Mendelian randomization of lipids, diabetes and coronary disease

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Abstract (250 words)

Background Low-density lipoprotein cholesterol is causally related to coronary artery disease (CAD) and emerging evidence suggests that drugs that target LDL-C may also influence risk of type 2 diabetes (T2D). In contrast, the causal role of TG and HDL-C for CAD and T2D is less clear. We investigated the causal relationships of these three lipid fractions with CAD and T2D through Mendelian randomization (MR).

Methods Summary estimates for associations of common single nucleotide polymorphisms (SNPs) with LDL-C, TG and HDL-C were obtained from the Global Lipid Genetics Consortium, and for the same SNPs with CAD and T2D from CARDIoGRAMplusC4D and DIAGRAM consortia, respectively. We constructed genetic instruments for MR and applied them using three approaches: (i) conventional MR that does not account for pleiotropy; (ii) multivariate MR that adjusts for measured pleiotropic associations among the lipid fractions studied; (iii) MR analysis based on Egger regression (MR-Egger) that accounts for both measured and unmeasured pleiotropic associations of the genetic instruments. We used information on the proportion of variance of lipid trait explained and presence of pleiotropy to guide interpretation of causal estimates. We express all results as a genetically-instrumented 1-standard deviation (SD) increase in exposure.

Results Using a genetic instrument comprised of 130 SNPs that explained 7.4% of LDL-C variance, conventional MR in the absence of a pleiotropic instrument provided a causal estimate of (odds ratio; 95% confidence interval) (1.56; 1.38, 1.76) for CAD. For T2D, pleiotropy in the LDL-C genetic instrument was present and MR-Egger identified a protective causal effect on risk of T2D (0.73; 0.58, 0.97). Using 110 SNPs explaining 2.6% of HDL-C variation, the HDL-C genetic instrument was pleiotropic for CAD and MR-Egger yielded a non-causal estimate (0.95; 0.78, 1.14): however using all SNPs and/or limiting to non-pleiotropic SNPs showed a protective effect of HDL-C. For T2D, the HDL-C genetic instrument was non-pleiotropic and conventional MR identified a protective effect of HDL-C for T2D (0.78; 0.62, 0.97).Using 120 SNPs explaining 5.1% of its variance, the TG genetic instrument was pleiotropic for both CAD and T2D. MR-Egger for both estimates identified a casual effect of TG on increased risk of CAD (1.55; 1.42, 1.69) and no effect on T2D (0.99; 0.70, 1.42).

Conclusion The major lipid subfractions have divergent causal roles in CAD and T2D. LDL-C and TG robustly increase risk of CAD at a similar magnitude of effect whereas the protective role of HDL-C for CAD remains unclear. Higher concentrations of both HDL-C and LDL-C are both protective of T2D. These associations can be used to gauge expected findings from therapeutic interventions on blood lipid concentrations.

Keywords: lipids, diabetes, coronary artery disease, epidemiology, Mendelian randomization

Introduction

Understanding the interplay of lipids and their impact on risk of type 2 diabetes (T2D) and coronary artery disease (CAD) is gaining widespread interest, and is of considerable public health importance. For example, low-density lipoprotein cholesterol (LDL-C) is well recognized as causally related to CAD[1-3] and a causal role of triglycerides (TG) in CAD is gaining acceptance.[4, 5] In contrast, the causal role of high-density lipoprotein cholesterol (HDL-C) in CAD remains in doubt.[5-7]

Evidence is emerging that LDL-C reduction through statins results in a modest increase in risk of type 2 diabetes (T2D)[8, 9] that is outweighed by the benefit of statins in protecting from CAD. However, whether these effects on CAD and T2D are general properties of LDL-C (as opposed to effects of certain LDL-C pathways such as those mediated by HMG-coA reductase) remains unclear.[10] In contrast, the causal role of TG and HDL-C in diabetes is less clear.[11]

A better understanding of how lipids affects T2D and CAD is important in order to shed light on the causal relationships between these highly correlated traits. This would help our understanding of intervening on one exposure and the likely downstream consequences. Furthermore, characterizing the relationship of lipids with diabetes is critical to help inform on expected findings from ongoing drug development for lipid modification for cardiovascular disease.[12, 13]

While observational studies can shed light on the relationships of risk factors with one another and disease, residual confounding and reverse causality limit causal inference. Mendelian randomization (MR) permits unbiased investigations into causal roles of exposures. However, when investigating complex and potentially related traits such as lipids and diabetes, problems can arise with lack of specificity of individual or multiple genetic instruments for the risk factor of interest (termed "pleiotropy"). Such pleiotropy can be vertical (i.e. due to down-stream consequences of the instrumented phenotype) or horizontal (due to associations of SNPs with other traits, proximal to the phenotype of interest). While vertical pleiotropy is not problematic in MR (merely reflecting the causal pathway from risk factor through to disease), horizontal pleiotropy, particularly unbalanced horizontal pleiotropy, may invalidate one of the key assumptions of MR, that the genetic variant(s) only affects the outcome through the exposure of interest.[14]

Recent methodological advances in MR, including "multivariate"[15] and "MR-Egger",[16] provide routes to accounting for pleiotropy of genetic instruments. In multivariate MR, genetic associations of potential pleiotropic traits are incorporated into the model, thereby adjusting for them. However, this has the additional consequence of adjusting for both horizontal and vertical pleiotropy. For example, in the case of HDL-C and risk of CAD, adjusting for LDL-C and TG in a multivariate MR will genetically adjust not only for horizontal pleiotropy, but also for shared

pathways that are downstream of HDL-C that may be shared with LDL-C and TG; thereby adjusting for potential shared mediators. In contrast, MR-Egger corrects for unbalanced *horizontal* pleiotropy. Using the same example, MR-Egger for HDL-C and CAD would adjust for horizontal pleiotropy, while retaining shared pathways downstream of HDL-C that may causally result in CAD.

In this paper, we use summary data from multiple GWAS of cardiometabolic traits to investigate the causal relationships between lipids, diabetes and CAD using three MR approaches: (i) conventional (2-stage) MR that does not account for pleiotropy, (ii) multivariate MR, that accounts for both horizontal and vertical pleiotropy, and (iii) MR-Egger, that adjusts for unbalanced horizontal pleiotropy. We addressed the question of the design of the genetic instrument by considering a range of possible instruments and identifying the minimum sub-set of SNPs within available SNPs which gave maximal exposure variance explained.

Methods

Data sources

We used summary-level data for lipids from the Global Lipids Genetics Consortium (GLGC),[17] diabetes from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM), [18] and CAD from the Coronary ARtery DIsease Genome-wide Replication And Meta Analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics, collectively known as CARDIoGRAMplusC4D consortium.[19] Data were downloaded from their respective websites: GLGC: http://www.sph.umich.edu/csg/abecasis/public/lipids2013; GIANT consortium: https://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files; DIAGRAM consortium: http://diagram-consortium.org; and, CARDIoGRAMplusC4D consortium: http://www.cardiogramplusc4d.org. All datasets were limited to individuals of European ancestry. Beta coefficients and standard errors were obtained for the association of each SNP with all exposures and outcomes from these data sources. Where SNPs were not present in a data-set we used proxies (R^2>0.9) as indicated in Supplementary Table X [on the way].

Selection of SNPs

We used 185 lipid-associated SNPs identified by Willer et al,[17] together with 65 SNPs identified by Talmud et al[20] to generate a series of genetic instruments for each of the exposures: LDL-C, HDL-C and TG. This was conducted by first restricting the SNP-set to those with pairwise R^2<0.2 and then ordering the remaining SNPs by descending statistical significance of association with the corresponding lipid exposure and generating instruments comprising 1 to 200 SNPs. Thus for each exposure a series of up to 200 instruments (depending on the regression model) was created and taken forward to MR analysis.

