**Type of manuscript:** Original article

**Adiponectin and coronary artery disease risk: a bi-directional Mendelian randomization study**

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**Authors' contribution**

All two authors designed the study, wrote the analysis plan and interpreted the results. SLAY undertook analyses with feedback from CMS. SLAY wrote the first draft of the manuscript with critical feedback and revisions from CMS. All authors gave final approval of the version to be published.

**Acknowledgement**

Data on coronary artery disease have been contributed by CARDIoGRAMplusC4D investigators and have been downloaded from www.CARDIOGRAMPLUSC4D.ORG. Data on adiponectin have been contributed by ADIPOGen Consortium and have been downloaded from https://www.mcgill.ca/genepi/adipogen-consortium. Au Yeung SL was supported by the Health and Medical Research Fund Research Fellowship Scheme [#01150037], Food and Health Bureau, HKSAR,

China. The funder had no role in the design, analyses, interpretation of results or writing of the paper. We thank CARDIoGRAMplusC4D, ADIPOGen Consortium, Genetic Investigation of ANthropometric Traits (GIANT) and Global Lipids Genetics Consortium (GLGC) for making the summary statistics publicly available. We thank Benjamin J Cowling for helpful comments in the earlier drafts of the manuscript.

**Potential conflict of interests**

The authors report no relationships that could be construed as a conflict of interest.

**Keywords:** adiponectin; coronary artery disease; Mendelian randomization; bi-directional

Abstract: 249

Word Count: 2,810

Table: 2

Supplemental materials: 1 (6 tables)

**Abstract**

**Background:** Observational studies have found adiponectin inversely associated with cardiovascular disease thereby indicating a potential target of intervention, but genetically higher adiponectin appears unrelated to coronary artery disease (CAD). To clarify, we examined the role of genetically predicted adiponectin in CAD in a larger study and additionally examined the role of genetically predicted CAD in adiponectin using a bi-directional Mendelian randomization study.

**Methods:** We obtained estimates using inverse variance weighting (IVW) with multiplicative random effects, based on 21 single nucleotide polymorphism (SNPs) to predict adiponectin and 28 SNPs for CAD, using two large genome wide association studies of adiponectin (ADIPOGen Consortium (n=39,883)) and CAD CARDIoGRAMplusC4D 1000 Genomes based GWAS (n=60,801 CAD cases; n=123,504 controls). Sensitivity analyses included using MR-Egger, weighted median method, and exclusion of potentially invalid (pleiotropic) SNPs.

**Results:** Adiponectin was inversely associated with CAD (odds ratio 0.82 per unit increase log transformed adiponectin, 95% confidence interval (CI) 0.71 to 0.94) using IVW. However, the association was null using a weighted median method or MR-Egger, or after exclusion of pleiotropic SNPs acting on obesity related traits. CAD was not associated with adiponectin (-0.011 log transformed adiponectin unit per log odds CAD, 95% CI -0.039 to 0.017), with similar findings from MR-Egger, weighted median method or exclusion of pleiotropic SNPs.

**Conclusion:** Adiponectin is unlikely a cause of CAD although we cannot completely rule out the possibility. Previous observational studies are likely driven by factors driving both adiponectin and CAD, whose elucidation might provide new insights concerning interventions for CAD.

## Introduction

Adiponectin has been suggested as a potentially protective factor in coronary artery disease (CAD),[1, 2] possibly operating via improving insulin sensitivity and reducing hepatic triglyceride accumulation.[3] However, the relation of adiponectin with CAD appears inconsistent,[4] with some reports of positive relations between adiponectin and risk of CAD reoccurrence.[5] These discrepancies could be driven by confounding by factors, such as obesity, which affect both adiponectin and CAD,[6] or by selection bias in studies restricted to patients.[7] Furthermore, central and peripheral fat compartments appear to have different effects on adiponectin,[6] making adjustment difficult and possibly incomplete.

Mendelian randomization, which is more robust to confounding because it takes advantage of the random allocation of genetic material at conception, can clarify the causal role of adiponectin in CAD.[8] Previous genetic studies are conflicting,[7, 9] possibly due to selection bias,[7] and inability to take advantage of newly developed methods to assess the reliability of Mendelian randomization estimates.[7, 9, 10] A more recent Mendelian randomization study with more sensitivity analyses showed no relation between adiponectin and CAD,[11] although whether the study provided a definitive answer on the causal role of adiponectin in CAD has been questioned.[3] Previous studies also have not considered the complimentary question, i.e. whether lower adiponectin could be a symptom rather than a cause of CAD. In order to provide clarification as to the causal role of adiponectin in CAD and potential reverse causation, we assessed whether genetically higher adiponectin was associated with CAD using an updated CAD genome wide association study (GWAS) with more extensive genotyping and only an overlap of ~55% with the study used in the previous Mendelian randomization study (CARDIoGRAMplusC4D Metabochip),[9, 11, 12] and whether genetically higher CAD risk was associated with adiponectin in a bi-directional Mendelian randomization study.

