($P<10^{-6}$), in the *CETP* (cholesteryl ester transfer protein) gene. The *CETP* gene is a regulator of plasma high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and lipoprotein(a) levels, and it is a therapeutic drug target. The associations were replicated in the UK Biobank (subpopulation of N=13 888 individuals aged ≥69 years without prevalent ASCVD). Carriers of the identified *CETP* variants (versus noncarriers) had higher plasma high-density lipoprotein cholesterol levels, lower plasma low-density lipoprotein cholesterol levels, and reduced risk of incident ASCVD events during follow-up. Expression quantitative trait loci analysis predicted the identified *CETP* variants reduce *CETP* gene expression across various tissues. Previously reported associations between genetic *CETP* inhibition and increased risk of age-related macular degeneration were not observed among the 3917 ASPREE trial participants with retinal imaging and genetic data available.

CONCLUSIONS: Common genetic variants in the *CETP* gene region are associated with cardiovascular resilience during aging.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01038583.

Key Words: aging ■ cardioprotective variants ■ cardiovascular disease ■ genome-wide association study ■ lipid metabolism

During aging, the risk of atherosclerotic cardiovascular disease (ASCVD) increases sharply and becomes the leading cause of death.[1,2] However, despite high predicted risk, some "resilient" older individuals remain ASCVD event free beyond the age of 70 years. Such individuals may be more likely to carry naturally occurring cardioprotective genetic variants.

Previous genome-wide association studies (GWASs) of ASCVD[3,4] and genetic studies of blood cholesterol levels[5,6] have identified common ASCVD risk-associated

Correspondence to: Chenglong Yu, PhD, School of Public Health and Preventive Medicine, Monash University, Level 5, 553 St Kilda Rd, Melbourne VIC 3004, Australia. Email: chenglong.yu@monash.edu

This article was sent to Julie K. Freed, MD, PhD, Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.031459

For Sources of Funding and Disclosures, see page 13.

© 2023 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

JAHA is available at: www.ahajournals.org/journal/jaha

RESEARCH PERSPECTIVE

What Is New?

- This study uses an unconventional "controls-only" genome-wide association study design to identify candidate protective genetic variation contributing to the phenotype of cardiovascular disease resilience during aging.
- The study involved 12 031 healthy individuals, aged ≥70 years, with no history of atherosclerotic cardiovascular disease events, and identified 2 putatively protective risk-modifying loci in the *CETP* (cholesteryl ester transfer protein) gene.

What Question Should Be Addressed Next?

- Further investigation of genetic variation within the *CETP* gene is warranted, to better understand the consequences of genetic and therapeutic inhibition.

Nonstandard Abbreviations and Acronyms

AMD	age-related macular degeneration
ASPREE	Aspirin in Reducing Events in the Elderly
CETP	cholesteryl ester transfer protein gene
eQTL	expression quantitative trait loci
MAF	minor allele frequency
PC	principal component
SCORE2-OP	Systematic Coronary Risk Evaluation 2–Older People
TC	total cholesterol

variants. Rare loss-of-function variants in genes regulating low-density lipoprotein cholesterol (LDL-C) levels, including *PCSK9* and *APOB*, also demonstrate that single variants can reduce plasma LDL-C levels and thereby decrease ASCVD risk.[7] Rare variants that inhibit the function of these genes, however, are carried by few people in the general population (<2%) and do not account for all genetic ASCVD risk modification. Common variants (polymorphisms) are carried by larger subgroups of the population and can also explain variation in ASCVD outcomes, especially in aggregate, as shown with polygenic scores.[8–10]

Here, we use an unconventional "controls-only" GWAS approach as an alternative strategy to identify cardioprotective common risk-modifying variants for ASCVD. Our study leverages the unique ascertainment of >20 000 healthy ASCVD-free older individuals enrolled into the ASPREE (Aspirin in Reducing Events in the Elderly) trial[11,12] and the UK Biobank,[13] who at the time of providing DNA samples, had no prior clinically manifest ASCVD events. Both cohorts have healthy volunteer/survivorship bias, where participants, on average, were healthier at enrollment than the general population at equivalent ages.[14,15] This ascertainment bias (by design) was hypothesized to increase the power of our controls-only analytical method to discover candidate cardioprotective variants.

METHODS

Availability of Data and Materials

The ASPREE trial data can be accessed via https://aspree.org/aus/for-researchers/.[14] The UK Biobank data can be accessed via https://www.ukbiobank.ac.uk/.[13] The data that support the findings of this study are available from the corresponding author upon reasonable request.

For detailed methods, please see Data S1. The phenotype used for GWAS was a derived quantitative variable of 10-year predicted ASCVD risk,[16] calculated only in healthy asymptomatic older individuals without a history of diagnosed ASCVD events who have survived despite increasing age and risk. The risk prediction score (Systematic Coronary Risk Evaluation 2–Older People [SCORE2-OP]) estimates 10-year cardiovascular disease risk in individuals aged ≥70 years.[16] We modified the score to focus on ASCVD risk associated with lipid metabolism.

Study Samples

The discovery GWAS included healthy older individuals, aged ≥70 years, without a history of diagnosed ASCVD events enrolled into the ASPREE trial, a randomized double-blind placebo-controlled clinical trial to determine whether daily 100-mg aspirin extended disability-free survival in healthy older individuals.[14] The design and protocol of the ASPREE trial have been reported previously,[11] including genetic analyses.[10,12] Longitudinal analyses involved use of follow-up data from the ASPREE-XT (Extension) study.[17]

Of the 19 114 ASPREE trial participants, a total of 12 031 genotyped, unrelated participants aged ≥70 years with European ancestry (see Figure S1 for a principal component [PC] plot of genetic races and ethnicities) were included in the GWAS (Figure 1). Aspirin allocation was investigated as a covariate in sensitivity analysis. ASPREE trial participants provided written informed consent for genetic analysis. The study was approved by the Alfred Hospital Human Research Ethics Committee in Australia and site-specific institutional

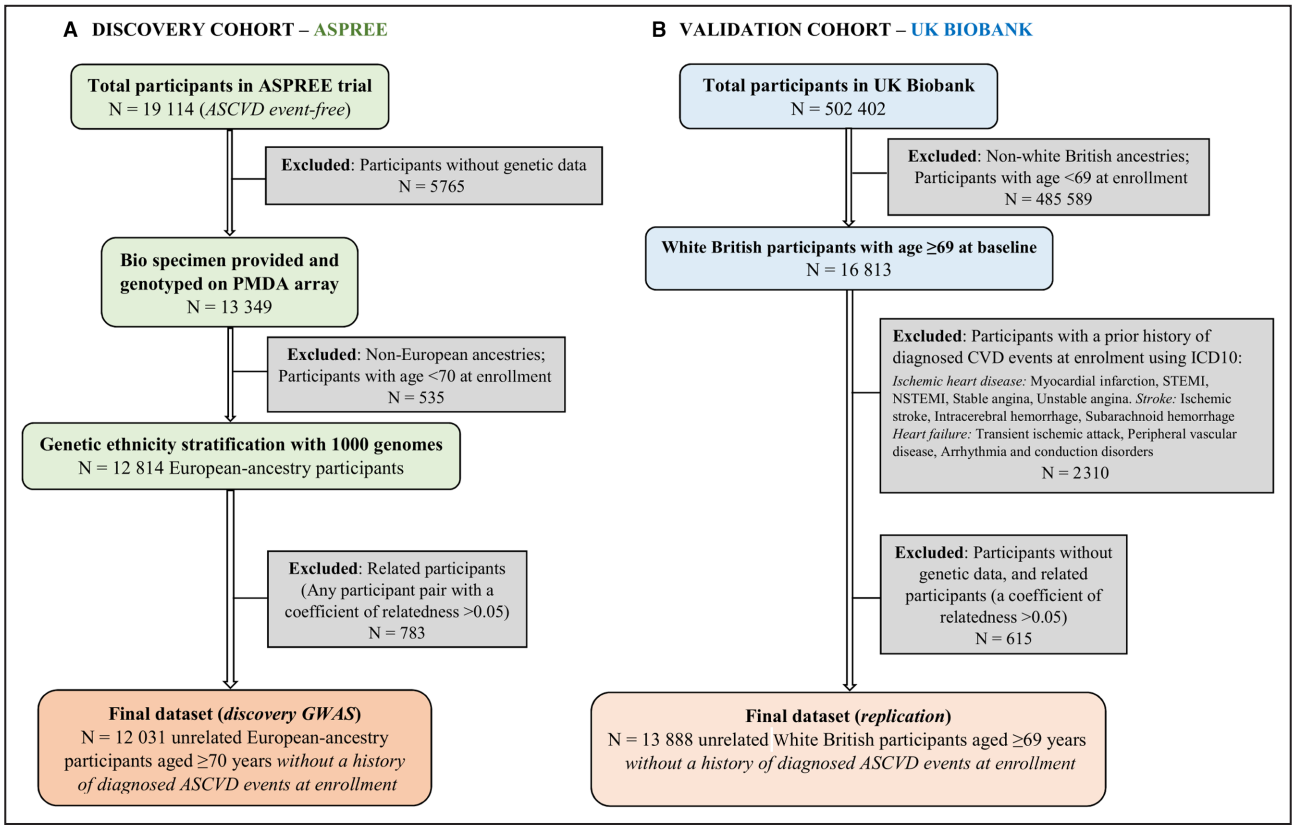

Figure 1. Flowchart of selection of the final data sets in the ASPREE trial (A) and UK Biobank (B) for the GWAS.
ASCVD indicates atherosclerotic CVD; ASPREE, Aspirin in Reducing Events in the Elderly; CVD, cardiovascular disease; GWAS, genome-wide association study; *ICD-10*, *International Classification of Diseases, Tenth Revision*; NSTEMI, non–ST-segment–elevation myocardial infarction; PMDA, precision medicine diversity array; and STEMI, ST-segment–elevation myocardial infarction.

review boards in the United States and registered on https://www.clinicaltrials.gov (NCT01038583).

