

Lipid and Metabolic Syndrome Traits in Coronary Artery Disease: A Mendelian Randomization Study

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Running Title: MR of lipid and metabolic syndrome traits in CAD

Nonstandard Abbreviations:

MR: Mendelian randomization

SBP: systolic blood pressure

IVW: inverse variance-weighted

UKBB : UK Biobank

MR-PRESSO: MR-pleiotropy residual sum and outlier

OR: odds ratio

CI: confidence interval

TRL: triglyceride-rich lipoprotein

MAF: minor allele frequency

Abstract

Mendelian randomization (MR) of lipid traits in coronary artery disease (CAD) has provided evidence for causal associations of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) in CAD, but many lipid trait genetic variants have pleiotropic effects on other cardiovascular risk factors that may bias MR associations. The goal of this study was to evaluate pleiotropic effects of lipid trait genetic variants and to account for these effects in MR of lipid traits in CAD. We performed multivariable MR using inverse variance-weighted (IVW) and MR-Egger methods in large ($n \geq 300,000$) GWAS datasets. We found that 30% of lipid trait genetic variants have effects on metabolic syndrome traits, including body mass index (BMI), type 2 diabetes (T2D), and systolic blood pressure (SBP). Nonetheless, in multivariable MR analysis, LDL-C, high-density lipoprotein cholesterol (HDL-C), TG, BMI, T2D, and SBP are independently associated with CAD, and each of these associations is robust to adjustment for directional pleiotropy. MR at loci linked to direct effects on HDL-C and TG suggests locus- and mechanism-specific causal effects of these factors on CAD.

Keywords: Human Genetics, Lipoproteins, LDL, HDL, Triglycerides, Mendelian Randomization, Coronary Artery Disease

Introduction

Mendelian randomization (MR) is a method used to infer the causal effect of a risk factor, or exposure, on a disease outcome (1). In MR, genetic variants affecting the exposure are considered to confer a randomized allocation of the exposure (2). The causal effect of changes in the exposure can then be assessed by comparing the disease outcome across genotypes. Typically, single nucleotide polymorphisms (SNPs) are used for MR, and their individual effects on exposures and outcomes can be collected from genome-wide association studies (GWAS) (3). However, in cases where exposure SNPs have pleiotropic effects on unmeasured traits, risk factors other than the exposure may be imbalanced in the groups selected by allocation of the exposure SNPs, leading to biased causal estimates in MR (2,4).

MR has been used to assess the role of the lipid traits low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) in coronary artery disease (CAD). SNPs at specific loci modifying LDL-C, HDL-C, and TG have been used in MR (5-7), but lipid trait SNPs often modify more than one lipid trait because they affect enzymes or disease states, such as insulin resistance, that control the remodeling of multiple lipoproteins. Multivariable MR has been used to adjust for effects of lipid trait SNPs on multiple lipid traits while integrating data from hundreds of SNPs (8,9). MR methods that account for measurement error in GWAS effect estimates, such as inverse-variance weighting (MR-IVW), and methods that estimate bias due to pleiotropy statistically, such as MR-Egger, have been developed and applied to MR of lipid traits in CAD (4,10,11).

In surveys of many GWAS, lipid traits share genetic determinants with other metabolic traits such as body mass index (BMI) and waist-hip ratio, which are also risk factors for CAD (12-15). Derangements of lipid and other metabolic traits co-occur in the metabolic syndrome, a clinical entity comprising abdominal obesity, dyslipidemia, hypertension, and insulin resistance (16). A few studies have attempted to adjust for the impact of metabolic syndrome trait pleiotropy in lipid trait MR by omitting BMI and blood pressure SNPs or regressing on residuals after adjustment for BMI and systolic blood pressure (SBP) (17,18). These

studies have concluded that there is a causal relationship of LDL-C and TG with CAD and have arrived at conflicting results with regard to the causal effect of HDL-C. MR-Egger analysis suggests that the apparent causal effects of both HDL-C and TG in CAD are mediated at least in part by pleiotropic effects of lipid trait SNPs on other traits (9,19); however, MR-Egger is known to have reduced power to detect causal effects compared to MR-IVW (4).

The availability of MR datasets of increasing size, including measurements of potential pleiotropic factors such as BMI, type 2 diabetes (T2D), and SBP provides the opportunity to readdress and adjust for the impact of pleiotropy in the association of lipid and metabolic syndrome traits with CAD. Using expanded datasets 2- to 6-fold larger than those previously used for pleiotropy adjustment in lipid trait MR, we found that 30% of lipid trait SNPs have pleiotropic effects on the metabolic syndrome traits BMI, SBP or T2D. We collected lipid and metabolic syndrome trait SNPs from large ($n \geq 300,000$) GWAS and modeled the effects of LDL-C, HDL-C, TG, BMI, T2D, and SBP on CAD in multivariable MR. We were also able to assess the impact of SNPs which directly modify CAD. We found that each of the studied lipid and metabolic syndrome traits is independently associated with CAD by MR. Nonetheless, MR analysis of representative SNPs at loci with direct effects on HDL-C and TG suggest that the causal association between these traits and CAD is locus- and mechanism-specific.

Materials and Methods

Ethical Review

All analyses were performed on anonymized summary statistics from published GWAS studies with appropriate institutional review board approval (20-24). Separate institutional review board approval was not required for this study.

Datasets

Lipid trait summary statistics were obtained from the Million Veteran Program GWAS ($n = 291,746$) (20). Lipid trait effect sizes are in s.d. from inverse normal transformed residuals of lipids after adjusting for age, sex, and 10 principal components of ancestry as described in Klarin et al. (20). BMI summary statistics were obtained from a meta-analysis of GIANT and UK Biobank (UKBB) datasets ($n = 806,834$) (21). BMI effect sizes are in s.d. from inverse normal transformed residuals of BMI adjusted for age, sex, recruitment center, genotyping batches, and 10 principal components as described in Pilit et al. (21). T2D summary statistics were obtained from a meta-analysis of DIAGRAM and UKBB datasets ($n = 71,124$ cases and 824,006 controls) (22). T2D effect sizes are expressed as log odds ratio adjusted for age, sex, array, and 6 principal components as described in Mahajan et al. (22). SBP summary statistics were obtained from a meta-analysis of ICBP and UKBB datasets ($n = 757,601$) (23). SBP effect sizes are in mm Hg (untransformed), adjusted for age, sex, genotyping array, and 10 principal components as described in Evangelou et al. (23). CAD summary statistics were obtained from a meta-analysis of CARDIoGRAMplusC4D and UKBB datasets ($n = 122,733$ cases and 424,528 controls) (24). CAD effect sizes are expressed as log odds ratio and adjusted for age, sex, and up to 30 principal components as described in van der Harst and Verweij (24).

