Genetic determinants of cholesterol and energy metabolism : implications for cardiometabolic health

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CETP concentration: a genome-wide association study followed by Mendelian randomization on coronary artery disease

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Circulation: Genomic and Precision Medicine 2018; 11(5):e002034

Abstract

Introduction

The genetic basis of circulating cholesteryl ester transfer protein (CETP) concentration is largely unknown. We aimed to identify independent genetic determinants of circulating CETP to assess causal effects of variation in CETP concentration on circulating lipid concentrations and cardiovascular disease risk.

Methods

A genome-wide association (GWA) discovery and replication study on serum CETP concentration were embedded in the Netherlands Epidemiology of Obesity (NEO) study. Based on the independent identified variants, Mendelian randomization was conducted on serum lipids (NEO study) and coronary artery disease (CAD) (CARDIoGRAMplusC4D consortium).

Results

In the discovery analysis (N=4,248), we identified three independent variants (rs247616, rs12720922 and rs1968905; all $P<5\times10^{-8}$) that determine serum CETP concentration. These SNPs were mapped to CETP, and replicated in a separate subpopulation (N=1,458). Per-allele increase (SE) in serum CETP was 0.32 (0.02) μg/mL for rs247616-C, 0.35 (0.02) μg/mL for rs12720922-A, and 0.12 (0.02) μg/mL for rs1968905-G. Combined, these variants explained 16.4% of the total variation in CETP concentration. One μg/mL increase in genetically-determined CETP concentration strongly decreased high-density lipoprotein (HDL) cholesterol (-0.23 mmol/L; 95% CI -0.26, -0.20), moderately increased low density lipoprotein (LDL) cholesterol (0.08 mmol/L; 0.00, 0.16), and was associated with an odds ratio of 1.08 (0.94, 1.23) for CAD risk.

Conclusion

This is the first large GWAS study identifying independent variants that largely determine serum CETP concentration. While HDL-C is not a causal risk factor for CAD, it has been unequivocally demonstrated that LDL-C lowering is proportionally associated with a lower CAD risk. Therefore, the results of our study are fully consistent with the notion that CETP concentration is causally associated with CAD through LDL-C.

Introduction

Cholesteryl ester transfer protein (CETP) facilitates the net flux of cholesteryl esters from high-density lipoproteins (HDL) towards (very-) low-density lipoproteins ((V)LDL), coupled to a net flux of triglycerides from (V)LDL to HDL.[1] As such, CETP contributes to an atherogenic lipoprotein profile (i.e. high LDL-cholesterol/HDL-cholesterol ratio), as has been extensively studied in both humans and in mice transgenic for human CETP.[2,3] Therefore, inhibition of CETP has long been regarded a promising therapeutic strategy to attenuate dyslipidaemia and ultimately prevent the development of cardiovascular disease (CVD).

Of the four clinical trials that have studied the effects of pharmacological CETP inhibition on CVD risk reduction, only the fourth and most recent REVEAL trial with anacetrapib did meet its primary endpoint, i.e. a reduction in major coronary events.[4] Contrary to expectations, the clinical trials with the CETP the inhibitors torcetrapib, dalcetrapib, and evacetrapib, were terminated: torcetrapib had off-target effects on blood pressure and caused an increase in cardiovascular events,[5] and both dalcetrapib and evacetrapib lacked efficacy in reducing cardiovascular events on top of statin therapy.[6,7] All of these CETP inhibitors caused a large increase in HDL-C, and a low to moderate decrease in LDL-C.[5–8] Although high HDL-C concentration is associated with a decreased risk of CVD in epidemiological studies,[9] Voight and colleagues[10] showed in a Mendelian randomization study that genetically-determined higher HDL-C concentrations do not decrease the risk of myocardial infarction, indicating that the association between HDL-C and CVD is not causal. This may be one of the explanations for the lack of efficacy of the three initial CETP inhibitors. Although the underlying reason for success of the fourth CETP inhibitor is not yet elucidated, anacetrapib showed the largest reduction in LDL-C concentration compared to the three initial CETP inhibitors,[4] which may possibly explain its beneficial effects on CVD risk reduction.

Recent evidence shows that serum CETP is largely derived from hepatic macrophages[11], but the genetic basis of serum the CETP concentration in the general population remains to be elucidated. A large genome-wide association study (GWAS) on circulating CETP has not been performed to date. With identification of the main genetic determinants of circulating CETP, the causal effects of variation in serum CETP concentration on circulating lipid concentrations and CVD risk can be assessed using Mendelian randomization. In the past, several Mendelian randomization studies with a comparable aim have been performed,[12–14] including a recent meta-Mendelian randomization analysis by Ference et al. with data from over 100,000 participants.[15] However, these studies used candidate SNPs rather than GWAS-identified SNPs, which may be less powerful genetic instruments for assessing the causal role of CETP concentration in cardiovascular disease.[16,17]

With the present study, we aim to identify independent genetic variants that determine circulating CETP concentration, using a genome-wide rather than a candidate gene approach. In addition, we aim to use these variants as genetic instruments in Mendelian randomization to assess the causal effects of variation in CETP on serum lipids and coronary artery disease (CAD) risk, which may assist in understanding the effectiveness of pharmaceutical CETP inhibition. To this end, we performed a GWAS on serum CETP concentration, using a discovery cohort (n=4,248) and a separate replication cohort (n=1,458) from the Netherlands Epidemiology of Obesity (NEO) study. Subsequently, we used the identified SNPs in Mendelian randomization analyses on serum lipid concentrations in the NEO study population and the Global Lipids Genetics Consortium (GLGC)[18], and on CAD using publically-available data from the CARDGIoGRAMplusC4D consortium.[19]

Materials and Methods

Study design and population

The NEO study is a population-based prospective cohort study of men and women aged between 45 and 65 years. From the greater area of Leiden, The Netherlands, all inhabitants with a self-reported body mass index (BMI) of 27 kg/m or higher were eligible to participate. In addition, inhabitants from one nearby municipality (Leiderdorp, The Netherlands) in the same age group were invited to participate regardless of their BMI, forming a reference population for BMI distribution. In total, 6,671 participants were included from September 2008 until September 2012. Participants visited the NEO study center for extensive physical examination. Venous blood samples were obtained from the antecubital vein after a 10 hour overnight fast. Research nurses recorded current medication use by means of a medication inventory. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information.

The NEO study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC), and all participants gave their written informed consent. Detailed information about the study design and data collection has been described elsewhere.[20]

Methods used for genotyping and biochemical analyses are described in detail in the Supplemental material 4.A.

Genome-wide association study

We conducted the GWAS on the unstandardized serum CETP concentration for all autosomal chromosomes. We divided the total NEO study population based on the graphical area of recruitment into a discovery cohort (n=4,248; greater area of Leiden, The Netherlands) and a replication cohort (n=1,458; Leiderdorp, The Netherlands). This was considered to

be valid as we recently showed, using data of the NEO study population, that serum CETP concentration was not associated with BMI nor with other measures of body fat.[21]

Additive (per-allele) linear regression analyses were conducted separately for the discovery and replication cohort in SNPTEST v2, adjusted for age, sex and the first four PCs. To identify variants that were independently associated with serum CETP concentration, we used conditional and joint analyses to perform a stepwise selection procedure using the genome-wide complex trait analysis (GCTA) tool version 1.24.4.[22] A conditioned P-value $<5\times10^{-8}$ was considered to be genome-wide significant, and a conditioned P-value $<1\times10^{-6}$ was considered a suggestive signal. Independent single nucleotide polymorphisms (SNPs) with a conditioned P-value $<1\times10^{-6}$ in the discovery analysis were validated in the replication sample. SNPs with a P-value <0.05 in the replication cohort were considered to be replicated. Upon identification of the lead SNPs, we determined whether the distribution of the coding alleles was similar in users and non-users of lipid-lowering drugs. More detailed information on this method is described in the Supplemental material 4.A.

As Taq1B (rs708272)[23] and -629C>A (rs1800775)[24] are the most studied variants in the CETP gene in literature, we specifically checked the GWAS results for their association with CETP concentration. In addition, we reported the linkage disequilibrium (NEO study) of the lead SNPs from the present GWAS with the eight CETP SNPs that were used in a recent meta-Mendelian randomization study on coronary heart disease and serum lipids by Ference et al.[15] (i.e. rs3764261, rs1800775, rs1864163, rs9929488, rs9989419, rs12708967, rs289714 and rs5880). This allows comparison between our genetic instrument composed of SNPs identified form a GWAS on serum CETP concentration and their genetic instrument composed of candidate SNPs.

The explained variance in serum CETP concentration for the independent variants was estimated in the replication cohort. For each individual SNP the explained variance was estimated as the partial R^2 from the linear regression model with the SNP as independent variable and serum CETP concentration as dependent variable. To estimate the total variance explained by all independent lead SNPs, a weighted genetic risk score (GRS) was calculated per individual. The GRS was constructed as the sum of the number of risk alleles on the lead SNPs weighted by their effect size on CETP concentration in the discovery cohort. The combined explained variance was the partial R^2 from the linear regression model, with the weighted GRS as independent variable and serum CETP concentration as dependent variable.