For the GWAS replication, we selected a subgroup of older ASCVD event-free individuals from the UK Biobank, aged ≥69 years at enrollment (73 years was the upper age limit of UK Biobank recruitment).[13] This included 13 888 genotyped, unrelated White British individuals, aged ≥69 years, with no personal history of diagnosed cardiovascular events at enrollment (no prevalent ASCVD) (Figure 1 and Data S1). Analysis of the UK Biobank was approved under project identifier 47061.

Predicted ASCVD Risk as a Derived Quantitative Variable for GWAS

We used the risk model SCORE2-OP recently developed and validated to estimate the 10-year risk of cardiovascular disease in individuals aged ≥70 years.[16] A person-specific SCORE2-OP score can be derived for each participant, using a weighted linear combination of age (per year), diabetes status (yes/no), current smoking status (yes/no), systolic blood pressure (per mm Hg), total cholesterol (TC) (per mmol/L), and high-density lipoprotein cholesterol (HDL-C) (per mmol/L) for men and women, separately (Table S1 and Data S1). We modified the SCORE-OP equation to focus on risk associated with lipid metabolism (SCORE2-OP-*Lipid*), including only the variables of age, TC, HDL-C, interaction between age and TC, and interaction between age and HDL-C (for men and women, separately) (Table S1). For details of laboratory measurement of TC, HDL-C, and LDL-C, see Data S1.

Genetic Data and GWAS

DNA samples provided by ASPREE trial participants were genotyped using the Axiom 2.0 Precision Medicine Diversity Research Array (Thermo Fisher Scientific, CA). Data were processed and analyzed following standard protocols (Data S1). Genotyping and imputation of UK Biobank samples have been described previously.[13] Using a linear regression model, adjusted for the first 20 ancestry PCs to account for population stratification (model 1), we tested for single-variant associations between minor alleles and the SCORE2-OP-*Lipid* score as a derived quantitative variable, using the additive genetic model. We performed sensitivity analyses using model 2 (adjusted for the first 20 PCs, lipid-lowering statin therapy [a statin reductase

inhibitor] use, and aspirin allocation in ASPREE trial), and model 3 (further adjusted for the other variables used in SCORE2-OP; ie, diabetes status, current smoking status, and systolic blood pressure) (Data S1). To explore the evidence of multiple independent signals within or surrounding the identified locus, we performed conditional analyses in which the top single-nucleotide polymorphism (SNP) was included as a covariate in the linear regression model.

We repeated the same GWAS analyses, including the calculation of the new variable SCORE2-OP-*Lipid*, in the UK Biobank population (ASCVD-free participants aged ≥69 years at enrollment only) to replicate the findings.

Variant Carrier Status, Plasma Lipid Levels, and Incident ASCVD Risk

After performing GWAS, we used linear regressions to examine associations between carrier status for the identified variants and baseline plasma TC, HDL-C, LDL-C, and non–HDL-C levels, adjusting for age, sex, first 20 PCs, and statin use at baseline (in ASPREE trial) or cholesterol-lowering medication (in the UK Biobank). We also tested for associations between carrier status and risk of incident ASCVD events (fatal and nonfatal myocardial infarction, and death attributable to coronary heart diseases) in the ASPREE trial population during a median of 6.4 years of follow-up, using Cox proportional hazards models and an additive genetic model (noncarriers=0, heterozygous carriers=1, and homozygous carriers=2) adjusting for the same covariates. We did not include aspirin treatment as a covariate as we found no evidence that aspirin was associated with risk of cardiovascular events in the ASPREE trial cohort,[11] and the aspirin treatment was only provided for a median of 4.7 years of follow-up, not applicable to the longer ASPREE-XT study.[17] In the UK Biobank population, we tested for associations between carrier status for the same variants and lifetime risk of ASCVD events for all White British individuals with genetic data available across all ages at enrollment (N=430 139), using logistic regression models and an additive genetic model (Data S1). We tested for associations between the identified GWAS SNPs and plasma apolipoprotein B (apoB) levels in the UK Biobank (apoB levels not yet measured in ASPREE trial).

Expression Quantitative Trait Loci Analysis

After performing GWAS, to investigate whether the identified SNPs were predicted to regulate gene expression levels (and if so, across what genes and tissues), we performed expression quantitative trait loci (eQTL) analysis using the Genotype-Tissue Expression portal, following standard protocols.[18]

Variant Carrier Status and Age-Related Macular Degeneration

Genetic *CETP* (cholesteryl ester transfer protein) gene inhibition has been reported to be associated with increased risk of self-reported age-related macular degeneration (AMD)[19,20]; however, clinical results have been conflicting.[21,22] We investigated associations between the lead SNPs identified by GWAS with the risk of prevalent AMD in the ASPREE trial population. In a subset of 3917 participants in whom both retinal imaging and genetic data were available,[23] we used logistic regression models, adjusted for age, sex, first 20 PCs, and smoking status, to test associations between carrier status for SNPs of interest and risk of AMD at baseline, detected on nonmydriatic 45° macular images, following methods described previously[23] (Data S1). A flowchart showing an overview of the study is shown in Figure 2.

RESULTS

Baseline Characteristics and Risk Scores

The baseline characteristics of the ASPREE trial and UK Biobank selected populations are shown in Table 1 (including variables used for calculating SCORE2-OP-*Lipid*). The mean (SD) age of ASPREE trial participants was 75.1 (4.2) years, and 6606 (54.9%) were women. The mean (SD) age of the UK Biobank subpopulation was 69.1 (0.3) years, and 7285 (52.4%) were women. The 2 populations had similar mean plasma blood lipid levels (TC, HDL-C, and LDL-C) at baseline.

The risk scores SCORE2-OP and SCORE2-OP-*Lipid* for the 12 031 ASPREE trial participants follow a bell-shaped distribution, with a shift rightward in the risk distribution toward men (Figure S2). Correlations between the distributions of SCORE2-OP, SCORE2-OP-*Lipid*, and 3 baseline plasma blood-lipid measurements (TC, HDL-C, and LDL-C) are shown in Figure S3 for the ASPREE trial population. SCORE2-OP and SCORE2-OP-*Lipid* distributions were highly correlated (r=0.85), yet distinctly different, because of the customization of SCORE-OP-*Lipid* toward risk conferred specifically by blood lipid levels.

Genome-Wide Association Study

The discovery GWAS identified 8 SNPs above the genome-wide significance threshold ($P<5\times10^{-8}$) for the SCORE2-OP-*Lipid* analysis (Figure 3 and Table 2). Sensitivity analyses (models 2 and 3) showed that the signals were not changed ($P<5\times10^{-8}$) when adding relevant covariates to the models, including aspirin

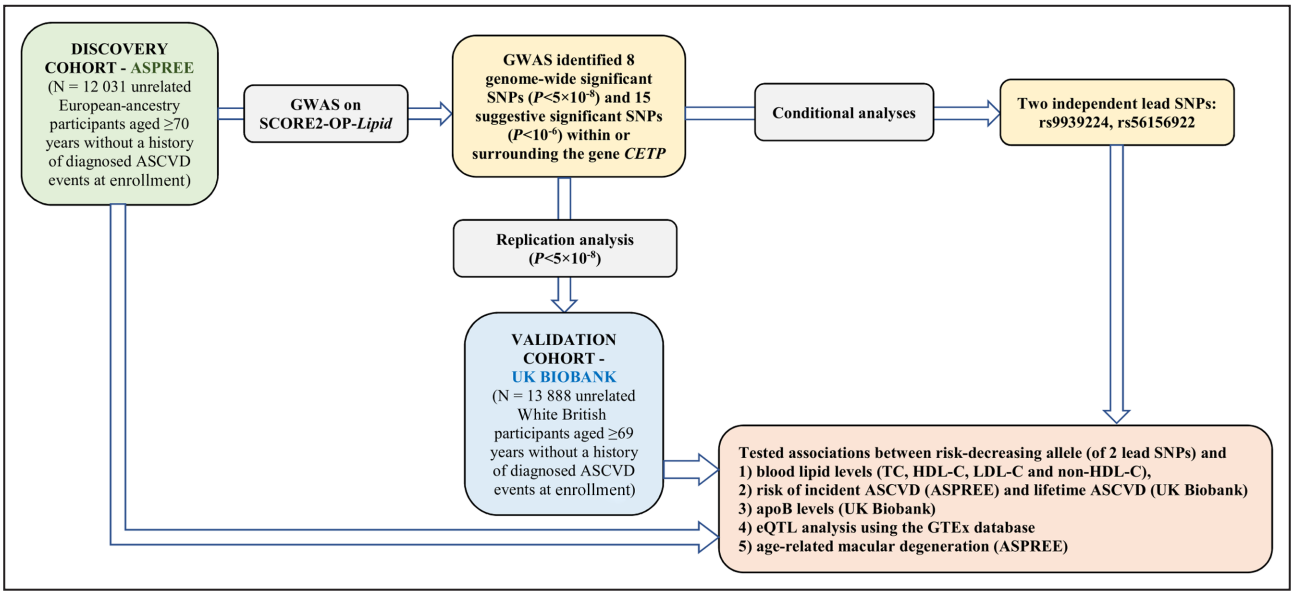

Figure 2. Flowchart of the main analysis.
apoB indicates apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; ASPREE, Aspirin in Reducing Events in the Elderly; eQTL, expression quantitative trait loci; GTEx, Genotype-Tissue Expression; GWAS, genome-wide association study; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SCORE2-OP, Systematic Coronary Risk Evaluation 2–Older People; SNP, single-nucleotide polymorphism; and TC, total cholesterol.

allocation (Figure 3 and Table S2). Quantile-quantile plots and genomic inflation factors (no evidence of inflation) for these GWASs are shown in Figure S4. No genome-wide significant signals were detected in the analysis of the phenotype SCORE2-OP in its full and original form, which included blood pressure, smoking status, and diabetes (Figure S5).