Selection of Instrumental Variables

We used a threshold for genome-wide significance of 1×10^{-8} to account for the presence of both common (minor allele frequency (MAF) $> 5\%$) and low-frequency variants ($5\% > MAF > 1\%$) and the large number

of imputed SNPs (25-27). We selected genome-wide significant SNPs ($P < 1 \times 10^{-8}$) for each trait and used the TwoSampleMR R package (28) to clump SNPs that are in LD ($R^2 > 0.01$) within a 10Mb interval. For locus-specific MR, we considered distinct, genome-wide significant SNPs within a 1Mb interval of the transcription start site of the selected gene.

Mendelian Randomization

For multivariable MR, we oriented the SNPs with respect to a positive effect on the exposure of interest as described (11) and modeled the effects of lipid traits or lipid and metabolic syndrome traits for each SNP. We considered two types of multivariable MR analysis. One is multivariable MR-IVW (10), and the second is multivariable MR-Egger (11). In both approaches, we used linear regression to model log odds ratio for CAD against lipid or lipid and metabolic syndrome trait effects and weighted each SNP by the inverse of the variance of its log odds ratio for the outcome. In MR-IVW, we set the intercept at zero, while in MR-Egger, the intercept is included in the model and estimated in the regression (4). The inclusion of the intercept allows the SNPs to have a direct effect on CAD without mediation through the modeled exposures, but may decrease statistical power when there is no unmeasured pleiotropic effect (4). For univariable MR at selected loci, we applied univariate MR-IVW on the set of SNPs at the selected locus (29). For single-SNP MR, we constructed 95% confidence intervals for univariable MR on individual SNPs as described (30).

Analysis of Heterogeneity

For each model, we report multivariable I^2 based on multivariable H^2 as described in Jackson et al., which measures the between-study heterogeneity in meta-analyses (31), and categorized I^2 as low, moderate, or high based on the reference values suggested by Higgins and Thompson (32). We used MR-pleiotropy residual sum and outlier (MR-PRESSO) (33) to identify SNPs with large, inconsistent CAD effects suggestive of direct effects on CAD and considered a SNP to be pleiotropic if the multivariable MR-PRESSO outlier test $P < 0.05$. We considered multivariable MR models with or without exclusion of SNPs

demonstrating evidence of pleiotropy by MR-PRESSO in the indicated analyses to produce more robust estimates.

Interpretation of Results

For pleiotropic effects, we used a Bonferroni-corrected significance threshold of $0.05/2338 = 2 \times 10^{-5}$. For MR analysis, we considered the results significant at a threshold of 0.05 for univariable MR, $0.05/3 = 0.0167$ for three-exposure multivariable MR, and $0.05/6 = 0.0083$ for six-exposure multivariable MR, reflecting Bonferroni correction for multiple testing in the setting of modeling 3 and 6 associations, respectively.

Results

Pleiotropic Effects of Lipid Trait SNPs on Metabolic Syndrome Traits

We extracted independent, genome-wide significant lipid trait SNPs from the Million Veteran Program GWAS of approximately 300,000 individuals (20). We obtained 164 LDL-C SNPs, 202 HDL-C SNPs, and 179 TG SNPs at a significance level of $P < 1 \times 10^{-8}$, representing 343 distinct loci with available CAD data. We compared lipid trait effect sizes at these SNPs to corresponding estimates from the earlier Global Lipid Genetics Consortium GWAS ($n = 188,577$) (34) and found that effect estimates from these two datasets are highly concordant, although estimates from the Million Veteran Program GWAS are slightly smaller in magnitude on average (Supplemental Figure S1), likely due to attenuation of upward bias due to the winner's curse effect (35). Effects of lipid trait SNPs on BMI, T2D and SBP were collected from large ($n > 700,000$), densely imputed meta-analyses of UKBB data with GIANT, ICBP, and DIAGRAM consortium datasets, respectively (21-23). We also assessed independent, genome-wide significant SNPs for metabolic syndrome traits and obtained 897 SNPs for BMI, 207 SNPs for T2D, and 689 SNPs for SBP.

A total of 2338 SNPs representing 1492 distinct loci modifying lipid and metabolic syndrome traits at genome-wide significance were assessed for pleiotropy. Among all lipid trait SNPs, 30% have pleiotropic effects on BMI, T2D, or SBP at a Bonferroni-corrected significance threshold of $P < 2 \times 10^{-5}$, including 20% of LDL-C SNPs, 31% of HDL-C SNPs, and 37% of TG SNPs (Table 1; Figure 1). Overlap between genetic determinants of HDL-C and TG is also widespread, with 47% of HDL-C SNPs affecting TG and 49% of TG SNPs affecting HDL-C (Table 1). As a result, only 36% of genome-wide significant HDL-C SNPs and 30% of genome-wide significant TG SNPs do not have effects on other lipid and metabolic syndrome traits (Figure 1). A similar degree of genetic overlap between lipid and metabolic syndrome traits is evident in the set of 185 lipid trait SNPs based on the Global Lipid Genetics Consortium GWAS (Supplemental Table S1) that has been used in previous multivariable MR of lipid traits in CAD (8).

Since many lipid trait SNPs carry pleiotropic lipid and metabolic syndrome trait effects, we first considered whether a restricted set of non-pleiotropic lipid trait SNPs could be generated by filtering out known pleiotropic SNPs. We generated prospective restricted sets of lipid trait-specific SNPs for HDL-C and TG, where pleiotropy is most widespread, by filtering out SNPs with evidence for pleiotropy at a stringent Bonferroni-corrected threshold of $P < 2 \times 10^{-5}$. However, the distribution of P-values for pleiotropic trait effects in these restricted sets is concentrated in the interval $P < 0.1$ (Supplemental Figure S2A-B), indicating that many of these variants have pleiotropic effects that are inefficiently estimated and not removed by filtering. Filtering out pleiotropic effects at $P < 0.05$ also yields a left-shifted distribution of P-values, suggesting that pleiotropic effects are still present (Supplemental Figure S2C-D). Filtering out pleiotropic effects at $P < 0.1$ yields only 6 HDL-C and 2 TG SNPs (Supplemental Figure S2E-F), an insufficient number for robust MR analysis. Thus, statistical filtering for non-pleiotropic lipid trait SNPs is limited by widespread pleiotropy, making multivariable adjustment a more appropriate approach for genome-wide MR of lipid traits in CAD.

To identify individual SNPs that modify CAD through lipid trait-independent mechanisms, we used the multivariable MR-PRESSO method (33) to test for large CAD effects that are not explained by effects on LDL-C, HDL-C, or TG. Multivariable MR-PRESSO identified 8 SNPs as pleiotropic in MR of lipid traits in CAD (Supplemental Table S2). Four of these have very large CAD effects and small lipid trait effects; these SNPs are in the *LPA* and 9p21 loci, which are well-known to have lipid trait-independent atherogenic effects (36,37). Three of the remaining 4 lipid trait SNPs identified as pleiotropic by MR-PRESSO have strong SBP associations; the last SNP is located in *DAGLB*, the gene encoding diacylglycerol lipase β , in which a coding variant is associated with waist-hip ratio (38). In multivariable MR-PRESSO analysis of lipid and metabolic syndrome traits in CAD, only 3 SNPs in the *LPA* and 9p21 loci are identified by MR-PRESSO as pleiotropic (Supplemental Table S2). This indicates that their CAD effects are not mediated via the lipid and metabolic syndrome traits considered here but more likely through other mechanisms.