To quantify the genome-wide cumulative effects of independent variants influencing various phenotypes, genetic correlations of serum CETP concentration with serum lipid concentra-

tions (i.e. HDL-C, LDL-C, triglycerides and total cholesterol), and BMI were calculated (Supplemental material 4.A).

Expression quantitative trait loci (eQTLs) analysis

To investigate whether the identified lead SNPs could explain serum CETP concentration via transcriptional gene regulation, we checked if these SNPs were eQTLs for CETP using data from the genotype-tissue expression (GTEx) project portal (V7)[25] and the Blood eQTL browser.[26]

Mendelian randomization

Based on the identified independent and replicated SNPs for serum CETP concentration in our study population, we conducted Mendelian randomization analyses on serum lipid concentrations in the NEO study population, and on the risk of CAD based on publically-available summary statistics data from the CARDGIoGRAMplusC4D 1000 Genomes study.[19] A detailed description of the Mendelian randomization analyses on CAD risk using data from the CARDIoGRAMplusC4D consortium can be found in the Supplemental material 4.A. Effect estimates for CAD risk were reported as odds ratio with corresponding 95% CI. We used a publically available tool[27] to conduct a power analysis for the Mendelian randomization analysis on CAD, which was based on the findings from the GWAS on serum CETP concentration, the explained variance of the SNPs used to compose the GRS, and the sample size of the CARDIoGRAMplusC4D 1000 Genomes study (60,801 cases; 123,504 controls).

In the NEO study population, we calculated the individual weighted GRS based on the identified SNPs and determined the effect of 1 μg/mL increase in genetically-determined CETP concentration on the concentrations of total cholesterol, HDL-C, triglycerides, LDL-C, total cholesterol/HDL-C ratio, LDL-C/HDL-C ratio and apolipoprotein B (ApoB), using linear regression analysis adjusted for age and sex. Beta coefficients and 95% CIs were reported.

In addition, for replication purposes, we extracted the independent leads SNPs from publically available datasets of the GLGC.[18] We also extracted the Taq1B (rs708272) and -629C>A (rs1800775) polymorphisms from the CARDGIoGRAMplusC4D 1000 Genomes and GLGC datasets.[18]

Results

Population characteristics

Characteristics of the discovery and replication cohorts are summarized in Table 4.1. Compared with the replication cohort, there were fewer women in the discovery cohort (50.6% versus 56.1%). Also, participants in the discovery cohort had a higher BMI (30.3 kg/m versus 25.6 kg/m) and more often used lipid-lowering drugs (17.5% versus 10.4%) than participants in the replication cohort. Serum CETP and lipid concentrations were comparable between both cohorts.

Table 4.1: Characteristics of the discovery and replication cohort from the Netherlands Epidemiology of Obesity (NEO) study.

Characteristics	Discovery cohort	Replication cohort
Number of participants	4,248[a]	1,458[b]
Age (year)	56 (51, 61)	57 (51, 61)
Women	2,148 (50.6%)	818 (56.1%)
Body mass index (kg/m^2)	30.3 (28.4, 33.0)	25.6 (23.2, 28.2)
Lipid-lowering drug users	745 (17.5%)	151 (10.4%)
Fasting serum concentrations		
CETP (μg/mL)	2.50 (0.67)	2.43 (0.64)
Total cholesterol (mmol/L)	5.66 (1.08)	5.69 (1.07)
LDL-cholesterol (mmol/L)	3.58 (0.99)	3.56 (0.98)
HDL-cholesterol (mmol/L)	1.38 (0.38)	1.58 (0.46)
Triglycerides (mmol/L)	1.34 (0.95, 1.87)	1.00 (0.71, 1.45)

Results are presented as median (inter quartile range) for not normally distributed data, mean (SD) or number (percentage).

[a] Missing data: n=13 for total cholesterol concentration, n=14 for HDL-cholesterol concentration, triglycerides and LDL-cholesterol concentration.

[b] No missing data.

Table 4.2: Summary statistics of the associations of the three independent lead SNPs that reached genome-wide significance and seven suggestive signals with serum CETP concentration.

								Discovery cohort (n=4,248)				Replication cohort (n=1,458)		
Chr	SNP	Position	Location	Closest gene	Coding/ non-coding allele	Coding allele frequency[a]	Imputation quality	Effect size per allele[b](μg/mL)	SE	P-value	Conditioned P-value[c]	Effect size per allele[b](μg/mL)	SE	P-value
Lead SNPs														
16	rs247616	56989590	Intergenic	*CETP*	C/T	0.67	1	0.32	0.015	3.98×10^{-100}	1.86×10^{-64}	0.31	0.024	1.24×10^{-37}
16	rs12720922	57000885	Intron	*CETP*	A/G	0.17	0.98	0.35	0.019	3.48×10^{-74}	6.68×10^{-13}	0.36	0.03	3.27×10^{-33}
16	rs1968905	57010948	Intron	*CETP*	G/T	0.82	0.85	0.12	0.02	4.12×10^{-9}	1.66×10^{-12}	0.098	0.031	1.80×10^{-3}
Suggestive SNPs														
2	rs185550357	50249349	Intron	*NRXN1*	C/T	0.007	0.7	0.63	0.12	3.94×10^{-7}	4.57×10^{-7}	0.064	0.15	0.67
3	rs6442310	12358230	Intron	*PPARG*	A/T	0.54	0.92	0.08	0.015	5.52×10^{-8}	6.13×10^{-8}	0.0044	0.024	0.85
8	chr8:19811023:I	19811023	Intron	*LPL*	ATG/A	0.12	0.87	0.12	0.023	1.45×10^{-7}	1.59×10^{-7}	-0.018	0.038	0.63
9	rs3094377	136312119	Intron	*ADAMTS13*	T/C	0.03	0.41	0.35	0.064	7.65×10^{-8}	9.58×10^{-8}	-0.18	0.11	0.098
10	rs12253367	62003462	Intron	*ANK3*	G/A	0.17	0.94	0.1	0.019	1.08×10^{-7}	1.18×10^{-7}	0.0051	0.031	0.87
16	rs117427818	57010486	Intron	*CETP*	T/C	0.05	0.75	0.46	0.038	3.26×10^{-33}	1.02×10^{-7}	0.54	0.061	1.93×10^{-18}
20	rs150904289	18845428	Intergenic		G/A	0.11	0.79	0.13	0.026	6.31×10^{-7}	6.87×10^{-7}	-0.0084	0.042	0.84

Threshold for genome-wide significance is 5×10^{-8}, based on the conditioned P-value[c]. Threshold for the suggestive signals is 1×10^{-6}. Threshold for replication is 0.05.

[a] In the discovery cohort.

[b] Beta coefficient expressed as the difference in serum CETP concentration.

[c] P-value conditioned on the top lead SNPs using step-wise conditional analysis

Chr, chromosome

Genome-wide association analysis

The -Log(P-value) plot for the GWAS is shown in Figure 4.1. The accompanying list of SNPs that reached genome-wide significance ($P<5\times10^{-8}$) is presented in Supplementary table 4.C.1. After conditioning on the lead SNPs, three independent variants reached genome-wide significance (conditioned $P<5\times10^{-8}$; Supplementary Figure 4.B.2) and seven suggestive signals were identified (conditioned $P<1\times10^{-6}$) in the discovery cohort (Table 4.2). The three genome-wide significant variants were all mapped to the *CETP* gene. Notably, these independent variants were rs247616 ($P=1.86\times10^{-64}$), rs12720922 ($P=6.68\times10^{-13}$) and rs1968905 ($P=1.66\times10^{-12}$), which had a per-allele increase (SE) in serum CETP of 0.32 (0.02) μg/mL (rs247616-C), 0.35 (0.02) μg/mL (rs12720922-A) and 0.12 (0.02) μg/mL (rs1968905-G). These three variants were all replicated in the replication analysis ($P<0.05$). In the NEO study, these variants together explained 16.4% of the serum CETP concentration. The distributions of the effect alleles of the three lead SNPs were similar for individuals taking lipid-lowering drugs and not taking lipid-lowering drugs (Supplementary table 4.C.2). A number of SNPs were suggestively associated with serum CETP concentration, including SNPs mapped to *ADAMTS3*, *PPARG* and *LPL*.

