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Role of Adiponectin in Coronary Heart Disease Risk

A Mendelian Randomization Study

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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. (*Circ Res*. 2016;119:491-499. DOI: 10.1161/CIRCRESAHA.116.308716.)**

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,[1] inflammatory response,[1,2] and atherogenesis.[2] In contrast to most adipokines, adiponectin secretion is downregulated in obese individuals.[3] Observational epidemiological studies support that hypoadiponectinemia is associated with cardiovascular risk factors[4,5] (eg, insulin resistance and dyslipidemia) and type 2 diabetes mellitus risk[6]; inconsistent findings have been observed on coronary heart disease (CHD)[7–10] and stroke risk.[9,11]

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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.[12] 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,[13] validate and prioritize novel drug targets,[14] and increase understanding of current therapies.[15]

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Nonstandard Abbreviations and Acronyms

BMI	body mass index
C4	conservative instrumental variable analysis approach
CARDIoGRAM	Coronary ARtery DIsease Genome-wide Replication And Meta- analysis
CARDIoGRAMplusC4D	CARDIoGRAMplusC4D Metabochip and GWAS
Metabochip	meta-analysis
CEU	Utah residents with Northern and Western European ancestry
CHD	coronary heart disease
CI	confidence interval
GIANT	Genetic Investigation of ANthropometric Traits
GLGC	Global Lipids Genetics Consortium
GWAS	genome-wide association studies
HDL-c	high-density lipoprotein cholesterol
IVW	inverse-variance weighted
L17	liberal instrumental variable analysis approach
LDL-c	low-density lipoprotein cholesterol
MAGIC	Meta-Analyses of Glucose and Insulin-Related Traits Consortium
OR	odds ratio
SNP	single-nucleotide polymorphisms
TAG	triacylglycerols
WC	waist circumference

There is evidence of a shared allelic architecture of circulating adiponectin with CHD risk and carotid intima–media thickness[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), body mass index (BMI), and BMI-adjusted waist circumference (WC).

Data Sources

Summary data on the association between SNPs and the phenotypes of interest were extracted from public databases of different consortia: ADIPOGen for adiponectin[18]; CARDIoGRAM (Coronary ARtery DIsease Genome-wide Replication And Meta-analysis)[19] and CARDIoGRAMplusC4D Metabochip (CARDIoGRAMplusC4D Metabochip and GWAS meta-analysis)[20] for CHD; MAGIC (Meta-Analyses of Glucose and Insulin-Related Traits Consortium) for glycohemoglobin[21] and fasting insulin[22]; GLGC (Global Lipids Genetics Consortium) for HDL-c, LDL-c, and TAG[23]; and GIANT (Genetic Investigation of ANthropometric Traits) for BMI[24] and WC.[25] Details about each data source are displayed in Online Table I. CARDIoGRAMplusC4D Metabochip includes data from CARDIoGRAM GWAS.

Instrumental Variables

The SNPs for our main instrumental variable analyses (n=17 SNPs) were selected from 145 SNPs strongly ($P<5\times10^{-8}$) associated with blood adiponectin levels in the European ancestry GWAS meta-analysis from the ADIPOGen consortium.[18] Independent SNPs were previously selected by Dastani et al[16] by linkage disequilibrium pruning of the genome-wide significant SNPs, retaining SNPs that explained most variance in adiponectin levels in each linkage disequilibrium block (linkage disequilibrium threshold: $R^2<0.05$ in HapMap CEU population [Utah residents with Northern and Western European ancestry]; Table 1).

We used 2 sets of instruments (Figure 1):

1. A conservative instrumental variable analysis, in which only SNPs within the *ADIPOQ* locus (±50 kb) were considered eligible (n=4 SNPs; C4). *ADIPOQ* is mainly expressed in adipose tissue and encodes adiponectin. We considered this approach unlikely to be biased by horizontal pleiotropy given the functional relationship of *ADIPOQ* to adiponectin levels.
2. A liberal analysis, in which independent SNPs from any locus that had reached a genome-wide significant association ($P<5*10^{-8}$) with adiponectin levels in the ADIPOGen consortia GWAS (n=17 SNPs), were included (L17), as previously reported by Dastani et al.[16] 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 perfect linkage disequilibrium ($R^2=1.0$ for CEU population) was used instead.