Multivariable MR of Lipid Traits in CAD

We used 343 genome-wide significant lipid trait SNPs to model multivariable MR of lipid traits in CAD using the Million Veteran Program lipid dataset and a recent meta-analysis of CARDIoGRAMplusC4D and UKBB datasets ($n = 122,733$ cases and 424,528 controls; 2-fold more CAD cases than CARDIoGRAMplusC4D alone) while excluding pleiotropic SNPs identified statistically by MR-PRESSO. The multivariable MR-IVW method accounts for effects of SNPs that modify multiple lipid traits. Multivariable MR-IVW analysis of lipid traits in CAD supports associations with CAD for LDL-C (OR 1.55; 95%CI 1.47-1.63; $P = 4.8 \times 10^{-47}$), HDL-C (OR 0.86; 95%CI 0.82-0.91; $P = 1.2 \times 10^{-7}$), and TG (OR 1.23; 95%CI 1.16-1.30; $P = 1.5 \times 10^{-11}$) (Table 2; Figure 2A-C).

Inclusion of 8 pleiotropic SNPs identified by MR-PRESSO does not change this pattern of associations but decreases the strength of all associations slightly and increases the proportion of variation attributable to heterogeneity in the size of the lipid trait-CAD association for different SNPs (I^2) by 19% (Supplemental Table S3; Figure 2D-F). Multivariable MR-Egger estimates and adjusts for the intercept predicted by a collection of SNPs (11). The MR-Egger intercept can be interpreted as the CAD effect suggested by these SNPs in the absence of a lipid effect, e.g. a global measure of the unmeasured pleiotropic effects of a set of SNPs (4). Multivariable MR-Egger analysis moderately attenuates the HDL-C and TG associations (Table 2). However, the MR-Egger intercept test does not indicate the presence of bias due to directional pleiotropy.

Multivariable MR of Lipid and Metabolic Syndrome Traits in CAD

Multivariable MR-IVW analysis of lipid and metabolic syndrome traits in CAD using 1492 independent, genome-wide significant lipid and metabolic syndrome trait SNPs reveals associations with CAD for LDL-C (OR 1.48; 95%CI 1.41-1.56; $P = 4.5 \times 10^{-51}$), HDL-C (OR 0.87; 95%CI 0.82-0.91; $P = 1.1 \times 10^{-7}$), TG (OR 1.17; 95%CI 1.10-1.23; $P = 4.8 \times 10^{-8}$), BMI (OR 1.33; 95%CI 1.25-1.41; 2.2×10^{-21}), T2D (OR 1.07;

95%CI 1.05-1.10; $P = 3.3 \times 10^{-8}$), and SBP (OR 1.037; 95%CI 1.034-1.041; $P = 3.5 \times 10^{-91}$) when the 3 pleiotropic SNPs identified by MR-PRESSO are excluded (Table 3; Figure 3A-F). Inclusion of 3 pleiotropic SNPs identified by MR-PRESSO does not change this pattern but decreases the strength of all associations slightly and increases the proportion of variation attributable to heterogeneity in the size of the lipid trait-CAD association for different SNPs (I^2) by 7% (Supplemental Table S4).

Multivariable MR-Egger analysis of lipid and metabolic syndrome traits in CAD also suggests associations of LDL-C, HDL-C, TG, BMI, T2D, and SBP with CAD. The MR-Egger intercept test does not indicate the presence of bias due to directional pleiotropy for HDL-C, TG, T2D, and SBP. However, it does indicate the presence of bias due to directional pleiotropy for LDL-C and BMI, and MR-Egger adjustment moderately increases the OR for LDL-C and BMI. This effect suggests that the LDL-C and BMI estimates are affected by pleiotropic effects of the modeled SNPs that are still unaccounted for, and that the magnitude of these associations may be moderately larger if these factors were modeled.

Monogenic Univariable MR of Lipid Traits in CAD

In multivariable MR of lipid and metabolic syndrome traits, a moderate to high degree of statistical heterogeneity remains ($I^2 = 70\%$), likely reflecting distinct pathways by which different SNPs modify lipid and metabolic syndrome traits and consequent variation in the respective CAD associations (Table 3). To characterize this heterogeneity, we performed monogenic univariable MR at lipid trait-modifying loci where the mechanism of the lipid trait effect is well-defined, facilitating the causal interpretation of monogenic MR at these loci (39,40). We focused on HDL-C and TG for this analysis because the causal effect of LDL-C in CAD is well-established and the effects of LDL-C SNPs on CAD are relatively homogeneous (Figure 2A); monogenic univariable MR of mechanistically well-defined LDL-C SNPs of large lipid trait effect size, e.g. *PCSK9* and *LDLR* (Supplemental Table S5), has been previously reported (41).

We first considered all loci carrying HDL-C and TG SNPs with large lipid trait effect sizes, which are listed in Supplemental Table S5. Of these loci, *LIPG*, *CETP*, *LPL*, and *ANGPTL4* have well-defined mechanisms linked to a single enzymatic activity, while SNPs at the *CD300LG* and *APOA1-C3* loci do not. Variants in *CETP* were previously studied and have been suggested to modify CAD risk via disparate effects on multiple lipid traits (42-45), so we omitted this locus from monogenic univariable MR. In order to interrogate the basis of heterogeneity of HDL-C and TG associations with CAD, we also selected mechanistically well-defined loci carrying SNPs with moderate lipid trait effects and CAD effects that are inconsistent with the CAD effects of large effect SNPs, which occurred at *LCAT* and *LIPC* for HDL-C, and *MLXIPL* and *FADS1* for TG.

Locus-specific MR at HDL-C-modifying Genes

We performed monogenic univariable MR of HDL-C in CAD at *LIPG*, *LCAT*, and *LIPC*. The *LIPG* locus encodes endothelial lipase, which modifies HDL-C by catabolizing HDL phospholipids; this activity decreases HDL stability and may cause the resulting phospholipid-poor HDL to be a poorer cholesterol acceptor (46-48). The rs77960347 SNP in *LIPG* encoding the N396S mutation confers a large increase in HDL-C and has been previously studied in a monogenic MR experiment (6). In MR based on CAD data from 20,913 CAD cases and 95,407 controls, a null association of *LIPG* N396S with CAD was reported (6), with an OR for CAD of 0.99 (95%CI 0.88-1.11; $P = 0.85$) for the G allele encoding the missense mutation. In a subsequent CAD GWAS comprising 60,801 CAD cases and 123,504 controls (49), *LIPG* N396S is associated with a possible CAD effect with an OR of 0.90 (95%CI 0.82-1.00; $P = 0.05$). In the present CAD meta-analysis of 122,733 CAD cases and 424,528 controls, *LIPG* N396S is associated with a CAD effect with an OR of 0.90 (95%CI 0.86-0.95; $P = 8.8 \times 10^{-5}$). Consequently, univariable MR on this single SNP reveals a causal protective effect of increased HDL-C conferred by decreased endothelial lipase activity in the present study (Table 4; Figure 4A).