## Methods

Mendelian randomization, as instrumental variable analysis with genetic instruments, has three stringent assumptions. First, strong genetic predictors of exposure are required where the strength of instrument is proportional to the strength of the relation between genetic variants and exposure, given by the F-statistic. We obtained the F-statistic using an approximation.[13] Second, the relation of the genetic predictors of exposure with the outcome should not be confounded, likely achieved by taking advantage of random genetic allocation at conception. Third, the single nucleotide polymorphisms (SNP) predicting the exposures should only affect the outcome via the exposure, i.e., be free of pleiotropic effects, i.e., satisfy what is called the exclusion-restriction assumption. We checked for known pleiotropy by searching for reported effects of the genetic predictors of exposure and for unknown pleiotropy from the statistical evidence of heterogeneity of the genetic predictors.

### *Genetically higher adiponectin and CAD risk*

We obtained independent genetic predictors, i.e., SNPs, with $r^2 \leq 0.05$ to exclude SNPs in linkage disequilibrium strongly associated (p value $< 5 \times 10^{-8}$) with adiponectin (natural log transformed) from the largest available adiponectin genome wide association studies (GWAS).[9] The ADIPOGen Consortium is a meta-analysis of discovery and follow-up phases in people of mostly European descent (n=45,981), adjusted for age, sex, body mass index, study site (if appropriate) and family structure for family based cohorts, with genomic control (principal components of population stratification).[14] In this study, we extracted the genetic predictors of adiponectin from the analyses restricted to European descent (n=39,883).[9] We obtained associations of these genetic predictors of adiponectin with CAD from CARDIoGRAMplusC4D 1000 Genomes-based GWAS,[12] a meta-analysis of GWAS of CAD case-control studies of people of mainly European descent (77%) imputed using the 1000 Genomes phase 1 v3 training set with 38 million variants. The study interrogated 9.4 million variants and included 60,801

CAD cases and 123,504 controls.[12] CAD case status encompassed a diagnosis of myocardial infarction, acute coronary syndrome, chronic stable angina, or coronary stenosis >50%. Diagnoses were based on clinical diagnosis, procedures (coronary angiography results or by-pass surgery), use of medications or symptoms that indicate angina, or self-report of a doctor diagnosis, as described elsewhere.[12] CARDIoGRAMplusC4D 1000 Genomes-based GWAS adjusted for study-specific covariates (e.g. age and sex) and genomic control.

*Genetically higher CAD risk and adiponectin*

We obtained independent genetic predictors, SNPs, (with $R^2 \leq 0.05$) strongly associated (p value < $5 \times 10^{-8}$) with CAD from CARDIoGRAMplusC4D 1000 Genomes-based GWAS.[12] We obtained associations of these genetic prediction of CAD risk (odds ratios) with adiponectin from the ADIPOGen Consortium discovery phase (n=29,340).[14]

To obtain independent genetic predictors of each exposure correlated SNPs ($r^2$ of >0.05) were discarded based on larger effect size and smaller p value. Correlations were obtained from the 1000 Genomes project. We identified SNPs with potentially pleiotropic effects (i.e. reported effect of the SNP on the outcome other than via the exposure) from the GWAS Catalog (https://www.ebi.ac.uk/gwas/)[15] and Phenoscanner.[16] We defined a SNP as being potentially pleiotropic if the phenotype is potentially a cause of the outcome.

*Statistical analysis*

We obtained the F-statistic based on an $r^2$ of 5%, the variance of adiponectin explained by genetic predictors in the original GWAS.[13, 14] We obtained unconfounded estimates of genetically predicted exposure on outcome using inverse variance weighting (IVW) with multiplicative random effects, which is a weighted regression of SNP-outcome association on SNP-exposure associations, with the intercept constrained to zero. IVW with random effects assumes balanced pleiotropy, rather than using fixed effects which assumes no pleiotropy. Using multiplicative random effects has the advantage of being less susceptible to bias introduced by weaker SNP-exposure associations.[17] We ensured the same effect allele was used for both exposure and outcome based on reported effect allele and effect allele frequency. We assessed heterogeneity of the SNP-specific Wald estimates (SNP on outcome divided by SNP on exposure) from $I^2$ where high $I^2$ may indicate the presence of invalid pleiotropic SNPs.