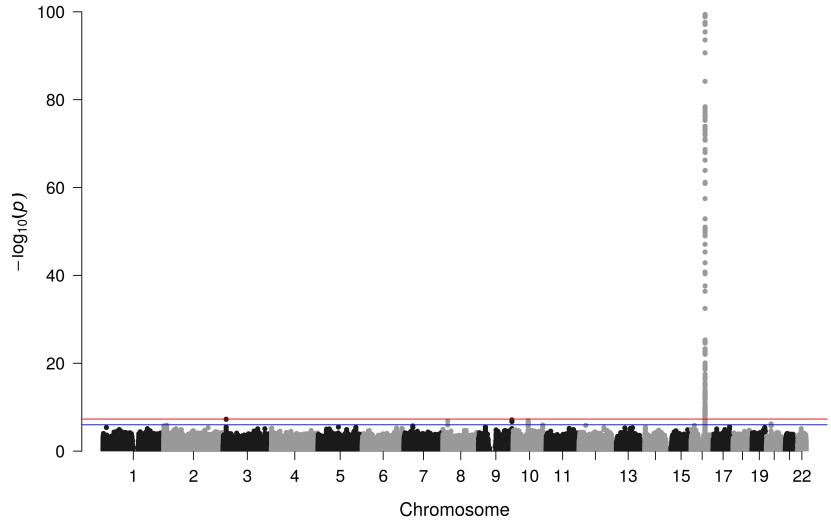

Figure 4.1: –Log(P-value) plot for the genome-wide association study in the discovery cohort (n=4,248). The *CETP* gene is located on chromosome 16. The red line represents the threshold for genome-wide significance ($P<5\times10^{-8}$). The blue line represents the threshold for suggestive signals ($P<1\times10^{-6}$).

The unconditioned per-allele effect size of the well-known Taq1B (rs708272) and -629C>A

(rs1800775) variants was 0.27 μg/mL for both SNPs (Supplementary table 4.C.1). Of the three lead SNPs, Taq1B was in high linkage disequilibrium with rs247616 (LD=0.55), as was -629C>A (LD=0.51). Taq1B and -629C>A were also in high linkage with each other (LD=0.83).

The linkage disequilibrium between the three lead SNPs and eight SNPs that were used as genetic instruments for a CETP GRS in a recent meta-Mendelian randomization analysis on coronary heart disease and serum lipids[15] are shown in Supplementary figure 4.B.3. The two strongest lead SNPs from the present GWAS, i.e. rs247616 and rs12720922, were in high linkage disequilibrium with the eight candidate SNPs of the GRS that was used in the study of Ference et al.,[15] with the highest linkage disequilibrium between rs247616 and rs3764261 (0.996), and between rs12720922 and rs1864163 (0.646).

The genetic correlation of serum CETP concentration with serum lipid concentrations and BMI is reported in Supplementary table 4.C.3. The genetic correlation of serum CETP concentration was highest with serum HDL-C concentration (0.17) and serum triglyceride concentration (-0.29), and lowest with serum total cholesterol concentration (-0.020), serum LDL-C concentration (0.074) and BMI (0.032).

eQTL analysis of the lead SNPs

Table 4.3 shows the eQTLs for the genetic variants rs247616, rs12720922 and rs1968905. The SNP that was most strongly associated with serum CETP concentration in the GWAS, i.e. rs247616, was identified as an eQTL for the *CETP* gene in several tissues (P-value range 6.3×10^{-10} to 4.1×10^{-5}). Rs12720922 was an eQTL for *NLRC5*, but not for CETP. However, we found rs1864163, which is in moderate linkage disequilibrium with rs12720922 (LD=0.65), to be an eQTL for CETP in whole blood (P=8.5×10^{-4}, effect size A-allele 3.33). The third lead SNP, i.e. rs1968905, was not identified as an eQTL for *CETP* in the studied tissues, neither were any variants in strong linkage disequilibrium with this SNP.

Table 4.3: Expression quantitative trait loci (eQTL) for the three GWAS-identified lead SNPs.

SNP	Assessed allele	Gene	Chr	P-value	Effect size	Tissue	Database
rs247616	C	*NLRC5*	16	9.5×10^{-14}	0.34	Transformed fibroblasts	GTEx
rs247616	C	*CETP*	16	6.3×10^{-10}	0.3	Lung	GTEx
rs247616	C	*CETP*	16	1.1×10^{-7}	0.41	Transverse colon	GTEx
rs247616	C	*CETP*	16	1.4×10^{-6}	0.45	Terminal Ileum	GTEx
rs247616	C	*CETP*	16	3.6×10^{-6}	0.32	Liver	GTEx
rs247616	C	*CETP*	16	7.9×10^{-6}	0.3	Esophagus (mucosa)	GTEx
rs247616	C	*CETP*	16	9.3×10^{-6}	0.41	Pancreas	GTEx
rs247616	C	*BBS2*	16	1.7×10^{-5}	0.37	Cerebellar Hemisphere	GTEx
rs247616	C	*CETP*	16	4.1×10^{-5}	4.1	Whole blood	Blood eQTL browser
rs12720922	A	*NLRC5*	16	1.9×10^{-6}	0.24	Transformed fibroblasts	GTEx
rs1968905	G	-	16	-	-	-	

Mendelian randomization

Figure 4.2 shows the results from the Mendelian randomization analyses on CAD risk and serum lipid and ApoB concentrations. We had a power of 0.90 to detect an odds ratio of 1.04 with conventional Mendelian randomization analyses (which makes use of a formal weighted genetic risk score), when taking into account an alpha of 0.05, the explained variance of the SNPs that compose the GRS (i.e. 16.4%), and the sample size of the CARDIoGRAMplusC4D 1000Genomes study. Per 1 μg/mL increase in genetically-determined serum CETP concentration the odds ratio for CAD risk was 1.08 (95% CI: 0.94, 1.23). For the lead SNPs separately, odds ratios were 1.11 (95% CI: 1.03, 1.17) for rs247616, 1.08 (95% CI: 1.01, 1.15) for rs12720922, and 0.89 (95% CI: 0.72, 1.09) for rs1968905. For Taq1B and -629C>A, odds ratios for CAD risk were 1.02 (95% CI 1.01, 1.04) and 1.03 (95% CI 1.01, 1.05), respectively (Supplementary table 4.C.5).

A 1 μg/mL increase in genetically-determined serum CETP concentration was associated with decreased total cholesterol concentration, i.e. -0.14 (95% CI: -0.22, -0.05) mmol/L, and HDL-C concentration, i.e. -0.23 (95% CI: -0.26, -0.20) mmol/L, while it was associated with increased serum LDL-C concentration, i.e. 0.08 (95% CI: 0.00, 0.16) mmol/L, and ApoB concentration, i.e. 0.02 (95% CI: 0.00, 0.03) g/L. Genetically-determined serum CETP concentration was not associated with serum triglycerides concentration, i.e. 0.02 (95% CI -0.05, 0.09) mmol/L. Supplementary table 4.C.4 shows the results from the Mendelian randomization analysis with data from GLGC. The results for total cholesterol, triglycerides and LDL-C concentrations from GLGC were comparable with the results from the NEO study. Effect sizes for HDL-C were larger in GLGC than in the NEO study.

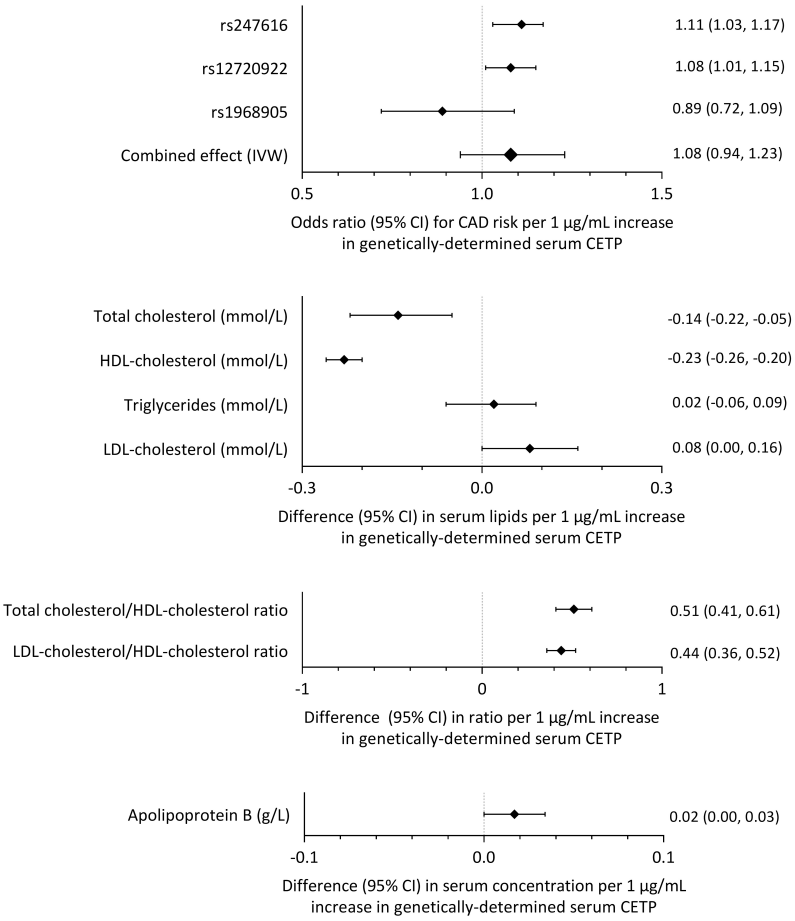

Figure 4.2: Results from the Mendelian randomization study on coronary artery disease in the CARDIoGRAMplusC4D 1000 Genomes Consortium (60,801 cases; 123,504 controls), and on serum lipid and lipoprotein B concentrations in the total Netherlands Epidemiology of Obesity (NEO) study population (n=5,706[a]).