Three SNPs in regulatory regions of the *LIPG* locus, including the UTR and upstream and downstream noncoding DNA, are associated with smaller HDL-C effects and concordant, small CAD effects. In univariable MR on these 3 regulatory variants in *LIPG*, increased HDL-C is associated with decreased CAD risk (Table 4; Figure 4B). Mutations in *LIPG* have effects on LDL-C at 25-35% the magnitude of their HDL-C effects, but these LDL-C effects are directionally discordant with the CAD effects of *LIPG* SNPs, e.g. inconsistent with mediation via a deleterious effect of LDL-C (Supplemental Table S6).

The *LCAT* locus encodes lecithin-cholesterol acyltransferase (LCAT), which modifies HDL-C by converting free cholesterol in HDL into the more neutral species cholestryl ester without affecting macrophage reverse cholesterol transport (50). SNPs in *LCAT* lead to isolated changes in HDL-C (Supplemental Table S6) and confer an increased risk of CAD per unit increase in HDL-C (Table 4; Figure 4C). The *LIPC* locus encodes hepatic lipase, which remodels HDL and apoB-containing lipoproteins (51) and modifies HDL-C by enhancing hepatic selective uptake of cholestryl esters without affecting macrophage reverse cholesterol transport (52-54). SNPs in *LIPC* primarily modify HDL-C, except for the LDL-C SNP rs2218260 (Supplemental Table S6). SNPs in *LIPC* confer an increased risk of CAD per unit increase in HDL-C; this effect is independent of rs2218260 (Table 4; Figure 4D).

Locus-specific MR at TG-modifying Genes

We next performed monogenic univariable MR of TG in CAD at *LPL*, *ANGPTL4*, *MLXIPL*, and *FADS1*. Nine distinct SNPs in *LPL* encoding lipoprotein lipase (LPL), which hydrolyzes TG in all lipoprotein classes (55), are associated with large and heterogeneous, but consistently deleterious, effects on CAD per unit increase in TG. Univariable MR on this set of 9 SNPs suggests that increased TG conferred by decreased LPL activity leads to increased CAD risk (Table 4; Figure 4E), as previously reported (7,56). SNPs at *ANGPTL4* encoding angiopoietin-like 4, an inhibitor of LPL (57), are also associated with large, deleterious effects on CAD per unit increase in TG. Univariable MR on 3 *ANGPTL4* SNPs suggests that increased TG conferred by *ANGPTL4*-mediated LPL inhibition leads to increased CAD risk (Table 4;

Figure 4F), consistent with prior studies (7,58). SNPs in both *LPL* and *ANGPTL4* are associated with large HDL-C effects in addition to their TG effects (Supplemental Tables S5 and S7), likely as a consequence of increased cholesteryl ester transfer protein activity driven by availability of TG (59).

We also considered TG SNPs at the transcriptional regulator *MLXIPL* (also known as carbohydrate response element binding protein), which controls expression of genes involved in hepatic TG biosynthesis (60). When considered in univariable MR, 4 SNPs in *MLXIPL* were not associated with an increased risk of CAD per unit increase in TG (Table 4; Figure 4G). The *FADS1* locus, which encodes a fatty acid desaturase that produces both substrates and endogenous regulators of TG biosynthesis (61), is represented by one distinct SNP, rs174537, which primarily modifies TG. In univariable MR, rs174537 is associated with decreased risk of CAD per unit increase in TG (Table 4; Figure 4H). SNPs in *MLXIPL* and *FADS1* have HDL-C effects at 25-50% of the magnitude of the TG effects which do not account for the lack of deleterious TG effects observed at these loci (Supplemental Table S7).

Discussion

In this study, data from large GWAS of lipid and metabolic traits reveals that numerous lipid trait SNPs also have effects on metabolic syndrome traits. These widespread pleiotropic effects invalidate the use of polygenic univariable MR using restricted sets of SNPs apparently specific for a single lipid trait, even though this approach was used in early MR studies of lipid traits in CAD (6,34,62). When pleiotropic effects are accounted for in multivariable MR, we show independent associations between LDL-C, HDL-C, TG, BMI, T2D, and SBP with CAD, and each of these associations is robust to adjustment for directional pleiotropy using the MR-Egger method. Our results suggest that the lipid and metabolic syndrome traits LDL-C, HDL-C, TG, BMI, T2D and SBP are causally linked to CAD, although HDL-C and TG appear to have locus- and mechanism-specific causal effects.

The CAD associations for LDL-C, TG, BMI, T2D, and SBP are consistent with the results of previous MR and observational studies (9,63-69). In previous smaller studies, MR of lipid traits in CAD revealed causal associations of LDL-C and TG with CAD and suggested that pleiotropic effects of lipid trait SNPs accounted for much of the apparent protective effect of HDL-C and part of the deleterious effect of TG (9,18,19). Here, we have systematically addressed pleiotropy due to co-occurrence of lipid and metabolic trait derangements in insulin resistance and the metabolic syndrome using multivariable analysis on large GWAS datasets, with the result that there is minimal evidence of effects of residual pleiotropy that would substantially alter our conclusions. Our finding that LDL-C, HDL-C, and TG are independently associated with CAD by MR builds on three recent reports showing independent MR associations of LDL-C, HDL-C, and TG with abdominal aortic aneurysm (19,20,70), suggesting that each of these factors is biologically active at the level of the arterial wall.

The locus- and mechanism-specific causal effects of HDL-C and TG may be expected given the biology of the lipid traits. While LDL-C is thought to directly measure the primary etiologic agent of CAD (71), the HDL hypothesis suggests that HDL-C is a proxy for an unmeasured causal variable, macrophage cholesterol

efflux and reverse cholesterol transport, which mediate protection from atherosclerosis (Figure 5) (72,73). Increased HDL-C conferred by decreased endothelial lipase activity is linked to protection from CAD, possibly because the change in HDL-C in this setting reflects a change in properties of HDL that correlate with increased cholesterol efflux and reverse cholesterol transport (47,48). In contrast, mutations in LCAT and LIPC that increase the cholesteryl ester content of HDL without concordantly modifying reverse cholesterol transport (50,53) lead to increased HDL-C but are associated with increased CAD.

