

Effects of Adiponectin on T2DM and Glucose Homeostasis: A Mendelian Randomization Study

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Zefeng Chen ¹⁻⁵
Yulan Bai¹⁻⁵
Xinyang Long¹⁻⁵
Qianqian Luo¹⁻⁵
Zheng Wen¹⁻⁵
Yuanfan Li¹⁻⁵
Shengzhu Huang¹⁻⁵
Yunkun Yan¹⁻⁵
Zengnan Mo^{1-4,6}

¹Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning 530021, Guangxi, People's Republic of China; ²Guangxi Key Laboratory for Genomic and Personalized Medicine, Nanning 530021, Guangxi, People's Republic of China; ³Guangxi Collaborative Innovation Center for Genomic and Personalized Medicine, Nanning 530021, Guangxi, People's Republic of China; ⁴Guangxi Key Laboratory of Colleges and Universities, Nanning 530021, Guangxi, People's Republic of China; ⁵School of Public Health, Guangxi Medical University, Nanning 530021, Guangxi, People's Republic of China; ⁶Institute of Urology and Nephrology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, People's Republic of China

Correspondence: Zengnan Mo
Center for Genomic and Personalized Medicine, Guangxi Medical University, 22 Shuangyong Road, Nanning 530021, Guangxi, People's Republic of China
Tel +86771-5353342
Email mozengnan@gxmu.edu.cn

Purpose: The associations of adiponectin with type 2 diabetes mellitus (T2DM), glucose homeostasis (including β -cell function index (HOMA- β), insulin resistance (HOMA-IR), fasting insulin (FI) and fasting glucose (FG)) have reported in epidemiological studies. However, the previous observational studies are prone to biases, such as reverse causation and residual confounding factors. Herein, a Mendelian Randomization (MR) study was conducted to determine whether causal effects exist among them.

Materials and Methods: Two-sample MR analyses and multiple sensitivity analyses were performed using the summary data from the ADIPOGen consortium, MAGIC Consortium, and a meta-analysis of GWAS with a considerable sample of T2DM (62,892 cases and 596,424 controls of European ancestry). We got eight valid genetic variants to predict the causal effect among adiponectin and T2DM and glucose homeostasis after excluding the probable invalid or pleiotropic variants.

Results: Adiponectin was not associated with T2DM (odds ratio (OR) = 1.004; 95% confidence interval (CI): 0.740, 1.363) when using MR Egger after removing the invalid SNPs, and the results were consistent when using the other four methods. Similar results existed among adiponectin and HOMA- β , HOMA-IR, FI, FG.

Conclusion: Our MR study revealed that adiponectin had no causal effect on T2DM and glucose homeostasis and that the associations among them in observational studies may be due to confounding factors.

Keywords: GWASs, adiponectin, Mendelian randomization, T2DM, glucose homeostasis

Introduction

Type 2 diabetes mellitus (T2DM), affecting no less than 400 million people all over the world,¹ is a classic example of the polygenic disease. The prevalence and morbidity of T2DM are rapidly increasing worldwide.^{2,3} The prevalence of diabetes and prediabetes was estimated to be 11.6% and 50.1%, respectively, in China, in 2013.⁴ The people with diabetes mellitus are evaluated to increase to 42.3 million by 2030.^{5,6} T2DM is also one of the critical risk factors of nonalcoholic fatty liver disease, which is considered the most common chronic liver disease globally.⁷ The risk of T2DM consists of two parts, genetic factors and unhealthy living,⁸ and the heritability estimates are moderate to high (0.47–0.77).⁹ The inheritance of adiponectin is ethnically specific, whose variants at CDH13, ADIPOQ, PDE3A, RFC4, EIF4A2, and so on exhibited significant association ($P < 5 \times 10^{-8}$) in the European ethnicity.^{10,11} In contrast, the variants at CDH13, KNG1-ADIPOQ^{12–14} ($P < 5 \times 10^{-8}$), and WDR11-FGFR2 ($P = 3 \times 10^{-14}$) exhibited significant association in Asians.¹⁵ Besides, there also provided suggestive evidence for a locus on chromosome 12 near OR8S1-LALBA ($P = 1.2 \times 10^{-7}$) in

Asians.¹⁵ Adiponectin, which is an anti-inflammatory adipocytokine secreted by adipocytes, has anti-atherogenic and anti-inflammatory properties and was implicated in comprehensive biological pathways associated with insulin resistance (HOMA-IR).^{16–18} Inflammation has been considered the potential cause of T2DM.^{19,20} The destruction of the β -cell function index (HOMA- β) leads to an absolute lack of insulin, and impaired HOMA- β and HOMA-IR are vital determinants of T2DM.²¹ Cross-sectional epidemiological studies have shown that low serum adiponectin is connected to HOMA- β ,²² HOMA-IR, T2DM, and obesity.^{23,24} In longitudinal studies, hypoadiponectinemia was able to forecast the development of HOMA-IR, T2DM, low-density lipoprotein cholesterol, and triglyceride.^{24–27} Increasing adiponectin is connected with a lower risk of T2DM,^{24,28} and inversely relates to changes in HOMA- β .²² However, whether this correlation is causal or reverse causal or caused by confounding factors remains unknown.

Mendelian randomization (MR) utilizes genetic variants as instrumental variables (IVs) and explore the causal effect between exposure and outcome, but has to meet that the IVs associated with the exposure (IV1, $P < 5E-8$) and the IVs do not affect the outcome through other paths other than the exposure of interest (IV2) and are not associated with confounders (IV3) (Figure 1). Any SNPs that go against these assumptions should be served as invalid IVs and removed.^{29,30} MR can overcome the weakness of classical epidemiological studies and provide available evidence to support or reject causal hypotheses using the existing summary data of genome-wide association studies (GWAS).³⁰ MR is not vulnerable to confounding factors because the genotype of an individual is determined at gamete formation and cannot be altered later on. Therefore, reverse causation is impossible.²⁶ MR analyses have been conducted to explore the causal relationship between adiponectin and HOMA-IR, but the conclusion is controversial.^{23,28,31} Studies have shown that no causal relationship exists among adiponectin and fasting glucose (FG), fasting insulin (FI).³¹ Nonetheless, the result was disappointing because either the sample size was too small or multiracial issues were involved.²³ Here, we will evaluate the effects of adiponectin on T2DM and glucose homeostasis utilizing the summary data of European ancestry.