*Power calculation*

The variance in adiponectin explained by the genetic variants was 5%.[14] As such, our study is adequately powered to detect an odds ratio of 0.95 per 1 SD of log transformed adiponectin. Similarly, assuming the variance explained by the adiponectin SNPs subset on CAD was 1%, the study would be adequately powered to detect an effect size of 0.17.[18]

*Sensitivity analyses*

To assess the possibility of unknown pleiotropy (violation of the exclusion-restriction assumption), we used different methods with different assumptions for validity, i.e., a leave one out IVW analysis, a weighted median method and MR-Egger. If all the SNPs are valid instruments, the leave one out IVW estimates should be directionally consistent. The weighted median estimate uses the weighted median of the SNP-specific Wald estimates and is valid if valid SNPs contribute more than 50% of the weight.[10] The

MR-Egger method give valid estimate as long as the instrument strength is independent of the direct effect,[19] but has wide confidence intervals, and may not be reliable if pleiotropic effects are mediated by an exposure-outcome confounder.[19] A zero intercept from MR-Egger provides some evidence of absence of direct effects on the outcome from SNPs not via the exposure (horizontal pleiotropy).[19] Lastly, we repeated all these analyses excluding known pleiotropic SNPs such as SNPs acting via obesity which is a determinant of adiponectin, and hence a confounder,[6] or acting via lipids. For adiponectin on CAD we also restricted the estimate to SNPs from the adiponectin gene (*ADIPOQ*), as in the previous Mendelian randomization study.[11]

All analyses were performed using R Version 3.3.2 (R Development Core Team, Vienna, Austria) with the R package (TwosampleMR). This study only used publicly available data and hence no ethical approval was required to conduct this study.

**Results**

Of the 162 SNPs predicting adiponectin at genome wide significance in the ADIPOGen Consortium, 21 SNPs (Supplemental table 1) remained after excluding correlated SNPs, with an F statistic of 99.9 suggesting little evidence for weak instrument bias. Of 21 SNPs, 13 SNPs had known potentially pleiotropic effects related to obesity traits and 4 SNPs were related to activated partial thromboplastin time (Supplemental table 2).

Table 1 shows that genetically higher adiponectin was associated with lower CAD risk based on the overall IVW estimate (odds ratio (OR) 0.82 per natural log transformed adiponectin unit, 95% confidence interval (CI) 0.71 to 0.94) and the leave out one IVW estimates (Supplemental Table 3). However, the $I^2$

of 61% and the significant MR-Egger intercept (-0.016; p value 0.009) suggested the presence of potentially pleiotropic SNPs and hence the IVW estimate could be invalid. Other sensitivity analyses including MR-Egger and the weighted median method which are more robust to the inclusion of invalid SNPs showed little association of adiponectin with CAD. After excluding 13 obesity related trait SNPs, the association was attenuated to the null with little evidence of directional pleiotropy (-0.005, p value: 0.63), with an $I^2$ of 0%. Similar results were found when we further excluded 4 SNPs related to activated partial thromboplastin time. Similar findings were observed when we restricted our instruments to potentially functional SNPs (*ADIPOQ*).

Among the 58 loci predicting CAD in previous GWAS,[12, 20-23] 4 were indels (i.e. insertion/deletion mutations). Among these loci, only 44 SNPs reached genome wide significance in the CARDIoGRAMplusC4D 1000 Genomes based GWAS in an additive model. One SNP, rs4252185, was correlated with another SNP and hence was discarded. Of the remaining 43 SNPs, only 19 were available in the ADIPOGen Consortium which included two SNPs (rs4593108 and rs7568458) with ambiguous effect alleles (palindromes). To replace missing and ambiguous SNPs, we identified 11 non-palindromic proxy SNPs ($r^2$=1)[24] which were available in both CARDIoGRAMplusC4D 1000 Genomes based GWAS and ADIPOGen Consortium, leaving 28 SNPs to assess the effect of CAD on adiponectin (Supplemental table 4). Among these 28 SNPs, 17 had known potentially pleiotropic effects, primarily on lipids, blood pressure, obesity related traits, and white blood cells attributes (Supplemental table 5). Amongst these SNPs, rs2891168 was associated with glioma and glaucoma whereas rs17293632 was associated with irritable bowel disease and Crohn's disease although these phenotypes unlikely alter adiponectin (Supplemental table 5).