All of the 8 identified SNPs above the genome-wide significance threshold for SCORE2-OP-*Lipid* were located within noncoding regions of the *CETP* gene, on chromosome 16, position 56 961 923 to 56 983 845 in GRCh38 (Table 2 and Figure 4). All of the minor *CETP* alleles were positively associated with SCORE2-OP-*Lipid* (β>0; Table 2), meaning the major (more common) alleles were enriched in frequency and associated with lower SCORE2-OP-*Lipid* scores, therefore inferred to be associated with lower ASCVD risk (putatively cardioprotective).

To perform an independent replication of the GWAS, we repeated the analysis in the UK Biobank–selected subpopulation of ASCVD event-free individuals aged ≥69 years. All 8 identified genome-wide significant *CETP* SNPs from the discovery GWAS were validated above the genome-wide statistical significance threshold in the UK Biobank population (Table S2). One SNP (rs118060412) in chromosome 11 was identified above the genome-wide significance threshold in the ASPREE trial in model 1 (P=4.8×10^{-8}) but not in models 2 and 3 (Figure 3), and was not replicated in the UK Biobank (P=0.71).

Table 1. Baseline Characteristics of Study Participants

Characteristics	ASPREE trial (n=12 031)	UK Biobank (n=13 888)
Age, y		
Mean (SD)	75.1 (4.2)	69.1 (0.3)
Minimum, maximum	70, 96	69, 73
Sex, n (%)		
Men	5426 (45.1)	6603 (47.5)
Women	6606 (54.9)	7285 (52.4)
TC, mean (SD), mmol/L	5.3 (1.0)	5.7 (1.2)
HDL-C, mean (SD), mmol/L	1.6 (0.5)	1.5 (0.4)
LDL-C, mean (SD), mmol/L	3.1 (0.9)	3.5 (0.9)

ASPREE indicates Aspirin in Reducing Events in the Elderly; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and TC, total cholesterol.

Conditional Analysis of GWAS SNPs

We expanded the analysis to include 15 additional SNPs in linkage disequilibrium r^2≥0.1 with the lead GWAS SNP (rs9939224). These additional SNPs were not genome-wide significant (P<5×10^{-8}) but passed a lower/suggestive statistical significance threshold of P<10^{-6} (Table 2). We explored whether the 23 GWAS and additional SNPs were independent or conditional on the lead GWAS SNP (rs9939224). Using rs9939224 as a covariate, we found that 13 of the 23 SNPs, in which minor alleles were negatively associated with the SCORE2-OP-*Lipid* (β<0), were independent of

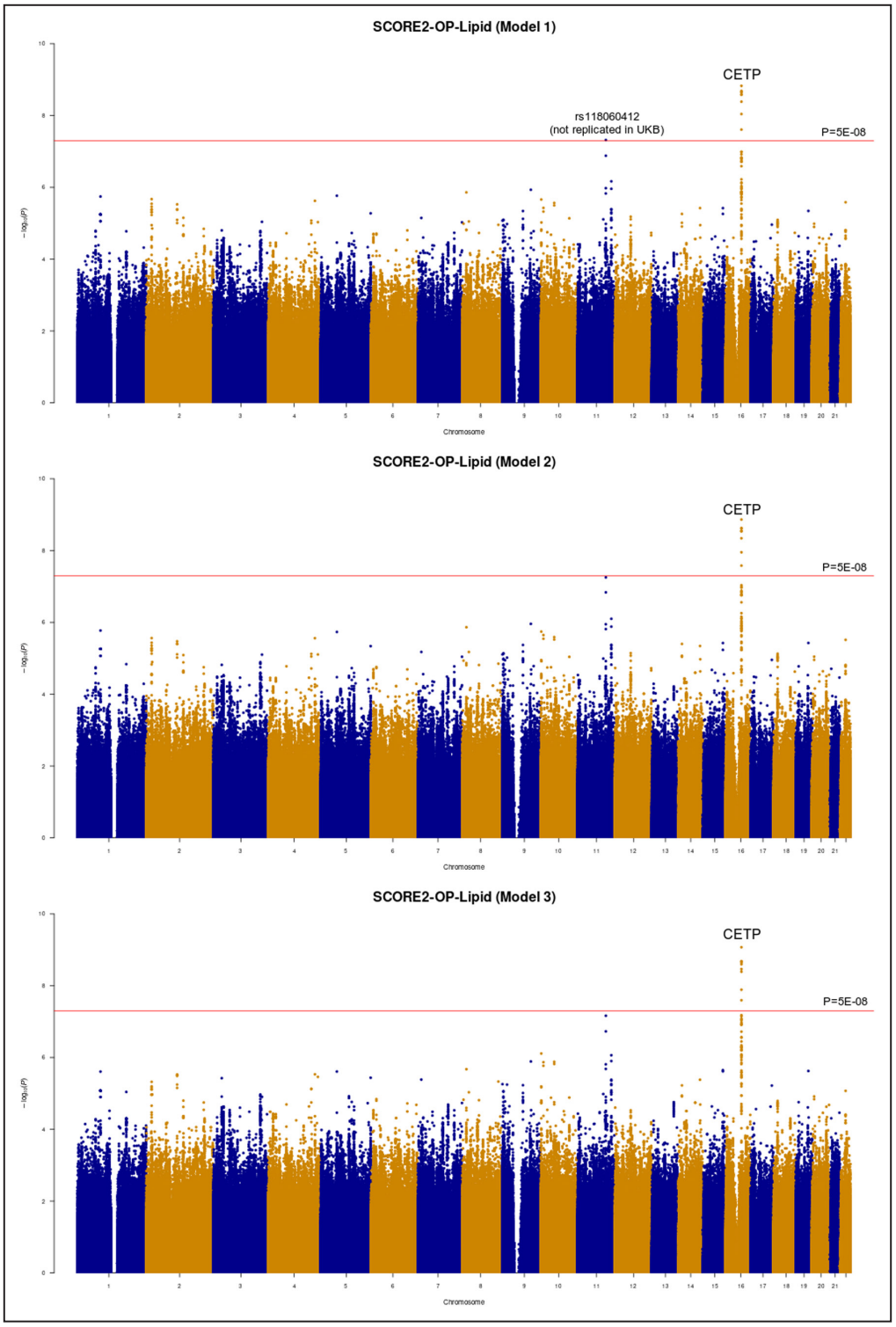

Figure 3. Genome-wide Manhattan plots displaying genome-wide association study results for SCORE2-OP-*Lipid* as a derived quantitative variable in the ASPREE trial population.
Linear regression models were used to test for associations between minor alleles and SCORE2-OP-*Lipid* scores with additive effect. Model 1 was adjusted for the first 20 genetic principle components. As sensitivity analyses, model 2 was adjusted for the first 20 genetic principle components, statin use, and aspirin allocation in the ASPREE trial, and model 3 was further adjusted for diabetes status, current smoking status, and systolic blood pressure. The horizontal red line denotes a genome-wide significance level (P=5×10^{-8}). ASPREE indicates Aspirin in Reducing Events in the Elderly; SCORE2-OP, Systematic Coronary Risk Evaluation 2–Older People; and UKB, UK Biobank.

Table 2. Genome-Wide Significant ($P<5\times10^{-8}$) and Suggestive Significant ($P<10^{-6}$) Variants in the *CETP* Gene Region in the ASPREE Trial Population

Chromosome	Position	SNP identifier	REF (major)	ALT (minor)	Gene region	MAF in gnomAD*	MAF in ASPREE trial	β	SE	*P* value	*P* value conditional on rs9939224	*P* value conditional on rs56156922
16	56968820	rs9939224	G	T	*CETP*-intron	0.21	0.20	0.098	0.016	1.49×10^{-9}	…	
16	56966973	rs12720922	G	A	*CETP*-intron	0.18	0.18	0.103	0.017	2.21×10^{-9}	0.25	
16	56965346	rs7203984	A	C	*CETP*-intron	0.20	0.19	0.100	0.017	2.26×10^{-9}	0.15	
16	56972678	rs7499892	C	T	*CETP*-intron	0.18	0.17	0.104	0.017	2.59×10^{-9}	0.22	
16	56966784	rs8045855	T	A	*CETP*-intron	0.19	0.18	0.102	0.017	2.65×10^{-9}	0.26	
16	56972466	rs11076175	A	G	*CETP*-intron	0.18	0.17	0.102	0.017	4.11×10^{-9}	0.31	
16	56972917	rs289713	A	T	*CETP*-intron	0.19	0.18	0.097	0.017	9.10×10^{-9}	0.28	
16	56963321	rs1864163	G	A	*CETP*-intron	0.26	0.26	0.084	0.015	2.47×10^{-8}	0.06	
16	56953457	rs56156922	T	C	None	0.33	0.33	−0.074	0.014	1.02×10^{-7}	5.26×10^{-4}	…
16	56953853	rs56228609	C	T	None	0.32	0.32	−0.075	0.014	1.04×10^{-7}	5.26×10^{-4}	0.56
16	56960616	rs17231506	C	T	*CETP* 2-kilobases upstream	0.32	0.33	−0.074	0.014	1.21×10^{-7}	6.70×10^{-4}	0.93
16	56959412	rs3764261	C	A	None	0.32	0.33	−0.073	0.014	1.49×10^{-7}	7.80×10^{-4}	0.87
16	56959249	rs12149545	G	A	None	0.31	0.32	−0.074	0.014	1.52×10^{-7}	6.94×10^{-4}	0.76
16	56955678	rs247616	C	T	None	0.32	0.33	−0.073	0.014	1.54×10^{-7}	8.01×10^{-4}	0.69
16	56956804	rs247617	C	A	None	0.32	0.33	−0.073	0.014	1.78×10^{-7}	8.87×10^{-4}	0.55
16	56957451	rs183130	C	T	None	0.32	0.33	−0.072	0.014	1.94×10^{-7}	9.48×10^{-4}	0.49
16	56953103	rs12446515	C	T	None	0.32	0.33	−0.072	0.014	1.95×10^{-7}	9.31×10^{-4}	0.37
16	56967026	rs118146573	G	A	*CETP*-intron	0.12	0.12	0.103	0.020	2.58×10^{-7}	0.32	
16	56965006	rs12720926	A	G	*CETP*-intron	0.43	0.43	−0.066	0.013	6.01×10^{-7}	1.04×10^{-2}	0.13
16	56973539	rs289714	A	G	*CETP*-intron	0.19	0.17	0.086	0.017	7.36×10^{-7}	0.09	
16	56954132	rs173539	C	T	None	0.33	0.33	−0.068	0.014	7.70×10^{-7}	1.35×10^{-3}	0.25
16	56970977	rs7205804	G	A	*CETP*-intron	0.42	0.42	−0.065	0.013	9.21×10^{-7}	1.49×10^{-2}	0.20
16	56967304	rs4784741	C	T	*CETP*-intron	0.44	0.44	−0.065	0.013	9.91×10^{-7}	1.41×10^{-2}	0.17