Materials and Methods

Study Design

We researched the relationship between adiponectin and T2DM and four related glucose homeostasis traits

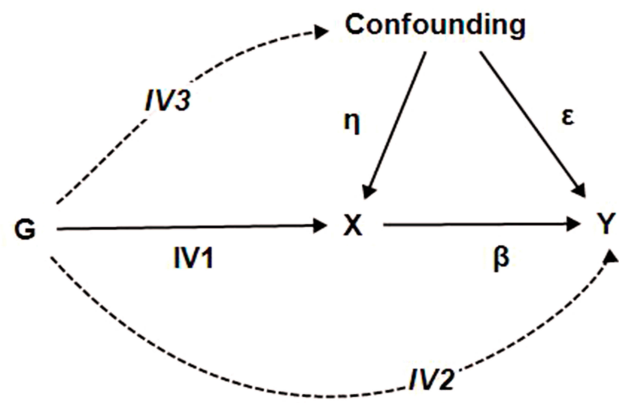


Figure 1 A directed acyclic graph representing the MR framework.

Notes: IV1: The G associates with the X ($P < 5E-8$); IV2: The G should not straightforwardly associate with Y; IV3: The G should not associate with any confounding factors. **Abbreviations:** MR, Mendelian randomization; G, multiple single nucleotide polymorphisms; X, exposure; Y, outcome; η , the correlation coefficient of confounding factors and exposure; ϵ , the correlation coefficient of confounding factors and outcome; β , the causal effect estimate of the exposure and outcome.

(HOMA- β , HOMA-IR, FI, FG) using MR. The hypotheses and study design were shown in Figure 2.

Selection of IVs and Datasets

Adiponectin and Glucose Homeostasis

We identified 162 candidate variants (Table S1, <https://www.mcgill.ca/genepi/adipogen-consortium>) that had genome-wide significant ($P < 5E-8$) associations with adiponectin in the ADIPOGen consortium, which conducted the meta-analysis of GWASs of adiponectin levels (European ancestry, $n = 39883$).^{10,11,16} When the F statistic is 10, it is equivalent to the p -value of 0.001, and 30 corresponds to $5E-8$.³² So our IVs are thus compelling. We removed 25 SNPs for being palindromic and 119 SNPs for being palindromic linkage disequilibrium (LD). The calculation of LD utilized the European population data of 1000 Genomes Project (CEU (Utah Residents from North and West Europe), FIN (Finnish in Finland), GBR (British in England and Scotland), IBS (Iberian population in Spain) and TSI (Toscani in Italia)) at the standard of $R^2 \geq 0.1$ online (https://asia.ensembl.org/Homo_sapiens/Tools/). The calculation of LD was conducted in the same chromosome, and the one that had the minimal P -value was reserved when two or more SNP is LD.

The MR study requires that IVs with known pleiotropy should be eliminated.³³ In the context of adiponectin and T2DM or glucose homeostasis relationship, obesity-related traits is most likely a significant confounder.^{34–38} Then, the pleiotropy of 18 remaining candidate IVs (Table S2)

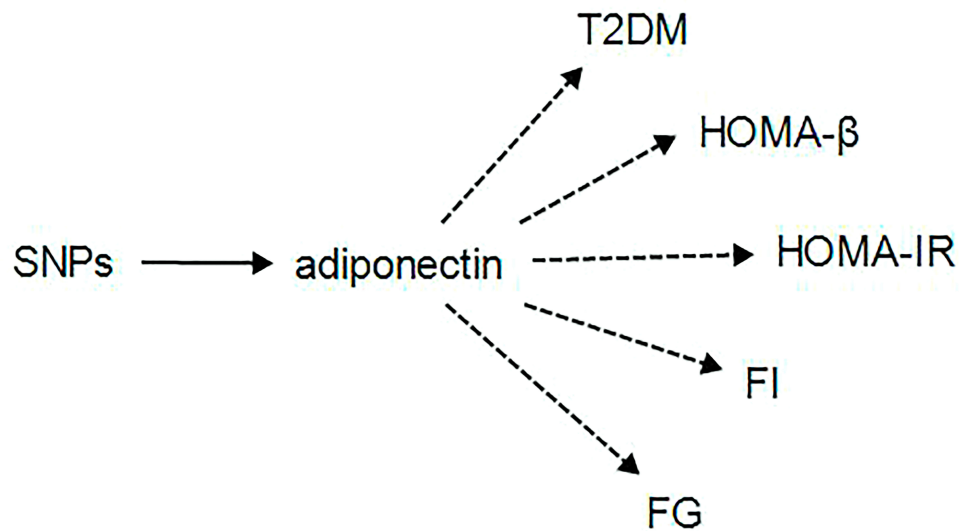


Figure 2 The unidirectional flow chart showing the relationship between adiponectin and outcomes.

Notes: To explore whether adiponectin regulated by the SNPs is the cause of T2DM and glucose homeostasis. The solid arrow represents the causal effect, and the dotted arrow may have a causal relationship.

Abbreviations: SNPs, single-nucleotide polymorphisms; T2DM, type 2 diabetes mellitus; HOMA-B, β -cell function index; HOMA-IR, insulin resistance; FI, fasting insulin; FG, fasting glucose.