[a] Missing data: n=13 for total cholesterol concentration, n=14 for HDL-cholesterol concentration, triglycerides and LDL-cholesterol concentration, n=41 for apolipoprotein B concentration. Results were adjusted for age and sex. CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; IVW, inverse-variance weighted.

Discussion

With this first large GWAS on serum CETP concentration, we identified and replicated three independent SNPs, all mapping to the *CETP* region. These three variants, notably rs12720922, rs247616 and rs1968905, explained 16.4% of the total variation in serum CETP concentration. Effect sizes of all lead SNPs were large, with rs12720922-A having the largest effect on serum CETP: +0.35 μg/mL per additional risk allele. Also, we showed

that genetically-determined variation in circulating CETP associates with a stepwise substantial decrease in HDL-C concentration, a moderate increase in LDL-C and ApoB concentration, and a concordant 8% increase in CAD risk.

We found three independent SNPs in the *CETP* region that largely explained CETP concentration. The association of the rs12720922 variant with circulating CETP, blood lipids or risk of CAD has, to the best of our knowledge, never been described before. In the eQTL studies that we considered,[25,26] rs12720922 was not reported as an eQTL for *CETP*. However, rs1864163, which is in moderate linkage disequilibrium with rs12720922 (LD=0.65), was found to be an eQTL for *CETP* in whole blood,[26] although not in the liver.[25] Despite the absence of a direct association between rs12720922 and *CETP* mRNA levels, it is not ruled out that rs12720922 affects CETP levels via transcriptional regulation. Possibly, we were not able to identify eQTLs of rs12720922 for *CETP* due to the low sample size of eQTL studies in liver tissue, and the dilution that is introduced by considering whole liver expression, since *CETP* is specifically expressed by hepatic macrophages (i.e. Kupffer cells).[11] The second independent lead SNP, rs247616, is located in the promotor region of the *CETP* gene.[28] This SNP has also not been associated with serum CETP concentration before, but it has previously been shown that the minor allele of this variant (rs247616-T) is associated with decreased *CETP* mRNA expression in human liver and increased HDL-C concentrations[28–31], which is in line with our findings.

The third identified variant, rs1968905, was reported by one study to associate with HDL-C concentration specifically in Africans, but has not been linked to serum CETP concentration previously.[32] Of note, rs1968905 is in strong linkage disequilibrium (LD=0.89) with rs1801706 (i.e. G84A), which has been reported as a risk factor for CAD in South Indians.[33] Interestingly, rs1801706 is located in the 3' untranslated region (3' UTR) of the CETP gene, suggesting involvement in posttranscriptional regulation.[34] In addition to these lead SNPs, we found a suggestive signal (i.e. rs117427818) that could be a potential fourth hit in the *CETP* gene. Its statistical significance was, however, largely reduced upon conditional analyses. Indeed, this SNP is in linkage disequilibrium with (one of) the lead SNPs and not completely independently associated with serum CETP concentration (highest linkage disequilibrium with rs12720922; LD=0.20).

To obtain insight in the role of LDL-C and HDL-C in the causal association between serum CETP and CAD risk, we performed Mendelian randomization analyses on serum lipid concentrations. A higher CETP GRS was associated with a large decrease in HDL-C concentration and a moderate increase in LDL-C concentration. The strong association of genetically-determined serum CETP concentration with HDL-C concentration is probably partially explained by a shared genetic background of these two phenotypes, as the ge-

netic correlation between serum CETP concentration and HDL-C concentration was relatively high, which indicates pleiotropy. It should be noted, however, that HDL-C has been observationally, but not causally associated with CVD risk.[10] Thus, although a genetically-determined increase in serum CETP is causally associated with a decrease in HDL-C concentration, this likely does not explain the association between serum CETP and CAD risk. On the other hand, a causal, proportional, log-linear association between LDL-C concentration and CAD risk has been firmly established.[35] In a previously performed Mendelian randomization study using an LDL-C GRS, it was shown that the odds ratio for CAD risk was 1.68 (1.51-1.87) per 1 SD increase in LDL-C (i.e.0.98 mmol/L).[36] To compare, we showed that per 1 μg/mL increase in serum CETP, LDL-C concentration increased with 0.08 mmol/L and the odds ratio for CAD risk was 1.08. Thus, expressed per 0.98 mmol/L increase in LDL-C concentration, we observed 1.98 times increase in CAD risk using the CETP GRS, which is comparable with the effect estimate found with the LDL-C GRS.[36] Taken together, our study suggests that the causal association between CETP concentration and CAD risk may be explained by effects on LDL-C concentration. Interestingly, a recent large meta-Mendelian randomization analysis by Ference et al.,[15] indicated that ApoB concentration is an even more important causal link between CETP and CAD risk than LDL-C concentration. This implies that an increase in the absolute number of VLDL, IDL and LDL (i.e. non-HDL) particles, as reflected by ApoB concentration, due to increased circulating CETP may explain the association with CAD risk, rather than the amount of cholesterol in LDL particles.

Our findings are in line with this recent meta-Mendelian randomization analysis, including over a 100,000 participants that showed comparable effects for a CETP GRS on CAD risk, LDL-C and ApoB concentration.[15] In that meta-analysis, a CETP GRS was composed of eight candidate SNPs selected from the *CETP* gene with a forward conditional regression analysis on HDL-C concentration. In the present study, we identified three different *CETP* SNPs that independently determine circulating CETP concentration by using a hypothesis-free approach (i.e. GWAS). These GWAS-identified SNPs are therefore direct genetic instruments to study the causal effects of CETP in Mendelian randomization. Although none of the lead SNPs that we identified with GWAS were included in the GRS composed by Ference et al.[15], we observed that the two strongest lead SNPs from our GWAS were in high (rs247616) to moderate (rs12720922) linkage disequilibrium with the eight candidate SNPs of that GRS. This indicates that the GRS composed of candidate *CETP* SNPs is a reliable genetic instrument to study the causal effects of CETP, and our results therefore extend this recent meta-Mendelian randomization analysis.[15]

Although we showed a causal association between CETP concentration and CAD risk, the

three initial CETP inhibitors did not reduce the risk of cardiovascular events when given in addition to statin treatment. In fact, clinical trials with those CETP inhibitors were even terminated due to off-target effects (torcetrapib) or a lack of efficacy (dalcetrapib and evacetrapib).[5–7] Dalcetrapib had minimal effects in LDL-C concentration possibly explaining its futility.[7] Evacetrapib did significantly reduce LDL-C concentration, but did not evoke a concordant decrease in ApoB,[37] indicating unfavourable LDL particle remodelling rather than removal from the circulation.[35,38] This explanation for the failure of the evacetrapib trial is in line with a recent meta-Mendelian randomization study by Ference et al.[15] Data from that study indicate that the success of CETP inhibitors when prescribed on top of statin treatment is dependent on their capability to reduce the absolute number non-HDL particles as reflected by a reduction in ApoB concentration. A reduction in the LDL-C concentration through CETP inhibition may thus only be beneficial when a concordant reduction in ApoB concentration is achieved.[15] Indeed, anacetrapib did show a concordant reduction in non-HDL and ApoB concentration of -18%, which was accompanied by a reduced rate ratio for major coronary events of 0.91 (95% CI 0.85, 0.97).[4]

Our study may have had insufficient statistical power to identify additional variants with small effects on serum CETP concentration or with low allele frequencies. As we did not replicate our GWAS findings in additional heterogeneous populations, caution should be taken when extrapolating the results to other populations. Also, despite the similar associations between the lead SNPs and LDL-C concentrations in GLGC and the NEO study, effect sizes for HDL-C concentrations were higher in GLGC. A possible explanation might involve differences in the composition of the study populations, as GLGC is a meta-analysis of a wide variety of cohorts.

In conclusion, with a GWAS, we identified and replicated three independent SNPs mapping to the *CETP* gene that together explained 16.4% of the total variation in serum CETP concentration, which shows that serum CETP concentration is strongly genetically determined. Using Mendelian randomization, we showed that 1 μg/mL increase in serum CETP causally associates with a large decrease in HDL-C cholesterol of -0.23 mmol/L, moderate increases in LDL-C concentration of 0.08 mmol/L and ApoB concentration of 0.02 g/L, and an odds ratio of 1.08 for CAD risk. While HDL-C is not a causal risk factor for CAD, it has been unequivocally demonstrated that LDL-C lowering is proportionally associated with a lower CAD risk. Therefore, the results of our study are fully consistent with the notion that CETP concentration is causally associated with CAD through LDL-C.

Acknowledgements

We express our gratitude to all individuals who participate in the NEO study. We are grateful to all participating general practitioners for inviting eligible participants. We furthermore thank Pat van Beelen and all research nurses for collecting the data, Petra Noordijk and her team for laboratory management and DNA isolation, and Ingeborg de Jonge for all data management of the NEO study. We sincerely thank Chris van der Bent for performing the serum CETP concentration measurements. The genotyping in the NEO study was supported by the Centre National de Génotypage (Paris, France), headed by Jean-Francois Deleuze.

