Role of Adiponectin in Coronary Heart Disease Risk
A Mendelian Randomization Study

Maria Carolina Borges, Debbie A. Lawlor, Cesar de Oliveira, Jon White, Bernardo Lessa Horta, Aluísio J.D. Barros

Rationale: Hypoadiponectinemia correlates with several coronary heart disease (CHD) risk factors. However, it is unknown whether adiponectin is causally implicated in CHD pathogenesis.

Objective: We aimed to investigate the causal effect of adiponectin on CHD risk.

Methods and Results: We undertook a Mendelian randomization study using data from genome-wide association studies consortia. We used the ADIPOGen consortium to identify genetic variants that could be used as instrumental variables for the effect of adiponectin. Data on the association of these genetic variants with CHD risk were obtained from CARDIoGRAM (22,233 CHD cases and 64,762 controls of European ancestry) and from CARDIoGRAMplusC4D Metabochip (63,746 cases and 130,681 controls; ≈ 91% of European ancestry) consortia. Data on the association of genetic variants with adiponectin levels and with CHD were combined to estimate the influence of blood adiponectin on CHD risk. In the conservative approach (restricted to using variants within the adiponectin gene as instrumental variables), each 1 U increase in log blood adiponectin concentration was associated with an odds ratio for CHD of 0.83 (95% confidence interval, 0.68–1.01) in CARDIoGRAM and 0.97 (95% confidence interval, 0.84–1.12) in CARDIoGRAMplusC4D Metabochip. Findings from the liberal approach (including variants in any locus across the genome) indicated a protective effect of adiponectin that was attenuated to the null after adjustment for known CHD predictors.

Conclusions: Overall, our findings do not support a causal role of adiponectin levels in CHD pathogenesis.

Key Words: adiponectin ▪ cardiovascular disease ▪ coronary artery disease ▪ mendelian randomization analysis ▪ obesity

Adiponectin, a 30 kDa protein produced mainly by mature adipocytes, has been implicated in a wide spectrum of biological pathways related to peripheral insulin sensitivity, inflammatory response, and atherogenesis. In contrast to most adipokines, adiponectin secretion is downregulated in obese individuals. Observational epidemiological studies support that hypoadiponectinemia is associated with cardiovascular risk factors (eg, insulin resistance and dyslipidemia) and type 2 diabetes mellitus risk; inconsistent findings have been observed on coronary heart disease (CHD) and stroke risk.

Mendelian randomization studies make use of genetic variants as instrumental variables to investigate the effect of environmental exposures and biomarkers on outcomes. Because alleles are randomly allocated during gametogenesis and genotype is a fixed exposure, Mendelian randomization studies are not as vulnerable to confounding and reverse causality and can substantially improve causal inference from observational data. Mendelian randomization is regarded as nature’s analogue of randomized controlled trials and has successfully been used in cardiovascular research to investigate potential etiologic mechanisms, validate and prioritize novel drug targets, and increase understanding of current therapies.

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From the Postgraduate Program in Epidemiology, Federal University of Pelotas, Pelotas, Brazil (M.C.B., B.L.H., A.J.D.B.); MRC Integrative Epidemiology Unit (D.A.L.); School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom (D.A.L.); and Epidemiology and Public Health, Institute of Epidemiology and Health Care (C.d.O.) and UCL Genetics Institute, Division of Biosciences, Faculty of Life Sciences (J.W.), University College London, London, United Kingdom.

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Correspondence to Maria Carolina Borges, Postgraduate Program in Epidemiology, Federal University of Pelotas, Rua Marechal Deodoro, 1160-3° Piso, Centro Pelotas, RS, Brazil 96020-220. E-mail carolina.borges.mcb@gmail.com

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There is evidence of a shared allelic architecture of circulating adiponectin with CHD risk and carotid intima-media thickness\(^\text{16,17}\); however, it remains unanswered if these findings implicate a causal effect of adiponectin on CHD risk or merely shared pleiotropic factors. Our aim was to investigate the causal effect of adiponectin on CHD risk using Mendelian randomization.

**Methods**

**Study Design**

We performed a 2-sample Mendelian randomization analysis using summary data from genome-wide association studies (GWAS) consortia. Single-nucleotide polymorphisms (SNPs), previously reported to be associated with blood adiponectin levels, were used as instrumental variables for testing the causal effect of adiponectin on CHD risk. Data on the association of SNPs with (1) adiponectin levels (first sample) and (2) CHD risk (second samples) were combined to estimate the influence of blood adiponectin on CHD risk. To investigate the presence of potential bias (horizontal pleiotropy) or mediation of the effect of adiponectin on CHD via other CHD risk factors (vertical pleiotropy; Online Figure I), we also analyzed data on the association of the selected adiponectin-related SNPs with a range of CHD risk factors: glycohemoglobin, fasting insulin, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triacylglycerols (TAG), BMI, and WC. Where we found evidence of an effect of the SNP on these risk factors, estimates of the association between adiponectin and CHD were adjusted for these risk factors to reduce the possibility that horizontal pleiotropy biased our findings\(^\text{27}\). These 17 SNPs included the four SNPs within the ADIPOQ locus.

Ten of the 17 selected SNPs could be found in CARDIoGRAMplusC4D Metabochip data, 3 of which were proxy SNPs (\(R^2>0.95\) for CEU population). For the remaining 7 SNPs, data from CARDIoGRAM GWAS was used. As the SNP rs1108842 could not be found in GLGC data, a proxy SNP (rs13083798) in peri-ADIPOQ locus (\(P=5\times10^{-8}\)) with adiponectin levels in the ADIPOGen consortium was included (L17), as previously reported by Dastani et al\(^\text{16}\). These 17 SNPs included the four SNPs within the ADIPOQ locus.

**Validation of Instrumental Variable Assumptions**

Validity of Mendelian randomization analyses results can be compromised if the instrumental variable assumptions are violated. In Online Table II, we described the 3 core assumptions of instrumental variable analysis and the strategies used to address these.