Likewise, TG is a proxy for the concentration and composition of TG-rich lipoproteins (TRL), which promote atherosclerosis via several mechanisms, including deposition of cholesterol and apolipoprotein B in atherosclerotic lesions (Figure 5) (74,75). SNPs in *LPL* and *ANGPTL4* that increase TG by decreasing the turnover of TRL have large, deleterious effects on CAD, but SNPs in *MLXIPL* and *FADS1* that modify TG biosynthesis have minimal or opposite effects on CAD. Our conclusion that HDL-C and TG are associated with locus- and mechanism-specific causal effects on CAD is not in disagreement with the negative results of some randomized controlled trials of HDL-C-raising and TG-lowering therapies, including fibrates, niacin, and cholesteryl ester transfer protein inhibitors (76-79), as the reported MR associations represent a composite of hundreds of distinct lipid-modifying mechanisms (17).

Certain characteristics of the datasets used in this study are unique. Using densely imputed GWAS of 300,000 individuals, we identified twice as many lipid trait SNPs compared to previous lipid trait MR and estimated their CAD effects from a dataset over twice as large as the CAD datasets used in previous lipid trait MR (8,9,18,19). Metabolic syndrome trait effects for lipid and metabolic syndrome trait SNPs were estimated from GWAS of >700,000 individuals, and we used this data to adjust for metabolic syndrome trait effects and reduce confounding due to pleiotropic effects of lipid trait SNPs. Using MR-PRESSO, we screened for SNPs with large, exposure-independent CAD effects, including SNPs in the *LPA* and 9p21 loci. Exclusion of these SNPs reduced the potential for bias due to direct CAD effects of lipid trait SNPs,

decreased statistical heterogeneity, and permitted more robust multivariable MR of lipid and metabolic syndrome traits.

Our study also has several limitations. We adjusted for pleiotropic effects of lipid SNPs at the level of metabolic syndrome traits and pathways leading to large, inconsistent CAD effects, but the moderate to high degree of heterogeneity in our final multivariable MR model suggests that genetic overlap with unmeasured traits is present. MR-Egger analysis suggests that pleiotropic effects of these SNPs do not bias the modeled associations for HDL-C, TG, T2D, and SBP, while adjustment for directional pleiotropy slightly increases the magnitude of the LDL-C and BMI associations and does not change our conclusions. We used large datasets to model lipid and metabolic syndrome traits and CAD, but the quality of effect size estimates is likely to improve with the incorporation of additional data. This is particularly evident for CAD, as illustrated by the improvement in precision over successive CAD datasets for the LIPG N396S mutation. Finally, while MR may provide insights into causal effects of the exposure traits, it may not accurately estimate the magnitude of those effects (2,17,40), and thus may have limited utility in assessing the impact of therapeutic intervention on the clinical outcome.

Data Availability

All GWAS summary statistics used for the included MR analyses and analysis of pleiotropy have been deposited and published on Mendeley: <https://data.mendeley.com/datasets/dz9w684x8p/1> (doi: 10.17632/dz9w684x8p.1). R scripts for these analyses are available on Github: <http://www.github.com/dgt2109/bio-script/> (doi: 10.5281/zenodo.3986183).

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Disclosures

A.R.T. is a consultant to CSL and Amgen and on the scientific advisory boards of Staten Biotechnology and Fortico Biotechnology.

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Table 1. Genetic Overlap Between Lipid and Metabolic Syndrome Traits.

	Pleiotropic Trait						BMI,T2D, or SBP
Exposure	LDL-C	HDL-C	TG	BMI	T2D	SBP	
LDL-C	100%	17%	21%	12%	7%	6%	20%
HDL-C	18%	100%	47%	14%	18%	14%	31%
TG	18%	49%	100%	17%	21%	19%	37%
BMI	3%	4%	4%	100%	11%	9%	100%
T2D	3%	16%	21%	29%	100%	19%	100%
SBP	2%	4%	5%	10%	6%	100%	100%

Percent of SNPs that are genome-wide significant ($P < 1 \times 10^{-8}$) for the exposure trait with a secondary (pleiotropic trait) exposure that is significant after Bonferroni correction ($P < 2 \times 10^{-5}$).

Table 2. Multivariable MR of lipid traits in CAD.

Multivariable MR-IVW						
	OR	CI	P	I ²		
LDL-C	1.55	1.47 - 1.63	4.8×10^{-47}	71%		
HDL-C	0.86	0.82 - 0.91	1.2×10^{-7}	71%		
TG	1.23	1.16 - 1.30	1.5×10^{-11}	71%		
Multivariable MR-Egger						
	OR	CI	P	I ²	Int.	Int. P
LDL-C	1.55	1.46 - 1.65	5.0×10^{-35}	71%	0.00011	0.92
HDL-C	0.89	0.83 - 0.95	0.00089	71%	-0.0016	0.13
TG	1.19	1.11 - 1.28	1.5×10^{-6}	71%	0.0017	0.10

Results of multivariable regression of CAD effect size on LDL-C, HDL-C, and TG effect size with the intercept fixed at zero (multivariable MR-IVW), or with estimation of the intercept (multivariable MR-Egger). Eight pleiotropic SNPs identified by MR-PRESSO have been excluded; analysis with inclusion of these SNPs is presented in Supplemental Table S3.

MR-IVW: Mendelian randomization, inverse-variance weighted

MR-Egger: Mendelian randomization-Egger regression

OR: odds ratio per unit change in exposure trait

CI: 95% confidence interval

Int.: MR-Egger Intercept

Table 3. Multivariable MR of lipid and metabolic syndrome traits in CAD.

Multivariable MR-IVW						
	OR	CI	P	I ²		
LDL-C	1.48	1.41 - 1.56	4.5×10^{-51}	70%		
HDL-C	0.87	0.82 - 0.91	1.1×10^{-7}	70%		
TG	1.17	1.10 - 1.23	4.8×10^{-8}	70%		
BMI	1.33	1.25 - 1.41	2.2×10^{-21}	70%		
T2D	1.07	1.05 - 1.10	3.3×10^{-8}	70%		
SBP	1.037	1.034 - 1.041	3.5×10^{-91}	70%		
Multivariable MR-Egger						
	OR	CI	P	I ²	Int.	Int. P
LDL-C	1.56	1.48 - 1.65	3.8×10^{-54}	70%	-0.0015	1.0×10^{-5}
HDL-C	0.88	0.83 - 0.93	1.4×10^{-5}	70%	-0.00053	0.19
TG	1.18	1.11 - 1.26	6.2×10^{-8}	70%	-0.00041	0.30
BMI	1.44	1.32 - 1.57	3.3×10^{-16}	70%	-0.0012	0.013
T2D	1.06	1.03 - 1.09	0.00020	70%	0.00071	0.13
SBP	1.037	1.032 - 1.042	1.3×10^{-50}	70%	7.7×10^{-5}	0.86

Results of multivariable regression of CAD effect size on LDL-C, HDL-C, TG, BMI, T2D, and SBP effect size with the intercept fixed at zero (multivariable MR-IVW), or with estimation of the intercept (multivariable MR-Egger). Three pleiotropic SNPs identified by MR-PRESSO have been excluded; analysis with inclusion of these SNPs is presented in Supplemental Table S4.