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 β 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.[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[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.[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.

Table 1. Characteristics of SNPs Selected for Each Analytic Approach

SNP	Chr	Position*	Closest Gene	EA	NEA	EAF†	C4	L17
rs1415293	1	219730006	ZC3H11B	T	A	0.25	…	√
rs1108842	3	52720080	GNL3	C	A	0.49	…	√
rs6810075	3	186548565	ADIPOQ	T	C	0.61	√	√
rs16861209	3	186563114	ADIPOQ	A	C	0.08	√	√
rs17366568	3	186570453	ADIPOQ-AS1, ADIPOQ	G	A	0.93	√	√
rs3774261	3	186571559	ADIPOQ-AS1, ADIPOQ	A	G	0.50	√	√
rs998584	6	43757896	VEGFA	C	A	0.54	…	√
rs2980880	8	126480972	TRIB1	A	G	0.71	…	√
rs7955516	12	20498036	PDE3A	C	A	0.28	…	√
rs601339	12	123174743	HCAR2	G	A	0.25	…	√
rs6488898	12	124203832	ATP6V0A2	A	G	0.98	…	√
rs7978610	12	124468572	ZNF664, FAM101A	C	G	0.27	…	√
rs2925979	16	81534790	CMIP	C	T	0.71	…	√
rs7200895	16	82644606	CDH13	T	C	0.69	…	√
rs8047711	16	82667671	CDH13	G	A	0.92	…	√
rs12929479	16	82997853	CDH13	G	A	0.42	…	√
rs731839	19	33899065	PEPD	A	G	0.54	…	√

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, $P<5\times10^{-8}$); and NEA, noneffect allele.

*Genome Reference Consortium Human Build 37.

†1000 Genomes.

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.[31] 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 rs6488898) 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

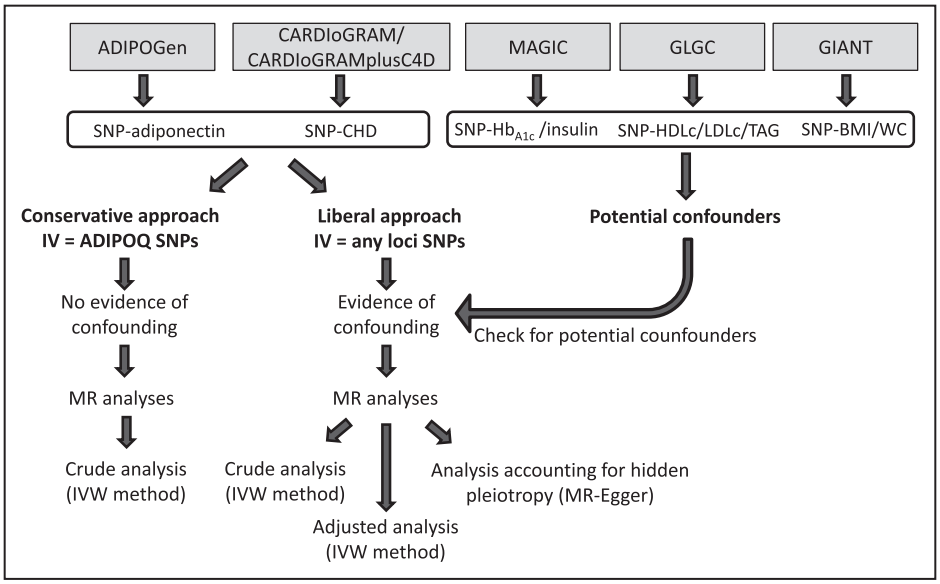

Figure 1. Analysis plan. Summary data from the association of single-nucleotide polymorphism (SNP) with phenotypes were extracted from genome-wide association study (GWAS) consortia data sets (ADIPOGen, CARDIoGRAM, C4D, MAGIC, GLGC, and GIANT). The effect of adiponectin on CHD was estimated using a conservative 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; Hb_{A1c}: glycohemoglobin; HDL, high-density lipoprotein; IV, instrumental variable; LDL, low-density lipoprotein; MAGIC, Meta-Analyses of Glucose and Insulin-related traits Consortium; MR, Mendelian randomization; SNP, single-nucleotide polymorphism; TAG, triacylglycerol; and WC, waist circumference.