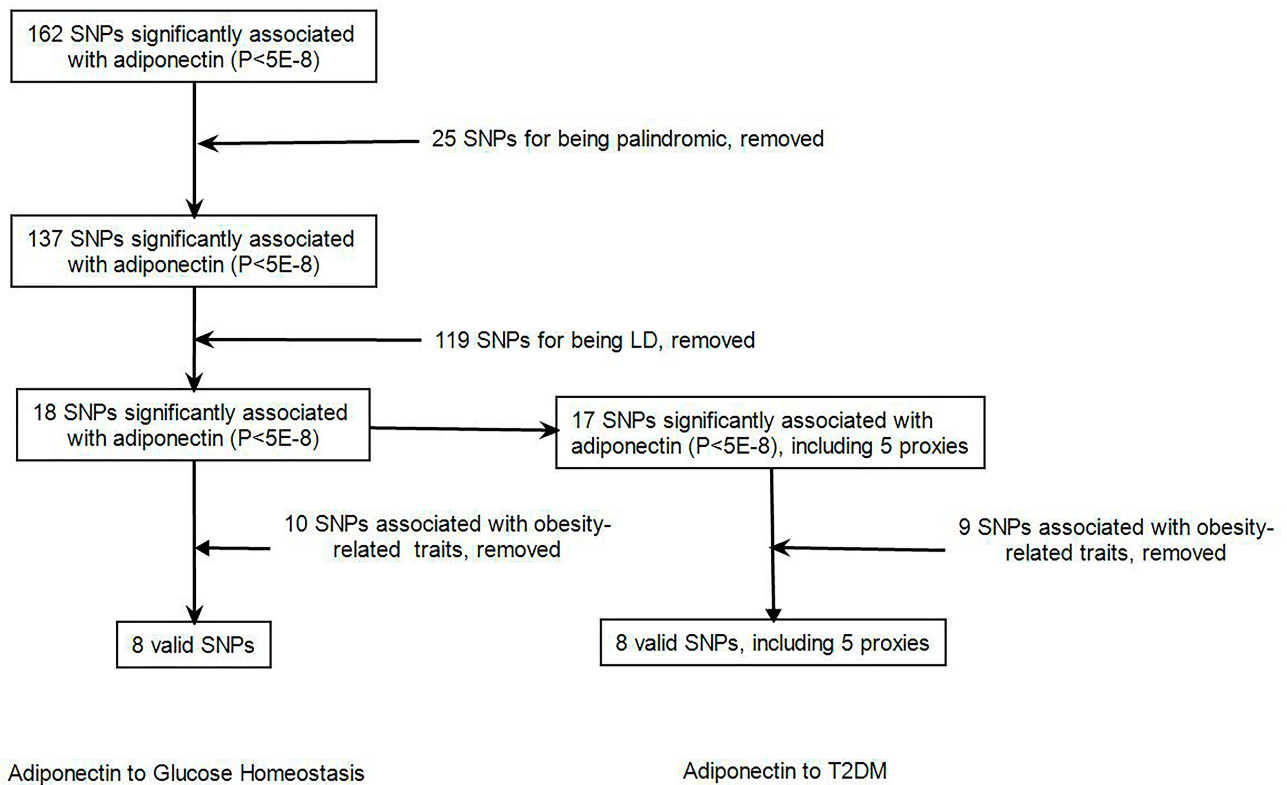


Figure 3 The flow chart of instrumental variables selection.

Notes: The 162 candidate variants that had genome-wide significant ($P < 5E-8$) associations with adiponectin ($P < 5E-8$) were from the ADIPOGen consortium.

Abbreviations: SNPs, single-nucleotide polymorphisms; LD, linkage disequilibrium.

Table 1 The Results of MR Analysis of Adiponectin and T2DM, HOMA-B, HOMA-IR, FI, FG Before and After Removed the Pleiotropic SNPs

Method	N	P-value	SE	Beta	Beta_95%LCI	Beta_95%UCI	OR	OR_95%LCI	OR_95%UCI
Adiponectin to T2DM									
Egger	17 ^a	0.282	0.230	-0.257	-0.708	0.195	0.774	0.493	1.215
IVW	17 ^a	0.058	0.146	0.277	-0.009	0.563	1.319	0.991	1.757
WM	17 ^a	0.150	0.077	0.111	-0.040	0.262	1.117	0.961	1.300
PWM	17 ^a	0.151	0.077	0.110	-0.040	0.261	1.117	0.960	1.298
MBE	17 ^a	0.557	0.062	0.037	-0.085	0.160	1.038	0.919	1.173
Egger	8 ^b	0.978	0.156	0.004	-0.301	0.310	1.004	0.740	1.363
IVW	8 ^b	0.615	0.078	0.039	-0.113	0.191	1.040	0.893	1.211
WM	8 ^b	0.580	0.077	0.042	-0.116	0.200	1.043	0.891	1.222
PWM	8 ^b	0.595	0.080	0.042	-0.108	0.192	1.043	0.898	1.212
MBE	8 ^b	0.415	0.091	0.079	-0.094	0.252	1.082	0.910	1.287
Adiponectin to HOMA-β									
Egger	18 ^a	0.745	0.030	0.010	-0.048	0.068	1.010	0.953	1.071
IVW	18 ^a	0.020	0.020	0.047	0.008	0.087	1.048	1.008	1.091
WM	18 ^a	0.196	0.026	0.033	-0.017	0.084	1.034	0.983	1.087
PWM	18 ^a	0.183	0.025	0.033	-0.016	0.081	1.033	0.985	1.085
MBE	18 ^a	0.206	0.025	0.033	-0.016	0.083	1.034	0.984	1.086
Egger	8 ^b	0.360	0.033	0.032	-0.032	0.096	1.033	0.969	1.101
IVW	8 ^b	0.156	0.021	0.030	-0.011	0.072	1.031	0.989	1.074
WM	8 ^b	0.340	0.027	0.025	-0.027	0.078	1.026	0.973	1.081
PWM	8 ^b	0.301	0.024	0.025	-0.025	0.076	1.026	0.975	1.079
MBE	8 ^b	0.586	0.028	0.016	-0.044	0.076	1.016	0.957	1.079
Adiponectin to HOMA-IR									
Egger	18 ^a	0.803	0.039	0.010	-0.067	0.087	1.010	0.935	1.091
IVW	18 ^a	0.003	0.029	0.086	0.029	0.142	1.089	1.030	1.152
WM	18 ^a	0.096	0.030	0.050	-0.009	0.109	1.051	0.991	1.115
PWM	18 ^a	0.111	0.031	0.050	-0.012	0.112	1.051	0.989	1.118
MBE	18 ^a	0.093	0.028	0.050	-0.005	0.106	1.052	0.995	1.111
Egger	8 ^b	0.360	0.033	0.032	-0.032	0.096	1.033	0.969	1.101
IVW	8 ^b	0.156	0.021	0.030	-0.011	0.072	1.031	0.989	1.074
WM	8 ^b	0.340	0.027	0.025	-0.027	0.078	1.026	0.973	1.081
PWM	8 ^b	0.301	0.024	0.025	-0.026	0.076	1.026	0.975	1.079
MBE	8 ^b	0.586	0.028	0.016	-0.041	0.073	1.016	0.960	1.076
Adiponectin to FI									
Egger	18 ^a	0.721	0.039	0.014	-0.062	0.090	1.014	0.940	1.095
IVW	18 ^a	0.001	0.029	0.093	0.037	0.150	1.098	1.038	1.162
WM	18 ^a	0.037	0.029	0.060	0.000	0.120	1.062	1.001	1.128
PWM	18 ^a	0.039	0.029	0.060	0.002	0.118	1.062	1.002	1.125
MBE	18 ^a	0.062	0.029	0.058	0.000	0.116	1.060	1.001	1.123
Egger	8 ^b	0.170	0.041	0.063	-0.016	0.143	1.065	0.984	1.154
IVW	8 ^b	0.034	0.026	0.055	0.004	0.106	1.057	1.004	1.112
WM	8 ^b	0.067	0.031	0.057	-0.005	0.119	1.059	0.995	1.127
PWM	8 ^b	0.064	0.031	0.057	-0.004	0.118	1.059	0.996	1.126
MBE	8 ^b	0.148	0.035	0.057	-0.015	0.130	1.059	0.985	1.139

(Continued)

Table I (Continued).