Table 2 shows that genetically higher CAD risk was not associated with adiponectin using an IVW estimate (-0.011 log transformed adiponectin unit per log odds CAD, 95% CI -0.039 to 0.017). The $I^2$ (29%), IVW leave out one analysis (Supplemental Table 6) and the MR-Egger intercept (-0.004; p value: 0.23) all indicated little unknown pleiotropy. After excluding potentially pleiotropic SNPs, the IVW estimate remained null consistent with the MR-Egger and weighted median estimates.

**Discussion**

Consistent with a previous Mendelian randomization study, but using a study with different participants (~55% difference between CARDIoGRAMplusC4D Metabochip and 1000 Genomes) and using additional sensitivity analysis, adiponectin remained an unlikely a cause of CAD.[11] Our study adds by showing, for the first time, that CAD was also not associated with adiponectin. As such, the inverse association between adiponectin and CAD seen in previous observational studies is likely to be driven by residual confounding. This study suggests that instead of adiponectin being a target of intervention to prevent CAD, an unknown cause of CAD which lowers adiponectin exists and might instead be a target to combat CAD.

A previous Mendelian randomization study showed that adiponectin was not associated with CAD risk and the association was unlikely a result of lack of power, with 81% power to detect an odds ratio of 0.90 per log adiponectin.[11] Our study had slightly more power than the previous study because we used more genetic predictors of adiponectin (21 compared to 17) and we obtained all the genetic associations based on a larger sample (60,801 cases and 123,504 controls for all SNPs, rather than 22,233 cases, and 64,762 controls for 7 of 17 SNPs), both of which contribute to power.[25] We also conducted additional sensitivity analyses with different assumptions.[10, 26] Hence, our study adds by suggesting the null finding in the

previous Mendelian randomization was possibly genuine given the consistency of our results compared with the previous study.[11]

Observational and animal studies have suggested an inverse association of adiponectin with CAD. However, these observations could be due to residual confounding by abdominal fat,[6] androgens,[27] and possibly conjugated linoleic acid,[28] which are known to reduce adiponectin and may affect CAD or statins which increases adiponectin[29] and decreases CAD. Although the observed association in previous studies could be due to reverse causality, i.e. CAD affecting adiponectin,[30] our study suggests this explanation is also unlikely given the lack of association of genetically predicted CAD risk with adiponectin (Table 2). The discrepancies between animal studies and studies in humans may be due to differences in metabolic pathways and disease latency across species or methodological flaws such as incomplete blinding.[31] As such, the lack of consistency across studies,[5] and the difference between observational and Mendelian randomization studies,[11] suggests the existence of causal factors that have opposite effects on adiponectin and CAD, as described previously.[32, 33] Whether any of these factors are driving the inverse association of adiponectin with CAD in observational studies may shed light on the identification of novel targets for interventions to reduce CAD risk. One approach to identify potential targets is to use publicly available GWAS summary statistics. We have searched the gene-phenotype associations in other GWAS such as adiposity and lipids.[34-36] Specifically amongst the SNP associations reaching nominal significance ($p<0.05$), the adiponectin increasing alleles in 7 SNPs were associated with increasing BMI, and the adiponectin increasing alleles in 10 SNPs were associated with decreasing WHR, which was similar to what has been reported in a previous Mendelian randomization study.[6] However, the adiponectin increasing alleles were not clearly related to lipid profile. Future studies should also explore the paradoxical observations between higher adiponectin and higher mortality in CVD patients, which is not well understood but perhaps due to confounding by underlying CVD condition which may induce

adiponectin resistance, atrial NP (ANP) or brain NP (BNP), and reduced kidney function,[37] or due to selection bias.[38]