**Estimation of Causal Effect**

For both liberal and conservative approaches, the \(\beta\) coefficient (log odds ratio of CHD per one natural log greater adiponectin level) and its SE were calculated using the inverse-variance weighted (IVW) method as described by Burgess et al\(^\text{26}\) (See Online Data Supplement).

For the liberal approach, we also used the IVW method to estimate the combined effect of adiponectin levels on cardiovascular risk factors (glycohemoglobin, fasting insulin levels, HDL-c, LDL-c, TAG, BMI, and WC). Where we found evidence of an effect of the SNPs on these risk factors, estimates of the association between adiponectin and CHD were adjusted for these risk factors to reduce the possibility that horizontal pleiotropy biased our findings\(^\text{27}\) (See Online Data Supplement).

**Sensitivity Analyses**

Assuming that all valid instrumental variables identify the same causal parameter, substantial heterogeneity would be suggestive of pleiotropic SNPs. We evaluated heterogeneity in our IVW estimates using standard tools from the meta-analysis literature: forest plot of per SNP ratio estimate, Cochran Q test, and \(I^2\) values.\(^\text{28–30}\) In addition, to identify overly influential SNPs, additional meta-analyses were performed by removing 1 SNP at a time and recalculating the overall instrumental variable estimates.
Adiponectin and Coronary Heart Disease Risk

Even after adjusting for cardiovascular risk factors associated with our instrument, the liberal approach estimates could still be biased by unknown horizontal pleiotropic pathways that link the adiponectin genetic instrumental variable to CHD independently of path through adiponectin. To explore the presence of this possible bias, the MR-Egger regression method was used. See Online Data Supplement for a description of this method.

We also undertook a positive control analysis that consisted of a Mendelian randomization analysis in which LDL-c was the biomarker of interest and CHD risk was the outcome (using the IVW and MR-Egger method) because of its established causal role in CHD development (see Online Data Supplement).

Results

Association of the Genetic Instrument With Adiponectin and CHD Risk

Figure 2 shows the associations of SNPs, used as instrumental variables in the conservative (n=4 SNPs within ADIPOQ gene) and liberal analyses (n=17 SNPs across the genome), with adiponectin levels and CHD risk. For the conservative approach, each adiponectin-increasing allele was associated with 2.3% reduction in CHD risk (95% confidence interval [CI], −4.1 to −0.4) in CARDIoGRAM data and 0.6% reduction in CHD risk (95% CI, −1.9 to 1.0) in CARDIoGRAMplusC4D Metabochip. For the liberal approach, each adiponectin-increasing allele was associated with 2.3% reduction in CHD risk (95% CI, −3.2 to −1.5) in CARDIoGRAM data and 1.7% reduction in CHD risk (95% CI, −2.3 to −1.1%) in CARDIoGRAMplusC4D Metabochip. Of the 17 SNPs, there was some evidence of heterogeneity (P<0.05) between studies that contributed to each consortium for 3 SNPs: 2 SNPs in CARDIoGRAM (rs1108842 and rs6488989) and 1 SNP in CARDIoGRAMplusC4D Metabochip (rs3774261).

Association of the Genetic Instruments With CHD Risk Factors

More than 50% of individual SNPs were associated with one or more CHD risk factor (glycohemoglobin, fasting insulin levels, HDL-c, LDL-c, TAG, BMI, and WC), and none of these SNPs were located within ADIPOQ gene (±50 kb; Table 2). In general, adiponectin-increasing variants were not associated with CHD risk factors in the conservative approach but were related to lower fasting insulin, higher HDL-c, lower TAG, lower WC, and higher BMI in the liberal approach (Figure 3).

Effect of Blood Adiponectin Concentration on CHD Risk

Figure 4 shows the results of all Mendelian randomization analyses assessing the association of genetically predicted adiponectin with CHD risk. Using the conservative approach (including only the 4 SNPs within ADIPOQ gene), each unit increase in log adiponectin concentration was associated with an odds ratio for CHD of 0.83 (95% CI, 0.68–1.01) in CARDIoGRAM and 0.97 (95% CI, 0.84–1.12) in CARDIoGRAMplusC4D Metabochip data set. Using the liberal approach (including 17 SNPs), the odds ratio (OR) for the effect of each unit increase in log adiponectin concentration on CHD was 0.76 (95% CI, 0.65–0.89) in CARDIoGRAM and 0.83 (95% CI, 0.74–0.93) in CARDIoGRAMplusC4D Metabochip.

Table 1. Characteristics of SNPs Selected for Each Analytic Approach

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<th>Position*</th>
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<th>NEA</th>
<th>EAF†</th>
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C4 indicates the 4 SNPs used in the conservative analyses; Chr indicates chromosome; EA, effect allele; EAF, effect allele frequency; L17, 17 SNPs used in the liberal analyses (SNPs selected on the basis of reaching genome-wide significant levels in association with adiponectin, P<5×10−8); and NEA, non-effect allele.

*Genome Reference Consortium Human Build 37.

†1000 Genomes.
Metabochip. When we adjusted these liberal approach results for the CHD risk factors associated with the genetic instrument (fasting insulin, HDL-c, TAG, WC, and BMI), the OR was 0.88 (95% CI, 0.75–1.03) in CARDIoGRAM and 1.00 (95% CI, 0.90–1.12) in CARDIoGRAMplusC4D Metabochip.

Sensitivity Analyses
There was substantial heterogeneity in IVW estimates among the 17 SNPs from the liberal approach in both CARDIoGRAM (I² = 65.2; P = 1×10⁻⁴) and CARDIoGRAMplusC4D Metabochip (I² = 72.4; P = 2×10⁻⁶) data (Online Figure II). The effect of removing one SNP at a time on the overall estimate showed that no SNP could explain the observed protective effect in the liberal analysis. The inclusion of the SNPs rs17366568 and rs8047711 slightly underestimated findings from the IVW method in CARDIoGRAM data set (Online Figure III).