Method	N	P-value	SE	Beta	Beta_ 95%LCI	Beta_ 95%UCI	OR	OR_ 95%LCI	OR_ 95%UCI
Adiponectin to FG									
Egger	18 ^a	0.674	0.035	-0.015	-0.084	0.054	0.985	0.919	1.056
IVW	18 ^a	0.249	0.022	0.026	-0.018	0.069	1.026	0.982	1.071
WM	18 ^a	0.813	0.030	0.007	-0.051	0.065	1.007	0.950	1.068
PWM	18 ^a	0.804	0.028	0.007	-0.049	0.063	1.007	0.953	1.065
MBE	18 ^a	0.899	0.029	0.004	-0.054	0.061	1.004	0.948	1.063
Egger	8 ^b	0.820	0.040	0.010	-0.069	0.089	1.010	0.933	1.093
IVW	8 ^b	0.861	0.025	0.004	-0.045	0.054	1.004	0.956	1.056
WM	8 ^b	0.932	0.030	0.003	-0.057	0.062	1.003	0.945	1.064
PWM	8 ^b	0.933	0.031	0.003	-0.057	0.062	1.003	0.945	1.064
MBE	8 ^b	0.976	0.033	0.001	-0.070	0.072	1.001	0.932	1.075

Note: ^aThe instrumental variables without removing the pleiotropic single nucleotide polymorphisms (SNPs); ^bThe instrumental variables removed the pleiotropic SNPs. **Abbreviations:** T2DM, type 2 diabetes mellitus; HOMA-B, β -cell function index; HOMA-IR, insulin resistance; FI, fasting insulin; FG fasting glucose; Egger, MR Egger regression; IVW, Inverse variance weighting; WM, Wweighted median; PWM, Penalized WM; MBE, Mode-based estimate; N, the numbers of instrumental variables; Beta, beta coefficient; SE, standard error; OR, odds ratio; 95% CI, 95% confidence interval; LCI and UCI are lower and upper 95% confidence intervals, respectively.

was analyzed online (PhenoScanner V2: <http://www.phenoscanter.medschl.cam.ac.uk/>; GWAS Catalog: <https://www.ebi.ac.uk/gwas/>). We excluded 10 SNPs associated with obesity-related traits (Table S3) from the 18 SNPs associated with adiponectin. Lastly, eight valid SNPs were acquired (Figure 3). And the summary data of glucose homeostasis were obtained from a meta-analysis of 21 GWASs (46 186 European non-diabetic patients) (<https://www.magicinvestigators.org/downloads/>).²¹

Summary data used in this study were freely accessible online and did not require ethical approval.

Adiponectin and T2DM

Summary data of T2DM cannot match the six SNPs (rs12051272, rs1870843, rs16861209, rs266743, rs7615090, and rs998584) so that they needed proxies. Firstly, the proxy should exist between the summary data of exposure and outcome. Secondly, the proxy and the SNP that need proxy are in LD and has a larger Pearson's correlation.³² The proxies found were rs8047711, rs8046196, rs266719, rs2062632, and rs187868, respectively, except that rs998584 cannot find out proxy (Table S4). The process of valid IVs selection was shown in Figure 3. The summary data of T2DM were from the meta-analysis of GWASs by combining 3 GWAS data sets: Diabetes Genetics Replication and Meta-analysis (DIAGRAM), Genetic Epidemiology Research on Aging (GERA), and the UK Biobank (UKBB) (62892 T2DM cases and 596 424 controls of European ancestry) (<http://cnsgenomics.com/data.html>).³⁹ Following the formula used previously,^{18,40} we estimated the

phenotypic variance explained by a given SNP (R^2). Eight IVs approximatively had R^2 of 3% for T2DM and 4% for glucose homeostasis.³²

Statistical Analysis

Statistical Analyses for MR

There were five MR methods used in two-sample MR analyses: (1) Inverse variance weighting (IVW) is to execute an IVW meta-analysis to acquire the MR estimate, and the weight is the inverse of the variance of SNP-outcome effect.¹⁰ When all genetic variants are valid IV, IVW is an effective method of analysis. However, even only one genetic variant is invalid, it will be biased. IVW estimates will remain biased even if there are an infinite number of IVs because all IVs would contribute to overall IVW estimate;²⁹ (2) MR Egger regression (MR-Egger) relaxes the IV2 assumption of "no horizontal pleiotropy" and returns unbiased causal estimate. The IV2 is violated by all SNPs but meets that the hypothesis of the horizontal pleiotropic effects is uncorrelated with the SNP-exposure effects (ie, INSIDE assumption).^{29,30,41} If the pleiotropy of all IVs is associated with the same confounding, the InSIDE assumption will be violated;²⁹ (3) Weighted median (WM), whose effect estimate would gain by weighting the contribution of each SNP using the inverse variance of its association with outcome.³⁰ WM would acquire an unbiased causal estimate when half of the SNPs are valid IVs (ie, meeting the needs of three assumptions). The type I error rate of WM is lower than that of the IVW method. WM is also complementary to

Table 2 The Results of Cochran's Heterogeneity Test and MR Egger Intercept of Adiponectin and T2DM, HOMA-B, HOMA-IR, FI, FG Before and After Removed the Pleiotropic SNPs

Outcome	N	Cochran's Heterogeneity Test (IVW) – Q	Cochran's Heterogeneity Test (IVW) – Q_pval	Cochran's Heterogeneity Test (Egger) – Q	Cochran's Heterogeneity Test (Egger) – Q_pval	Intercept (Egger)	se	p-val
T2DM	17 ^a	145.798	<0.001	97.082	<0.001	0.028	0.010	0.015
T2DM	8 ^b	13.313	0.065	13.161	0.041	0.002	0.009	0.801
HOMA-β	18 ^a	19.953	0.277	17.028	0.384	0.003	0.002	0.117
HOMA-β	8 ^b	2.603	0.919	2.595	0.858	<0.001	0.003	0.930
HOMA-IR	18 ^a	25.539	0.083	18.310	0.306	0.005	0.002	0.023
HOMA-IR	8 ^b	0.337	>0.999	0.269	>0.999	-0.001	0.003	0.803
FI	18 ^a	27.316	0.054	19.032	0.267	0.005	0.002	0.018
FI	8 ^b	0.556	0.999	0.485	0.998	-0.001	0.003	0.799
FG	18 ^a	10.344	0.889	8.160	0.944	0.003	0.002	0.159
FG	8 ^b	1.734	0.973	1.707	0.945	<-0.001	0.003	0.875

Notes: ^aThe instrumental variables without removing the pleiotropic SNPs; ^bThe instrumental variables removed the pleiotropic SNPs.