MR-IVW: Mendelian randomization, inverse-variance weighted

MR-Egger: Mendelian randomization-Egger regression

OR: odds ratio per unit change in exposure trait

CI: 95% confidence interval

Int.: MR-Egger Intercept

Table 4. Monogenic Univariable MR of HDL-C and TG in CAD.

Locus	Trait	Univariable MR-IVW			
		OR	CI	P	I ²
LIPG N396S [†]	HDL-C	0.64	0.51 - 0.80	0.00013	-
3 regulatory <i>LIPG</i> SNPs	HDL-C	0.87	0.77 - 0.98	0.035	0%
2 <i>LCAT</i> SNPs	HDL-C	1.28	1.19 - 1.37	0.014	0%
6 <i>LIPC</i> SNPs	HDL-C	1.28	1.13 - 1.44	0.0032	8%
9 <i>LPL</i> SNPs	TG	1.60	1.33 - 1.93	0.00043	72%
3 <i>ANGPTL4</i> SNPs	TG	1.79	1.08 - 2.97	0.038	50%
4 <i>MLXIPL</i> SNPs	TG	1.10	0.93 - 1.31	0.16	0%
1 <i>FADS1</i> SNP	TG	0.77	0.65 - 0.92	0.0038	-

Results of univariable MR on selected SNPs at 7 representative loci for genes directly modifying HDL-C or TG. The heterogeneity statistic I² is reported for analyses involving more than one SNP.

MR-IVW: Mendelian randomization, inverse-variance weighted

[†]: Based on CAD data from the meta-analysis of CARDIoGRAMplusC4D and UKBB data

OR: odds ratio per unit change in HDL-C or TG

CI: 95% confidence interval

Figure Legends

Figure 1: Pleiotropic Effects of Lipid Trait SNPs on Metabolic Syndrome Traits

In this chord plot, the thickness of the arc leaving each exposure trait represents the percentage of the genome-wide significant exposure trait SNPs that are associated with the pleiotropic trait receiving the arc at a Bonferroni-corrected significance level of 2×10^{-5} . The size of the parabola that arcs from each exposure trait to itself represents the percentage of SNPs with no pleiotropic associations detected at a Bonferroni-corrected significance level of 2×10^{-5} .

Figure 2: MR of Lipid Traits in CAD

Points represent genome-wide significant lipid trait SNPs without missing data. Regression lines for multivariable MR-IVW and multivariable MR-Egger are shown. (A-C) Plot of LDL-C (A), HDL-C (B), or TG (C) effect size in s.d. units against lipid covariate-adjusted CAD risk, excluding 8 pleiotropic SNPs identified by MR-PRESSO. (D-F) Plot of LDL-C (D), HDL-C (E), or TG (F) effect size in s.d. units against lipid covariate-adjusted CAD risk in log odds ratio, including 8 pleiotropic SNPs identified by MR-PRESSO.

Figure 3: MR of Lipid and Metabolic Syndrome Traits in CAD

Points represent genome-wide significant lipid and metabolic syndrome trait SNPs without missing data. Regression lines for multivariable MR-IVW and multivariable MR-Egger are shown. (A-F) Plot of LDL-C (A), HDL-C (B), TG (C), BMI (D), T2D (E), or SBP (F) effect size in s.d. units (LDL-C, HDL-C, TG, BMI), log odds ratio (T2D), or untransformed (SBP) against lipid and metabolic syndrome covariate-adjusted CAD risk in log odds ratio, excluding 3 pleiotropic SNPs identified by MR-PRESSO.

Figure 4: Locus- and Mechanism-Specific MR Associations for HDL-C and TG

Forest plots of locus- or SNP-specific MR estimates for the indicated genes. (A) MR of HDL-C in CAD based on the LIPG N396S mutation in successive CAD datasets of increasing size. (B) MR of HDL-C in

CAD based on 3 noncoding SNPs in the *LIPG* locus. (C) MR of HDL-C in CAD based on 2 SNPs in the *LCAT* locus. (D) MR of HDL-C in CAD based on 6 SNPs in the *LIPC* locus. (E) MR of TG in CAD based on 9 SNPs in the *LPL* locus. (F) MR of TG in CAD based on 3 SNPs in the *ANGPTL4* locus. (G) MR of TG in CAD based on 4 SNPs in the *MLXIPL* locus. (H) MR of TG in CAD based on 1 SNP in the *FADS1* locus.

Figure 5: Proposed Causal Diagram of Lipid and Metabolic Syndrome Trait Effects in CAD

Directed acyclic graph representing proposed causal relationships between lipid traits and CAD, unobserved causal factors mediating HDL-C and TG effects, and confounding by metabolic syndrome traits. Putative effect of genetic intervention at *LIPG*, *LCAT*, *LIPC*, *LPL*, *ANGPTL4*, *MLXIPL*, and *FADS1* is indicated.