By using the MR-Egger method with our liberal instrument, we observed further evidence of directional pleiotropy, that is, the instrument was associated with a decreased log odds of CHD independently of its effect on adiponectin in CARDIoGRAM (log OR, −0.03; 95% CI: −0.05 to −0.02 for the intercept) and in CARDIoGRAMplusC4D Metabochip (log OR, −0.03; 95% CI, −0.05 to −0.02 for the intercept; Online Figure IV). According to Mendelian randomization estimates using the MR-Egger method, each unit increase in log adiponectin concentration was associated with an OR for CHD of 1.25 (95% CI, 0.96–1.63) in CARDIoGRAM and 1.30 (95% CI, 1.06–1.58) in CARDIoGRAMplusC4D Metabochip data set (Figure 4).

Discussion
Taken together, our results are not supportive of a protective causal effect of adiponectin on CHD risk. First, we found no consistent evidence that genetic predisposition to elevated blood adiponectin levels is associated to reduced risk of CHD in the analysis restricted to ADIPOQ SNPs (conservative approach). Second, in the more liberal analysis, using variants associated with adiponectin across the genome, there was evidence of a protective effect, but this was because of horizontal pleiotropy. This conclusion regarding horizontal pleiotropy resulting in a biased apparent protective effect with our liberal approach is supported by both multivariable Mendelian randomization and MR-Egger. Some of the variants strongly associated with circulating adiponectin, in our liberal analysis,

Figure 1. Analysis plan. Summary data from the association of single-nucleotide polymorphism (SNP) with phenotypes were extracted from genome-wide association study (GWAS) consortia data sets (ADIPOGen, CARDIoGRAM, C4D, MAGIC, GLGC, and GIANT). The effect of adiponectin on CHD was estimated using a conservative Mendelian randomization approach (instrumental variable: SNPs within ADIPOQ locus [±50 kb]) and a liberal approach (instrumental variable: SNPs in any locus). For the conservative approach, inverse-variance weighted (IVW) method was used. For the liberal approach, IVW method was used in both crude and adjusted analysis for known pleiotropic factors and MR-Egger regression in the analysis accounting for hidden pleiotropy (sensitivity analysis). BMI indicates body mass index; CARDIoGRAM, Coronary Artery Disease Genome-wide Replication and Meta-analysis; CARDIoGRAMplusC4D Metabochip, CARDIoGRAMplusC4D Metabochip meta-analysis; GIANT, genetic investigation of anthropometric traits; GLGC, Global Lipids Genetics Consortium; HbA1c: glycohemoglobin; HDL, high-density lipoprotein; IV, instrumental variable; LDL, low-density lipoprotein; MAGIC, Genetic Investigation of Anthropometric Traits; MR, Mendelian randomization; SNP, single-nucleotide polymorphism; TAG, triacylglycerol; and WC, waist circumference.
are related to loci of potential importance for LDL-c signaling in endothelial cells (CDH13) and for vascular biology (eg, TRIB1 and VEGFA), which might explain their pleiotropic effects regarding CHD pathogenesis.\textsuperscript{18} Last, our results are strengthened by the consistent strong positive associations of LDL-c with CHD when we use the same methods used for adiponectin to test this known causal effect.

Few previous studies have conducted Mendelian randomization analysis to investigate the effect of adiponectin on metabolic diseases. Two smaller studies found evidence that genetically raised adiponectin levels were positively associated with insulin sensitivity.\textsuperscript{32,33} However, a larger study did not provide evidence of a causal role of adiponectin in insulin resistance or type 2 diabetes mellitus\textsuperscript{34} but found that genetically raised insulin levels are associated with lower adiponectin levels, suggesting that the association was possibly because higher insulin levels caused lower adiponectin, rather than the other way round.

We have undertaken the first large Mendelian randomization study of the causal effect of adiponectin on cardiovascular disease risk using GWAS consortia data from CARDIoGRAM (22,233 CHD cases and 64,762 controls) and CARDIoGRAMplusC4D Metabochip (63,746 cases and 130,681 controls) with detailed phenotyping of coronary artery disease, myocardial infarction, or both. We applied a rigorous analyses plan to assess the validity and consistency of our findings. This included (1) adopting a systematic prespecified approach to selecting SNPs for our instrumental variables; (2) exploring different scenarios from the plausibly valid (but less well powered) conservative MR approach.

### Figure 2. Forest plots of mean difference in log adiponectin levels and odds ratio of coronary heart disease per allele of single-nucleotide polymorphism (SNP) according to the conservative (A) and liberal (B) approaches.

#### A Conservative approach

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>ADIPOGen</th>
<th>CARDIoGRAM</th>
<th>CARDIoGRAMplusC4D Metabochip</th>
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<tr>
<td>rs610075</td>
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<td></td>
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<td>Overall</td>
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#### B Liberal approach

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<tr>
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<tr>
<td>Overall</td>
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</table>
SNPs within the ADIPOQ gene (±50 kb) are identified by an asterisk (*). After Bonferroni correction, only P values lower than 4.2×10^{-4} (0.05/17 SNPs/7 phenotypes) were considered statistically significant. BMI indicates body mass index; HbA1c, glycohemoglobin; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; SNP, single-nucleotide polymorphism; TAG, triacylglycerols; and WC, waist circumference.