Handling of SNPs

We matched SNPs across the data sources by aligning them to the same effect allele. Effect allele frequencies were checked for concordance.

Causal framework

We considered the causal effects of lipid traits on risk of diabetes and CAD.

Mendelian randomization analyses

We used three MR approaches.

First, we used conventional 2-sample instrumental variable (IV) analyses, which does not make any allowance for pleiotropy. This was implemented as a linear regression, of outcome over exposure betas weighted by the inverse variance of the outcome beta. Critically the regression is forced to pass through the origin, and this equates to the method first proposed by Johnson.[21]Second, we conducted multivariate MR (MVMR) analyses, which statistically adjusts for pleiotropy with identified additional phenotypes.[22] MVMR is an extension of the conventional weighted regression in which the betas for additional (pleiotropic) phenotypes are included as covariates. In

this case we used all three lipid traits in the model (e.g. for the HDL-C instrument we included XXX???, thereby adjusting for, LDL-C and TG). Third, we used the recently published technique of MR-Egger,[16] which accounts for unbalanced horizontal pleiotropy. MR-Egger is a linear regression of estimated SNP effects (for the exposure-raising allele) on exposure against the corresponding estimates of the SNP on outcome, weighted by the inverse variance of the SNP on outcome effect estimates. This differs from conventional 2-sample Mendelian randomization in that the regression is not forced through the origin. Bowden et al[16] show that this estimate is unaffected by unmeasured pleiotropy and, indeed, the presence of unmeasured pleiotropy can be inferred if the intercept term is non-zero.For MR-Egger, the standard error (SE) for each exposure-outcome estimate was obtained by bootstrapping the distributions of the SNP effect estimates for both exposure and outcome 100,000 times. Otherwise results were obtained from the lm() function in R.

Selection of optimal number of SNPs in genetic instrument

We quantified the proportion of variance (R^2) of the exposure explained by a genetic instrument comprising n SNPs using the R package gtx(). We then made a similar estimate for an instrument with n+50 SNPs and presented this as a ratio. In parallel, we tested (by bootstrapping the summary statistics for the two models 5000 times) the null hypothesis that adding the $(n+1)^{th}$ SNP did not increase the value of R^2, this was tested as a binomial probability $\sim B_{5000,0.5}$ To decide upon the optimal number of SNPs, (without prior reference to the MR results) we identified the point where the slope for the P-value (of no increase in R^2) leveled out – and checked to see that this was the point where the ratio or R^2/R^2+50 plateaued. Two authors (JW and MVH) checked this independently and arose at a consensus as to the number of SNPs to include for each lipid.

Selection of MR model to derive causal estimate

Once we determined the optimal number of SNPs, we used the following decision-tree to select the optimal model from which to derive the causal estimate:

(i) if there was no evidence of unbalanced pleiotropy using the intercept derived from MR-Egger, we used the conventional (2-sample) IV as the causal estimate (as it retains maximal power and has fewest assumptions)

(ii) if there was evidence of unbalanced pleiotropy, we used the causal estimate from MR-Egger

(iii) in cases where there was discordance between conventional MR and MR-Egger, we used MVMR to inform whether differences could arise from pathways shared between the three lipids traits

For HDL-C, where the evidence based in CAD was less clear, we took the additional step of generating a "non-pleiotropic" instrument. This was conducted as per the steps above, but when addition of a SNP to the instrument generated unbalanced pleiotropy, as tested by the intercept of MR-Egger, we excluded the SNP from the model. This therefore forced only non-unbalanced-pleiotropic SNPs into the model.

We used an overly-conservative Bonferroni-adjusted P-value threshold to infer significance. As there were 6 tests (three lipid traits and two outcomes), our threshold for significance was 0.05/6=0.0083.

The inSIDE assumption

The assumption that the IV effect estimate is independent of the exposure effect estimate is an important assumption made during MR analysis (the so-called "inSIDE" rule.[16] We tested the null hypothesis that the ratios of outcome to exposure effect estimates for the SNPs in an instrument were independent of the exposure effect estimates for the same SNPs.

Results

The optimal number of SNPs for each of the lipid traits was 130 for LDL-C (explaining 7.4% of its variance), 130 for HDL-C (accounting for 2.6% of HDL-C variance) and 120 for TG (5.1% of TG variance) (**Supplementary Figures 1-6**).

LDL-C

All three MR models provided very strong evidence of a causal effect of LDL-C and risk of CAD. At 125 SNPs, there was no evidence for unbalanced pleiotropy, and the causal estimates ranged from OR 1.44 (95%CI: 1.33, 1.55) from MVMR to OR 1.52 (95%CI: 1.42, 1.63) for MR-Egger per 1-SD genetically-instrumented increase in LDL-C (equivalent to 1.03 mmol/l) (**Figure 1** and **Supplementary Figure 1**).

For diabetes, there was evidence for pleiotropy as the number of SNPs in the instrument increased. At the optimal number of SNPs (n=130), in the presence of pleiotropy, MR-Egger provided a causal estimate of OR 0.73 (95%CI: 0.58, 0.97) per 1-SD increase in LDL-C, which was consistent with IV estimates derived from conventional and MVMR approaches (**Figure 1** and **Supplementary Figure 2**).

HDL-C

A genetically instrumented 1-SD increase in HDL-C (equivalent to 0.46 mmol/l) did not provide conclusive evidence of a causal relationship of HDL-C with risk of CAD. At 110 SNPs, there was evidence for unbalanced pleiotropy and the corresponding MR-Egger provided an estimate of OR 0.95 (95%CI: 0.78, 1.14) for CAD. This was at odds with the multivariate MR estimate (adjusted for LDL-C and TG) of 0.80 (95%CI: 0.71, 0.91), suggesting that the unbalanced pleiotropy could arise from additional sources not fully accounted for by LDL-C and HDL-C (**Figure 1** and **Supplementary Figure 3A**). Using all available SNPs (n=200), there was no unbalanced pleiotropy and all three MR approaches yielded an estimate indicative of a protective effect of HDL-C on CAD, with the estimate from MR-Egger being OR 0.84 (95%CI: 0.71, 0.98). Pruning the genetic instrument to include only non-pleiotropic SNPs, at 173 SNPs there was a similar proportion of variance of HDL-C explained as the "optimal instrument" (that included unbalanced pleiotropic SNPs), evidence for a causal association emerged using all three models with MR-Egger yielding an OR of 0.82 (95%CI: 0.70, 0.98) **(Supplementary Figure 3A, 3B and Figure 2)**.

For diabetes, at 110 SNPs there was no evidence for unbalanced pleiotropy of the genetic instrument. The two-sample MR provided a causal estimate of OR 0.78 (95%CI: 0.62, 0.97), which was directionally consistent with both MVMR and MR-Egger (although the 95%CI for both these models included the null) (**Supplementary Figure 4**).

TG

At 120 SNPs, in the presence of pleiotropy, a genetically instrumented 1-SD increase in TG yielded a causal OR from MR-Egger of 1.55 (95%CI: 1.42, 1.69), which was of greater magnitude that the estimates derived from 2-sample or MVMR approaches. For diabetes, again there was presence of unbalanced pleiotropy and the MR-Egger estimate did not provide evidence of a causal effect of TG on risk of T2D (OR 0.99; 95%CI: 0.70, 1.42) (**Figure 1** and **Supplementary Figures 5 and 6**).