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 (I^2=65.2; P=1×10^{-4}) and CARDIoGRAMplusC4D Metabochip (I^2=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). In the influence meta-analysis, in which we removed 1 of the 17 SNPs at a time from the pooled estimates, all of the results for the remaining 16 SNPs were in the same (positive) direction, but the magnitude of this varied somewhat (Online Figure III).

To investigate any differences between CARDIoGRAM and CARDIoGRAMplusC4D Metabochip, we compared Mendelian randomization results of the effect of LDL-c on CHD risk (positive control analysis). The OR for CHD for each standardized unit increase in LDL-c was 1.70 (95% CI, 1.54–1.88) in CARDIoGRAM and 1.57 (95% CI, 1.47–1.67) in CARDIoGRAMplusC4D Metabochip. After accounting for unknown horizontal pleiotropy (MR-Egger method), estimates were 1.96 (95% CI, 1.59–2.33) for CARDIoGRAM and 1.92 (95% CI, 1.65–2.17) for CARDIoGRAMplusC4D Metabochip.

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,

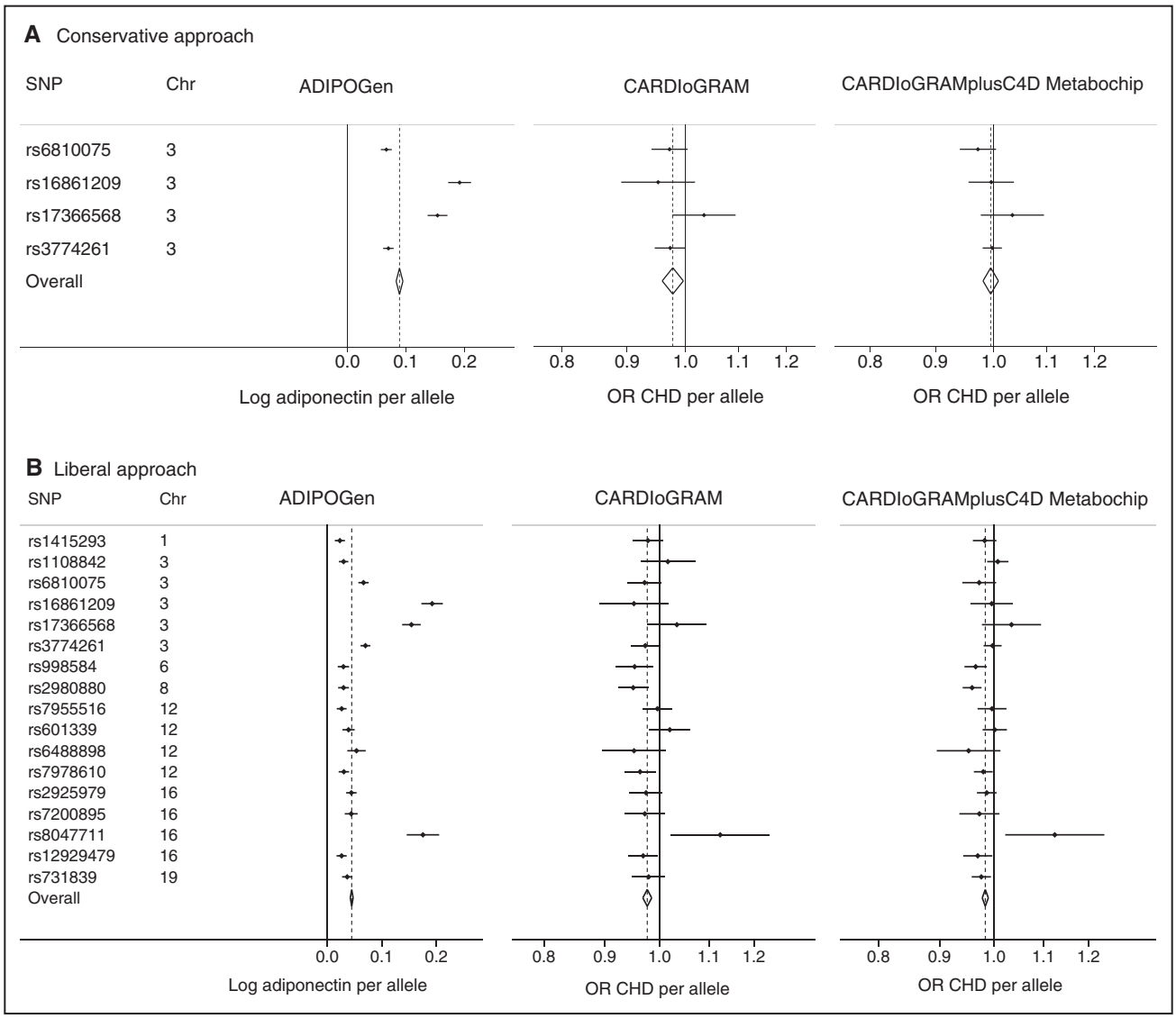

Figure 2. Forest plots of mean difference in log adiponectin levels and odds ratio of coronary heart disease per allele of single-nucleotide polymorphism (SNP) according to the conservative (A) and liberal (B) approaches. A, Conservative approach including 4 SNPs within *ADIPOQ* gene associated with adiponectin at genome-wide significant levels ($P<5\times10^{-8}$; C4). **B**, Liberal approach including 17 SNPs across the genome associated with adiponectin at genome-wide significant levels ($P<5\times10^{-8}$; L17). CHD indicates coronary heart disease; Chr, chromosome; and OR, odds ratio. Results for log adiponectin included 29 347 individuals from ADIPOGen consortium and for CHD risk included 86 995 individuals (22 233 CHD cases) from CARDIoGRAM and 194 427 individuals (63 746 CHD cases) from CARDIoGRAMplusC4D Metabochip consortium.