Abbreviations: T2DM, type 2 diabetes mellitus; HOMA-B, β-cell function index; HOMA-IR, insulin resistance; FI, fasting insulin; FG fasting glucose; Egger, MR Egger regression; IVW, Inverse variance weighting; N, the numbers of instrumental variables; SE, standard error; SNPs, single nucleotide polymorphisms.

MR-Egger,²⁹ (4) Penalized weighted median (PWM), which is similar to WM. The accuracy of PWM is slightly worse than that of IVW but is marginally better in some cases. The method is consistent and more accurate than the MR-Egger method, with a standard error reduction of approximately 30%-50%;²⁹ (5) Mode-based estimate (MBE) based on the similarity of IVs' causal effects, MBE clusters the SNPs into different groups and returns the causal effect estimation based on the cluster with the largest number of SNPs. If the SNPs within the maximum cluster are valid, the MBE returns an unbiased causal effect.⁴¹ We managed the MR analyses utilizing R (version 3.5.3) with the R package "TwosampleMR". Two-tail $P < 0.05$ was considered statistically significant.

Heterogeneity and Sensitivity Tests

The heterogeneity test acts as an indicator of potential horizontal pleiotropy utilizing the Cochran's Q test,^{30,42} and it was considered statistically significant (indicating no horizontal pleiotropy) with a P -value of > 0.1 . The MR Egger intercept can be worked as the estimate of directional pleiotropy,⁴³ and the P -value < 0.05 indicates SNPs with directional pleiotropy. Furthermore, a "leave-one-out" analysis was used to assess whether the causal estimate was driven by a single SNP that may have great pleiotropy by re-estimating the effect by sequentially dropping one SNP at a time.³⁰ Single SNP analysis was used to value the contribution of the causal effect of each SNP. We also conducted data harmonization to prevent probable bias.³²

Power Calculation

We also evaluated the power for our MR analyses utilizing the statistics of the observational study using an online calculator tool mRnd (<http://cnsgenomics.com/shiny/mRnd/>) (Table S5).⁴⁴

Results

MR Results

Before removed the potential pleiotropic IVs, the four methods (IVW, WM, PWM, and MBE) showed a causal effect of adiponectin on FI (odds ratio (OR) = 1.098, 95% confidence interval (95% CI): 1.038–1.162, $P = 0.001$; OR = 1.062, 95% CI: 1.001–1.128, $P = 0.037$; OR = 1.062, 95% CI: 1.002–1.125, $P = 0.039$; OR = 1.060, 95% CI: 1.001–1.123, $P = 0.062$; respectively) (Table 1). Meanwhile, There showed a causal effect of adiponectin on HOMA-β and HOMA-IR by the IVW method (OR = 1.048, 95% CI: 1.008–1.091, $P = 0.020$; OR = 1.089, 95% CI: 1.030–1.152, $P = 0.003$; respectively) and no causal effect existed among adiponectin and T2DM and FG (Table 1). After remove the ten pleiotropic IVs, there showed no causal effect among adiponectin and T2DM, HOMA-β, HOMA-IR, FG, whereas the results of IVW method showed a causal effect of adiponectin on FI (OR = 1.057; 95% CI: 1.004, 1.112; $P = 0.034$) (Table 1).

Heterogeneity and Sensitivity Tests

Before removed the ten pleiotropic SNPs, Cochran's Q test (IVW method) and MR Egger intercept revealed showed

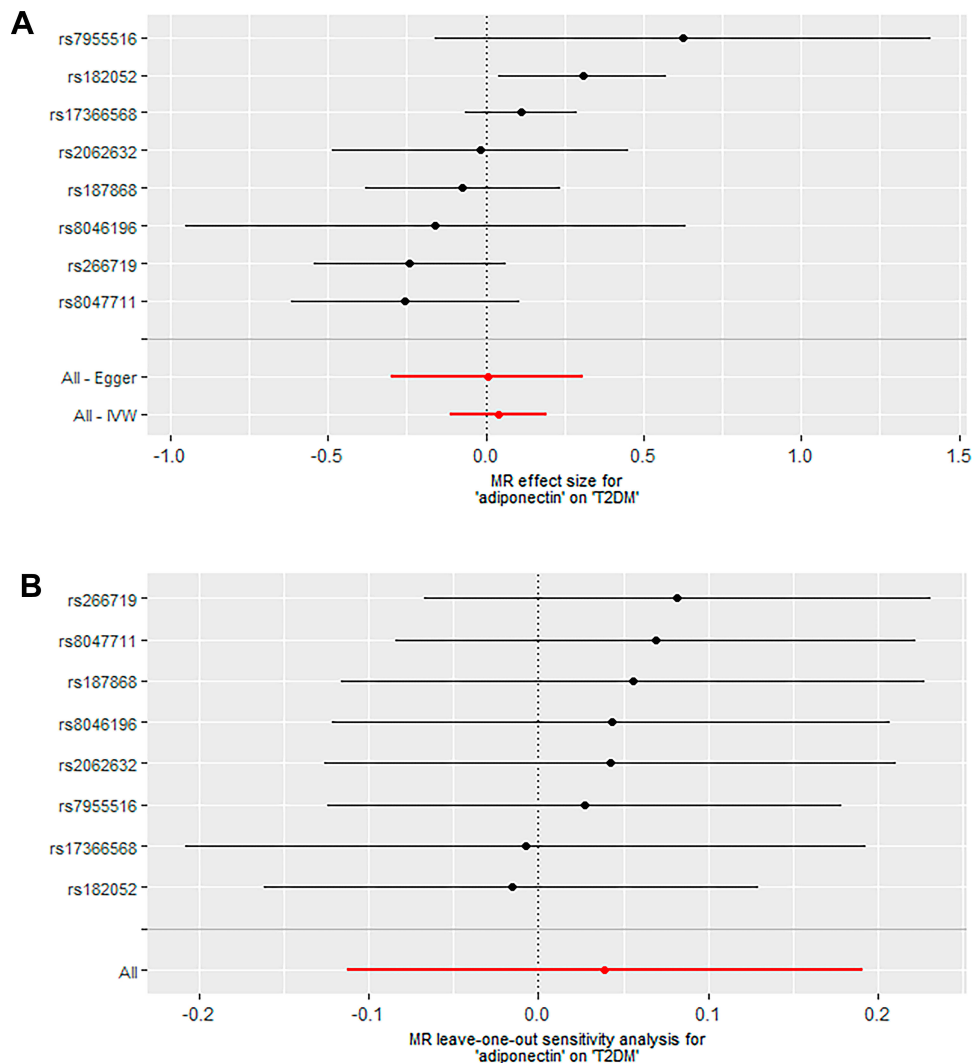


Figure 4 The forest plot of single SNP analysis and leave-one-out analysis depicting the relationship between adiponectin and T2DM with eight valid SNPs.