Data on coronary artery disease have been contributed by CARDIoGRAMplusC4D investigators and have been downloaded from www.cardiogramplus c4d.org. The NEO study was supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'. L.L. Blauw was supported by a grant from the Board of Directors of the Leiden University Medical Center. Y. Wang is supported by the Dutch Science Organization [ZonMW-VENI Grant 91617027]. D. van Heemst was supported by the European Commission funded project HUMAN [Health-2013-INNOVATION-1-602757]. D. Mook-Kanamori is supported by the Dutch Science Organization [ZonMW-VENI Grant 91614023]. P.C.N. Rensen is an Established Investigator of the Dutch Heart Foundation [2009T038].

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Appendix

4.A. Expanded methods

Genotyping and imputation

DNA was isolated from venous blood samples. Genotyping was performed using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, United States of America). Participants were excluded (Supplementary Figure 4.B.1) in the process of quality control when 1) the sample call rate was <98%, 2) there was a sex mismatch, 3) heterozygosity rate was not within ±3 SD of mean heterozygosity rate, 4) participants widely diverged based on the first two principal components (PCs) (±3.5 SD), 5) samples were duplicates, and 6) concordance with another DNA sample was >0.25 (related individuals). Genetic variants were excluded when 1) genotype call rate was <98%, and 2) variants were not in Hardy-Weinberg equilibrium (P-value $<1\times10^{-6}$). Subsequently, genotypes were imputed to the 1000 Genome Project reference panel (v3 2011)[39] using IMPUTE (v2.2) software.[40] All genetic variants with an imputation quality below 0.4 or a minor allele frequency below 0.01 were not considered for the analyses in the present study.

Biochemical analyses

After centrifugation, aliquots of plasma and serum were stored at -80°C. From 11 April until 16 July 2014 CETP concentrations were measured in serum that had undergone one previous freeze-thaw cycle with enzyme-linked immune sorbent assay (ELISA) kits according to the manufacturer's instructions (DAIICHI CETP ELISA, Daiichi, Tokyo, Japan; coefficient of variation (CV) 11.7%). We measured CETP concentration instead of exogenous CETP activity, both of which are highly correlated.[41–43] Participants with missing data on serum CETP concentration (n=65) and participants with a serum CETP concentration beyond four SD from the mean (n=3) were excluded for analyses (Supplementary figure 4.B.1).

Fasting serum total cholesterol and triglyceride concentrations were measured with enzymatic calorimetric assays (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany; CV <2% and CV <3%, respectively) and fasting serum HDL-C concentrations with third generation homogenous HDL-C methods (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany; CV <3%). Fasting LDL-C concentrations were calculated using the Friedewald equation.[28]

Nuclear magnetic resonance (NMR) spectroscopy

Serum apolipoprotein B (ApoB) concentration was determined with a high-throughput proton NMR metabolomics platform.[30] Details of the experimentation and applications of the NMR metabolomics platform have been described previously.[30]

Genome-wide association study

We used conditional and joint analyses to perform a stepwise selection procedure using the genome-wide complex trait analysis (GCTA) tool version 1.24.4.[8] This method uses summary statistics from the discovery phase and linkage disequilibrium correlations between SNPs estimated from the entire NEO study population. The step-wise selection of independent loci starts with the genetic variant with the lowest P-value in the summary statistics dataset, and performs association analyses to identify the next independent single nucleotide polymorphisms (SNPs) with the lowest conditioned P-value. This procedure is repeated until no more independent SNPs are identified.

Genetic correlations

To quantify the genome-wide cumulative effects of independent variants influencing various phenotypes, genetic correlations of serum CETP concentration with serum lipid concentrations (i.e. HDL-C, LDL-C, triglycerides and total cholesterol) and BMI were calculated. We used the bivariate genomic-relatedness-based restricted maximum-likelihood (GREML) approach implemented in genome-wide complex trait analysis (GCTA) to estimate the genetic correlations.[44] The genetic variance-covariance matrix between two phenotypes was estimated by the bivariate linear mixed model.[44] To take the effects of lipid-lowering drugs on blood lipid measurements into account, the total cholesterol concentration was divided by 0.8 for those individuals using lipid-lowering drugs.[45] Natural logarithmic transformation was used to obtain a normal distribution for serum triglyceride concentration.[45]

Mendelian randomization CARDIoGRAMplusC4D consortium

The genome-wide association meta-analysis of the CARDIoGRAMplusC4D consortium included 60,801 CAD cases and 123,504 controls of mainly European ancestry populations. CAD was defined as having a myocardial infarction, acute coronary syndrome, chronic stable angina or coronary stenosis of >50%.[19] The dataset contains the summary level meta-analysis data of the GWAS, comprising the additive (per-allele) beta estimates from logistic regression analyses of the SNPs on CAD risk, accompanying standard errors and effect alleles. For the Mendelian randomization on CAD, we combined the individual genetic variants for CETP concentration to estimate the causal effect of CETP concentration

on CAD risk. Analogous to pooling estimates from different observational studies with conventional meta-analysis using inverse-variance weighing, we weighted this combined effect estimate of the CETP SNPs on CAD by the inverse of the variance for each individual additive (per-allele) effect on CAD risk, and incorporated the individuals additive effects of the genetic instruments on CETP concentration. Effect estimates were reported as odds ratio with corresponding 95% CI.

4.B. Supplementary figures

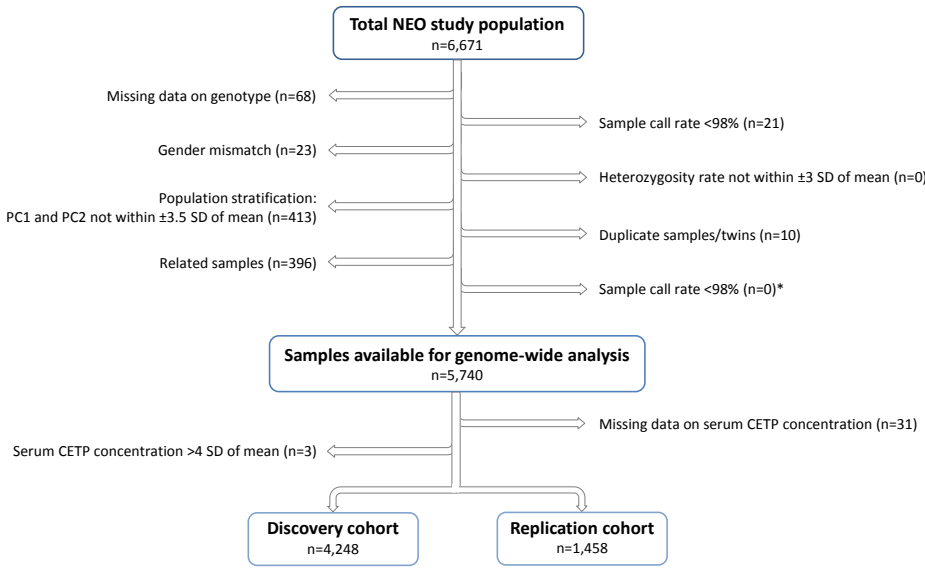

Figure 4.B.1: Quality control steps and exclusion criteria for the genome-wide association study (GWAS) on serum CETP concentration in the Netherlands Epidemiology of Obesity (NEO) study.

[a] Sample call rate was checked a second time, as it can change after removing samples on the basis of prior quality control steps.
PC, principal component.