*Limitations*

Although we used a Mendelian randomization study design which is less susceptible to confounding, limitation exists. The validity of our Mendelian randomization study depends on how well the assumptions are satisfied. First, we only used genetic predictors strongly and independently associated with the exposures. Second, we used genetic instruments which were randomly allocated during conception. Although we were unable to show a lack of association between genetic variants and potential confounders given lack of access to individual data, genetic predictors of exposure are unlikely to be confounded by lifestyle or sociodemographic factors, as has been empirically illustrated previously.[39] Ethnicity may also have confounded our analysis, although the GWAS included had applied genomic control to correct for population stratification. The absence of horizontal pleiotropy of the SNPs is difficult to assess and if violated, the IVW estimate would be biased. We used various approaches which are more robust to the violation of this assumption. Specifically, we used MR-Egger and a weighted median method which have different assumptions for valid estimation.[17] We also searched for potential pleiotropy of the SNPs using two sources (GWAS catalog and Phenoscanner) and repeated the analyses without these potentially pleiotropic SNPs. The results without these SNPs were consistent with other methods such as MR-Egger and weighted median method which relied on other assumptions, as well as the conservative approach which used SNPs functionally related to adiponectin. Such triangulation of evidence makes it unlikely that the adiponectin is a cause of CAD or that CAD influences adiponectin level (i.e. reverse causality). The null associations could be driven by weak instrument bias but the F-statistics were high. Second, we only included a subset of genetic predictors for CAD when assessing the relation of CAD with adiponectin because the ADIPOGen consortium GWAS assessed fewer SNPs (28

SNPs),[14] which may impact the overall statistical power in detecting the causal effect of CAD on adiponectin. Nevertheless, the estimates were all close to null for CAD on adiponectin in more than 29,000 participants. Our results would be biased if the excluded SNPs had distinctly different association with adiponectin than the ones included in this study. On the other hand, we cannot rule out the possibility of residual pleiotropic SNPs which may invalidate our analyses.[40] Nevertheless, future Mendelian randomization studies with potentially greater statistical power, such as using data with more dense genotyping for adiponectin (e.g. through summary-level imputation via DISTMIX)[41] or through the use of genetic risk scores in large biobanks (e.g. UK Biobank) with individual exposure data[42] may help verify the null findings in this study. However, assuming the SNPs available for adiponectin are a random subset of the SNPs predicting CAD, the estimate should be similar using a greater number of SNPs. The underlying GWAS mainly include people of European descent, so the estimates might not extend to other population, such as Asians, although causes are likely to be consistent across different populations. Lastly, we were unable to examine potential sex-specific effects or non-linear effects of adiponectin, which could be investigated in the UK Biobank.[42]

This Mendelian randomization corroborates the null findings from the previous Mendelian randomization study and adds by showing limited evidence for reverse causation. As such, adiponectin is unlikely to be a cause of CAD and factors that drive adiponectin may be overlooked causes of CAD.

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**Table 1: The association of adiponectin on coronary artery disease risk using Mendelian randomization**

| | Inverse variance weighting | | MR-Egger method | | | Weighted median method | |
|---|---|---|---|---|---|---|---|
| Selection of SNPs | *Odds ratio | 95% CI | Odds ratio | 95% CI | Intercept (p value) | Odds ratio | 95% CI |
| All SNPs (21) | 0.82 | 0.71 to 0.94 | 1.02 | 0.85 to 1.24 | -0.016 (0.009) | 0.92 | 0.81 to 1.03 |
| Exclusion of obesity related traits SNPs (8) | 0.94 | 0.82 to 1.07 | 1.01 | 0.74 to 1.39 | -0.005 (0.63) | 0.95 | 0.82 to 1.12 |
| Exclusion of all pleiotropic SNPs (4) | 0.90 | 0.73 to 1.12 | 1.07 | 0.48 to 2.35 | -0.009 (0.71) | 0.92 | 0.72 to 1.19 |
| †Conservative approach (5) | 0.96 | 0.83 to 1.10 | 1.02 | 0.73 to 1.43 | -0.007 (0.69) | 0.97 | 0.84 to 1.13 |

*Per log transformed increase in adiponectin

†Only include SNPs in ADIPOQ gene, as in the previous study[11]

**Table 2: The association of coronary artery disease risk on adiponectin using Mendelian randomization**

| | Inverse variance weighting | | MR-Egger method | | | Weighted median method | |
|---|---|---|---|---|---|---|---|
| Selection of SNPs | *Beta | 95% CI | Beta | 95% CI | Intercept (p value) | Beta | 95% CI |
| All SNPs (28) | -0.011 | -0.039 to 0.017 | 0.030 | -0.041 to 0.102 | -0.004 (0.23) | 0.013 | -0.024 to 0.049 |
| Exclusion of obesity related SNPs (25) | -0.013 | -0.041 to 0.015 | 0.023 | -0.048 to 0.095 | -0.004 (0.29) | 0.013 | -0.024 to 0.050 |
| Exclusion of pleiotropic SNPs (13) | -0.011 | -0.050 to 0.028 | 0.011 | -0.080 to 0.103 | -0.002 (0.60) | -0.009 | -0.059 to 0.032 |