Table 2. Standardized Mean Difference (and P values) of Cardiovascular Risk Factors Per Allele of SNPs Used in Mendelian Randomization Analyses

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<tr>
<th>Phenotype</th>
<th>HbA1c</th>
<th>Insulin</th>
<th>HDL-c</th>
<th>LDL-c</th>
<th>TAG</th>
<th>WC</th>
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<td>0.888</td>
<td>-0.009</td>
<td>0.321</td>
<td>0.000</td>
<td>0.813</td>
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<td>rs17366568*</td>
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<td>0.984</td>
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<td>0.009</td>
<td>0.292</td>
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<tr>
<td>rs3774261*</td>
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<td>0.932</td>
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<td>0.781</td>
<td>-0.006</td>
<td>0.108</td>
<td>-0.001</td>
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<tr>
<td>rs9985840</td>
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<td>0.045</td>
<td>-0.002</td>
<td>0.657</td>
<td>0.026</td>
<td>2×10^{-11}</td>
<td>-0.001</td>
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<tr>
<td>rs29808800</td>
<td>0.014</td>
<td>0.030</td>
<td>0.000</td>
<td>0.967</td>
<td>0.043</td>
<td>1×10^{-20}</td>
<td>-0.040</td>
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<td>rs7955516</td>
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<td>rs601339</td>
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<td>0.528</td>
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<td>rs7978610</td>
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<td>0.032</td>
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<td>rs2989579*</td>
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<td>0.649</td>
<td>0.010</td>
<td>0.887</td>
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<td>rs12929479*</td>
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<td>0.764</td>
<td>-0.006</td>
<td>0.141</td>
<td>-0.008</td>
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<td>rs731839</td>
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<td>0.009</td>
<td>0.022</td>
<td>3×10^{-9}</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Figure 3. Standardized mean difference (and 95% confidence interval [CI]) in cardiovascular risk biomarkers per 1 U increase in genetically instrumented log adiponectin levels. A, Conservative approach including 4 SNPs within ADIPOQ gene associated with adiponectin at genome-wide significant levels (P<5×10^{-8}; C4). B, Liberal approach including 17 SNPs across the genome associated with adiponectin at genome-wide significant levels (P<5×10^{-8}; L17). BMI indicates body mass index; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TAG, triacylglycerols; and WC, waist circumference.
the consistency of our findings by performing influence meta-
alysis and a positive control analysis; and (7) using 2-sam-
ple Mendelian randomization to avoid statistical overfitting in
comparison to Mendelian randomization where all analyses
are conducted in the same participants35 (in a 1-sample setting,
results could be biased in the presence of weak instruments
because of genetic variants correlating with confounders by
chance).

Some limitations of this study should be considered. First,
we were not able to test for effect modification by sex, age,
or previous disease because of the use of summary data only.
In observational studies, the association between adiponectin
levels and CHD outcomes is modified by factors such as the
type of event (incident versus prevalent)10 and age of the par-
ticipant.36 Surprisingly, we did find a positive association be-
tween circulating adiponectin and CHD risk in the MR-Egger
analysis with CARDioGRAMplusC4D Metabochip data set,
which is likely to be reflecting a false-positive finding because
it was generally inconsistent with results from the conserva-
tive approach. We aimed to estimate the causal effect of total
adiponectin concentrations, but high-molecular-weight adipo-
nectin is thought to be the biologically active fraction, and we
are not able to specifically assess its effect. Although we have
explored possible violation of the assumptions of Mendelian
randomization (Online Table II), we cannot rule out bias be-
cause of possible compensatory mechanisms, known as ca-
nalization (eg, counter-regulation of adiponectin receptors
expression because of variations in blood adiponectin concen-
tration). That said, we are not aware of any evidence that this
might be the case.

The 2-sample Mendelian randomization assumes
that both samples come from comparable populations.
For our discovery analyses, this was the case, whereas in
CARDioGRAMplusC4D Metabochip, although the major-
ity of the participants were of European ancestry (the same
as in ADIPOGen), 9% were from other ethnic backgrounds.
However, we think it is unlikely that this will have resulted in a
major source of bias. First, double genomic control for ethnic-
ity was undertaken in CARDioGRAMplusC4D Metabochip
to control for confounding by population stratification. Second,
we found little evidence of heterogeneity in the association
of SNPs with CHD in the 2 consortia, which suggests that
(strong) effect modification by genomic ancestry is unlikely.
Last, in a positive control study, we showed that 2-sample
Mendelian randomization produced similar evidence for the
expected positive causal effect of LDL-c on CHD.

Adiponectin concentration in the blood ranges from 1
to 30 ng/mL in healthy adults, which is ≈103- to 106-folds
higher than the concentration of many hormones and cyto-
kines.37 Blood adiponectin concentration is a modifiable risk
factor that can be efficiently targeted by lifestyle modifica-
tions, mainly weight loss and dietary changes.38 Our results
reinforce that Mendelian randomization studies can be helpful
in prioritizing potential drug or lifestyle targets, which could
substantially reduce the high costs associated with the devel-
opment and evaluation of large numbers of compounds or life-
style changes that fail along the development process.

Overall, our findings are not supportive of a protective role
of adiponectin in CHD and indicate that the association of ge-
etically increased adiponectin levels and lower risk of CHD is
mainly driven by horizontal pleiotropy.

Acknowledgments

We thank Frank Dudbridge (London School of Hygiene and
Tropical Medicine, UK) and Alexandre Pereira (Heart Institute,
University of Sao Paulo, Brazil) for the helpful comments on the
study design and analysis. Data on adiponectin have been contrib-
uted by ADIPOGen Consortium and have been downloaded from
https://www.mcgill.ca/genepli/adipogen-consortium. Data on coro-
nary artery disease/myocardial infarction have been contributed
by CARDioGRAMplusC4D investigators and have been downloaded
from www.CARDIOGRAMPLUSC4D.ORG. Data on glycemic
traits have been contributed by MAGIC investigators and have been
downloaded from www.magicinvestigators.org. Data on lipid traits
have been contributed by Global Lipids Genetics Consortium and have been downloaded from http://csg.sph.umich.edu/abecasis/pub-
llic/lipids2013/. Data on anthropometric traits have been contributed
by Genetic Investigation of ANthropometric Traits (GIANT) consor-
tium and have been downloaded from http://www.broadinstitute.org/
collaboration/giant/index.php/GIANT_consortium_data_files. All