Putting the pieces together: causal framework

Using the above estimates, we illustrate the causal effects of lipids, diabetes and CAD under two models: a very conservative estimate (using only MR-Egger estimates) and an optimal approach that selected the MR model based on whether unbalanced pleiotropy was present (**Figure 3**). Using the most conservative effects from MR-Egger at the optimal number of SNPs, we identified LDL-C to be unique among the lipid subfractions in raising risk of CAD and protecting against T2D in both the conservative and optimal approaches. In contrast, TG only affected CAD. Under the conservative approach, HDL-C had no effect on either CAD or T2D. In the optimal model, LDL-C and TG were unchanged, whereas a protective effect of HDL-C on risk of T2D emerged.

inSIDE assumption

We were unable to reject the null hypothesis of independence for any outcome exposure pair (**Supplementary Table 1**). Thereby, the "inSIDE" assumption was satisfied for all models tested.

Discussion

We exploited data from multiple GWAS to conduct Mendelian randomization analyses in order to explore the causal relationships between lipids and risk of T2D and CAD. Our findings reveal a complex series of causal relationships that will help inform on potential downstream consequence of therapeutic modification of lipid levels.

LDL-C and TG have robust causal effects on risk of CAD, however the evidence for HDL-C was far less convincing. Using the optimal instrument for HDL-C, there was evidence of unbalanced pleiotropy of the genetic instrument and the estimate derived from MR-Egger (the most conservative and reliable estimate in the presence of unbalanced pleiotropy) did not provide evidence for a causal effect. However, when we widened the net to include all 200 lipid-associated loci, the estimates from all three MR approaches converged on a potential causal role of HDL-C. This was bolstered by an alternative approach in which we excluded pleiotropic SNPs and again identified evidence for a causal effect of HDL-C on risk of CAD by all three approaches. While it is tempting to suggest that two out of three approaches yielded evidence for a causal effect for HDL-C, this should be tempered for the following reasons: (i) using all lipid-associated SNPs as an instrument for HDL-C could result in model over-fitting, meaning that the estimate is biased; and, (ii) selecting only non-pleiotropic SNPs could introduce selection bias in the genetic instrument by focusing on a subset of SNPs that is not representative of any meaningful proxy of HDL-C. Therefore, the pragmatic interpretation is that HDL-C is unlikely to play a causal role in the aetiology of CAD, which is in keeping with prior MRs[5, 6] and findings from recent studies of therapeutics targeting HDL-C.[7, 23]

The association of TG with CAD recapitulates estimates derived from several prior MR and genetic studies.[4, 5] Of note, the strength of evidence using the MR approach that is least prone to bias (MR-Egger) in this study provides a causal estimate of CAD for a 1-SD increase in TG that is similar in magnitude for an equivalent difference in LDL-C (both OR ~1.5 per 1-SD increment in either lipid subfraction). This suggests that clinical trials targeting TG should be expected to have as large magnitudes of effect as therapeutics that lower LDL-C, and should be prioritized.

Both LDL-C and HDL-C had a protective effect on risk of T2D. This causal, yet protective, effect of LDL-C is worthy of further consideration. HMG-coA reductase inhibitors (statins) that reduce LDL-C and CAD risk are noted for their on-target effect of increased risk of diabetes,[8, 9] which fits with our data. However, although the interpretation of our findings is that LDL-C "as a whole" is protective of diabetes, it does not necessarily mean that all LDL-C lowering drugs will increase risk of diabetes. For example, it is possible that specific druggable loci that alter LDL-C and CAD risk (such as therapeutics targeting PCSK9 and apolipoprotein B) will have no net effect on T2D, whereas drugs that result in raised HDL-C and lower LDL-C (such

as CETP inhibitors) may have a beneficial effect on diabetes, as has been reported in prior clinical trials.[24, 25] Large-scale genetic and clinical investigations examining individual therapeutic targets are urgently needed to clarify the expected downstream consequences of intervening on specific therapeutic targets to gauge dysglycaemic associations.[26]

This study has several advantages. First, we use the most up to date data available for lipids, providing the most comprehensive genetic instruments available. Second, we used different MR models, from the most conventional method to recently developed approaches, to facilitate interpretation of the stability of the causal estimate under different models. Third, use of MR-Egger allowed us to include all GWAS-identified lipid-related SNPs in the genetic instruments, irrespective of whether they are pleiotropic, which increases power. Fourth, using summary-level data from different sources represents an efficient study design to facilitate original investigations such as these without the cost or need for *de novo* pheno/genotyping. Fifth, the robust positive association of LDL-C with CAD seen in all three Mendelian randomization approaches replicates prior findings and validates our approach.

Some limitations are also worthy of note. First, despite using all available GWAS data, it is possible that some associations were null due to lack of power, especially given that while MR-Egger is the least biased, it is also the least powered of the three MR approaches.[27] This could account for the lack of association of HDL-C with T2D when using MR-Egger, an association that was present using the conventional MR approach that is permissible given the lack of unbalanced pleiotreopy. Second, MR-Egger relies upon the "InSIDE rule",[16] which rests on the assumption that that the correlation between genetic associations with the exposure and the direct effect of the genetic variants on the outcome is zero. However, we show that none of the genetic models violates inSIDE rule. Third, associations could be diminished by treatment of individuals with lipid-lowering drugs, so-called "canalisation".

In conclusion, our comprehensive Mendelian randomization study identified distinct causal associations between the three major lipid subfraction and T2D and CAD. LDL-C and TG increase risk of CAD, and LDL-C and HDL-C are protective of diabetes. These findings help shed light on the underlying causal pathways, and may inform on expected downstream consequences of intervening on lipid traits.