Notes: (A) The black point showed the causal effect estimate (beta coefficient) of adiponectin and T2DM utilizing a certain SNP, and the black line indicated the 95% CI of the estimate. The red point showed the causal effect estimate of adiponectin and T2DM with the eight valid SNPs using the Egger or IVW method, and the red lines indicated the 95% CI of the estimate. (B) Leave-one-out analysis depicted adiponectin-to-T2DM MR results (IVW method) by sequentially re-evaluating the causal estimate after discarding one IV at a time, which helped determine whether the overall effect was driven by one specific genetic variant. The black point was the causal effect estimate of adiponectin and T2DM after discarding a certain IV, and the black line indicated the 95% CI of estimate. The red point was the causal effect estimate of adiponectin and T2DM with the eight valid SNPs using the IVW methods, and the red line indicated the 95% CI of the estimate.

Abbreviations: SNPs, single-nucleotide polymorphisms; T2DM, type 2 diabetes mellitus; MR, Mendelian randomization; Egger, MR Egger regression; IVW, Inverse variance weighting; IV, instrumental variable; CI, confidence interval.

certain heterogeneity and directional pleiotropy existed among adiponectin and T2DM, HOMA-IR, FI, and no heterogeneity and directional pleiotropy existed among adiponectin and HOMA- β , FG (Table 2). After removed the ten pleiotropic SNPs, Cochran's Q test showed there were no heterogeneity ($P_{MR-Egger} = 0.858$, $P_{IVW} = 0.919$; $P_{MR-Egger} > 0.99$, $P_{IVW} > 0.999$; $P_{MR-Egger} = 0.998$, $P_{IVW} = 0.999$; $P_{MR-Egger} = 0.945$, $P_{IVW} = 0.943$; respectively) and directional pleiotropy (intercept < -0.001 , $P = 0.930$; intercept $= -0.001$, $P = 0.803$; intercept $= -0.001$, $P = 0.799$; intercept $= -0.0005$,

$P = 0.875$, respectively) existed among adiponectin and HOMA- β , HOMA-IR, FI, FG, but showed the heterogeneity existed between adiponectin and T2DM ($P_{MR-Egger} = 0.041$; $P_{IVW} = 0.065$) (Table 2). For T2DM, not all SNP analyses were meaningless, (eg, rs182052, Figure 4A), while "leave-one-out" analysis was symmetrical (Figure 4B); accordingly, excluding any SNP would not induce significant changes, namely, no obvious outliers existed. Single SNP analyses were meaningless, that's, no outliers (Figure 5A-C), and "leave-one-out" analysis was symmetrical for HOMA- β ,

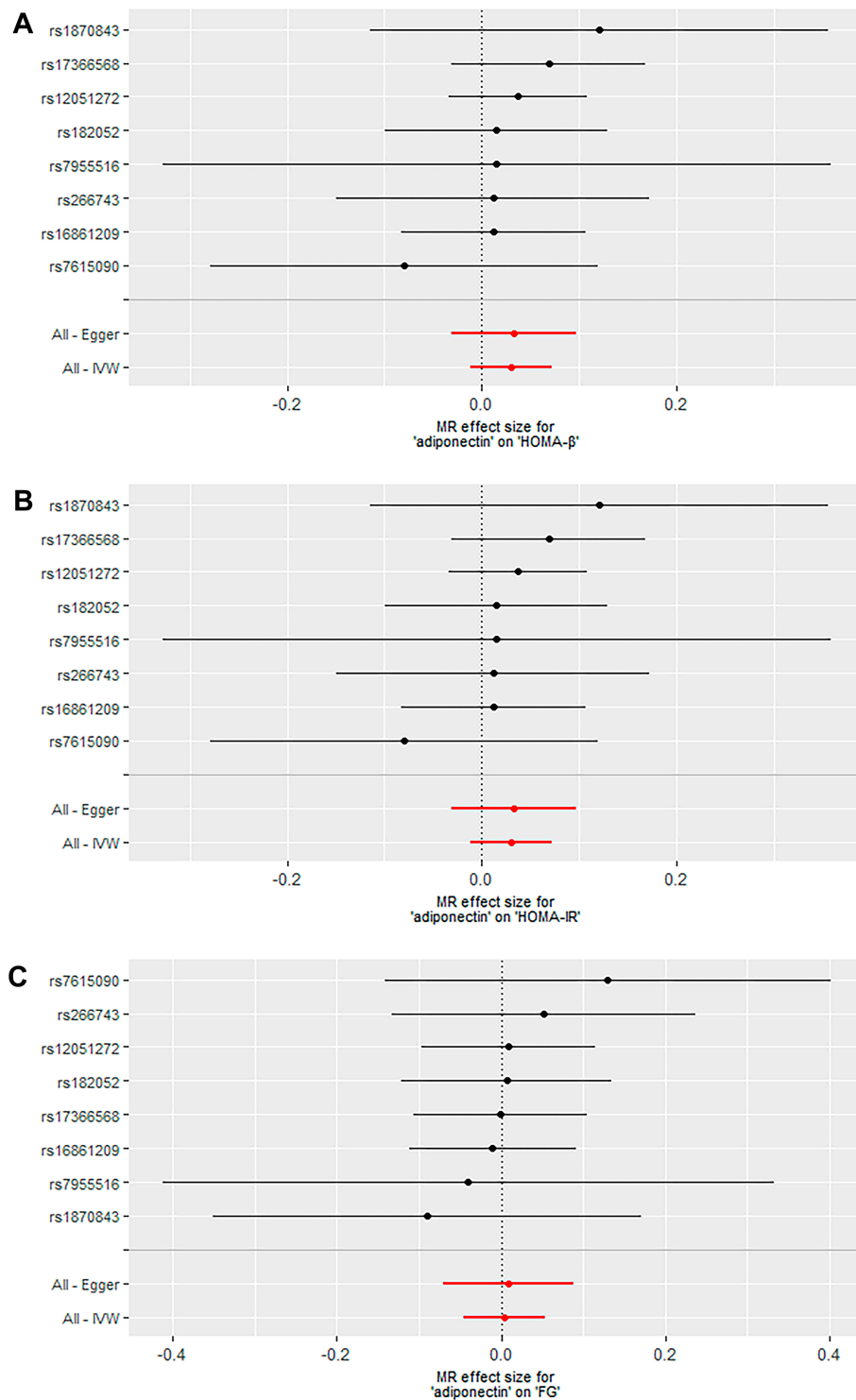


Figure 5 The forest plot of single SNP analysis depicting the relationship among adiponectin and HOMA- β , HOMA-IR, FG with eight valid SNPs.