<table>
<thead>
<tr>
<th>Approach</th>
<th>SNPs</th>
<th>Method</th>
<th>Adjusted</th>
<th>OR CHD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARDioGRAM</td>
<td>4</td>
<td>IVW</td>
<td>No</td>
<td>0.63 (0.70, 1.09)</td>
</tr>
<tr>
<td>Liberal</td>
<td>17</td>
<td>IVW</td>
<td>No</td>
<td>0.76 (0.65, 0.89)</td>
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<tr>
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<td>IVW</td>
<td>Yes</td>
<td>0.88 (0.75, 1.03)</td>
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<tr>
<td>Liberal</td>
<td>17</td>
<td>MR–Egger</td>
<td>Yes</td>
<td>1.25 (0.96, 1.63)</td>
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<tr>
<td>CARDioGRAMplusC4D</td>
<td>4</td>
<td>IVW</td>
<td>No</td>
<td>0.97 (0.84, 1.12)</td>
</tr>
<tr>
<td>Liberal</td>
<td>17</td>
<td>IVW</td>
<td>No</td>
<td>0.83 (0.74, 0.93)</td>
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<tr>
<td>Liberal</td>
<td>17</td>
<td>IVW</td>
<td>Yes</td>
<td>1.00 (0.90, 1.12)</td>
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<tr>
<td>Liberal</td>
<td>17</td>
<td>MR–Egger</td>
<td>Yes</td>
<td>1.30 (1.06, 1.58)</td>
</tr>
</tbody>
</table>
the data used are publicly available (Online Table I). Those people acknowledged here and who have made their genome-wide data available to scientist may not necessarily agree with comments made in this article, and the authors take full responsibility for the contents of this article.

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Author Contributions: M.C. Borges, D.A. Lawlor, C. de Oliveira, B.L. Horta, and A.J.D. Barros designed the study. M.C. Borges, D.A. Lawlor, J. White, and A.J.D. Barros conceived the analysis plan. M.C. Borges, D.A. Lawlor, C. de Oliveira assisted in data acquisition (from public data basis). M.C. Borges performed analyses. M.C. Borges wrote first draft of article. D.A. Lawlor, C. de Oliveira, J. White, B.L. Horta, and A.J.D. Barros were responsible for critical comments and contributions to final writing of article.

Disclosures

None.

References

Role of Adiponectin in Coronary Heart Disease Risk: A Mendelian Randomization Study
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SUPPLEMENTAL MATERIAL
Supplemental Methods

Inverse variance weighted (IVW) method

For unadjusted and adjusted Mendelian randomization analyses, the inverse variance weighted (IVW) method was used to derive the beta coefficient (log odds ratio of CHD per 1 natural log greater adiponectin level) and its standard error by using the following formulas:

\[
\hat{\beta}_{IVW} = \frac{\sum_{k=1}^{K} X_k Y_k \sigma_{yk}^{-2}}{\sum_{k=1}^{K} X_k^2 \sigma_{yk}^{-2}}
\]

\[
SE_{\hat{\beta}_{IVW}} = \sqrt{\frac{1}{\sum_{k=1}^{K} X_k^2 \sigma_{yk}^{-2}}}
\]

Where \(X_k\) is the mean change in log adiponectin level per additional effect allele of SNP k and \(Y_k\) is the mean change in log odds of CHD per additional effect allele of SNP k with standard error \(\sigma_{yk}\).

The IVW method was also used to estimate the effect of adiponectin on cardiovascular risk factors (HbA1c, fasting insulin levels, HDL-c, LDL-c, TAG, BMI, and WC) \((X_k,: mean change in log adiponectin level per additional effect allele of SNP k, Y_k: mean change in the risk factor per additional effect allele of SNP k; \sigma_{yk}: standard error of Y_k)\).

To estimate the association of genetically raised adiponectin and CHD in the model adjusted for cardiovascular risk factors, we used betas for SNP-CHD association as the dependent variable, betas for SNP-adiponectin and SNP-cardiovascular risk factors as independent variables and inverse variance weights (with no intercept). This method is equivalent to IVW method when there is only one independent variable.
**MR-Egger regression method**

The Egger regression has been used for almost two decades to detect small study bias (which may be due to publication bias) in meta-analyses of randomized clinical trials. In this method, the ratio of the effect estimate by its standard error is regressed against the estimate’s precision (the inverse of the standard error). Bowden et al. recently proposed an adaptation of the Egger regression to test for bias from horizontal pleiotropy in Mendelian randomization studies.

While the IVW estimate is equivalent to the slope of the best fitting line through the observations that pass through the origin, the MR-Egger estimate would be the best fitting line through the observations in a model that allows the intercept to vary. In this method, the intercept will reflect the average pleiotropic effect across genetic variants (e.g. log odds CHD per allele when difference in adiponectin per allele is zero) and the slope coefficient will provide an estimate of the causal effect provided that the InSIDE (Instrument Strength Independent of Direct Effect) assumption holds, which requires that there is no correlation between SNP-exposure association and direct effects of SNP on outcome.

Bootstrapping (10,000 iterations) was used to derive corrected 95% confidence intervals for MR-Egger intercept and slope using the percentile method.

**Positive control analysis**

The positive control consisted of a Mendelian randomization analysis in which LDL-c was the biomarker of interest and CHD risk was the outcome.
SNPs were reported as strongly associated with LDL-c in GLGC consortium\textsuperscript{4}. Of there, 38 could be found in both Cardiogram and CARDIoGRAMplusC4D Metabochip dataset and, thus, were used as the instrumental variable for LDL-c. The crude IVW method was used to estimate the association of LDL-c with CHD risk. Since many SNPs were also associated with other lipid traits (ex: HDL-c, TAG and total cholesterol), the MR-Egger method was also used.
Online Tables
## Online Table I. Characteristics of the data sources