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Conflicts of Interest

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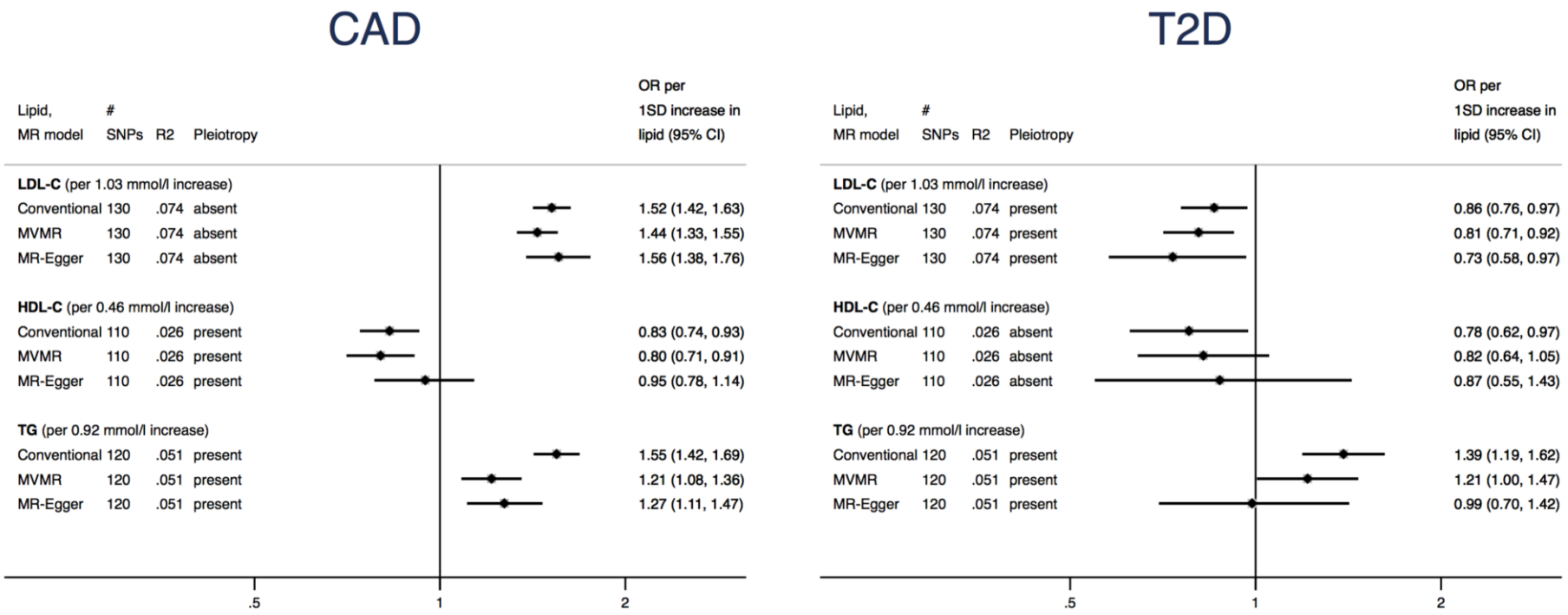
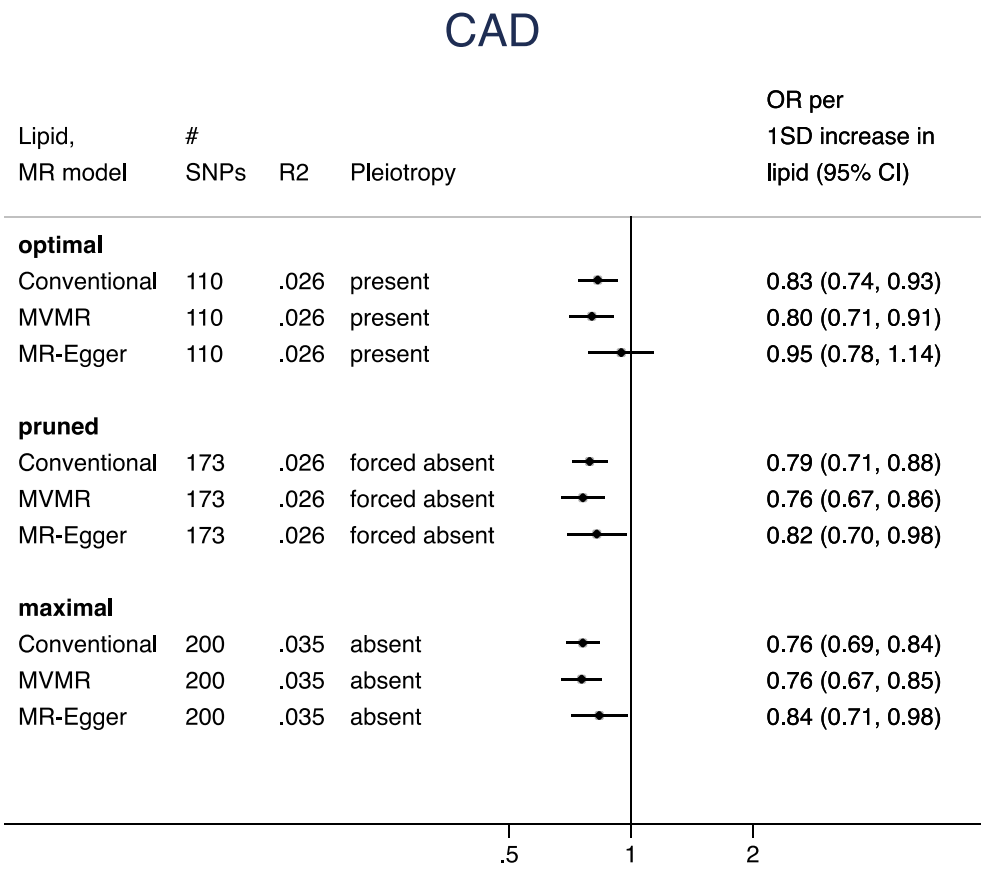
Figure 1. Mendelian randomization associations of lipid traits with risk of coronary artery disease (CAD) and type 2 diabetes (T2D).



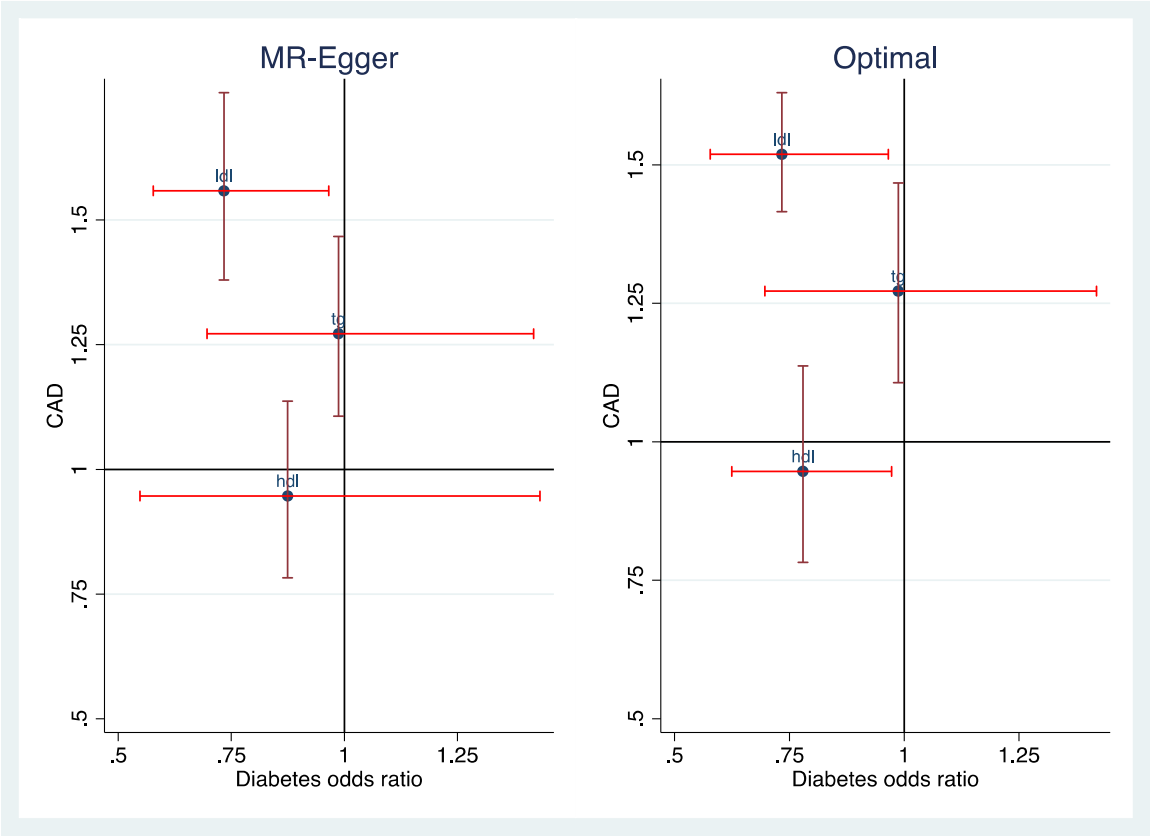
See Methods for description of the three Mendelian randomization (MR) models. Multivariate MR includes adjustment for other lipid traits.