Notes: (A) The black point showed the causal effect estimate (beta coefficient) of adiponectin and HOMA- β utilizing a certain SNP, and the black line indicated the 95% CI of the estimate. The red point showed the causal effect estimate of adiponectin and HOMA- β with the eight valid SNPs using the Egger or IVW method, and the red lines indicated the 95% CI of the estimate. (B) The black point showed the causal effect estimate (beta coefficient) of adiponectin and HOMA-IR utilizing a certain SNP, and the black line indicated the 95% CI of the estimate. The red point showed the causal effect estimate of adiponectin and HOMA-IR with the eight valid SNPs using the Egger or IVW method, and the red lines indicated the 95% CI of the estimate. (C) The black point showed the causal effect estimate (beta coefficient) of adiponectin and FG utilizing a certain SNP, and the black line indicated the 95% CI of the estimate. The red point showed the causal effect estimate of adiponectin and FG with the eight valid SNPs using the Egger or IVW method and the red lines indicated the 95% CI of the estimate.

Abbreviations: SNPs, single-nucleotide polymorphisms; Egger, MR Egger regression; IVW, Inverse variance weighting; HOMA-B, β -cell function index; HOMA-IR, insulin resistance; FG, fasting glucose; CI, confidence interval.

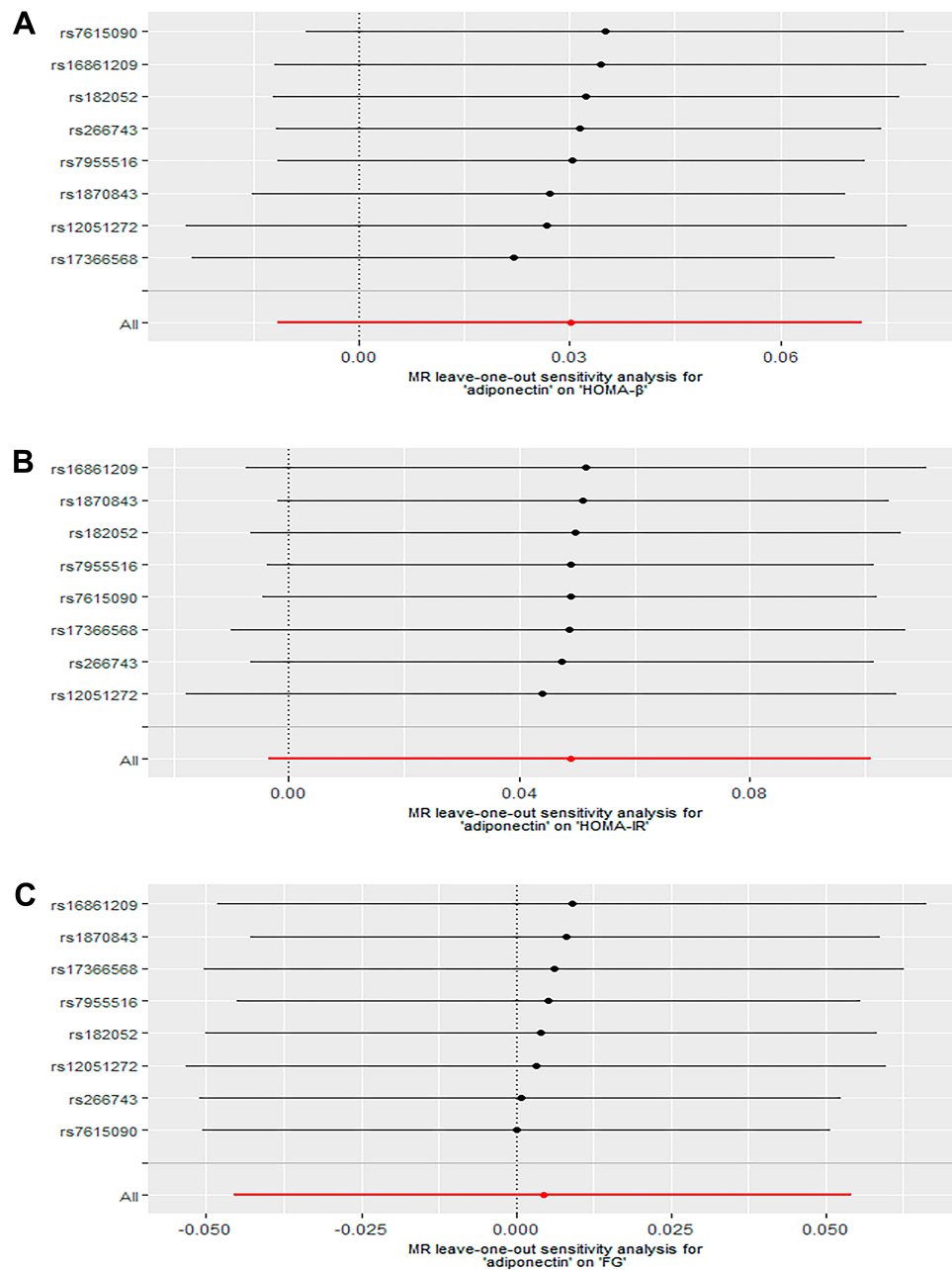


Figure 6 The forest plot of leave-one-out analysis depicting the relationship among adiponectin and HOMA- β , HOMA-IR, FG with eight valid SNPs.

Notes: Leave-one-out analysis depicted adiponectin-to-outcome MR results (IVW method) by sequentially re-evaluating the causal estimate after discarding one IV at a time, which helped determine whether the overall effect was driven by one specific genetic variant. **(A)** The black point was the causal effect estimate of adiponectin and HOMA- β after discarding a certain IV, and the black line indicated the 95% CI of the estimate. The red point was the causal effect estimate of adiponectin and HOMA- β with the eight valid SNPs using the IVW methods, and the red line indicated the 95% CI of the estimate. **(B)** The black point was the causal effect estimate of adiponectin and HOMA-IR after discarding a certain IV, and the black line indicated the 95% CI of estimate. The red point was the causal effect estimate of adiponectin and HOMA-IR with the eight valid SNPs using IVW methods, and the red line indicated the 95% CI of the estimate. **(C)** The black point was the causal effect estimate of adiponectin and FG after discarding a certain IV, and the black line indicated the 95% CI of estimate. The red point was the causal effect estimate of adiponectin and FG with the eight valid SNPs using IVW methods, and the red line indicated the 95% CI of the estimate.