<table>
<thead>
<tr>
<th>Consortium</th>
<th>Use</th>
<th>Studies</th>
<th>Study population</th>
<th>Imputation</th>
<th>QC criteria†</th>
<th>Model</th>
<th>Adjustments</th>
<th>Data download</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADIPOGen</td>
<td>SNP-log adiponectin</td>
<td>16 cohort studies with GWAS data</td>
<td>29,347 individuals of European ancestry</td>
<td>IMPUTE, MACH, BIMBAM or Beagle (reference: Phase II CEU HapMap population)</td>
<td>Call rate &gt; 0.95; MAF &gt; 0.01; p (_{\mathrm{M}}) &gt; 10(^{-6}); and quality measures for imputed SNPs (r(^2) ≥ 0.3, or proper info ≥ 0.4)</td>
<td>additive</td>
<td>Age, sex, BMI, principal components of genomic ancestry, study site (where appropriate), family structure (one family-based study) and genomic control inflation factor ((\lambda))</td>
<td><a href="http://www.mcgill.ca/genepi/adipogen-consortium">http://www.mcgill.ca/genepi/adipogen-consortium</a></td>
</tr>
<tr>
<td>CARDIoGRAM§</td>
<td>SNP-log odds CHD</td>
<td>14 case-control or cohort studies with GWAS data</td>
<td>22,233 CHD cases and 64,762 controls of European ancestry</td>
<td>IMPUTE, MACH or BIMBAM (reference: CEU HapMap population)</td>
<td>Sample call rate &gt; 0.97-0.98; SNP call rate &gt; 0.95-0.99; MAF &gt; 0.01; p (_{\mathrm{M}}) ≤ 10(^{-5}); 10(^{-6}); ethnic outliers. Quality measures for imputed SNPs: NR*</td>
<td>additive</td>
<td>Age, sex and genomic control inflation factor ((\lambda))</td>
<td><a href="http://www.cardiogramplusc4d.org/downloads">http://www.cardiogramplusc4d.org/downloads</a></td>
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<tr>
<td>CARDIoGRAMplus C4D Metabochip§</td>
<td>SNP-log odds CHD</td>
<td>48 case-control or cohort studies with GWAS data</td>
<td>63,746 CHD cases and 130,681 controls of European (~91%) and Asian ancestry</td>
<td>NA, Minimac or IMPUTE (reference: HapMap 2/3 or 1000 Genomes Project phase 1)</td>
<td>Sample call rate &gt; 0.98; MAF &gt; 0.01; p (_{\mathrm{M}}) &gt; 10(^{-6}); and other study-specific criteria</td>
<td>additive</td>
<td>Age, sex and genomic control inflation factor ((\lambda))</td>
<td><a href="http://www.cardiogramplusc4d.org/downloads">http://www.cardiogramplusc4d.org/downloads</a></td>
</tr>
<tr>
<td>MAGIC</td>
<td>SNP-HbA(_1c) (%)</td>
<td>23 cohort studies with GWAS data</td>
<td>35,920 individuals of European ancestry</td>
<td>IMPUTE or MACH (reference: CEU HapMap population)</td>
<td>Sample call rate &gt; 0.95-0.97; SNP call rate &gt; 0.90-0.95; MAF &gt; 0.01; p (_{\mathrm{M}}) &gt; 10(^{-4}-10(^{-5}); sex mismatch between genotyped and reported sex; outliers as assessed by population structure analysis; and quality measures for imputed SNPs (r(^2) ≥ 0.3, or proper info ≥ 0.4, and MAF &gt; 0.01)</td>
<td>additive</td>
<td>Age, sex, other cohort-specific variables as applicable, and genomic control inflation factor ((\lambda))</td>
<td><a href="http://www.magicinvestigators.org/downloads">http://www.magicinvestigators.org/downloads</a></td>
</tr>
<tr>
<td>GLGC</td>
<td>SNP-log fasting insulin</td>
<td>20 cohort studies with GWAS data</td>
<td>38,238 individuals of European ancestry</td>
<td>IMPUTE, MACH or BIMBAM (reference: CEU HapMap population)</td>
<td>Sample call rate &gt; 0.94-0.99; SNP call rate &gt; 0.90-0.95; MAF &gt; 0.01-0.05; p (_{\mathrm{M}}) &gt; 10(^{-4}-10(^{-5}); and quality measures for imputed SNPs (r(^2) ≥ 0.3, proper info ≥ 0.4 or observed/expected variance ratio &gt; 0.3)</td>
<td>additive</td>
<td>Age, sex, principal components of genomic ancestry (some studies), and genomic control inflation factor ((\lambda)). Individuals taking lipid-lowering medications were excluded.</td>
<td><a href="http://csg.sph.umich.edu/abecasis/public/lipids2013/">http://csg.sph.umich.edu/abecasis/public/lipids2013/</a></td>
</tr>
<tr>
<td>GIANT</td>
<td>SNP-HDLc</td>
<td>60 cohort and case control studies with GWAS or Metabochip data</td>
<td>188,577 European-ancestry individuals</td>
<td>MACH (reference: CEU HapMap population)</td>
<td>Quality control: NR*</td>
<td>additive</td>
<td>Age, sex, and study specific variables (e.g. principal components of genomic ancestry), and genomic control inflation factor ((\lambda))</td>
<td>[<a href="http://www.broadinstitute.org/collaborations/GIANT">http://www.broadinstitute.org/collaborations/GIANT</a> Consortium/Data_files](<a href="http://www.broadinstitute.org/collaborations/GIANT">http://www.broadinstitute.org/collaborations/GIANT</a> Consortium/Data_files)</td>
</tr>
<tr>
<td></td>
<td>SNP-LDLc</td>
<td>up to 322,154 individuals of European ancestry</td>
<td>IMPUTE, MACH or BIMBAM (reference: Phase II CEU HapMap population)</td>
<td>Sample call rate &gt; 0.80-0.98; SNP call rate &gt; 0.90-0.99; MAF &gt; 0.01-0.05; p (_{\mathrm{M}}) &gt; 10(^{-4}-10(^{-5}); and quality measures for imputed SNPs (r(^2) ≥ 0.3, proper info ≥ 0.4, or no filtering)</td>
<td>additive</td>
<td>Age, sex, and study specific variables (e.g. principal components of genomic ancestry), and genomic control inflation factor ((\lambda))</td>
<td>[<a href="http://www.broadinstitute.org/collaborations/GIANT">http://www.broadinstitute.org/collaborations/GIANT</a> Consortium/Data_files](<a href="http://www.broadinstitute.org/collaborations/GIANT">http://www.broadinstitute.org/collaborations/GIANT</a> Consortium/Data_files)</td>
<td></td>
</tr>
</tbody>
</table>
### Online Table I. Characteristics of the data sources (continued)