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.[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.[32,33] However, a larger study did not provide evidence of a causal role of adiponectin in insulin resistance or type 2 diabetes mellitus[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

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

	Hb_{A1c}		Insulin		HDL-c		LDL-c		TAG		WC		BMI	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
rs1415293	0.004	0.513	−0.016	1×10^{-4}	0.015	0.009	−0.012	0.063	−0.014	0.019	−0.006	0.200	0.004	0.283
rs1108842	−0.003	0.586	−0.009	0.027	0.008	0.077	0.010	0.024	−0.009	0.037	−0.020	2×10^{-8}	0.011	0.001
rs6810075*	−0.011	0.123	−0.005	0.214	−0.003	0.813	0.005	0.562	−0.004	0.350	0.003	0.530	−0.001	0.884
rs16861209*	−0.002	0.888	−0.009	0.321	0.000	0.813	0.009	0.245	−0.001	0.610	−0.004	0.570	0.000	0.995
rs17366568*	0.000	0.984	−0.005	0.478	0.009	0.292	0.012	0.343	−0.004	0.587	−0.007	0.400	0.000	1.000
rs3774261*	0.001	0.932	−0.001	0.781	−0.006	0.108	−0.001	0.863	0.001	0.707	0.000	0.910	−0.005	0.100
rs998584	−0.014	0.045	−0.002	0.657	0.026	2×10^{-11}	−0.001	0.936	−0.029	3×10^{-15}	−0.029	6×10^{-15}	0.017	9×10^{-7}
rs2980880	0.014	0.030	0.000	0.967	0.043	1×10^{-26}	−0.040	6×10^{-22}	−0.067	2×10^{-82}	0.001	0.790	0.007	0.026
rs7955516	−0.013	0.051	0.001	0.910	0.019	0.001	−0.003	0.650	−0.007	0.096	0.006	0.210	0.007	0.069
rs601339	0.005	0.528	−0.011	0.036	0.030	3×10^{-6}	0.007	0.284	−0.016	0.013	−0.017	0.003	0.004	0.414
rs6488898	0.027	0.050	−0.004	0.642	0.026	0.007	0.016	0.198	−0.023	0.048	−0.007	0.430	0.021	0.005
rs7978610	0.000	0.959	−0.003	0.432	0.032	2×10^{-9}	−0.020	0.001	−0.029	2×10^{-8}	−0.021	3×10^{-6}	0.013	0.002
rs2925979	−0.001	0.853	−0.005	0.236	0.035	1×10^{-19}	0.003	0.630	−0.021	2×10^{-7}	−0.011	0.003	0.001	0.721
rs7200895	0.000	0.966	0.005	0.347	0.006	0.278	−0.002	0.985	0.005	0.720	−0.001	0.850	−0.002	0.697
rs8047711	−0.020	0.329	−0.005	0.649	0.010	0.887	−0.011	0.482	−0.001	0.678	−0.006	0.700	0.000	0.982
rs12929479	0.002	0.764	−0.006	0.141	−0.008	0.530	−0.010	0.088	−0.004	0.380	−0.011	0.013	−0.016	1×10^{-4}
rs731839	−0.007	0.288	−0.011	0.009	0.022	3×10^{-9}	0.002	0.517	−0.022	3×10^{-9}	0.007	0.059	0.007	0.038