*Per log odds increase in coronary artery disease risk

**Supplemental table 1: Information on 21 single nucleiotide polymorphisms (SNPs) used to assess the effect of adiponectin on coronary artery disease (CAD) risk[1, 2]**

| Data sources | | | ADIPOGen Consortium (n=39,883) | | CARDIoGRAMplusC4D 1000 Genomes based GWAS (n=184,305) | |
|---|---|---|---|---|---|---|
| SNP | Effect allele | Other allele | Beta for adiponectin (log transformed) | Standard error | Log odds for CAD | Standard error |
| rs1108842 | C | A | 0.03 | 0.004 | 0.001244 | 0.0092866 |
| rs12051272 | G | T | 0.26 | 0.017 | -0.04967 | 0.0225913 |
| rs16861209 | A | C | 0.16 | 0.009 | -0.003395 | 0.0193086 |
| rs17366568 | G | A | 0.13 | 0.008 | -0.003076 | 0.016229 |
| rs1870843 | G | A | 0.03 | 0.004 | -0.007137 | 0.0098949 |
| rs187868 | G | A | 0.05 | 0.004 | -0.000015 | 0.0092703 |
| rs266743 | C | T | 0.05 | 0.006 | -0.007385 | 0.0112365 |
| rs2925979 | C | T | 0.04 | 0.005 | -0.016408 | 0.0103283 |
| rs2980879 | T | A | 0.03 | 0.005 | -0.041556 | 0.0100253 |
| rs3001032 | C | T | 0.02 | 0.004 | -0.006898 | 0.0100024 |
| rs601339 | G | A | 0.03 | 0.005 | -0.000786 | 0.0125585 |
| rs6444175 | A | G | 0.06 | 0.005 | -0.003769 | 0.0104359 |

| | | | | | | |
|---|---|---|---|---|---|---|
| rs6488898 | A | G | 0.05 | 0.008 | -0.002437 | 0.0201124 |
| rs731839 | A | G | 0.03 | 0.004 | -0.027621 | 0.0096809 |
| rs7615090 | T | G | 0.06 | 0.008 | -0.004887 | 0.0136013 |
| rs7649121 | A | T | 0.06 | 0.007 | -0.007757 | 0.0131005 |
| rs7955516 | C | A | 0.02 | 0.004 | -0.013912 | 0.0098406 |
| rs7978610 | C | G | 0.03 | 0.005 | -0.027537 | 0.0101719 |
| rs822355 | C | T | 0.04 | 0.005 | -0.001782 | 0.0108682 |
| rs863750 | C | T | 0.02 | 0.004 | -0.027788 | 0.0093898 |
| rs998584 | C | A | 0.03 | 0.005 | -0.041945 | 0.0098862 |

**Supplemental table 2: Pleiotropic effects of single nucleotide polymorphisms associated with adiponectin as reported in the GWAS Catalog and Phenoscanner[3, 4]**

| SNP associated with adiponectin | Traits |
|---|---|
| rs1108842 | Obesity related traits/ Schizophrenia |
| rs12051272 | Obesity related traits |
| rs16861209 | Activated partial thromboplastin time |
| rs17366568 | Obesity related traits |
| rs187868 | Activated partial thromboplastin time |
| rs266743 | Activated partial thromboplastin time |
| rs2925979 | Obesity related traits, lipids, type 2 diabetes |
| rs2980879 | Obesity related traits, CAD |
| rs3001032 | Obesity related traits |
| rs601339 | Obesity related traits, HDL |
| rs6488898 | Obesity related traits |
| rs731839 | Lipids, obesity related traits, insulin |
| rs7955516 | Obesity related traits |
| rs7978610 | Obesity related traits, lipids |
| rs822355 | Activated partial thromboplastin time |
| rs863750 | Obesity related traits; lipids |
| rs998584 | Obesity related traits; lipids |