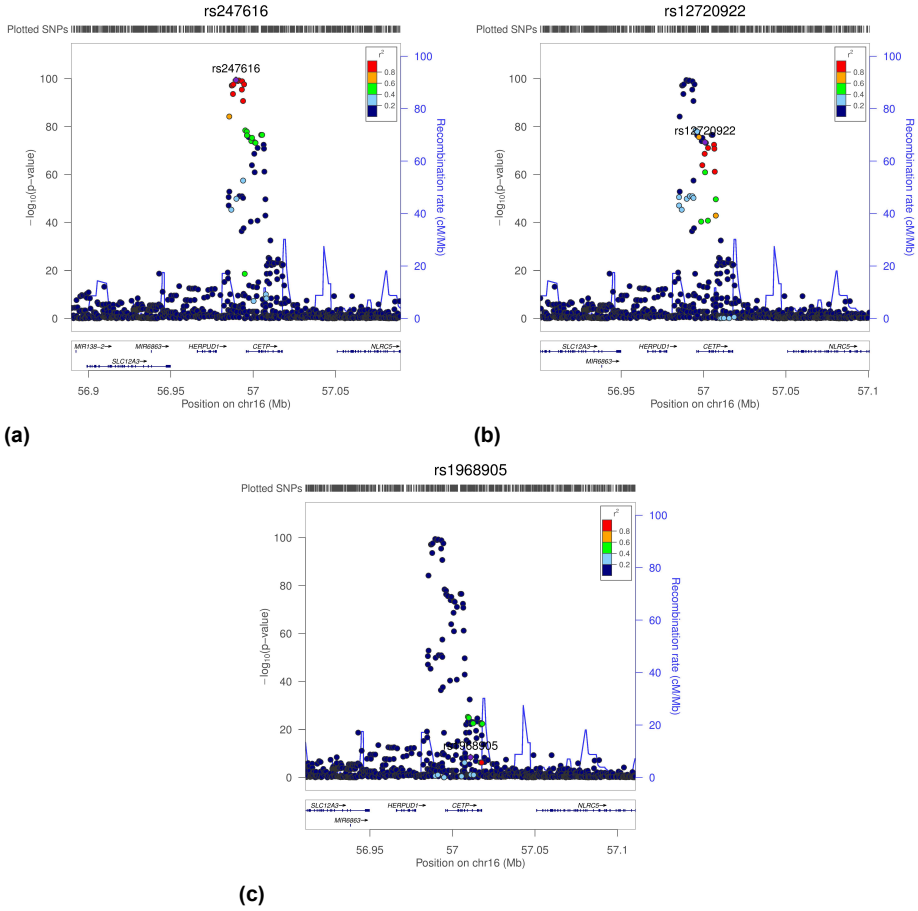

Figure 4.B.2: Regional association plots for the three independent genome-wide significant single nucleotide polymorphisms (SNPs), i.e. (**a**) rs247616, (**b**) rs12720922 and (**c**) rs1968905. The purple diamond indicates the lead SNP for the locus.

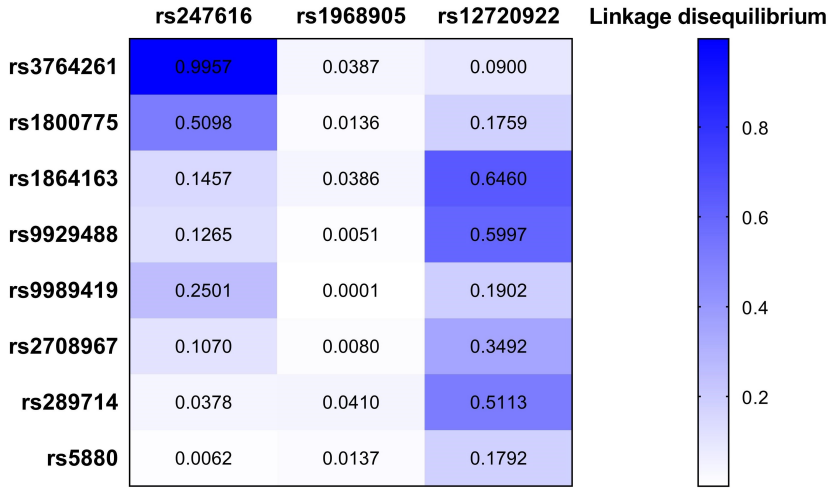

Figure 4.B.3: Linkage disequilibrium between the three GWAS-identified lead single nucleotide polymorphisms (SNPs) from the present study (i.e. rs247616, rs12720922 and rs1968905) and eight candidate SNPs that were used as genetic instruments for a CETP genetic risk score in a recent meta-Mendelian randomization analysis[a] on coronary heart disease and serum lipids (i.e. rs3764261, rs1800775, rs1864163, rs9929488, rs9989419, rs12708967, rs289714 and rs5880). Linkage disequilibrium was determined in the Netherlands Epidemiology of Obesity (NEO) study population.

[a] Ference BA, et al. Jama. 2017;318:947-956.

4.C. Supplementary tables

Table 4.C.1: Summary statistics of all single nucleotide polymorphisms (SNPs) that reached genome-wide significance in the discovery cohort, before conditioning on the independent lead SNPs.

SNP	CHR	POS	EA	NEA	EAF	BETA	SE	PVALUE
rs77712507	16	56742475	A	G	0.064	0.184	0.031	3.45E-9
rs185539847	16	56745071	C	T	0.081	-0.192	0.032	1.29E-9
rs4583235	16	56758596	A	G	0.102	0.137	0.024	1.06E-8
rs77050717	16	56767381	A	G	0.024	0.338	0.055	1.02E-9
rs76233589	16	56769902	G	A	0.103	0.134	0.024	1.71E-8
rs147726069	16	56790343	G	A	0.103	0.134	0.024	1.69E-8
rs60107605	16	56802246	G	T	0.102	0.133	0.024	2.40E-8
rs76477146	16	56814325	T	A	0.103	0.134	0.024	1.57E-8
rs8052978	16	56814847	A	G	0.376	-0.092	0.015	6.12E-10
rs77755067	16	56818259	C	A	0.103	0.134	0.024	1.58E-8
rs16962014	16	56819919	T	C	0.091	0.145	0.025	5.90E-9
rs79334440	16	56826362	C	A	0.103	0.134	0.024	1.58E-8
rs9938953	16	56826766	C	T	0.388	-0.088	0.015	1.51E-9
rs4783959	16	56834096	G	A	0.387	-0.089	0.015	1.24E-9
rs79984435	16	56834234	A	G	0.091	0.146	0.025	4.45E-9
rs79600951	16	56834254	G	C	0.091	0.146	0.025	4.45E-9
rs9929577	16	56836108	C	T	0.376	-0.092	0.015	5.32E-10
rs1803870	16	56839439	T	C	0.102	0.135	0.024	1.79E-8
rs7187512	16	56840171	G	A	0.387	-0.089	0.015	1.20E-9
chr16:56842612:D	16	56842612	T	TA	0.383	-0.107	0.016	9.78E-12
rs12444217	16	56843443	A	G	0.091	0.147	0.025	3.97E-9
rs16962399	16	56846804	T	C	0.09	0.146	0.025	4.50E-9
rs59542880	16	56848871	A	G	0.09	0.146	0.025	4.67E-9
rs1529929	16	56849496	A	G	0.389	-0.088	0.015	1.52E-9
rs9939678	16	56850602	G	A	0.389	-0.088	0.015	1.44E-9
rs1561139	16	56852822	T	G	0.386	-0.089	0.015	1.48E-9
rs80261911	16	56855192	T	C	0.09	0.146	0.025	4.57E-9
chr16:56855549:D	16	56855549	T	TC	0.09	0.146	0.025	4.58E-9
rs2895432	16	56856955	A	G	0.09	0.146	0.025	4.51E-9
rs7199480	16	56859595	A	G	0.389	-0.088	0.015	1.42E-9
rs2099536	16	56861995	G	C	0.39	-0.09	0.015	9.38E-10
chr16:56862143:D	16	56862143	G	GA	0.384	-0.086	0.015	6.00E-9
rs60310821	16	56863229	C	T	0.093	0.139	0.025	1.53E-8
rs11865000	16	56865374	A	G	0.093	0.142	0.025	8.43E-9
rs2241770	16	56866196	C	T	0.093	0.141	0.025	1.05E-8
rs3764266	16	56872824	A	G	0.093	0.14	0.025	1.29E-8
chr16:56873613:I	16	56873613	TA	T	0.093	0.14	0.025	1.27E-8
rs16962767	16	56873789	C	T	0.093	0.14	0.025	1.28E-8
rs1865830	16	56874197	A	G	0.384	-0.088	0.015	1.77E-9
rs2007432	16	56874857	T	C	0.387	-0.089	0.015	1.06E-9
chr16:56875272:I	16	56875272	AT	A	0.387	-0.089	0.015	1.06E-9
rs11860701	16	56876845	G	T	0.093	0.14	0.025	1.27E-8
rs2399562	16	56880158	G	A	0.388	-0.09	0.015	1.00E-9
rs8045306	16	56883709	C	G	0.391	-0.083	0.015	1.16E-8
rs735144	16	56883924	G	A	0.388	-0.089	0.015	9.97E-10