SNPs within *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; Hb_{A1c}, glycohemoglobin; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TAG, triacylglycerols; and WC, waist circumference.

(restricted to SNPs within adiponectin locus) to the well-powered (but vulnerable to horizontal pleiotropy) liberal MR approach (using SNPs across the genome); (3) extensively investigating the presence of bias because of horizontal pleiotropy by using data from other CHD-related phenotypes (eg, glycemic and lipid and anthropometric traits) and methods to account for it (adjusted IVW method and MR-Egger method); (4) testing our hypotheses in 2 data sets (CARDIoGRAM and CARDIoGRAMplusC4D Metabochip); (5) using a very large sample size that provides us with 100% power to detect an odds ratio of 0.80 and 81% to detect and odds ratio of 0.90 with a 0.05% type 1 error rate (Online Table III); (6) checking

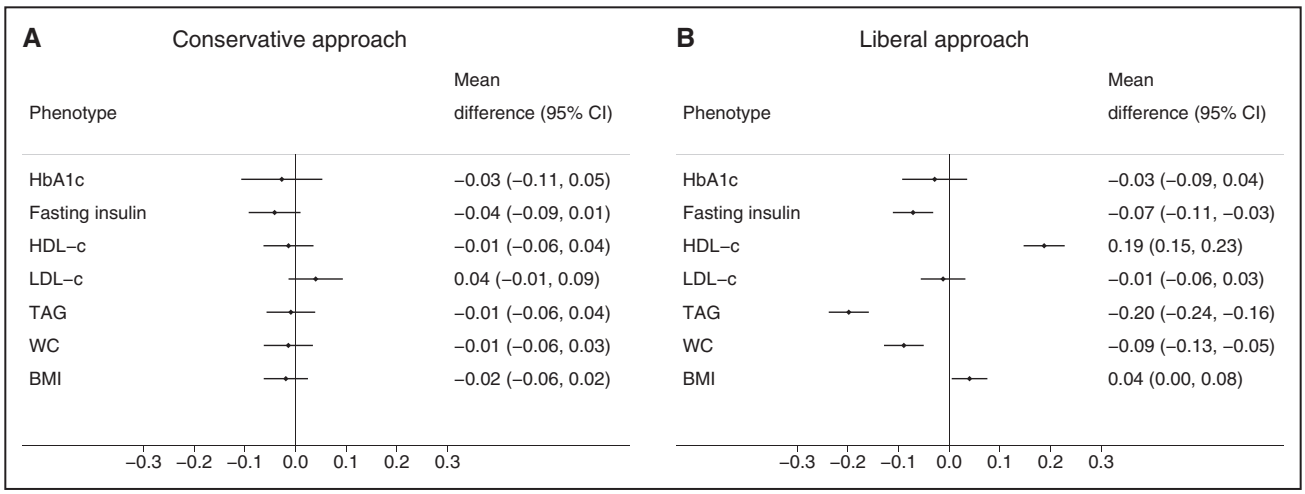

Figure 3. Standardized mean difference (and 95% confidence interval [CI]) in cardiovascular risk biomarkers per 1 U increase in genetically instrumented log adiponectin levels. A, Conservative approach including 4 SNPs within *ADIPOQ* gene associated with adiponectin at genome-wide significant levels ($P<5\times10^{-8}$; C4). **B**, Liberal approach including 17 SNPs across the genome associated with adiponectin at genome-wide significant levels ($P<5\times10^{-8}$; L17). BMI indicates body mass index; Hb_{A1c}, glycohemoglobin; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; SNP, single-nucleotide polymorphism; TAG, triacylglicerols; and WC, waist circumference.