**Supplemental Table 3: Leave one out analysis for the association of adiponectin on coronary artery disease risk using Mendelian randomization**

| Excluded single nucleotide polymorphism | Odds ratio per log transformed adiponectin | Lower 95% Confidence Interval | Upper 95% Confidence Interval |
|---|---|---|---|
| rs1108842 | 0.814776 | 0.704875 | 0.941813 |
| rs12051272 | 0.816437 | 0.689899 | 0.966184 |
| rs16861209 | 0.795898 | 0.684028 | 0.926062 |
| rs17366568 | 0.797902 | 0.686022 | 0.928028 |
| rs1870843 | 0.819593 | 0.708559 | 0.948025 |
| rs187868 | 0.80891 | 0.698424 | 0.936875 |
| rs266743 | 0.817238 | 0.70543 | 0.946767 |
| rs2925979 | 0.824387 | 0.712769 | 0.953484 |
| rs2980879 | 0.837018 | 0.737928 | 0.949414 |
| rs3001032 | 0.819968 | 0.709501 | 0.947634 |
| rs601339 | 0.817353 | 0.70715 | 0.944731 |
| rs6444175 | 0.811053 | 0.699253 | 0.940728 |
| rs6488898 | 0.817447 | 0.70715 | 0.944947 |
| rs731839 | 0.830724 | 0.723488 | 0.953854 |
| rs7615090 | 0.815065 | 0.703822 | 0.943891 |
| rs7649121 | 0.816465 | 0.704689 | 0.945972 |
| rs7955516 | 0.822414 | 0.712557 | 0.949209 |
| rs7978610 | 0.829551 | 0.721994 | 0.953131 |
| rs822355 | 0.815449 | 0.704841 | 0.943416 |

| | | | |
|---|---|---|---|
| rs863750 | 0.828031 | 0.723168 | 0.948099 |
| rs998584 | 0.837749 | 0.739354 | 0.949238 |
| Without exclusion (Main analysis) | 0.819002 | 0.711588 | 0.942631 |

**Supplemental table 4: Information on 28 single nucleiotide polymorphisms (SNPs) used to assess the effect of coronary artery disease (CAD) risk on adiponectin level[2, 5]**

| Data sources | | | CARDIoGRAMplusC4D 1000 Genomes based GWAS (n=184,305) | | ADIPOGen Consortium (Discovery stage) (n=29,340) | |
|---|---|---|---|---|---|---|
| SNP | Effect allele | Other allele | Log odds for CAD | Standard error | Beta for adiponectin (log transformed) | Standard error |
| *rs10793514 | C | T | 0.072332 | 0.0096266 | -0.003328 | 0.004676 |
| rs11191416 | T | G | 0.079249 | 0.0135252 | -0.001471 | 0.007189 |
| rs11206510 | T | C | 0.074519 | 0.0133438 | $-8.30 \times 10^{-5}$ | 0.006176 |
| *rs11226029 | A | G | -0.060966 | 0.0100133 | 0.004401 | 0.004855 |
| rs11556924 | C | T | 0.072569 | 0.0110605 | -0.015777 | 0.00481 |
| rs1412444 | C | T | -0.066812 | 0.0096809 | 0.002416 | 0.005519 |
| *rs1429142 | T | C | 0.06251 | 0.0113462 | -0.002265 | 0.005849 |
| *rs1535616 | C | T | 0.065839 | 0.0099492 | 0.009698 | 0.00482 |
| rs16986953 | G | A | -0.08516 | 0.0150265 | -0.008676 | 0.008264 |
| rs17087335 | G | T | -0.060764 | 0.0111159 | -0.002168 | 0.005597 |
| *rs17293632 | C | T | 0.069682 | 0.0119632 | -0.003692 | 0.004999 |
| rs17678683 | T | G | -0.098786 | 0.0166548 | 0.008148 | 0.008194 |
| rs2107595 | G | A | -0.073415 | 0.0112951 | 0.001504 | 0.006221 |
| *rs2133189 | T | C | 0.077472 | 0.0110551 | -0.019949 | 0.009545 |
| rs2487928 | G | A | -0.062633 | 0.0095049 | 0.003798 | 0.004351 |
| rs2681472 | A | G | -0.074114 | 0.0113331 | -0.00705 | 0.00606 |
| rs2891168 | A | G | -0.193401 | 0.0091877 | -0.002549 | 0.004343 |
| rs3184504 | C | T | -0.064208 | 0.0105173 | -0.00042 | 0.004456 |
| rs4420638 | A | G | -0.091906 | 0.0140977 | -0.012239 | 0.006936 |
| *rs5760293 | G | T | -0.155575 | 0.0258034 | 0.029102 | 0.02296 |
| *rs6427658 | C | T | -0.053986 | 0.0092573 | 0.002175 | 0.004485 |
| *rs6511720 | G | T | 0.125298 | 0.0169449 | 0.001568 | 0.007785 |
| rs663129 | G | A | -0.058163 | 0.0105173 | 0.007744 | 0.00538 |
| *rs7182716 | C | T | 0.077006 | 0.0095169 | 0.003468 | 0.005346 |
| rs7528419 | A | G | 0.11453 | 0.011482 | 0.001592 | 0.006334 |
| *rs7692387 | G | A | 0.067897 | 0.0117416 | -0.00718 | 0.005717 |
| rs9349379 | A | G | -0.131836 | 0.0096527 | -0.002176 | 0.005059 |
| rs9970807 | C | T | 0.12575 | 0.016695 | -0.01382 | 0.007412 |