rs735145	16	56884081	C	G	0.094	0.14	0.025	1.37E-8
rs77850047	16	56885915	T	C	0.094	0.14	0.025	1.37E-8
rs12445993	16	56886109	G	C	0.094	0.14	0.025	1.36E-8
rs12443821	16	56886203	C	T	0.094	0.14	0.025	1.39E-8
rs80072323	16	56892769	A	G	0.096	0.137	0.025	2.37E-8
rs1436424	16	56895034	T	G	0.406	-0.09	0.015	6.16E-10
rs12599065	16	56896036	C	T	0.392	-0.093	0.015	1.79E-10
rs3829502	16	56896730	A	G	0.389	-0.091	0.014	3.20E-10
rs62035923	16	56898198	G	A	0.639	-0.096	0.015	3.23E-10
rs4784733	16	56899006	T	C	0.667	-0.098	0.015	5.33E-11
rs13306690	16	56899007	C	G	0.094	0.149	0.025	2.48E-9
rs1968493	16	56900100	G	A	0.633	-0.105	0.016	1.37E-11
rs13306673	16	56900931	T	C	0.09	0.154	0.026	4.07E-9
rs77188937	16	56909598	T	C	0.087	0.219	0.029	6.40E-14
rs13306677	16	56926195	A	G	0.08	-0.148	0.027	4.33E-8
rs9929408	16	56929944	G	A	0.475	0.091	0.015	3.88E-9
rs1138429	16	56942921	T	A	0.113	0.231	0.026	2.12E-19
rs59515242	16	56945049	A	C	0.214	0.124	0.018	7.55E-12
rs56079121	16	56948292	G	A	0.098	-0.137	0.024	1.69E-8
rs9925265	16	56950210	A	G	0.455	0.09	0.014	2.87E-10
rs718620	16	56950643	T	C	0.097	-0.137	0.024	1.79E-8
rs9921780	16	56952098	G	A	0.454	0.09	0.014	2.95E-10
rs12924331	16	56953261	G	A	0.454	0.09	0.014	2.82E-10
rs55958623	16	56955110	T	C	0.141	0.156	0.021	3.54E-14
rs9931252	16	56959019	T	C	0.098	-0.136	0.024	2.17E-8
rs75429044	16	56960147	A	G	0.097	-0.136	0.024	2.23E-8
rs247606	16	56961204	A	G	0.144	0.149	0.02	2.63E-13
rs28495885	16	56961932	T	C	0.096	-0.135	0.024	3.26E-8
rs193693	16	56962169	G	A	0.144	0.147	0.02	3.75E-13
rs247607	16	56963322	A	G	0.144	0.147	0.02	3.85E-13
rs247608	16	56963468	T	C	0.144	0.147	0.02	3.76E-13
rs2518055	16	56963643	A	T	0.144	0.147	0.02	3.93E-13
rs9932164	16	56964445	T	G	0.097	-0.136	0.024	1.99E-8
rs1366544	16	56964719	A	G	0.144	0.148	0.02	2.80E-13
rs2518058	16	56966554	C	T	0.142	0.146	0.02	8.17E-13
rs2217332	16	56969148	A	G	0.142	0.144	0.02	1.38E-12
rs72786781	16	56970210	A	T	0.026	-0.316	0.053	2.30E-9
rs9938413	16	56972250	T	C	0.108	-0.141	0.023	9.96E-10
rs2562126	16	56972713	A	G	0.144	0.144	0.02	1.42E-12
rs3903056	16	56974791	G	A	0.142	0.146	0.02	6.17E-13
chr16:56974859:I	16	56974859	GAC	G	0.109	-0.141	0.023	8.56E-10
rs952439	16	56975277	C	A	0.148	0.131	0.02	8.85E-11
rs881598	16	56976744	A	G	0.142	0.147	0.02	5.15E-13
rs112952893	16	56980135	A	G	0.113	-0.146	0.023	1.89E-10
rs193694	16	56982549	T	C	0.138	0.178	0.022	6.49E-16
rs9938160	16	56984590	C	T	0.276	-0.17	0.019	7.23E-20
rs247615	16	56984763	G	A	0.206	0.181	0.021	2.22E-17
rs9989419	16	56985139	G	A	0.627	-0.252	0.017	2.71E-51
rs193695	16	56985156	G	A	0.659	-0.252	0.017	8.39E-48
rs72786786	16	56985514	A	G	0.327	-0.312	0.016	6.69E-85
rs12448528	16	56985555	G	A	0.792	-0.308	0.02	1.34E-53

rs1122390	16	56986045	T	G	0.756	-0.113	0.017	8.50E-11
rs7203286	16	56986762	T	G	0.429	0.213	0.015	4.72E-46
rs12446515	16	56987015	T	C	0.335	-0.319	0.015	7.63E-98
rs56156922	16	56987369	C	T	0.335	-0.319	0.015	6.84E-98
rs56228609	16	56987765	T	C	0.324	-0.316	0.015	2.59E-94
rs173539	16	56988044	T	C	0.336	-0.318	0.015	2.55E-98
rs247616	16	56989590	T	C	0.333	-0.321	0.015	3.98E-100
rs12923459	16	56989830	A	G	0.421	0.22	0.015	1.33E-50
rs247617	16	56990716	A	C	0.333	-0.32	0.015	1.02E-99
rs247618	16	56990803	A	G	0.752	-0.107	0.017	3.02E-10
rs183130	16	56991363	T	C	0.333	-0.321	0.015	5.92E-100
rs28888131	16	56991624	A	G	0.177	0.298	0.02	1.06E-51
rs12934632	16	56991741	T	C	0.177	0.298	0.02	1.10E-51
rs12920974	16	56993025	T	G	0.27	0.207	0.016	4.19E-37
rs12149545	16	56993161	A	G	0.321	-0.318	0.015	3.93E-96
rs12708967	16	56993211	C	T	0.177	0.297	0.02	1.18E-51
rs3764261	16	56993324	A	C	0.333	-0.32	0.015	1.36E-99
rs821840	16	56993886	G	A	0.287	-0.335	0.017	2.17E-91
rs36229786	16	56993901	C	A	0.172	0.316	0.021	5.93E-51
rs711751	16	56993909	C	A	0.553	-0.243	0.015	3.37E-58
rs12447839	16	56993935	T	C	0.756	-0.107	0.017	2.30E-10
rs12447924	16	56994192	T	C	0.755	-0.111	0.017	5.38E-11
rs12720918	16	56994212	C	T	0.273	0.212	0.016	2.49E-38
chr16:56994244:I	16	56994244	TA	T	0.332	-0.322	0.015	8.05E-100
rs17231506	16	56994528	T	C	0.328	-0.323	0.015	2.54E-98
rs4783961	16	56994894	A	G	0.508	-0.133	0.015	2.45E-19
rs4783962	16	56995038	C	T	0.756	-0.106	0.017	2.93E-10
rs1800775	16	56995236	A	C	0.491	-0.267	0.014	4.05E-79
rs3816117	16	56996158	C	T	0.492	-0.267	0.014	1.34E-78
rs711752	16	56996211	A	G	0.446	-0.267	0.014	4.70E-77
rs708272	16	56996288	A	G	0.446	-0.267	0.014	4.83E-77
chr16:56996645:D	16	56996645	G	GCC	0.448	-0.271	0.014	5.57E-78
rs1864163	16	56997233	A	G	0.232	0.321	0.017	2.46E-76
chr16:56997349:I	16	56997349	CA	C	0.233	0.305	0.018	5.89E-67
rs4587963	16	56997369	T	A	0.755	-0.1	0.017	2.98E-9
rs4369653	16	56997551	T	C	0.698	-0.12	0.016	1.81E-14
rs9929488	16	56998572	C	G	0.252	0.223	0.017	4.21E-41
rs12720926	16	56998918	G	A	0.442	-0.263	0.014	1.19E-74
rs7203984	16	56999258	C	A	0.186	0.317	0.019	1.29E-64
rs11508026	16	56999328	T	C	0.437	-0.265	0.014	5.05E-76
chr16:56999778:D	16	56999778	C	CG	0.169	0.348	0.019	1.17E-74
rs708273	16	56999949	G	A	0.7	-0.117	0.016	6.74E-14
rs8045855	16	57000696	A	T	0.176	0.344	0.02	2.22E-69
rs12720922	16	57000885	A	G	0.168	0.348	0.019	3.48E-74
rs118146573	16	57000938	A	G	0.117	0.379	0.023	1.17E-61
rs4784741	16	57001216	T	C	0.44	-0.26	0.014	5.12E-74
chr16:57001254:I	16	57001254	TCACA	T	0.169	0.345	0.019	6.75E-74
chr16:57001274:D	16	57001274	A	AC	0.43	-0.264	0.015	1.13E-72
rs12444012	16	57001438	A	G	0.439	-0.26	0.014	6.28E-74
chr16:57001579:D	16	57001579	A	AAAAAC	0.28	-0.264	0.018	1.01E-49
chr16:57001580:D	16	57001580	A	AAAAC	0.404	-0.261	0.015	1.02E-68