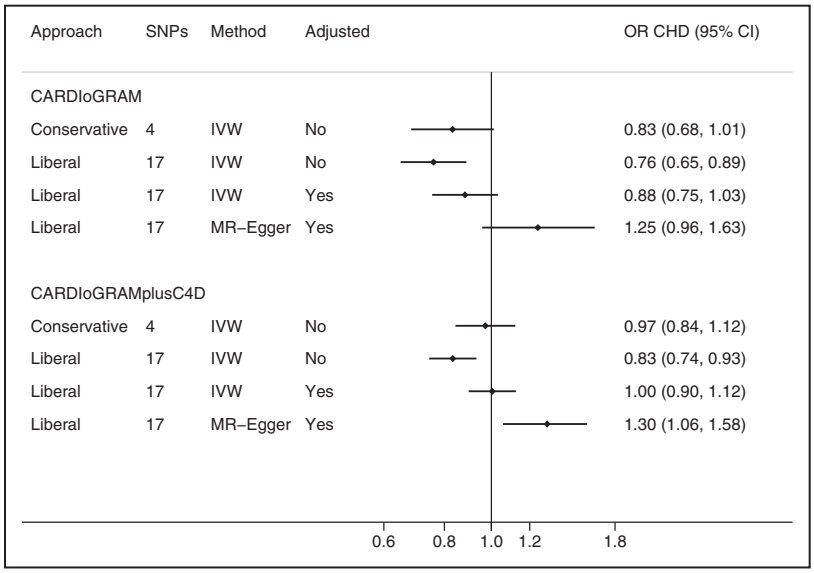

Figure 4. Mendelian randomization estimates of odds ratio (and 95% confidence interval [CI]) of coronary heart disease risk per 1 U increase in genetically instrumented log adiponectin levels. CHD indicates coronary heart disease; IVW, inverse-variance weighted; MR-Egger, Mendelian randomization-Egger method; OR, odds ratio; and SNP, single-nucleotide polymorphism.

the consistency of our findings by performing influence meta-analysis and a positive control analysis; and (7) using 2-sample Mendelian randomization to avoid statistical overfitting in comparison to Mendelian randomization where all analyses are conducted in the same participants[35] (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 participant.[36] Surprisingly, we did find a positive association between 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 conservative approach. We aimed to estimate the causal effect of total adiponectin concentrations, but high-molecular-weight adiponectin 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 because of possible compensatory mechanisms, known as canalization (eg, counter-regulation of adiponectin receptors expression because of variations in blood adiponectin concentration). 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 majority 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 ethnicity 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 $\approx 10^3$- to 10^6-folds higher than the concentration of many hormones and cytokines.[37] Blood adiponectin concentration is a modifiable risk factor that can be efficiently targeted by lifestyle modifications, 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 development and evaluation of large numbers of compounds or lifestyle 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 genetically increased adiponectin levels and lower risk of CHD is mainly driven by horizontal pleiotropy.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- Adiponectin is a protein produced mainly by mature adipose cells.
- Higher circulating adiponectin levels are associated with lower cardiometabolic risk.
- Some genetic variants are associated with both circulating adiponectin and coronary heart disease risk.

What New Information Does This Article Contribute?

- Our findings do not support a causal effect of circulating adiponectin levels on the risk of coronary heart disease (CHD).
- Genetic variants that are associated with both circulating adiponectin levels and CHD have pleiotropic effects and do not reflect a direct role of circulating adiponectin in CHD development.

Higher circulating adiponectin levels are associated with better cardiometabolic profile; however, it is unknown whether this association is causal or merely correlative because of confounding factors. We used genetic variants associated with circulating adiponectin levels to test whether adiponectin is causally involved in CHD development, a technique known as Mendelian randomization. Overall, our findings do not support a causal effect of adiponectin on CHD risk, indicating that primary perturbation of circulating adiponectin is unlikely to be a major cause of CHD. Interventions targeting total circulating adiponectin might not be appropriate therapeutic strategies for primary CHD prevention.