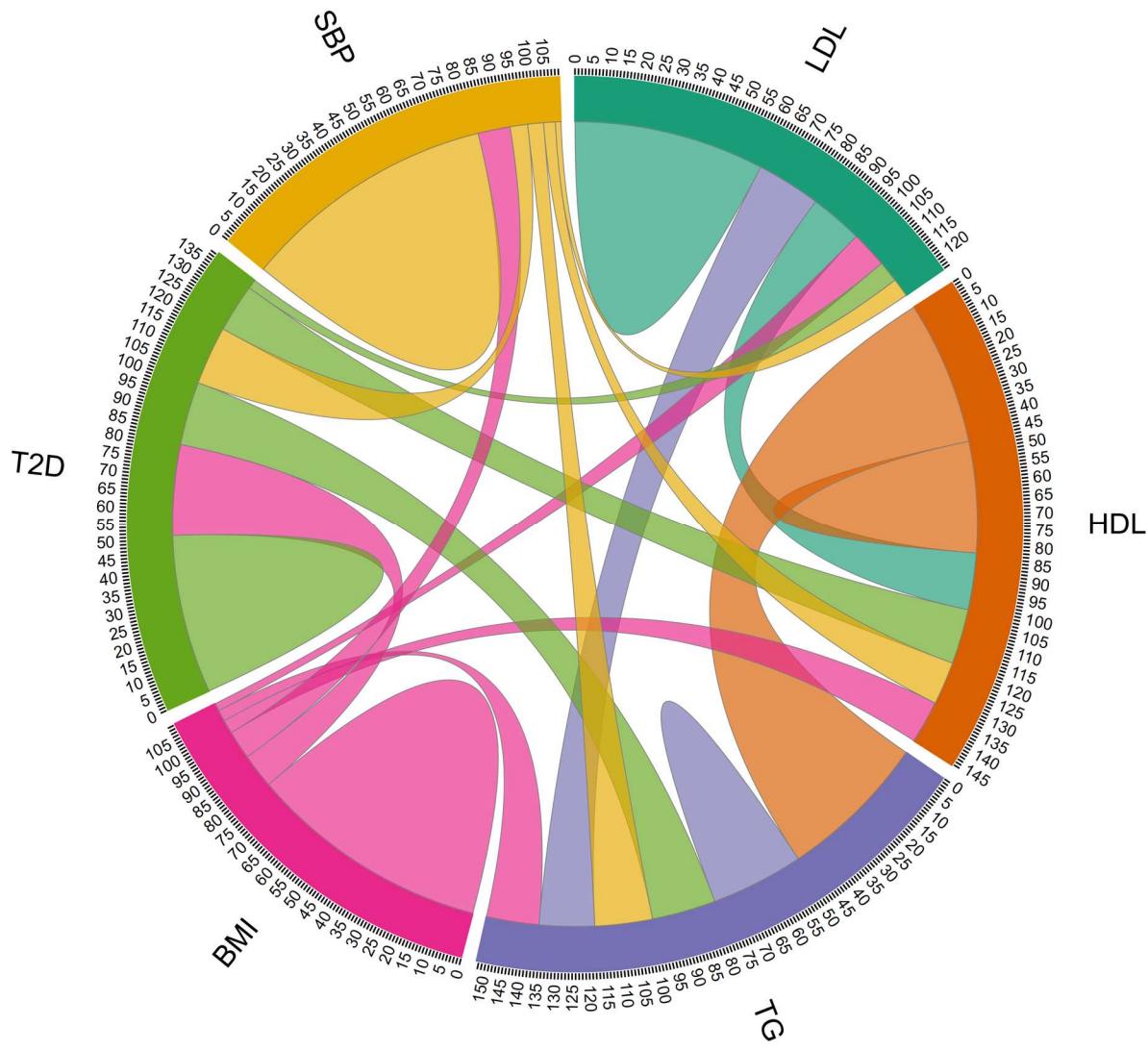
Figure 1

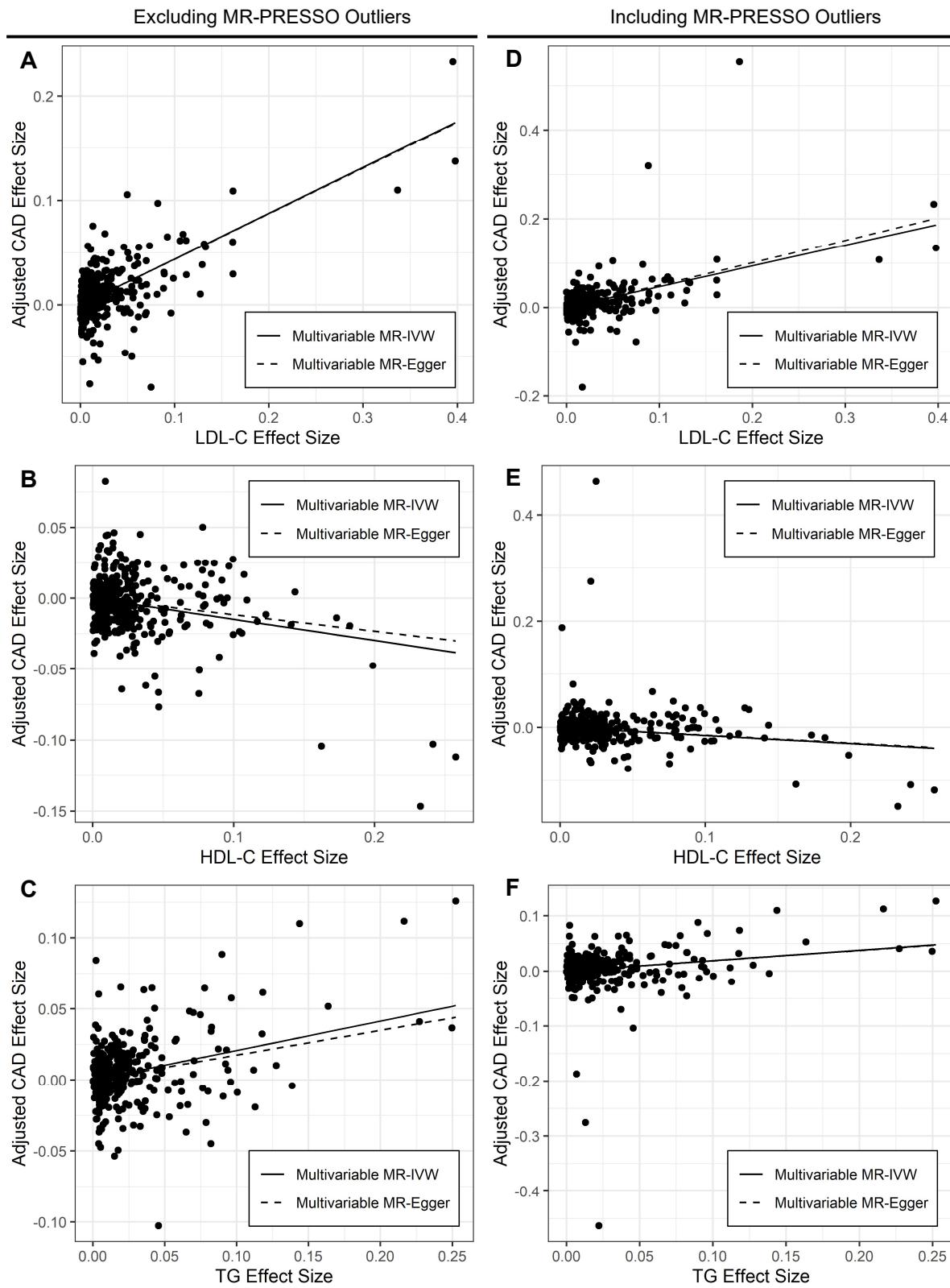
Figure 2