Figure 2. Relationship of HDL-C with CAD using different genetic instruments.



Optimal = selection of model where slope for P-value of increase in R2 plateaus; Maximal = using all available SNPs; Pruned = selecting only SNPs to avoid generating an unbalanced pleiotropic instrument from MR-Egger

Figure 3. Cross-hair plot of a 1-SD increase in lipids and risk of diabetes and coronary artery disease.



All estimates derived from MR-Egger (most conservative approach) for left panel, and optimized according to presence of pleiotropy (on right panel). For the optimized approach, in the absence of pleiotropy the two-sample (conventional) MR estimate was used.

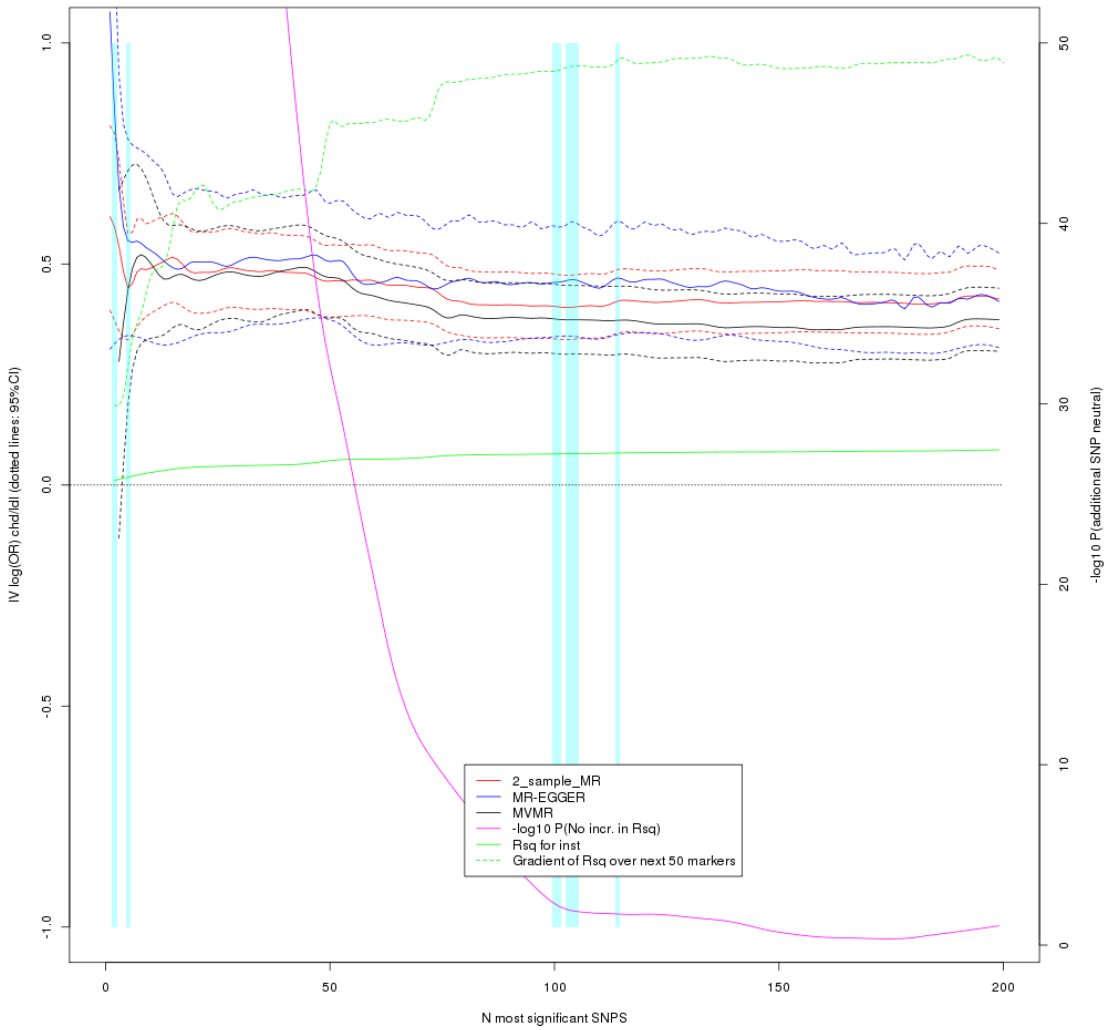
Supplementary Tables

Supplementary Table 1. The correlation and (*P* value) for an association test between the IV estimates and exposure estimates for the SNPs in the chosen instruments.

	CHD	T2D
LDL-C	0.082(*0.365*)	0.084(*0.355*)
HDL-C	-0.017(*0.861*)	0.028(*0.773*)
TG	-0.025(*0.79*)	-0.057(*0.54*)

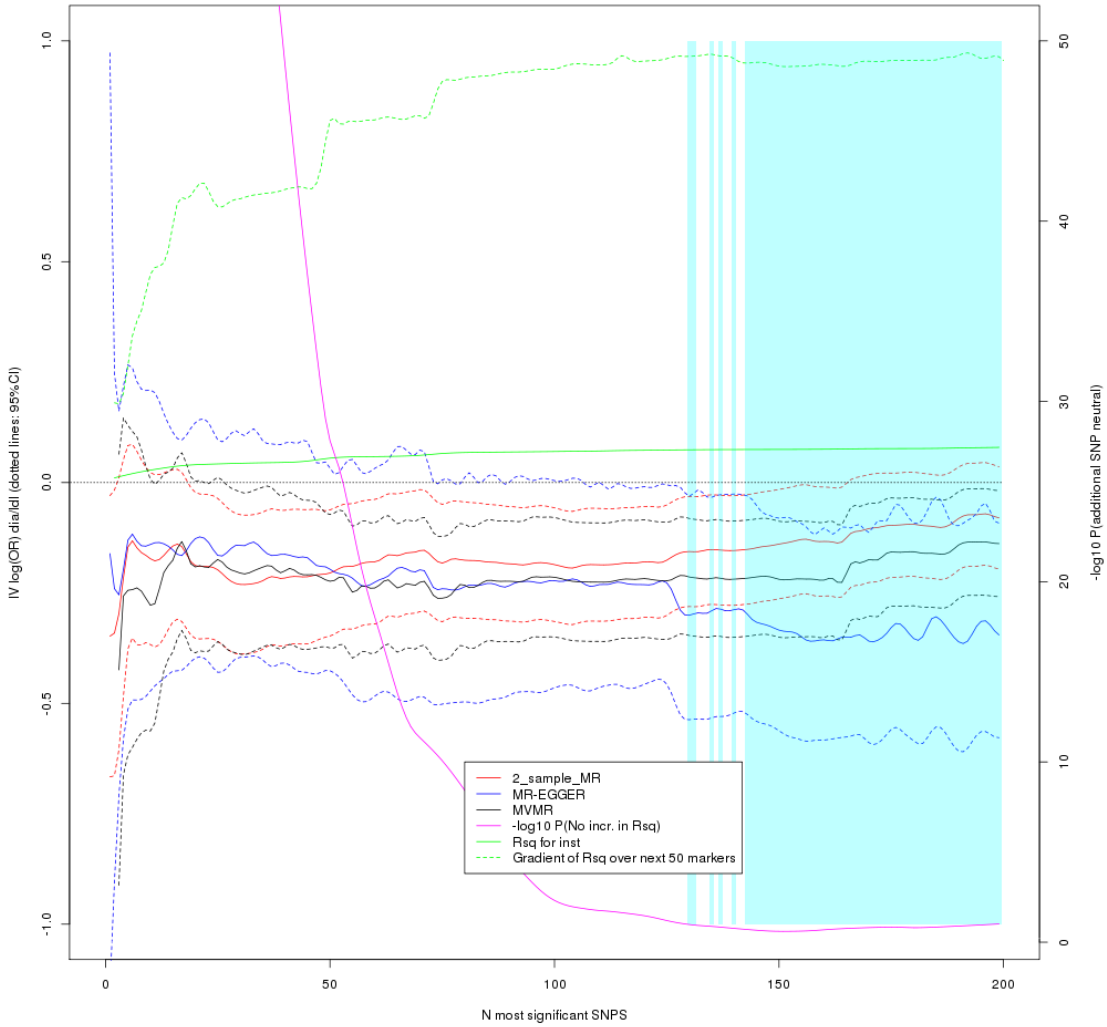
Supplementary Figures

Supplementary Figure 1: LDL-C and CAD – plot to show proportion of variance of LDL-C explained, presence of unbalanced pleiotropy and causal estimates derived from three MR approaches.