Linear regression models were used to test for associations between minor alleles and Systematic Coronary Risk Evaluation 2–Older People–*Lipid* scores with additive effects, adjusted for the first genetic principle components (model 1). Conditional analyses for 2 lead SNPs were performed using the same model. ALT indicates alternative allele; ASPREE, Aspirin in Reducing Events in the Elderly; gnomAD, Genome Aggregation Database; MAF, minor allele frequency; REF, reference allele; and SNP, single-nucleotide polymorphism.

*Denotes MAF in European (non-Finnish) in gnomAD, version 3.1.2.

rs9939224 (conditional $P<0.05$, considered marginal significance). These 13 SNPs were also replicated above the genome-wide statistical significance threshold in the UK Biobank population (Table S3). For the 13 SNPs, we further performed conditional analyses using their lead SNP (rs56156922) as a covariate but did not find any new independent signal (conditional $P>0.05$; Table 2). We, therefore, focused subsequent analyses on the 2 independent lead SNPs (rs9939224 and rs56156922; Figure 4).

CETP Allele Carrier Status and Blood Lipid Levels

We examined associations between carrier status for the 2 lead *CETP* SNPs and baseline plasma lipid levels in the ASPREE trial (TC, HDL-C, LDL-C, and non–HDL-C) (Table 3). For the rs9939224-G major allele, carrier status was associated with significantly higher plasma HDL-C (β=0.1, SE=0.007, $P=1.66\times10^{-48}$) and significantly lower LDL-C (β=−0.07, SE=0.013, $P=1.76\times10^{-7}$) levels, suggesting a favorable shift in cholesterol metabolism related to ASCVD risk modification. Carrier status for the second SNP (rs56156922-C) minor alleles was also significantly associated with higher plasma HDL-C levels and lower LDL-C levels (Table 3). Most associations between carrier status and HDL-C and LDL-C levels were replicated in the UK Biobank population in the same direction ($P<0.05$), with the exception of rs9939224-G ($P=0.613$).

CETP Allele Carrier Status and Incident ASCVD Risk

During a median of 6.4 years of follow-up in the ASPREE trial (N=12 031), 401 incident ASCVD events occurred. This included 325 fatal and nonfatal myocardial infarctions and 76 cases of other coronary heart disease death. We examined associations between the 2 risk prediction scores (SCORE2-OP and SCORE2-OP-*Lipid*) and risk of these incident events. The ASCVD events were more associated with SCORE2-OP-*Lipid*

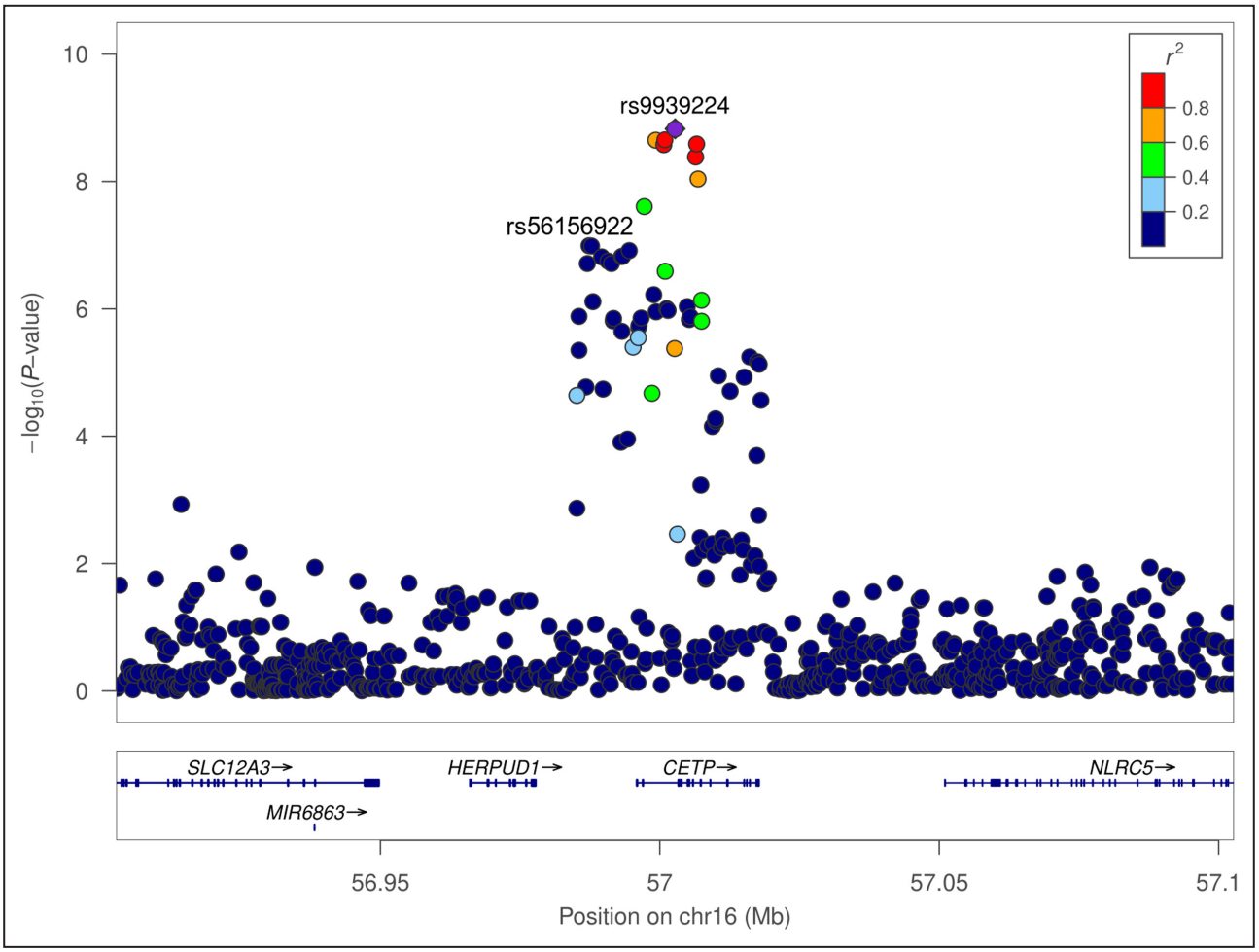

Figure 4. Locus zoom plot showing genome-wide significant and suggestive significant SNPs (including 2 lead SNPs) by model 1 within or surrounding the gene *CETP* region.
r^2 Denotes the linkage disequilibrium of variants with the lead SNP, rs9939224. Chr indicates chromosome; and SNPs, single-nucleotide polymorphisms.

(hazard ratio [HR]=18.84 [95% CI, 9.31–38.15]; P=3.41×10^{-16}) compared with the original SCORE2-OP score (HR=2.76 [95% CI, 1.91–4.00]; P=6.54×10^{-8}), suggesting a role driven by lipids in ASCVD risk.

The proportions (percentages) of ASCVD events per genotype at the 2 lead *CETP* SNPs are shown in Table 4. We found that carrier status for the 2 *CETP* variants of interest was associated with lower incident ASCVD risk, versus noncarriers (Table 4). This included an almost 15% risk reduction for rs9939224-G carriers (HR=0.86 [95% CI, 0.73–1.02]; P=0.08) and rs56156922-C carriers (HR=0.84 [95% CI, 0.73–0.98]; P=0.03). We tested for genotype-by-age and genotype-by-sex interactions in these associations but found no statistically significant interactions (P>0.05; Table S4). After adjustment for baseline plasma lipid levels (TC, HDL-C, and LDL-C), the associations between *CETP* allele carrier status and ASCVD risk were no longer statistically significant (Table S5). This indicates that the *CETP* variants and blood lipid levels are not independent and that the effects of the *CETP* variants in modifying ASCVD risk are likely to be mediated via blood lipid levels.

A cumulative analysis of the additive dosage effect of carrying between 0 and 4 of the identified *CETP* alleles (from the lead 2 SNPs) for incident ASCVD risk (Table 4) identified a clear downward trend (P=0.019 in a linear trend test; Figure 5) in lower ASCVD risk as more cumulative risk-decreasing alleles were carried. Participants who carried all 4 *CETP* alleles (N=1295, homozygous at each SNP, rs9939224-GG and rs56156922-CC) had the most benefit and were associated with significantly lower risk of incident ASCVD events than those who carried 0 of the alleles (N=492) (HR=0.51 [95% CI, 0.24–0.82]; P=0.019; Figure 5).