*Proxy SNPs (rs1535616 for rs12202017; rs5760293 for rs180803; rs10793514 for rs1870634; rs11226029 for rs2128739; rs7182716 for rs4468572; rs1429142 for rs4593108; rs17293632 for rs56062135; rs6511720 for rs56289821; rs6427658 for rs6689306; rs2133189 for rs67180937; and rs7692387 for rs72689147)

**Supplemental table 5: Pleiotropic effects of single nucleotide polymorphisms associated with coronary artery disease (CAD) as reported in the GWAS Catalog and Phenoscanner[3, 4]**

| SNP associated with CAD | Traits |
|---|---|
| rs11191416 | Obesity related traits; blood pressure; Schizophrenia |
| rs11206510 | lipids |
| rs11556924 | White blood cell; platelet |
| rs1412444 | LIPA expression |
| rs17087335 | Height |
| *rs17293632 | Irritable Bowel Disease; Crohn' disease |
| rs2107595 | Blood pressure; stroke |
| rs2681472 | Blood pressure |
| *rs2891168 | Glioma; glaucoma |
| rs3184504 | Various traits such as lipids and blood cells attribute |
| rs4420638 | Various traits such as lipids and obesity |
| rs6427658 | IL6 |
| rs6511720 | Lipids |
| rs663129 | Obesity related traits; height; type 2 diabetes; lipids |
| rs7528419 | Lipids; height |
| rs7692387 | Blood pressure |
| rs9349379 | Migraine; blood pressure |

*Not excluded in the sensitivity analysis since the traits are possibly not related to adiponectin

**Supplemental Table 6: Leave one out analysis for the association of coronary artery disease risk on adiponectin using Mendelian randomization**

| Excluded single nucleotide polymorphism | Beta per log odds of CAD | Lower 95% Confidence Interval | Upper 95% Confidence Interval |
|---|---|---|---|
| rs10793514 | -0.01 | -0.03865 | 0.018664 |
| rs11191416 | -0.01109 | -0.03961 | 0.017434 |
| rs11206510 | -0.01143 | -0.04 | 0.017138 |
| rs11226029 | -0.00982 | -0.0382 | 0.018553 |
| rs11556924 | -0.00433 | -0.02902 | 0.020359 |
| rs1412444 | -0.01069 | -0.03923 | 0.017855 |
| rs1429142 | -0.01081 | -0.03929 | 0.017678 |
| rs1535616 | -0.01553 | -0.04232 | 0.011255 |
| rs16986953 | -0.01295 | -0.04093 | 0.015024 |
| rs17087335 | -0.01202 | -0.04044 | 0.016403 |
| rs17293632 | -0.01003 | -0.03858 | 0.018513 |
| rs17678683 | -0.00972 | -0.03801 | 0.018575 |
| rs2107595 | -0.01103 | -0.03959 | 0.017527 |
| rs2133189 | -0.00889 | -0.03576 | 0.017974 |
| rs2487928 | -0.00972 | -0.03823 | 0.018788 |
| rs2681472 | -0.01353 | -0.04146 | 0.014401 |
| rs2891168 | -0.02079 | -0.05343 | 0.011852 |
| rs3184504 | -0.01176 | -0.04044 | 0.016922 |
| rs4420638 | -0.01491 | -0.0421 | 0.012279 |

| | | | |
|---|---|---|---|
| rs5760293 | -0.01006 | -0.0379 | 0.01777 |
| rs6427658 | -0.01061 | -0.03914 | 0.01792 |
| rs6511720 | -0.01213 | -0.04088 | 0.016631 |
| rs663129 | -0.00916 | -0.03701 | 0.018687 |
| rs7182716 | -0.01293 | -0.04138 | 0.015522 |
| rs7528419 | -0.01244 | -0.04132 | 0.016435 |
| rs7692387 | -0.00929 | -0.03737 | 0.018794 |
| rs9349379 | -0.01418 | -0.0437 | 0.015345 |
| rs9970807 | -0.00701 | -0.03476 | 0.020734 |
| Without exclusion (Main analysis) | -0.01122 | -0.03897 | 0.016532 |

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