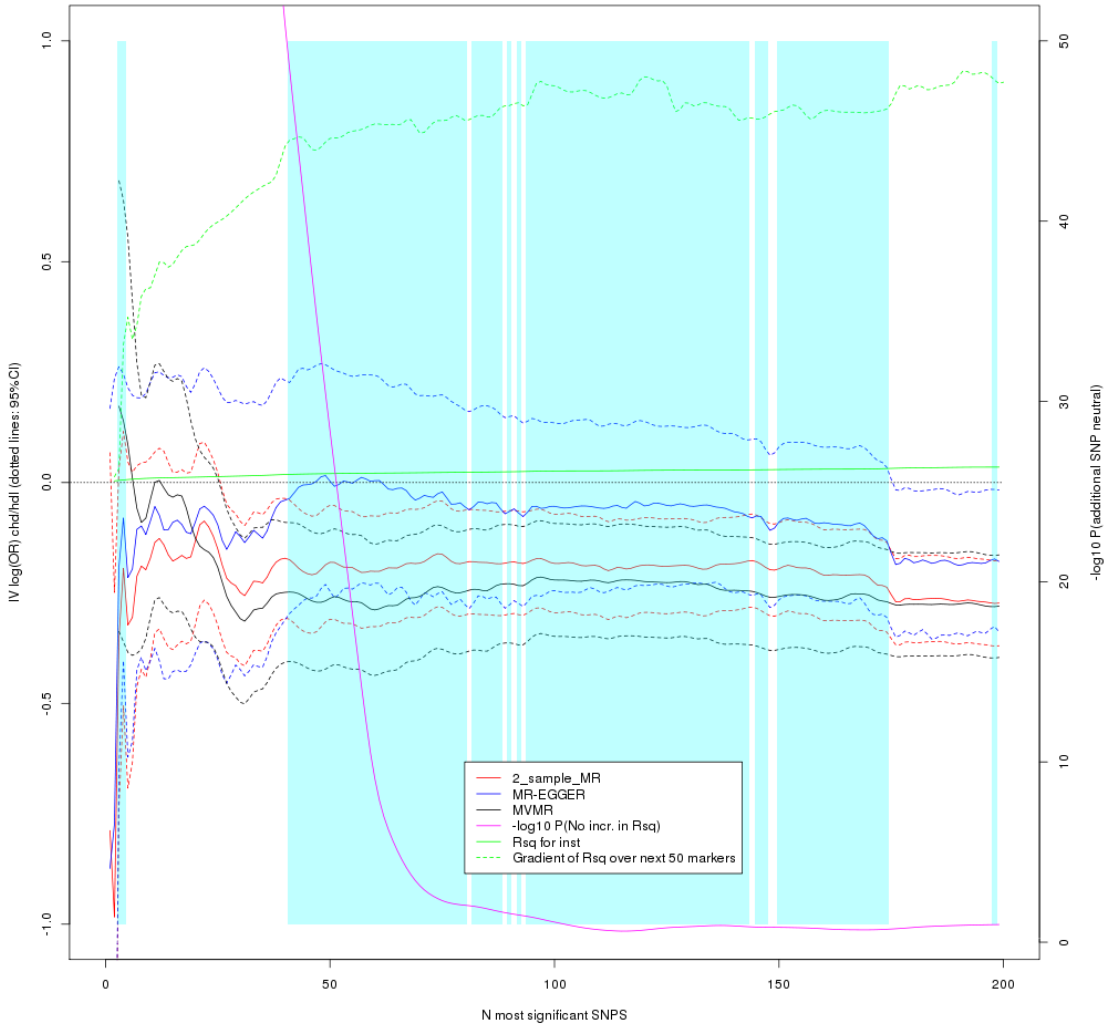
Blue-shading = presence of pleiotropy from MR-Egger. When pleiotropy is present, the most reliable causal estimate is from MR-Egger as conventional (2-sample) MR does not take into account unbalanced pleiotropy, and MVMR may not sufficiently remove the unbalanced nature of the pleiotropy.

Supplementary Figure 2: LDL-C and T2D – plot to show proportion of variance of LDL-C explained, presence of unbalanced pleiotropy and causal estimates derived from three MR approaches.



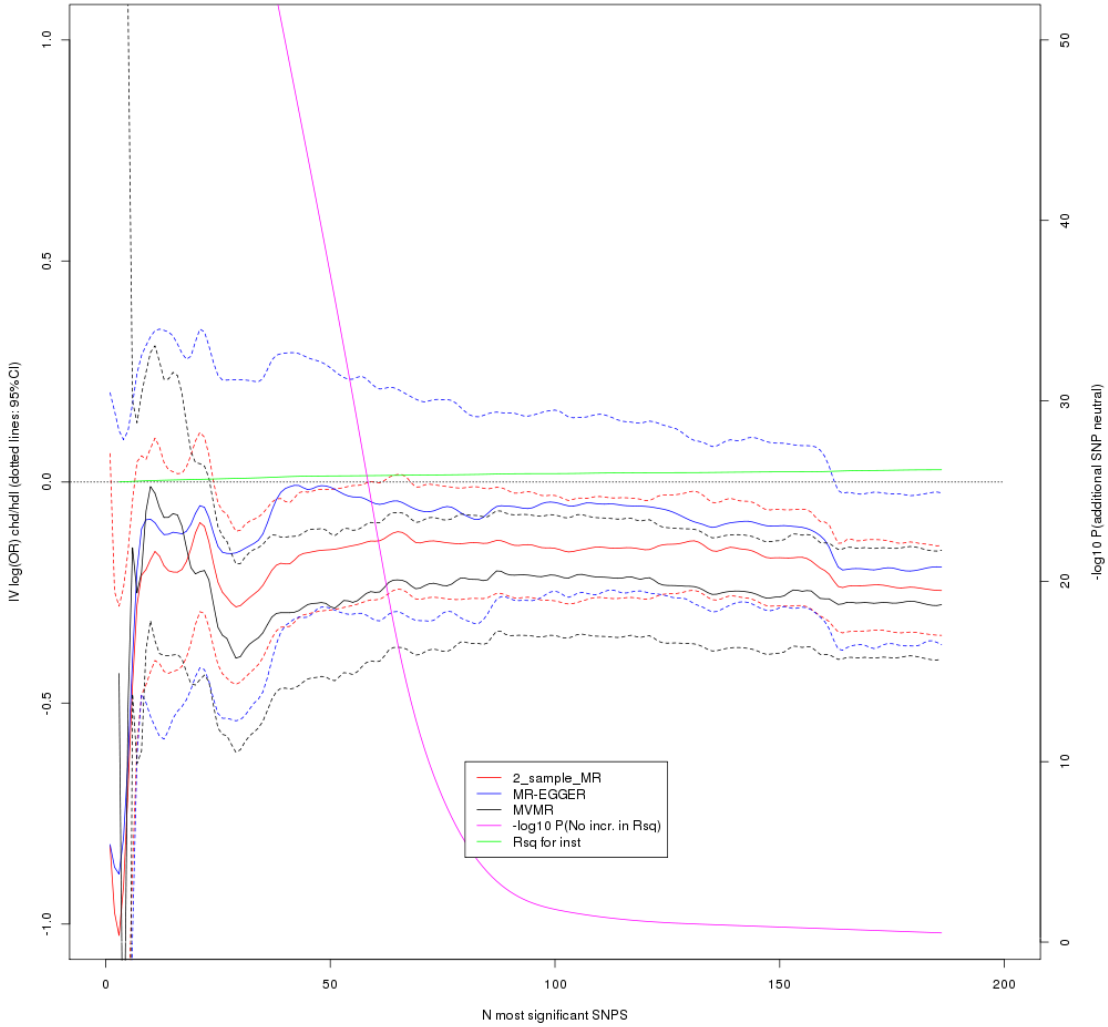
Blue-shading = presence of pleiotropy from MR-Egger. When pleiotropy is present, the most reliable causal estimate is from MR-Egger as conventional (2-sample) MR does not take into account unbalanced pleiotropy, and MVMR may not sufficiently remove the unbalanced nature of the pleiotropy.