We then examined the effects of *CETP* allele carrier status on lifetime risk of ASCVD in the UK Biobank population. We included all UK Biobank participants (White British ancestry) of all ages (N=430 139). In this

Table 3. Association of Carrier Status for 2 Putatively Risk-Decreasing *CETP* Alleles With Baseline Blood Lipid Levels for ASCVD Event-Free Older Individuals in the ASPREE Trial and UK Biobank Populations

SNP genotype	rs9939224			rs56156922		
	G*G*	G*T	TT	TT	TC*	C*C*
ASPREE trial						
No. of genotype carriers	7667	3860	504	5363	5347	1321
TC, mean (SD), per mmol/L	5.28 (0.97)	5.25 (0.98)	5.23 (0.98)	5.24 (0.97)	5.28 (0.98)	5.33 (0.97)
HDL-C, mean (SD), per mmol/L	1.63 (0.47)	1.53 (0.43)	1.43 (0.40)	1.52 (0.43)	1.61 (0.46)	1.73 (0.49)
LDL-C, mean (SD), per mmol/L	3.05 (0.87)	3.13 (0.88)	3.15 (0.86)	3.11 (0.87)	3.07 (0.88)	3.01 (0.86)
Non–HDL-C, mean (SD), per mmol/L	3.66 (0.93)	3.73 (0.94)	3.80 (0.94)	3.72 (0.93)	3.68 (0.94)	3.61 (0.93)
Association of TC with risk-decreasing allele	β (SE)=0.027 (0.015), P=0.063			β (SE)=0.043 (0.013), P=0.001		
Association of HDL-C with risk-decreasing allele	β (SE)=0.100 (0.007), $P=1.66\times10^{-48}$			β (SE)=0.096 (0.006), $P=2.16\times10^{-61}$		
Association of LDL-C with risk-decreasing allele	β (SE)=−0.070 (0.013), $P=1.76\times10^{-7}$			β (SE)=−0.046 (0.011), $P=6.26\times10^{-5}$		
Association of non–HDL-C with risk-decreasing allele	β (SE)=−0.075 (0.015), $P=2.71\times10^{-7}$			β (SE)=−0.052 (0.013), $P=3.08\times10^{-5}$		
UK Biobank						
No. of genotype carriers	8747	4552	589	6462	6033	1393
TC, mean (SD), per mmol/L	5.72 (1.21)	5.64 (1.21)	5.57 (1.22)	5.64 (1.21)	5.73 (1.20)	5.76 (1.23)
HDL-C, mean (SD), per mmol/L	1.50 (0.39)	1.42 (0.37)	1.33 (0.34)	1.41 (0.36)	1.49 (0.39)	1.61 (0.42)
LDL-C, mean (SD), per mmol/L	3.54 (0.91)	3.54 (0.92)	3.53 (0.92)	3.54 (0.92)	3.55 (0.90)	3.48 (0.91)
Non–HDL-C, mean (SD), per mmol/L	4.21 (1.11)	4.22 (1.11)	4.23 (1.12)	4.22 (1.12)	4.23 (1.10)	4.13 (1.12)
Association of TC with risk-decreasing allele	β (SE)=0.071 (0.017), $P=1.58\times10^{-5}$			β (SE)=0.063 (0.014), $P=1.10\times10^{-5}$		
Association of HDL-C with risk-decreasing allele	β (SE)=0.084 (0.006), $P=9.52\times10^{-52}$			β (SE)=0.093 (0.005), $P=1.41\times10^{-82}$		
Association of LDL-C with risk-decreasing allele	β (SE)=−0.006 (0.013), P=0.613			β (SE)=−0.022 (0.011), P=0.044		
Association of non–HDL-C with risk-decreasing allele	β (SE)=−0.017 (0.016), P=0.292			β (SE)=−0.032 (0.014), P=0.024		

In the ASPREE trial population, the associations of baseline blood lipid levels with putatively risk-decreasing alleles were assessed by linear regression, adjusted for age, sex, first 20 genetic principle components, and statin use at baseline. In the UK Biobank population, models were adjusted for age, sex, first 20 genetic principle components, and cholesterol-lowering medication at baseline. ASCVD indicates atherosclerotic cardiovascular disease; ASPREE, Aspirin in Reducing Events in the Elderly; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SNP, single-nucleotide polymorphism; and TC, total cholesterol.

*Denotes the putatively risk-decreasing alleles.

younger and larger population, there were N=80 488 ASCVD events, including hospitalized records in a lifetime, myocardial infarction reports, and coronary heart disease death events. Each of the identified *CETP* variants of interest was significantly associated with reduced risk of lifetime ASCVD events in the UK Biobank population (P<0.01), albeit with modest effect sizes (odds ratio>0.9) (Table 5).

CETP Allele Carrier Status and apoB Levels

Some studies suggest a clinical benefit in lowering LDL-C levels mediated by reduction in the concentration of apoB-containing particles.[24,25] Therefore, we examined the effects of the identified *CETP* alleles on plasma apoB levels in the UK Biobank population (N=430 139). We found that the 2 risk-decreasing *CETP* alleles (lead SNPs) were significantly associated with lower apoB levels in the UK Biobank (Figure S6 and Table S6). Measured apoB levels were not available for the ASPREE trial cohort.

eQTL Analysis

To investigate whether the 2 identified lead SNPs regulate *CETP* gene expression, and if so in what tissue, we performed eQTL analysis.[18] Our hypothesis was that the identified *CETP* variants would be associated with reduced *CETP* gene expression (suggesting genetic *CETP* deficiency). The eQTL analysis (Table S7) predicted rs9939224 reduces *CETP* mRNA levels in heart tissue (atrial appendage) ($P=1.1\times10^{-5}$) and *NLRC5* mRNA levels in cultured fibroblasts ($P=8.4\times10^{-11}$). The analysis also predicted rs56156922 reduces *CETP* mRNA levels across a range of different tissues (P values ranging from 4.8×10^{-12} to 1.2×10^{-4}), including the heart, lung, adipose tissue, stomach, and colon. The rs56156922 was predicted to reduce *NLRC5* mRNA levels in cultured fibroblasts ($P=9\times10^{-28}$).

CETP Allele Carrier Status and AMD

CETP inhibition has previously been reported to be associated with an increased risk of self-reported AMD[19,20]; however, results have been contrary to available clinical

Table 4. Association of Carrier Status for 2 Putatively Risk-Decreasing *CETP* Alleles With Incident ASCVD Risk in the ASPREE Trial Population

SNP	Genotype	No. in total (%) of incident ASCVD events	HR (95% CI), *P* value
rs9939224	G*G*	241 in 7667 (3.1)	0.863 (0.731–1.020), *P*=0.084
	G*T	140 in 3860 (3.6)	
	TT	20 in 504 (4.0)	
rs56156922	TT	191 in 5363 (3.6)	0.844 (0.726–0.981), *P*=0.027
	TC*	180 in 5347 (3.4)	
	C*C*	30 in 1321 (2.3)	
Combination of 2 SNPs	Cumulative dosage of 2 risk-decreasing alleles (category from 0 to 4)		0.889 (0.808–0.978), *P*=0.016
	0	20 in 492 (4.1)	
	1	81 in 2261 (3.6)	
	2	149 in 4207 (3.5)	
	3	121 in 3776 (3.2)	
	4	30 in 1295 (2.3)	

The HR (95% CI) and *P* value were estimated using the Cox model with additive allele effects, adjusted for age, sex, first 20 genetic principle components, and statin use at baseline. ASCVD indicates atherosclerotic cardiovascular disease; ASPREE, Aspirin in Reducing Events in the Elderly; HR, hazard ratio; and SNP, single-nucleotide polymorphism.

*Denotes the putatively risk-decreasing alleles.

evidence.[21,22] Therefore, we investigated associations between the 2 lead SNPs with risk of prevalent AMD in the ASPREE trial, as detected by nonmydriatic color digital images in a subset of 3917 participants in whom both retinal imaging and genetic data were available.[23] In this subcohort, there were 795 cases of early AMD, 623 cases of intermediate AMD, 46 cases of late AMD, and 2453 participants with no signs of AMD at baseline. We found no associations between *CETP* allele carrier status and any stage of AMD (all *P*>0.05) (Tables S8 and S9).

DISCUSSION

The main finding in this study was the implication of the *CETP* gene region in cardioprotection and ASCVD resilience. We identified 2 independent GWAS signals in the *CETP* gene region, implicating the *CETP* gene in ASCVD resilience. Our approach differed from previous studies[26–28] implicating the same region by focusing on clinically affected individuals with ASCVD. Carrier status for the identified *CETP* variants (presumed to be inhibiting *CETP* and causing genetic *CETP* deficiency[29]) was associated with beneficial outcomes, including higher plasma HDL-C levels, lower plasma LDL-C levels, lower apoB levels, and reduced risk of ASCVD events. The effects did not appear to be age or sex specific. In a subcohort of ASPREE trial participants based on retinal imaging data, carrier status for the 2 putatively protective *CETP* alleles was not associated with increased AMD risk. Although SCORE2-OP-*Lipid* has a nontrivial correlation with HDL-C by definition, the GWAS for SCORE2-OP-*Lipid* did not detect other known HDL-C–related genes, such as *LPL*, *LIPC*, and *ZPR1*,[30] suggesting the specificity of our GWAS design for ASCVD risk. Our study provides a novel GWAS method for the detection of protective variants and presents new evidence to inform the ongoing consideration of *CETP* as a potential drug target for inhibition in ASCVD.

Our main GWAS results implicating the *CETP* gene region in the phenotype of cardiovascular resilience are biologically plausible given prior evidence available from both animal[31–33] and human studies.[29,34] The *CETP* gene is a well-known and important regulator of LDL-C, HDL-C, and lipoprotein(a) levels[29,34] and is a contemporary drug target for inhibition in ASCVD.[21,22] The effects of *CETP* inhibition (either therapeutically or through naturally occurring genetic variation) have been well documented.[35–37] The most obvious explanation for our findings, therefore, is that genetic inhibition of *CETP* by the polymorphisms identified results in *CETP* deficiency, leading to increased plasma HDL-C and lower LDL-C levels, thereby reducing ASCVD risk. This hypothesis was supported by our eQTL analysis, which predicted the identified lead *CETP* SNPs to reduce *CETP* mRNA levels across various tissues.