| SNP-BMI-adjusted WC | 101 studies of multiple designs with GWAS or Metabochip data | up to 210,088 individuals of European ancestry | IMPUTE, MACH or Beagle (reference: Phase II CEU HapMap population) | Sample call rate > 0.85-0.98; SNP call rate > 0.90-0.99; MAF > 0.00-0.01; $p_{\text{HWE}} > 10^{-3-10^{-7}}$; and quality measures for imputed SNPs ($r^2 \geq 0.3$; proper info $\geq 0.4$, or no filtering) | Age, age$^2$, BMI and study specific variables (e.g. principal components of genomic ancestry), and genomic control inflation factor ($\lambda$) |

† Quality control criteria varied across studies; * NR: not reported in the main consortium publication. § CHD was defined as the presence of coronary artery disease or myocardial infarction. Detailed criteria for CHD definition for each study can be found in the Supplementary material of the main publications. QC: quality control; GWAS: genome-wide association study; CEU: Centre d’Etude du Polymorphisme Humain collected in Utah; CHD: coronary heart disease; MAF: minor allele frequency; SNP: single nucleotide polymorphism; BMI: body mass index; WC: waist circumference; NR: not reported; NA: not applicable. CARDIoGRAM: Coronary ARtery DIsease Genome-wide Replication And Meta-analysis; MAGIC: Meta-Analyses of Glucose and Insulin-Related Traits Consortium; GLGC: Global Lipids Genetics Consortium; GIANT: Genetic Investigation of ANthropometric Traits.
### Online Table II. Core instrumental variable assumptions and strategies to address them

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Graphical examples of assumption violation</th>
<th>Consequences of potential violation</th>
<th>Validation of assumption in the current analysis</th>
</tr>
</thead>
</table>
| 1. IV should be (strongly) associated with the exposure | ![Graph](image1) | A weak association between the IV and E can reduce precision and introduce weak instrument bias, which tends to bias the causal estimate towards the OLS estimate in one-sample MR | - The strength of SNPs-adiponectin association was explored using the F-statistic ($F > 20$ for every SNP)  
- In two-sample MR studies with non-overlapping datasets, any bias from weak instruments would be in the direction of the null and, thus, should not result in false positive findings |
| 2. IV should only affect the outcome through the exposure | ![Graph](image2) | Bias in MR estimate can result from horizontal pleiotropy (e.g. genetic variant itself or a correlated variant is associated with multiple pathways independent of the exposure); the direction and magnitude of this bias will depend on the direction and magnitude of the association path from IV to O that is not via E | Issues of horizontal pleiotropy were addressed by three different strategies:  
- The association of SNPs with known CHD risk factors was tested. In case of evidence of potential pleiotropy, this was accounted for in the analyses  
- By comparing the conservative and the liberal approach. In the conservative approach, horizontal pleiotropy is less likely given that variants in the ADIPOQ gene are more plausible valid instrumental variables for adiponectin levels. In the liberal approach, there is an increased likelihood of horizontal pleiotropy but also increased power, since more variants can be selected by this approach  
- Using methods that account for unknown directional pleiotropy (MR-Egger method) |
| 3. IV should be independent of exposure-outcome confounders | ![Graph](image3) | In cases of population stratification, there could be an spurious association between IV and phenotypes | - We cannot test for the absence of exposure-outcome confounders relating to the IV when summary-level data are used, but there is empirical evidence that this is unlikely  
- To reduce the possibility of bias due to population stratification, the analyses were restricted to only (or predominantly) European-ancestry individuals  
- All consortia adjusted for genomic control inflation factor |

IV: instrumental variable; E: exposure; O: outcome; U: unknown confounder; X: other phenotype; G: other genetic variant in LD; LD: linkage disequilibrium; CHD: coronary heart disease. A dashed arrow was used to indicate weak association between IV and E. *Adapted from Vanderweele*. 

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IV: instrumental variable; E: exposure; O: outcome; U: unknown confounder; X: other phenotype; G: other genetic variant in LD; LD: linkage disequilibrium; CHD: coronary heart disease. A dashed arrow was used to indicate weak association between IV and E. *Adapted from Vanderweele*. 
### Online Table III. Power simulations for the Mendelian randomization analyses

<table>
<thead>
<tr>
<th>Data source</th>
<th>Sample size</th>
<th>Proportion of cases</th>
<th>Type-I error rate (α)</th>
<th>Original OR</th>
<th>Equivalent standardized OR</th>
<th>R²ₕ₋ₐ</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARDIoGRAM</td>
<td>86,995</td>
<td>25.6%</td>
<td>0.05</td>
<td>0.70</td>
<td>0.80</td>
<td>5%</td>
<td>100%</td>
</tr>
<tr>
<td>CARDIoGRAM</td>
<td>86,995</td>
<td>25.6%</td>
<td>0.05</td>
<td>0.80</td>
<td>0.87</td>
<td>5%</td>
<td>97%</td>
</tr>
<tr>
<td>CARDIoGRAMplusC4D Metabochip</td>
<td>194,427</td>
<td>32.8%</td>
<td>0.05</td>
<td>0.70</td>
<td>0.80</td>
<td>5%</td>
<td>100%</td>
</tr>
<tr>
<td>CARDIoGRAMplusC4D Metabochip</td>
<td>194,427</td>
<td>32.8%</td>
<td>0.05</td>
<td>0.80</td>
<td>0.87</td>
<td>5%</td>
<td>100%</td>
</tr>
<tr>
<td>CARDIoGRAMplusC4D Metabochip</td>
<td>194,427</td>
<td>32.8%</td>
<td>0.05</td>
<td>0.90</td>
<td>0.94</td>
<td>5%</td>
<td>81%</td>
</tr>
</tbody>
</table>

OR: Assumed true odds ratio of CHD risk per standard deviation of the exposure variable

Conversion of original (per log adiponectin) to equivalent standardized OR (per standard unit of log adiponectin) was made using an external source of individual level data (1982 Pelotas Birth Cohort) with similar adiponectin distribution (adiponectin levels in ADIPOGen consortium: mean = 9.8 µg/ml (SD = 5.6); adiponectin levels in 1982 Pelotas Birth Cohort: mean = 9.3 µg/ml (SD = 5.7)).