Supplementary Figure 3A: HDL-C and CAD (all SNPs) – plot to show proportion of variance of HDL-C explained, presence of unbalanced pleiotropy and causal estimates derived from three MR approaches.



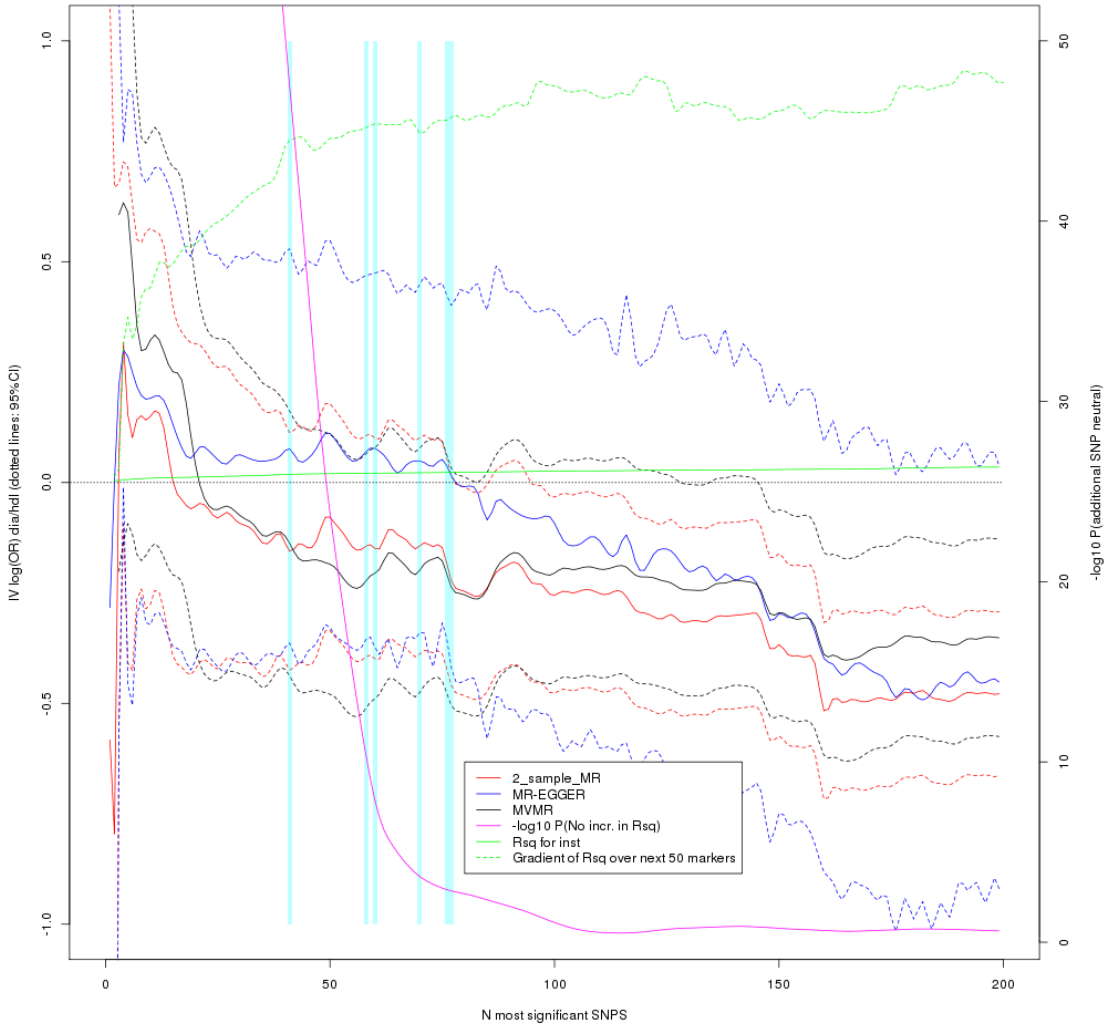
Blue-shading = presence of pleiotropy from MR-Egger. When pleiotropy is present, the most reliable causal estimate is from MR-Egger as conventional (2-sample) MR does not take into account unbalanced pleiotropy, and MVMR may not sufficiently remove the unbalanced nature of the pleiotropy.

Supplementary Figure 3B: HDL-C and CAD – (limited to non-pleiotropic SNPs hence no blue shading for unbalanced pleiotropy) - plot to show proportion of variance of HDL-C explained, presence of unbalanced pleiotropy and causal estimates derived from three MR approaches.