However, the precise mechanisms by which *CETP* inhibition modifies ASCVD risk are contentious. Some previous studies of *CETP* genotypes have only assessed ASCVD risk in relation to HDL-C and LDL-C levels,[34,35] and other studies suggest clinical benefit in lowering LDL-C levels may be determined by the corresponding absolute reduction in the concentration of apoB-containing particles.[24,25] Our results demonstrate that the 2 *CETP* lead SNPs were significantly associated with lower apoB levels in the UK Biobank (albeit with modest effect

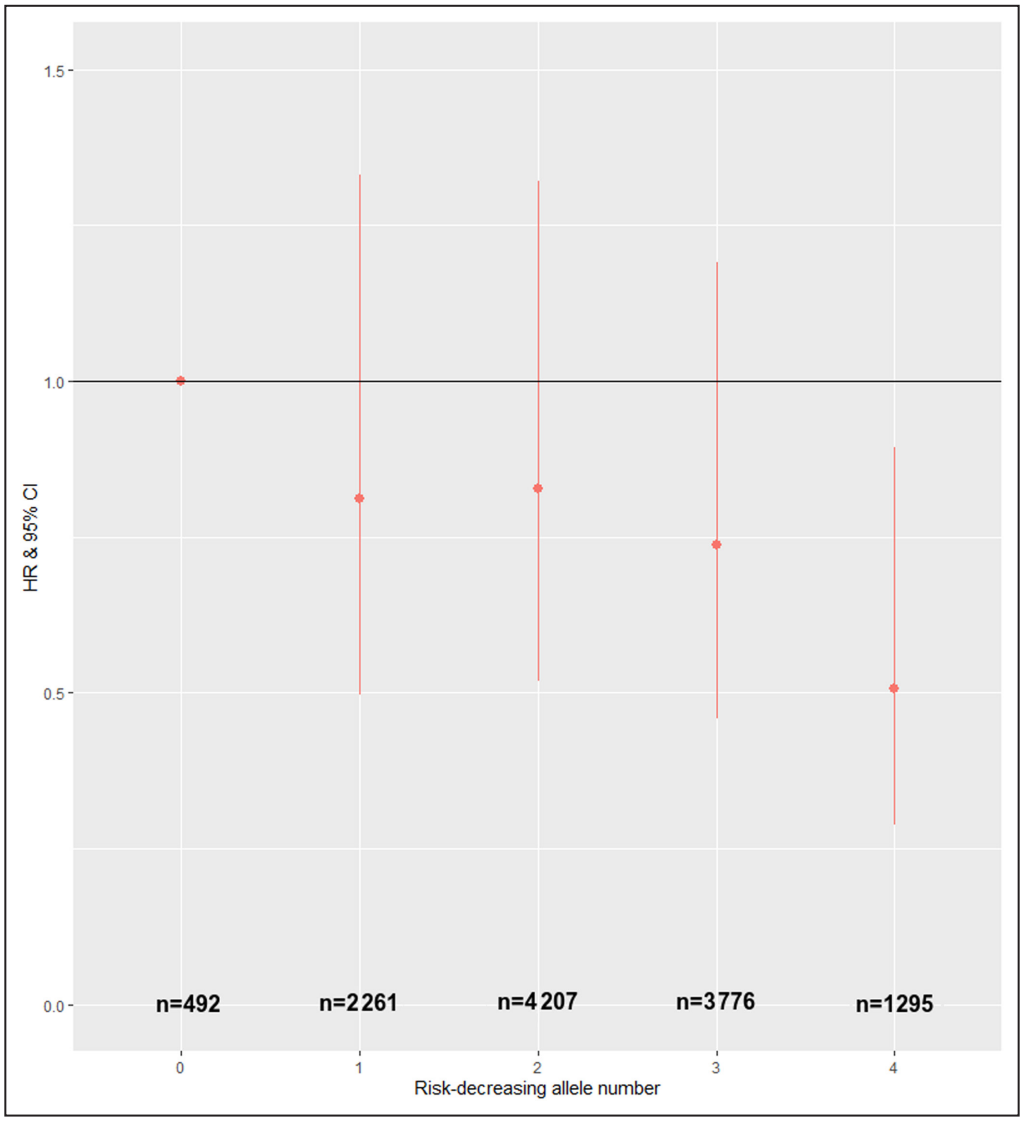

Figure 5. Association of carrier status for categories of cumulative dosage of 2 *CETP* risk-decreasing alleles with incident atherosclerotic cardiovascular disease risk in the ASPREE trial population.

The association (HR and 95% CI) was estimated using the Cox model, adjusted for age, sex, first 20 genetic principle components, and statin use at baseline. ASPREE indicates Aspirin in Reducing Events in the Elderly; HR, hazard ratio; and n, number of participants with a specific number of risk-decreasing alleles.

sizes), therefore providing evidence that genetic *CETP* inhibition may lead to modification of ASCVD risk through the regulation of apoB. However, modulation of lipoprotein(a) levels or other factors may also be contributing.

The clinical consequences of *CETP* therapeutic inhibition by newly developed drugs have been varied with regard to ASCVD risk modification.[34,38] The potential for *CETP* inhibition as a therapeutic strategy for ASCVD has been questioned[28,39] because of recent disappointing results of phase 3 clinical trials with reports of toxicity and clinical futility of new *CETP*-inhibiting therapeutic agents.[22,40–43] In addition, a recent Danish study suggested that genetic *CETP* deficiency, mimicking pharmacologic *CETP* inhibition, was associated with lower ASCVD risk but with an increased risk of AMD.[19] Our study could not replicate this finding, and our results do not suggest a relationship between genetic *CETP* inhibition and increased AMD risk (which is consistent with other recent reports[21,22]). Our analysis used retinal imaging data rather than self-reported AMD, providing a more robust measure of outcomes.

Other studies have reported genetic relationships between *CETP* inhibition and ASCVD risk, but using different epidemiologic approaches. For example, Ridker et al[44] reported associations between *CETP* polymorphisms and increased HDL-C levels, with reduced myocardial infarction risk in women, providing evidence to support our findings. The *CETP* variants

Table 5. Association of *CETP* Allele Carrier Status With Lifetime ASCVD Risk in the UK Biobank Population

SNP	Risk-decreasing allele	OR (95% CI)	*P* value
rs9939224	G (major)	0.980 (0.966–0.994)	4.60×10^{-3}
rs56156922	C (minor)	0.973 (0.961–0.985)	1.82×10^{-5}
Combination of 2 SNPs	Cumulative dosage of 2 risk-decreasing alleles (from 0 to 4)	0.982 (0.974–0.990)	1.91×10^{-5}

The association with OR (95% CI) and *P* value was estimated by the logistic models adjusted for age, sex, first 20 genetic principle components, and cholesterol-lowering medication. ASCVD indicates atherosclerotic cardiovascular disease; OR, odds ratio; and SNP, single-nucleotide polymorphism.

reported by Ridker et al[44] (eg, rs708272, rs4329913, and rs7202364) did not reach the suggestive significance threshold ($P<10^{-6}$) in our GWAS but were found to be in linkage disequilibrium with our 2 lead SNPs (rs9939224 or rs56156922). This suggests the underlying signal from both studies comes from the same *CETP* locus. More recently, a case-control study[45] using an exome chip detected a *CETP* variant (exm-rs1800775) associated with ASCVD risk ($P<5\times10^{-8}$). This SNP also did not reach the suggestive significance threshold in our GWAS but was also found to be in linkage disequilibrium with our lead SNP, rs56156922 (conditional $P=0.24$). These different studies implicating the same *CETP* locus provide evidence to support genetic *CETP* inhibition as a mechanism for ASCVD risk modification. Furthermore, Nomura et al,[46] in a meta-analysis of exome-sequencing studies, demonstrated that carriers of rare protein-truncating variants in the *CETP* gene, compared with noncarriers, had higher plasma HDL-C levels, lower LDL-C levels, and reduced ASCVD risk. This lends further support to the notion that naturally occurring (genetic) inhibition of *CETP* is associated with beneficial outcomes for ASCVD risk reduction.

The protective effects conferred by the identified *CETP* polymorphisms on plasma LDL-C, HDL-C, and apoB levels may be modest but would compound over the lifetime, even more than the longer-term beneficial effects of lipid-lowering therapy (such as statins). Further studies are warranted to better understand the relationship between naturally occurring genetic *CETP* inhibition and related mechanisms of cardioprotection.

Because there is currently no risk prediction score focusing on cardiovascular disease risk associated with lipid metabolism, we modified the original SCORE2-OP to propose the SCORE2-OP-*Lipid* to enrich the association signal for loci involved in the regulation of blood lipid levels. The ASCVD incident events in the ASPREE trial population were more associated with SCORE2-OP-*Lipid* (HR=18.84) compared with the original SCORE2-OP score (HR=2.76), suggesting a role driven by lipids in ASCVD risk. Further studies are warranted to recalibrate and validate this new score using external data to accurately estimate 10-year ASCVD risk in older adults (aged ≥70 years).

Strengths of our study include the availability of genetic data from 2 large, well-characterized populations of healthy older individuals, where the absence of diagnosed ASCVD events at enrollment was clinically verified. In the case of the ASPREE trial population, subsequent incident cardiovascular events were rigorously ascertained and formally adjudicated as part of a randomized trial. Furthermore, the availability of blood lipid levels and ASCVD outcomes in both cohorts enabled examination of the clinical and phenotypic effects of the identified *CETP* variants.