chr16:57001581:D	16	57001581	A	AAAC	0.44	-0.26	0.014	7.30E-74
rs60545348	16	57001985	C	A	0.299	0.115	0.016	1.02E-13
rs72771478	16	57002118	T	G	0.02	-0.401	0.064	4.74E-10
rs72771479	16	57002121	T	A	0.02	-0.4	0.064	5.19E-10
rs12597002	16	57002404	A	C	0.299	0.115	0.016	1.06E-13
rs9926440	16	57002663	G	C	0.731	-0.215	0.016	1.72E-41
rs9939224	16	57002732	G	T	0.81	-0.321	0.018	8.31E-72
rs11076174	16	57003146	C	T	0.071	0.236	0.029	7.92E-16
rs7205804	16	57004889	A	G	0.449	-0.264	0.014	2.94E-77
rs1532625	16	57005301	T	C	0.45	-0.264	0.014	2.78E-77
rs1532624	16	57005479	A	C	0.45	-0.264	0.014	2.98E-77
rs117040820	16	57005762	T	C	0.019	-0.356	0.062	1.06E-8
rs11076175	16	57006378	G	A	0.166	0.345	0.019	3.86E-73
rs7499892	16	57006590	T	C	0.166	0.34	0.019	1.71E-71
rs289713	16	57006829	A	T	0.817	-0.312	0.019	6.44E-62
rs11076176	16	57007446	G	T	0.168	0.305	0.02	2.09E-50
rs289714	16	57007451	A	G	0.821	-0.276	0.02	1.33E-43
rs158477	16	57007610	A	G	0.509	0.092	0.015	1.35E-9
rs158478	16	57007734	C	A	0.504	0.097	0.015	9.74E-11
rs158479	16	57008048	A	G	0.516	0.087	0.015	4.82E-9
rs158480	16	57008227	A	G	0.86	0.203	0.023	2.38E-19
rs158617	16	57008287	G	A	0.848	0.217	0.022	9.14E-23
rs289715	16	57008508	T	A	0.88	0.195	0.024	3.12E-16
rs289716	16	57009376	A	T	0.692	0.164	0.016	5.45E-26
rs289717	16	57009388	A	G	0.346	0.151	0.015	6.40E-24
chr16:57009651:D	16	57009651	T	TC	0.696	0.165	0.016	4.97E-26
chr16:57009657:D	16	57009657	C	CA	0.416	0.179	0.018	7.98E-24
rs736274	16	57009769	A	T	0.114	-0.193	0.024	2.81E-15
rs289718	16	57009932	T	C	0.7	0.165	0.016	1.27E-25
rs289719	16	57009941	C	T	0.701	0.165	0.016	1.29E-25
rs56208677	16	57010232	T	C	0.066	-0.301	0.033	1.87E-19
rs117427818	16	57010486	T	C	0.05	0.462	0.038	3.26E-33
rs1968905	16	57010948	G	T	0.824	0.117	0.02	4.12E-9
rs4784744	16	57011185	A	G	0.354	0.149	0.015	6.17E-23
rs291044	16	57011452	A	G	0.345	0.152	0.015	6.28E-24
chr16:57012559:I	16	57012559	AT	A	0.246	-0.15	0.017	1.27E-17
rs12720889	16	57012563	T	A	0.285	-0.16	0.016	2.82E-23
rs291043	16	57012699	G	A	0.341	0.153	0.015	9.20E-24
rs12447620	16	57014319	G	A	0.842	0.204	0.022	3.55E-20
rs12708983	16	57014411	C	T	0.039	-0.254	0.042	2.23E-9
rs12708985	16	57014610	C	T	0.842	0.19	0.023	4.43E-17
rs4784745	16	57014875	G	A	0.342	0.155	0.015	4.90E-24
rs5880	16	57015091	C	G	0.059	0.373	0.036	2.50E-25
rs5882	16	57016092	A	G	0.685	0.143	0.015	8.48E-21
rs1800777	16	57017319	A	G	0.047	0.417	0.042	1.91E-23
rs289741	16	57017474	A	G	0.706	0.156	0.016	8.17E-23
rs289742	16	57017762	G	C	0.869	0.199	0.023	2.86E-18
rs289743	16	57017796	A	G	0.707	0.158	0.016	3.74E-23
rs289744	16	57018102	T	G	0.707	0.16	0.016	4.23E-23
rs112039804	16	57018856	A	T	0.106	-0.204	0.027	1.83E-14
rs12720917	16	57019392	C	T	0.149	-0.14	0.024	6.21E-9

rs7198642	16	57032461	G	T	0.189	-0.115	0.021	3.87E-8
rs75911530	16	57049137	A	G	0.032	0.311	0.05	5.26E-10
rs61738710	16	57060097	T	C	0.068	0.205	0.033	5.32E-10

SNP, single nucleotide polymorphism; CHR, chomosome; POS, position; (N)EA, (non) effect allele; EAF, effect allele frequency; BETA, beta coefficient; SE, standard error.

Table 4.C.2: Distribution of coding alleles of the lead single nucleotide polymorphisms (SNPs) in lipid-lowering drug users and non-lipid-lowering drug users, in the total Netherlands Epidemiology of Obesity (NEO) study population (n=5,706).

Number of coding alleles	Non-users (n, %)	Lipid lowering drug users (n, %)
rs247616-C		
0	517 (11%)	113 (13%)
1	2,165 (45%)	373 (42%)
2	2,128 (44%)	410 (46%)
rs12720922-A		
0	3,326 (69%)	605 (68%)
1	1,341 (28%)	260 (29%)
2	143 (3%)	31 (3%)
rs1968905-G		
0	145 (3%)	38 (4%)
1	1,440 (30%)	237 (26%)
2	3,225 (67%)	621 (69%)

Table 4.C.3: Genetic correlation of serum CETP concentration with serum lipid concentrations (i.e. total cholesterol, HDL-cholesterol, triglycerides and LDL-cholesterol) and body mass index, in the total Netherlands Epidemiology of Obesity (NEO) study population (n=5,706).

Serum concentration	Genetic correlation with serum CETP concentration
Total cholesterol	-0.020
HDL-C	0.17
Triglycerides	-0.29
LDL-C	0.074
Body mass index	0.032

Table 4.C.4: Effects of genetic variation in the lead single nucleotide polymorphisms (SNPs) on circulating lipid concentrations, in the total Netherlands Epidemiology of Obesity (NEO) study population and the Global Lipids Genetics Consortium.

	Difference in concentration per additional serum CETP increasing allele	95% CI	N	P-Value
	rs247616 (C allele)			
NEO study				
Total cholesterol (mmol/L)	-0.03	-0.08, 0.01	5,693	0.109
HDL-C (mmol/L)	-0.09	-0.11, -0.08	5,692	1.18E-36
Triglycerides (mmol/L)	0.01	-0.03, 0.04	5,692	0.664
LDL-C (mmol/L)	0.05	0.01, 0.09	5,692	0.009
Global Lipids Genetics Consortium				
Total cholesterol (mmol/L)	-0.05	-0.06, -0.04	185,621	4.47E-32
HDL-C (mmol/L)	-0.24	-0.24, -0.25	185,471	0
Triglycerides (mmol/L)	0.04	0.03. 0.05	176,146	1.12E-25
LDL-C (mmol/L)	0.05	0.05, 0.06	171,458	2.57E-37
	rs12720922 (A allele)			
NEO study				
Total cholesterol (mmol/L)	-0.09	-0.14, -0.04	5,693	0.001
HDL-C (mmol/L)	-0.11	-0.13, -0.09	5,692	6.30E-32
Triglycerides (mmol/L)	0.01	-0.04, 0.05	5,692	0.808
LDL-C (mmol/L)	0.02	-0.03, 0.06	5,692	0.513
Global Lipids Genetics Consortium				
Total cholesterol (mmol/L)	-0.05	-0.06, -0.04	92,615	2.14E-14
HDL-C (mmol/L)	-0.26	-0.24, -0.25	92,714	1.67E-318
Triglycerides (mmol/L)	0.04	0.03, 0.05	86,712	3.24E-11
LDL-C (mmol/L)	0.05	0.04, 0.06	83,073	1.42E-13
	rs1968905 (G allele)			
NEO study				
Total cholesterol (mmol/L)	-0.03	-0.09, 0.02	5,693	0.193
HDL-C (mmol/L)	-0.02	-0.04, -0.00	5,692	0.030
Triglycerides (mmol/L)	0.01	-0.04, 0.05	5,692	0.808
LDL-C (mmol/L)	-0.02	-0.06, 0.03	5,692	0.490
Global Lipids Genetics Consortium				
Total cholesterol (mmol/L)	N/A	N/A	N/A	N/A
HDL-C (mmol/L)	N/A	N/A	N/A	N/A
Triglycerides (mmol/L)	N/A	N/A	N/A	N/A
LDL-C (mmol/L)	N/A	N/A	N/A	N/A

N/A, not available in the Global Lipids Genetics Consortium database.

Table 4.C.5: Effects of genetic variation in the Taq1B and -629C>A CETP single nucleotide polymorphisms (SNPs) on coronary artery disease (CAD) risk and circulating lipid concentrations, in the CARDIoGRAMplusC4D 1000 Genomes Consortium and the Global Lipids Genetics Consortium.

	Difference per additional serum CETP increasing allele	95% CI	P-Value
	Taq1B (rs708272-G)		
CARDIoGRAMplusC4D 1000 Genomes Consortium			
CAD risk (odds ratio)	1.02	1.01, 1.04	0.011
Global Lipids Genetics Consortium			
Total cholesterol (mmol/L)	N/A	N/A	N/A
HDL-C (mmol/L)	N/A	N/A	N/A
Triglycerides (mmol/L)	N/A	N/A	N/A
LDL-C (mmol/L)	N/A	N/A	N/A
	-629C>A (rs1800775-C)		
CARDIoGRAMplusC4D 1000 Genomes Consortium			
CAD risk (odds ratio)	1.03	1.01, 1.05	0.0027
Global Lipids Genetics Consortium			
Total cholesterol (mmol/L)	-0.04	-0.05, -0.04	2,10E-25
HDL-C (mmol/L)	-0.20	-0.21, -0.20	0
Triglycerides (mmol/L)	0.04	0.03, 0.05	1.33E-26
LDL-C (mmol/L)	0.04	0.03, 0.05	8.5E-24

N/A, not available in the Global Lipids Genetics Consortium database