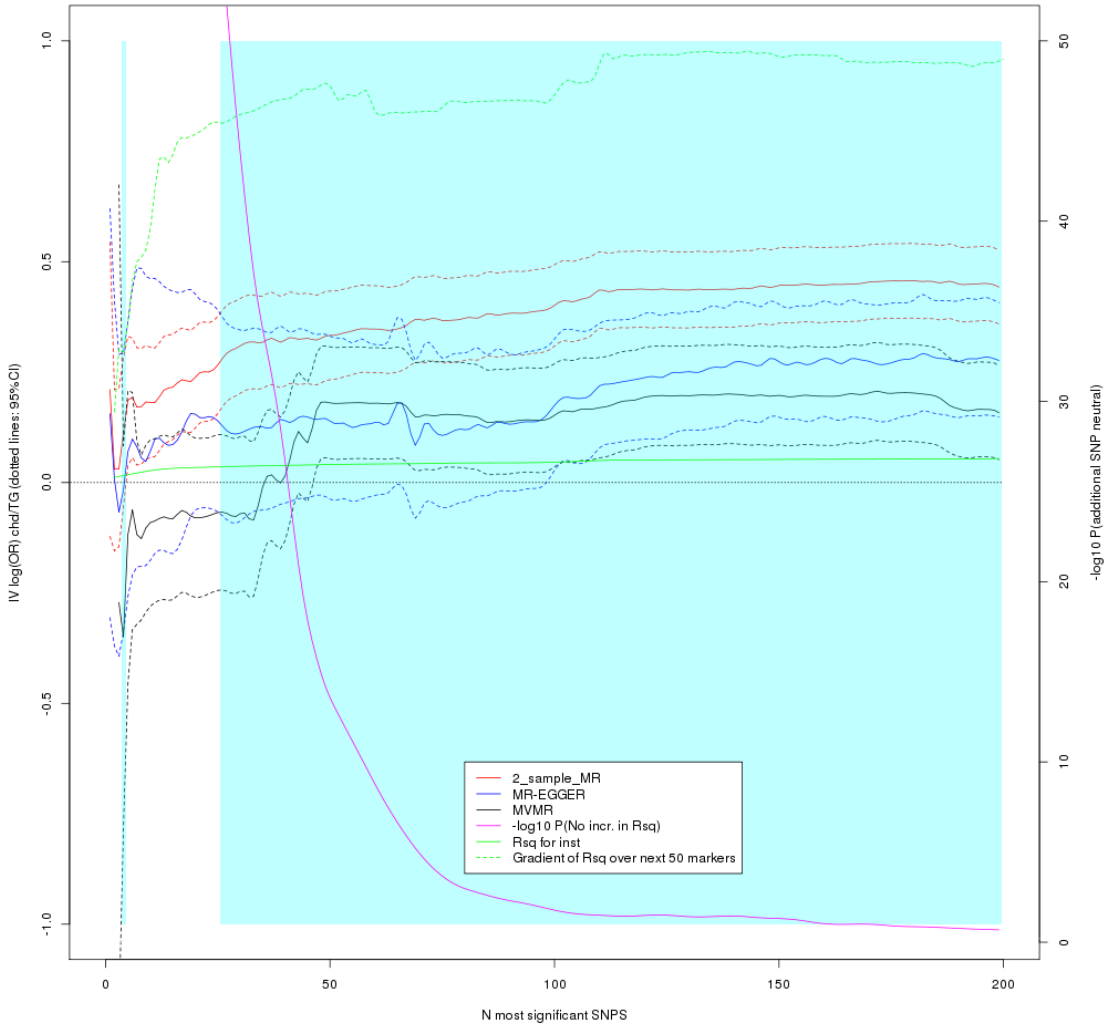
Blue-shading = presence of pleiotropy from MR-Egger. When pleiotropy is present, the most reliable causal estimate is from MR-Egger as conventional (2-sample) MR does not take into account unbalanced pleiotropy, and MVMR may not sufficiently remove the unbalanced nature of the pleiotropy.

Supplementary Figure 4: HDL-C and T2D – plot to show proportion of variance of HDL-C explained, presence of unbalanced pleiotropy and causal estimates derived from three MR approaches.



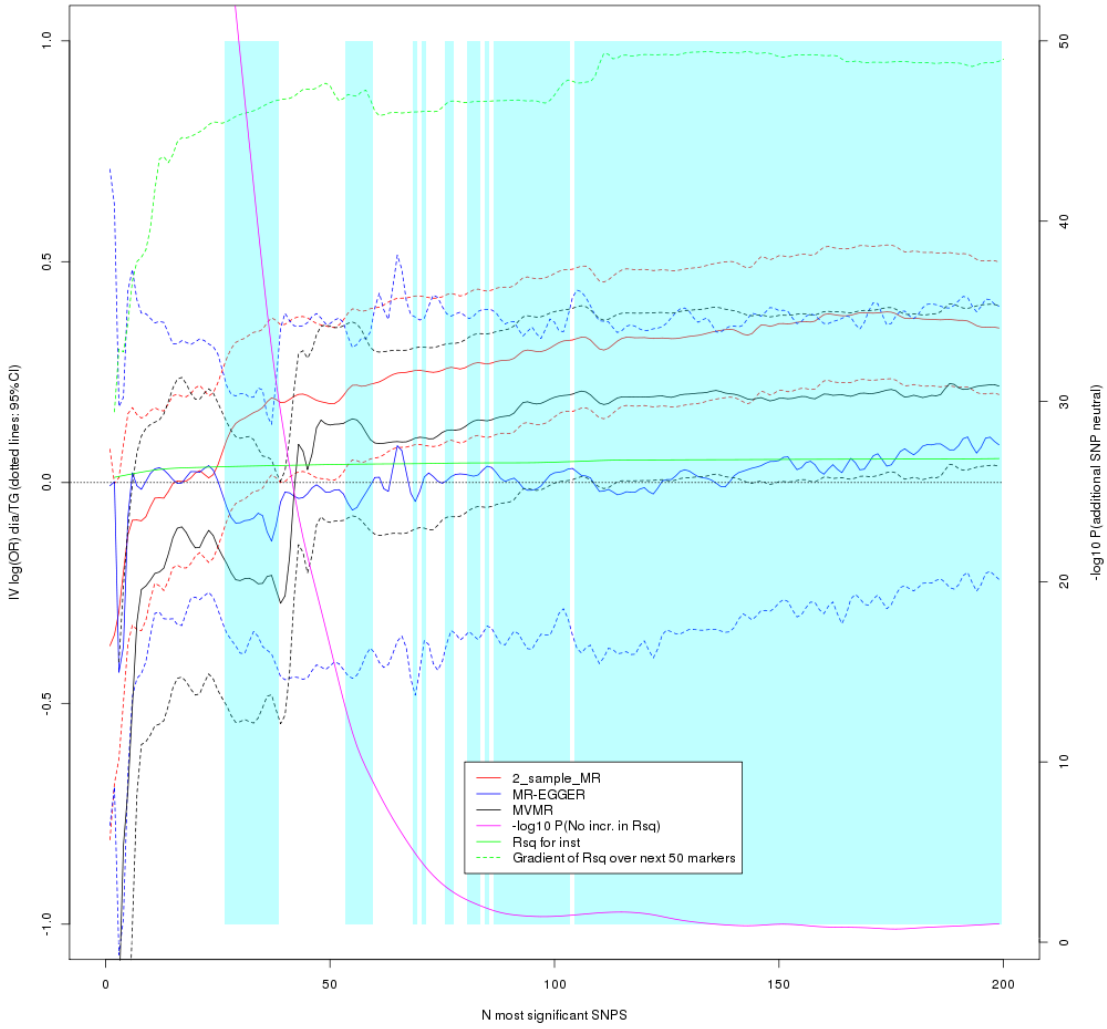
Blue-shading = presence of pleiotropy from MR-Egger. When pleiotropy is present, the most reliable causal estimate is from MR-Egger as conventional (2-sample) MR does not take into account unbalanced pleiotropy, and MVMR may not sufficiently remove the unbalanced nature of the pleiotropy.

Supplementary Figure 5: TG and CHD – plot to show proportion of variance of TG explained, presence of unbalanced pleiotropy and causal estimates derived from three MR approaches.



Blue-shading = presence of pleiotropy from MR-Egger. When pleiotropy is present, the most reliable causal estimate is from MR-Egger as conventional (2-sample) MR does not take into account unbalanced pleiotropy, and MVMR may not sufficiently remove the unbalanced nature of the pleiotropy.

Supplementary Figure 6: TG and T2D - – plot to show proportion of variance of TG explained, presence of unbalanced pleiotropy and causal estimates derived from three MR approaches.



Blue-shading = presence of pleiotropy from MR-Egger. When pleiotropy is present, the most reliable causal estimate is from MR-Egger as conventional (2-sample) MR does not take into account unbalanced pleiotropy, and MVMR may not sufficiently remove the unbalanced nature of the pleiotropy.