Study Limitations

Limitations of our study include the use of a modified SCORE2-OP-*Lipid* distribution as a GWAS quantitative trait, where weights from the original SCORE2-OP equation were not validated in the ASPREE trial or other external data, with regard to ASCVD risk prediction. We did not measure rare loss-of-function variants (including in the *CETP* gene region), which may further contribute to ASCVD risk reduction at the same loci. Also, we did not measure plasma *CETP* protein levels directly to examine whether the identified polymorphisms modified protein expression, nor did we measure plasma lipoprotein(a) levels to explore non–HDL-C/LDL-C–mediated effects. Although the GWAS for SCORE2-OP-*Lipid* did not detect any other HDL-C–related loci, we cannot exclude the possibility that the study was underpowered to detect these loci. Further investigations (eg, increasing sample size) are therefore warranted to understand the specificity of GWAS signals between HDL-C regulation and ASCVD risk modification. Finally, our GWAS was restricted to individuals of European descent, to avoid population stratification biases, which limits the generalizability of our findings.

CONCLUSIONS

Our study contributes new evidence implicating the *CETP* gene region in ASCVD risk modification during aging, produced without an a priori hypothesis on the *CETP* gene. This notion was generated by a new GWAS approach for identifying ASCVD risk-modifying common variants, leveraging the unique ascertainment of healthy older people. Our approach complements previous case-control GWASs and may also be applied to other complex diseases and sheds new light on the ongoing consideration of *CETP* inhibition as a therapeutic strategy for ASCVD.

ARTICLE INFORMATION

Received July 18, 2023; accepted October 5, 2023.

Affiliations

School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC, Australia (C.Y., A.B., L.R., R.L.W., R.W., L.T.T., J.J.M., A.M.T., S.J.N., P.L.); School of Medicine, University of Western Australia, Perth, WA, Australia (G.F.W.); Lipid Disorders Clinic, Cardiometabolic Service, Department of Cardiology, Royal Perth Hospital, Perth, WA, Australia (G.F.W.); Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY (A.E.R., B.F.-H., A.M.G.); Cardiovascular Research Center and Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA (P.N.); Program in Population and Medical Genetics and the Cardiovascular Disease Initiative, Broad Institute of Harvard and MIT, Cambridge, MA (P.N.); Department of Medicine (P.N.) and Preventive Medicine Division, Brigham and Women's Hospital (D.I.C.), Harvard Medical School, Boston, MA; Centre for Eye Research Australia, The University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia (L.R., R.G.); and Monash Cardiovascular Research Centre, Victorian Heart Institute, Monash University, Clayton, VIC, Australia (S.J.N.).

Acknowledgments

We thank the ASPREE (Aspirin in Reducing Events in the Elderly) trial staff and participants, and the general practitioners and staff of the medical clinics who cared for the participants. We also thank the UK Biobank participants and staff. Author contributions: Paul Lacaze initiated and conceived the project. Chenglong Yu performed all analyses. Paul Lacaze and Chenglong Yu drafted the manuscript.

Sources of Funding

The ASPREE (ASPirin in Reducing Events in the Elderly) trial Biobank is supported by a Flagship cluster grant (including the Commonwealth Scientific and Industrial Research Organisation, Monash University, Menzies Research Institute, Australian National University, University of Melbourne); and a grant (5U01AG29824-02) from the National Cancer Institute at the National Institutes of Health; and Monash University. The ASPREE project is supported by grants (U01AG029824 and U19AG062682) from the National Institute on Aging and the National Cancer Institute at the National Institutes of Health; and by grants (334047 and 1127060) from the National Health and Medical Research Council of Australia; and by Monash University and the Victorian Cancer Agency. Paul Lacaze is supported by a National Heart Foundation Future Leader Fellowship (102604). John J. McNeil is supported by a Leadership Fellowship from National Health and Medical Research Council of Australia (IG1173690). Robyn Guymer is supported by investigator grant 1194667 from National Health and Medical Research Council of Australia. Pradeep Natarajan is supported by grants from the National Heart, Lung, and Blood Institute at the National Institutes of Health (R01HL142711 and R01HL127564). Alan E. Renton, Brian Fulton-Howard, and Alison M. Goate are supported by a grant (U01AG058635) at the National Institutes of Health. The ASPREE-AMD (Age-Related Macular Degeneration) substudy was supported by the National Health and Medical Research Council of Australia (grant 1051625), National Eye Institute at the National Institutes of Health (grant R01EY026890), Monash University and Centre for Eye Research Australia.

Disclosures

A.M. Goate serves on the scientific advisory board at Genentech. Dr Tonkin has received research support from Bayer for materials in the ASPREE (Aspirin in Reducing Events in the Elderly) trial and honoraria for advisory board participation or lectures from Amgen, AstraZeneca, Boehringer-Ingelheim, and Pfizer. S.J. Nicholls has received research support from AstraZeneca, Amgen, Anthera, CSL Behring, Cerenis, Eli Lilly, Esperion, Resverlogix, Novartis, InfraReDx, and Sanofi-Regeneron and is a consultant for Amgen, Akcea, AstraZeneca, Boehringer Ingelheim, CSL Behring, Eli Lilly, Esperion, Kowa, Merck, Takeda, Pfizer, Sanofi-Regeneron, and Novo Nordisk. Dr Natarajan reports investigator-initiated grants from Amgen, Apple, AstraZeneca, Boston Scientific, and Novartis; personal fees from Apple, AstraZeneca, Blackstone Life Sciences, Foresite Labs, Novartis, and Roche/Genentech; is a cofounder of TenSixteen Bio; is a shareholder of geneXwell and TenSixteen Bio; and spousal employment at Vertex, all unrelated to the present work. R. Guymer reports honoraria for advisory board participation or lectures from Bayer, Novartis, Roche, Genentech, and Apellis. Bayer AG provided low-dose aspirin and placebo tablets for the clinical trial but had no other relationship with the work. The remaining authors have no disclosures to report.

Supplemental Material

Data S1
Tables S1–S9
Figures S1–S6
References 47–52

REFERENCES

1. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the global burden of disease study 2017. *Lancet*. 2018;392:1736–1788.
2. Christensen DM, Phelps M, Gerds T, Malmborg M, Schjerning AM, Strange JE, El-Chouli M, Larsen LB, Fosbøl E, Køber L, et al. Prediction of first cardiovascular disease event in 2.9 million individuals using Danish administrative healthcare data: a nationwide, registry-based derivation and validation study. *Eur Heart J Open*. 2021;1:oeab015. doi: 10.1093/ehjopen/oeab015
3. Tcheandjieu C, Zhu X, Hilliard AT, Clarke SL, Napolioni V, Ma S, Lee KM, Fang H, Chen F, Lu Y, et al. Large-scale genome-wide association study of coronary artery disease in genetically diverse populations. *Nat Med*. 2022;28:1679–1692. doi: 10.1038/s41591-022-01891-3
4. Koyama S, Ito K, Terao C, Akiyama M, Horikoshi M, Momozawa Y, Matsunaga H, Ieki H, Ozaki K, Onouchi Y, et al. Population-specific and trans-ancestry genome-wide analyses identify distinct and shared genetic risk loci for coronary artery disease. *Nat Genet*. 2020;52:1169–1177. doi: 10.1038/s41588-020-0705-3
5. Liu DJ, Peloso GM, Yu H, Butterworth AS, Wang X, Mahajan A, Saleheen D, Emdin C, Alam D, Alves AC, et al. Exome-wide association study of plasma lipids in >300,000 individuals. *Nat Genet*. 2017;49:1758–1766. doi: 10.1038/ng.3977
6. Liu DJ, Peloso GM, Yu H, Butterworth AS, Wang X, Mahajan A, Saleheen D, Emdin C, Alam D, Alves AC, et al. Whole genome sequence analysis of blood lipid levels in >66,000 individuals. *Nat Commun*. 2022;13:5995. doi: 10.1038/s41467-022-33510-7
7. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264–1272. doi: 10.1056/NEJMoa054013
8. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, Natarajan P, Lander ES, Lubitz SA, Ellinor PT, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*. 2018;50:1219–1224. doi: 10.1038/s41588-018-0183-z
9. Inouye M, Abraham G, Nelson CP, Wood AM, Sweeting MJ, Dudbridge F, Lai FY, Kaptoge S, Brozynska M, Wang T, et al. Genomic risk prediction of coronary artery disease in 480,000 adults: implications for primary prevention. *J Am Coll Cardiol*. 2018;72:1883–1893. doi: 10.1016/j.jacc.2018.07.079
10. Neumann JT, Riaz M, Bakshi A, Polekhina G, Thao LT, Nelson MR, Woods RL, Abraham G, Inouye M, Reid CM, et al. Prognostic value of a polygenic risk score for coronary heart disease in individuals aged 70 years and older. *Circ Genom Precis Med*. 2022;15:e003429. doi: 10.1161/CIRCGEN.121.003429
11. McNeil JJ, Wolfe R, Woods RL, Tonkin AM, Donnan GA, Nelson MR, Reid CM, Lockery JE, Kirpach B, Storey E, et al. Effect of aspirin on cardiovascular events and bleeding in the healthy elderly. *N Engl J Med*. 2018;379:1509–1518. doi: 10.1056/NEJMoa1805819
12. Lacaze P, Bakshi A, Riaz M, Polekhina G, Owen A, Bhatia HS, Natarajan P, Wolfe R, Beilin L, Nicholls SJ, et al. Aspirin for primary prevention of cardiovascular events in relation to lipoprotein(a) genotypes. *J Am Coll Cardiol*. 2022;80:1287–1298. doi: 10.1016/j.jacc.2022.07.027
13. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, et al. The UK biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562:203–209. doi: 10.1038/s41586-018-0579-z
14. McNeil JJ, Woods RL, Nelson MR, Murray AM, Reid CM, Kirpach B, Storey E, Shah RC, Wolfe RS, Tonkin AM, et al. Baseline characteristics of participants in the ASPREE (aspirin in reducing events in the elderly) study. *J Gerontol A Biol Sci Med Sci*. 2019;74:748. doi: 10.1093/gerona/gly278
15. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, Collins R, Allen NE. Comparison of sociodemographic and