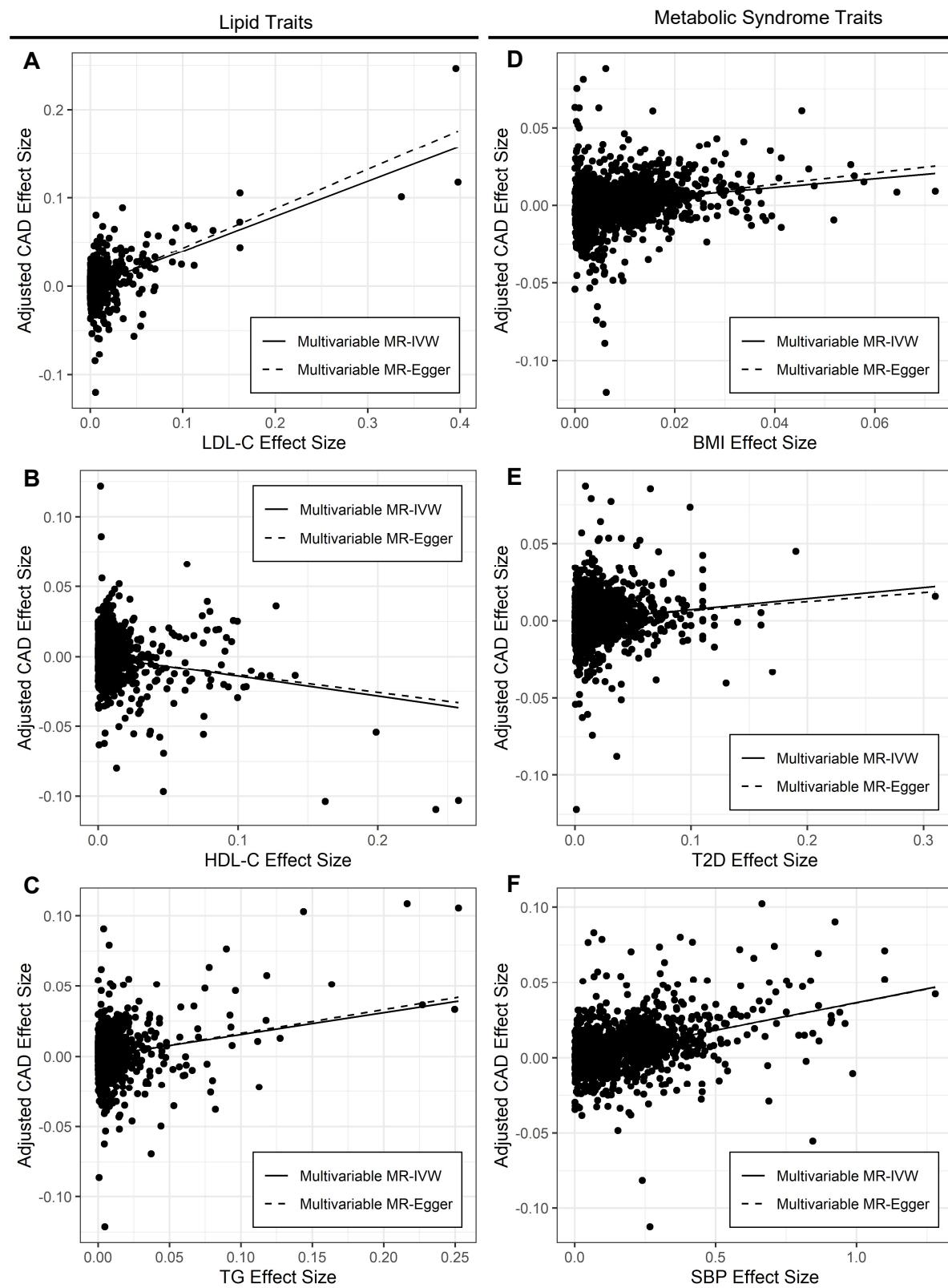
Figure 3

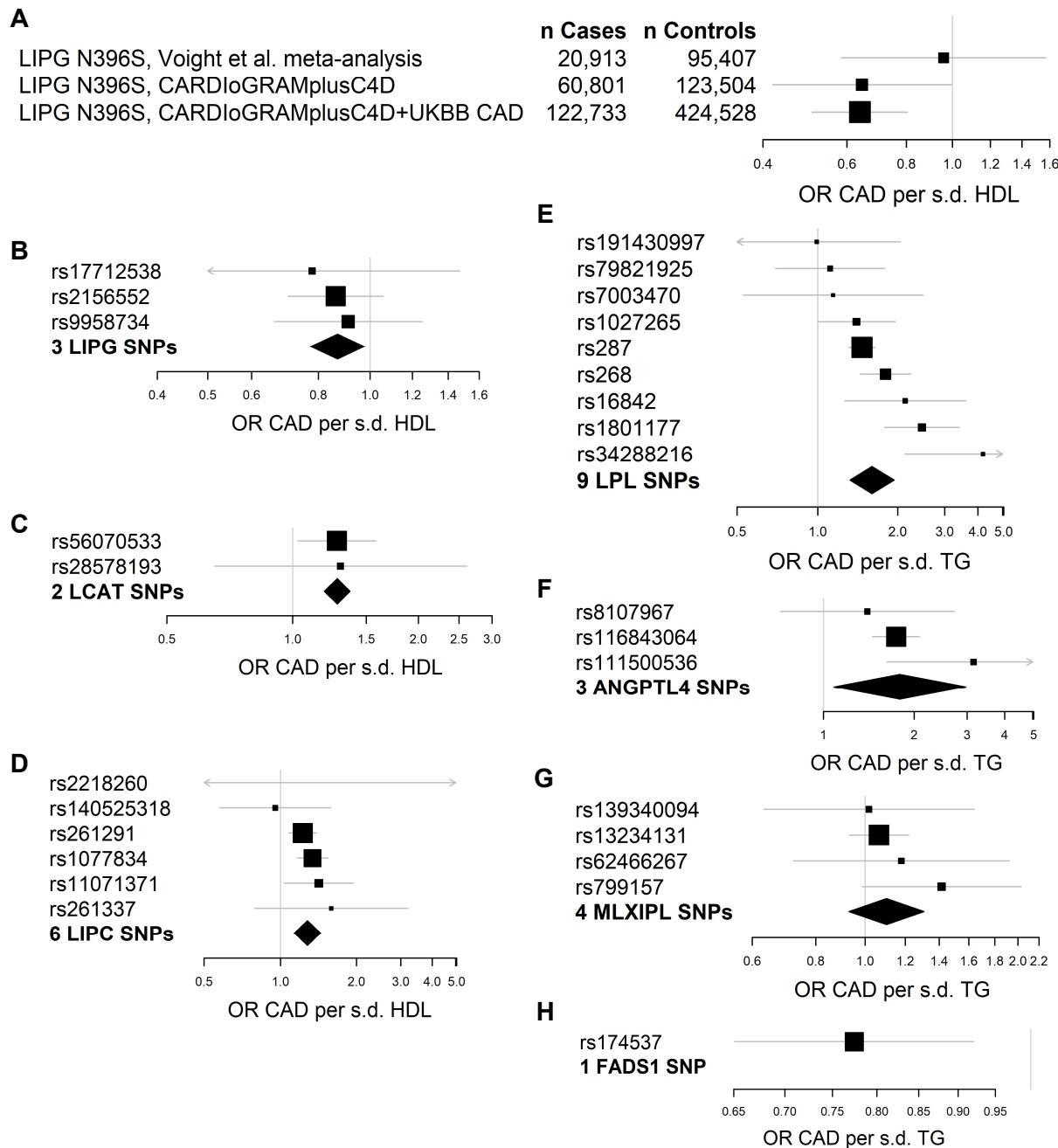
Figure 4

Figure 5