R²ₕ₋ₐ: proportion of variance explained for the association between the genetic instrument (Z) and adiponectin levels (X). Values approximate findings from Dastani et al.'s and Yaghootkar et al.'s studies.

Calculations were performed in the power calculator for Mendelian Randomization studies, available at [http://cnsgenomics.com/shiny/mRnd/](http://cnsgenomics.com/shiny/mRnd/), based on the publication by Brion et al. 2013.
Online Figures

Online Figure I. Graphical illustration of scenarios of (A) vertical pleiotropy (mediation) and (B) horizontal pleiotropy (bias) by CHD risk factors in the relation among SNPs, adiponectin levels and CHD risk. CHD: coronary heart disease; SNP: single nucleotide polymorphism.

Online Figure II. Meta-analysis and heterogeneity analysis of Mendelian randomization estimates of each SNP for the association of blood adiponectin levels with CHD risk. CHD: coronary heart disease; SNP: single nucleotide polymorphism, Chr: chromosome.

Online Figure III. Pooled odds ratio (and 95%CI) of coronary heart disease risk per unit increase in log adiponectin levels omitting one SNP at a time (influence meta-analysis) estimated by the IVW method and by the MR-Egger method. CHD: coronary heart disease; IVW: inverse-variance weighted method; MR-Egger: Mendelian randomization-Egger method; OR: odds ratio; SNP: single nucleotide polymorphism.

Online Figure IV. Log odds ratio of coronary heart disease and mean increase in log adiponectin levels per adiponectin raising allele in CARDIoGRAM and CARDIoGRAMplusC4D Metabochip. Each data point represents betas for SNP-log OR CHD (Y axis) and SNP-adiponectin (X axis) association (N = 17 SNPs). CHD: coronary heart disease; IVW: inverse-variance weighted method; MR-Egger: Mendelian randomization-Egger method; OR: odds ratio; SNP: single nucleotide polymorphism.
Online Figure I.
Online Figure II.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>CARDIoGRAM</th>
<th>SNP</th>
<th>Chr</th>
<th>CARDIoGRAMplusC4D Metabochip</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1415293</td>
<td>1</td>
<td><img src="image1" alt="CARDIoGRAM" /></td>
<td>rs1415293</td>
<td>1</td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
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<tr>
<td>rs1108842</td>
<td>3</td>
<td><img src="image1" alt="CARDIoGRAM" /></td>
<td>rs1108842</td>
<td>3</td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
</tr>
<tr>
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<td><img src="image1" alt="CARDIoGRAM" /></td>
<td>rs6810075</td>
<td>3</td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
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<tr>
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<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
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<tr>
<td>rs2980880</td>
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<td><img src="image1" alt="CARDIoGRAM" /></td>
<td>rs2980880</td>
<td>8</td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
</tr>
<tr>
<td>rs7955516</td>
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<td><img src="image1" alt="CARDIoGRAM" /></td>
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<tr>
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<td>16</td>
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<tr>
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<td><img src="image1" alt="CARDIoGRAM" /></td>
<td>rs7200895</td>
<td>16</td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
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<tr>
<td>rs8047711</td>
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<tr>
<td>rs12929479</td>
<td>16</td>
<td><img src="image1" alt="CARDIoGRAM" /></td>
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<td>16</td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
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<tr>
<td>rs731839</td>
<td>19</td>
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<td>rs731839</td>
<td>19</td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
</tr>
<tr>
<td>Overall (I-squared = 65.2%, p = 0.000)</td>
<td></td>
<td><img src="image1" alt="CARDIoGRAM" /></td>
<td>Overall (I-squared = 72.4%, p = 0.000)</td>
<td></td>
<td><img src="image2" alt="CARDIoGRAMplusC4D Metabochip" /></td>
</tr>
</tbody>
</table>
Online Figure III.

- SNP | CARDioGRAM – IVW method
---|---
rs1415293
rs1108842
rs6810075
rs16861209
rs17366568
rs3774261
rs998584
rs2980880
rs7955516
rs601339
rs6488898
rs7978610
rs2925979
rs7200895
rs8047711
rs12929479
rs731839

- SNP | CARDioGRAMplusC4D – IVW method
---|---
rs1415293
rs1108842
rs6810075
rs16861209
rs17366568
rs3774261
rs998584
rs2980880
rs7955516
rs601339
rs6488898
rs7978610
rs2925979
rs7200895
rs8047711
rs12929479
rs731839

- SNP | CARDioGRAM – MR-Egger method
---|---
rs1415293
rs1108842
rs6810075
rs16861209
rs17366568
rs3774261
rs998584
rs2980880
rs7955516
rs601339
rs6488898
rs7978610
rs2925979
rs7200895
rs8047711
rs12929479
rs731839

- SNP | CARDioGRAMplusC4D – MR-Egger method
---|---
rs1415293
rs1108842
rs6810075
rs16861209
rs17366568
rs3774261
rs998584
rs2980880
rs7955516
rs601339
rs6488898
rs7978610
rs2925979
rs7200895
rs8047711
rs12929479
rs731839
Online Figure IV.
Suplemental References


2. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629-634


Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet*. 2011;43:333-338