health-related characteristics of UK biobank participants with those of the general population. *Am J Epidemiol*. 2017;186:1026–1034. doi: 10.1093/aje/kwx246
16. SCORE2-OP working group and ESC Cardiovascular risk collaboration. SCORE2-OP risk prediction algorithms: estimating incident cardiovascular event risk in older persons in four geographical risk regions. *Eur Heart J*. 2021;42:2455–2467. doi: 10.1093/eurheartj/ehab312
17. Nunn JS, Sulovski M, Tiller J, Holloway B, Ayton D, Lacaze P. Involving elderly research participants in the co-design of a future multi-generational cohort study. *Res Involv Engagem*. 2021;7:23. doi: 10.1186/s40900-021-00271-4
18. GTEx Consortium. The genotype-tissue expression (GTEx) project. *Nat Gen*. 2013;45:580–585. doi: 10.1038/ng.2653
19. Nordestgaard LT, Christoffersen M, Lauridsen BK, Afzal S, Nordestgaard BG, Frikke-Schmidt R, Tybjærg-Hansen A. Long-term benefits and harms associated with genetic cholesteryl ester transfer protein deficiency in the general population. *JAMA Cardiol*. 2022;7:55–64. doi: 10.1001/jamacardio.2021.3728
20. Wang Y-F, Han Y, Zhang R, Qin L, Wang M-X, Ma L. CETP/LPL/LIPC gene polymorphisms and susceptibility to age-related macular degeneration. *Sci Rep*. 2015;5:15711. doi: 10.1038/srep15711
21. Nicholls SJ, Ditmarsch M, Kastelein JJ, Rigby SP, Kling D, Curcio DL, Alp NJ, Davidson MH. Lipid lowering effects of the CETP inhibitor obicetrapib in combination with high-intensity statins: a randomized phase 2 trial. *Nat Med*. 2022;28:1672–1678. doi: 10.1038/s41591-022-01936-7
22. HPS3/TIMI55-REVEAL Collaborative Group. Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med*. 2017;377:1217–1227. doi: 10.1056/NEJMoa1706444
23. Robman LD, Guymer RH, Wolfe R, Woods RL, Hodgson LA, Phung J, Makeyeva GA, Le-Pham YA, Orchard SG, Suleiman J, et al. Baseline characteristics and age-related macular degeneration in participants of the "ASPirin in reducing events in the elderly" (ASPREE)-AMD trial. *Contemp Clin Trials Commun*. 2020;20:100667. doi: 10.1016/j.conctc.2020.100667
24. Pencina MJ, D'Agostino RB, Zdrojewski T, Williams K, Thanassoulis G, Furberg CD, Peterson ED, Vasan RS, Sniderman AD. Apolipoprotein B improves risk assessment of future coronary heart disease in the Framingham heart study beyond LDL-C and non-HDL-C. *Eur J Prev Cardiol*. 2015;22:1321–1327. doi: 10.1177/2047487315569411
25. Wilkins JT, Li RC, Sniderman A, Chan C, Lloyd-Jones DM. Discordance between apolipoprotein B and LDL-cholesterol in Young adults predicts coronary artery calcification: the CARDIA study. *J Am Coll Cardiol*. 2016;67:193–201. doi: 10.1016/j.jacc.2015.10.055
26. Thomas T, Zhou H, Karmally W, Ramakrishnan R, Holleran S, Liu Y, Jumes P, Wagner JA, Hubbard B, Previs SF, et al. CETP (cholesteryl ester transfer protein) inhibition with anacetrapib decreases production of lipoprotein (a) in mildly hypercholesterolemic subjects. *Arterioscler Thromb Vasc Biol*. 2017;37:1770–1775. doi: 10.1161/ATVBAHA.117.309549
27. Nicholls SJ. CETP-inhibition and HDL-cholesterol: a story of CV risk or CV benefit, or both. *Clin Pharmacol Ther*. 2018;104:297–300. doi: 10.1002/cpt.1118
28. Nicholls SJ, Ray KK, Nelson AJ, Kastelein JJP. Can we revive CETP-inhibitors for the prevention of cardiovascular disease? *Curr Opin Lipidol*. 2022;33:319–325. doi: 10.1097/MOL.0000000000000854
29. Ference BA, Kastelein JJ, Ginsberg HN, Chapman MJ, Nicholls SJ, Ray KK, Packard CJ, Laufs U, Brook RD, Oliver-Williams C, et al. Association of genetic variants related to CETP inhibitors and statins with lipoprotein levels and cardiovascular risk. *JAMA*. 2017;318:947–956. doi: 10.1001/jama.2017.11467
30. Graham SE, Clarke SL, Wu KH, Kanoni S, Zajac GJ, Ramdas S, Surakka I, Ntalla I, Vedantam S, Winkler TW, et al. The power of genetic diversity in genome-wide association studies of lipids. *Nature*. 2021;600(7890):675–679. doi: 10.1038/s41586-021-04064-3
31. Sugano M, Makino N, Sawada S, Otsuka S, Watanabe M, Okamoto H, Kamada M, Mizushima A. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J Biol Chem*. 1998;273:5033–5036. doi: 10.1074/jbc.273.9.5033
32. Okamoto H, Yonemori F, Wakitani K, Minowa T, Maeda K, Shinkai H. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature*. 2000;406:203–207. doi: 10.1038/35018119
33. Rittershaus CW, Miller DP, Thomas LJ, Picard MD, Honan CM, Emmett CD, Pettey CL, Adari H, Hammond RA, Beattie DT, et al. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2000;20:2106–2112. doi: 10.1161/01.ATV.20.9.2106
34. Thompson A, Di Angelantonio E, Sarwar N, Erqou S, Saleheen D, Dullaart RP, Keavney B, Ye Z, Danesh J. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. *JAMA*. 2008;299:2777–2788. doi: 10.1001/jama.299.23.2777
35. Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhama Y, Mabuchi H, Tall AR. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med*. 1990;323:1234–1238. doi: 10.1056/NEJM199011013231803
36. Brousseau ME, Schaefer EJ, Wolfe ML, Bloedon LT, Digenio AG, Clark RW, Mancuso JP, Rader DJ. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *N Engl J Med*. 2004;350:1505–1515. doi: 10.1056/NEJMoa031766
37. Barter PJ, Brewer HB Jr, Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2003;23:160–167. doi: 10.1161/01.ATV.0000054658.91146.64
38. Johannsen TH, Frikke-Schmidt R, Schou J, Nordestgaard BG, Tybjaerg-Hansen A. Genetic inhibition of CETP, ischemic vascular disease and mortality, and possible adverse effects. *J Am Coll Cardiol*. 2012;60:2041–2048. doi: 10.1016/j.jacc.2012.07.045
39. Tybjaerg-Hansen A, Nordestgaard LT, Christoffersen M. Pharmacogenetics-guided CETP inhibition: an open question? *Eur Heart J*. 2022;43:3957–3959. doi: 10.1093/eurheartj/ehac398
40. Tall AR, Rader DJ. Trials and tribulations of CETP inhibitors. *Circ Res*. 2018;122:106–112. doi: 10.1161/CIRCRESAHA.117.311978
41. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med*. 2007;357:2109–2122. doi: 10.1056/NEJMoa0706628
42. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter LA, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med*. 2012;367:2089–2099. doi: 10.1056/NEJMoa1206797
43. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KA, Gibson CM, Granger C, Menon V, Montalescot G, et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. *N Engl J Med*. 2017;376:1933–1942. doi: 10.1056/NEJMoa1609581
44. Ridker PM, Paré G, Parker AN, Zee RYL, Miletich JP, Chasman DI. Polymorphism in the CETP gene region, HDL cholesterol, and risk of future myocardial infarction: Genomewide analysis among 18 245 initially healthy women from the Women's genome health study. *Circ Cardiovasc Genet*. 2009;2:26–33. doi: 10.1161/CIRCGENETICS.108.817304
45. Webb TR, Erdmann J, Stirrups KE, Stitziel NO, Masca NG, Jansen H, Kanoni S, Nelson CP, Ferrario PG, König IR, et al. Systematic evaluation of pleiotropy identifies 6 further loci associated with coronary artery disease. *J Am Coll Cardiol*. 2017;69:823–836. doi: 10.1016/j.jacc.2016.11.056
46. Nomura A, Won HH, Khera AV, Takeuchi F, Ito K, McCarthy S, Emdin CA, Klarin D, Natarajan P, Zekavat SM, et al. Protein-truncating variants at the cholesteryl ester transfer protein gene and Risk for coronary heart disease. *Circ Res*. 2017;121:81–88. doi: 10.1161/CIRCRESAHA.117.311145
47. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88:76–82. doi: 10.1016/j.ajhg.2010.11.011
48. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S, McGue M, et al. Next-generation genotype imputation service and methods. *Nat Genet*. 2016;48:1284–1287. doi: 10.1038/ng.3656
49. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4:7. doi: 10.1186/s13742-015-0047-8
50. Boughton AP, Welch RP, Flickinger M, VandeHaar P, Taliun D, Abecasis GR, Boehnke M. LocusZoom. js: interactive and embeddable visualization of genetic association study results. *Bioinformatics*. 2021;37:3017–3018. doi: 10.1093/bioinformatics/btab186
51. Turner SD. qqman: an R package for visualizing GWAS results using Q-Q and Manhattan plots. *J Open Source Softw*. 2018;3:731. doi: 10.21105/joss.00731
52. Ferris FL III, Wilkinson CP, Bird A, Chakravarthy U, Chew E, Csaky K, Sadda SR. Clinical classification of age-related macular degeneration. *Ophthalmology*. 2013;120:844–851. doi: 10.1016/j.ophtha.2012.10.036