Abbreviations: SNPs, single-nucleotide polymorphisms; Egger, MR Egger regression; IVW, Inverse variance weighting; HOMA-B, β -cell function index; HOMA-IR, insulin resistance; FG, fasting glucose; CI, confidence interval.

HOMA-IR, and FG (Figure 6A–C, respectively), which were consistent with their sensitivity evaluation. For FI, four outliers were determined (rs17366568, rs1870843, rs7955516,

7615090; Figure 7A), and the “leave-one-out” analysis showed the IVW causal association estimate was driven by a single SNP (Figure 7B).

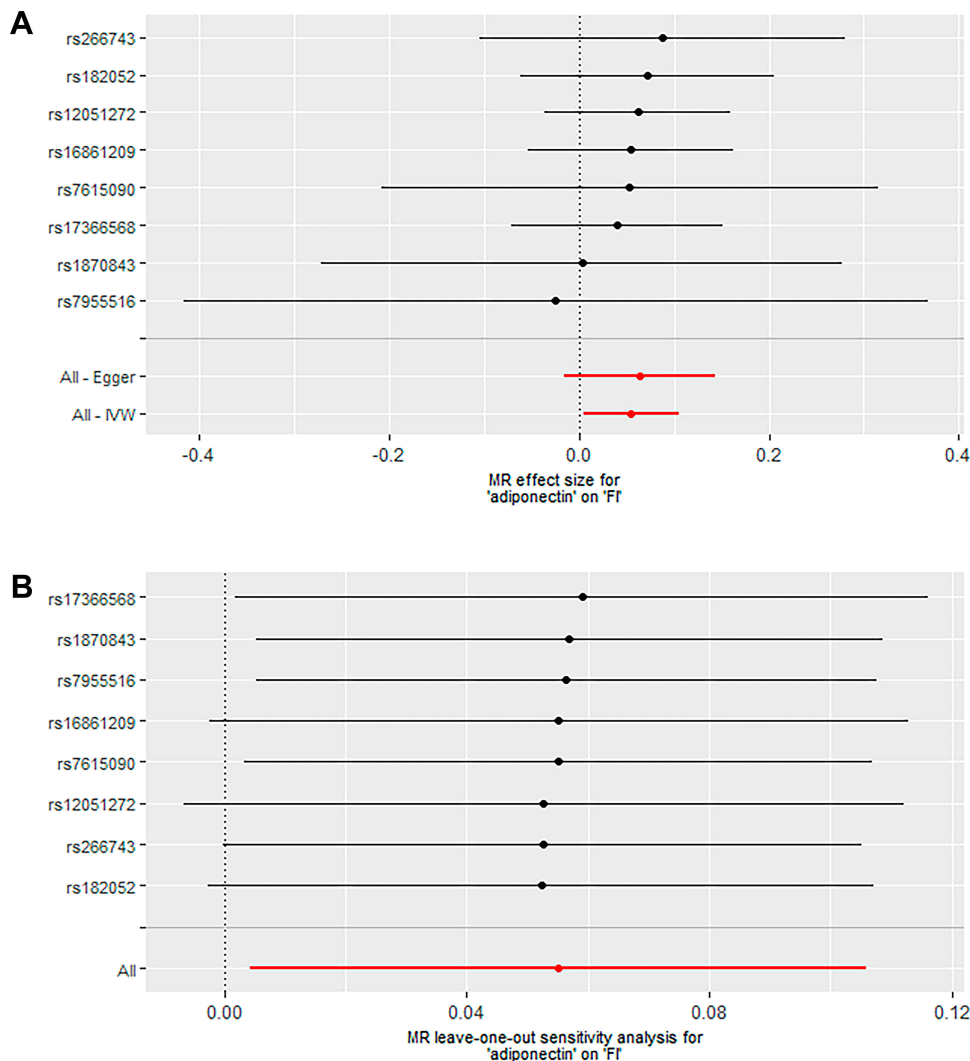


Figure 7 The forest plot of single SNP analysis and leave-one-out analysis depicting the relationship between adiponectin and FI with eight valid SNPs.

Notes: (A) The black point showed the causal effect estimate (beta coefficient) of adiponectin and FI utilizing a certain SNP, and the black line indicated the 95% CI of the estimate. The red point showed the causal effect estimate of adiponectin and FI with the eight valid SNPs using the Egger or IVW method, and the red lines indicated the 95% CI of the estimate. (B) Leave-one-out analysis depicted adiponectin-to-FI MR results (IVW method) by sequentially re-evaluating the causal estimate after discarding one IV at a time, which helped determine whether the overall effect was driven by one specific genetic variant. The black point was the causal effect estimate of adiponectin and FI after discarding a certain IV, and the black line indicated the 95% CI of the estimate. The red point was the causal effect estimate of adiponectin and FI with the eight valid SNPs using the IVW methods, and the red line indicated the 95% CI of the estimate.

Abbreviations: SNPs, single-nucleotide polymorphisms; Egger, MR Egger regression; IVW, Inverse variance weighting; FI, fasting insulin; CI, confidence interval.

Discussion

The relationship between adiponectin and HOMA-IR is controversial. People with extreme HOMA-IR have excessively high plasma adiponectin levels. Indeed, the inhibitory effect of hyperinsulinemia on plasma adiponectin occurred in females.^{45–48} Insulin infusion reduced plasma adiponectin in healthy volunteers⁴⁹ but elevated in patients with type 1 diabetes mellitus.⁵⁰ However, the hyperinsulinemia caused by HOMA-IR resulted in low plasma adiponectin levels rather than reverse using European summary data.⁵¹ Hence, an MR study regarding HOMA-

IR as the exposure and adiponectin as an outcome should be performed next.

In the present research, the association among adiponectin and HOMA- β , HOMA-IR, FI was causal before the elimination of pleiotropic SNPs; meanwhile, the Cochran's Q test and MR Egger regression revealed showed absolute heterogeneity and directional pleiotropy existed them. The results of five MR methods showed no causal effect, heterogeneity, and directional pleiotropy existed among adiponectin and HOMA- β , HOMA-IR, FI after the elimination of pleiotropic SNPs, suggesting that causal effect

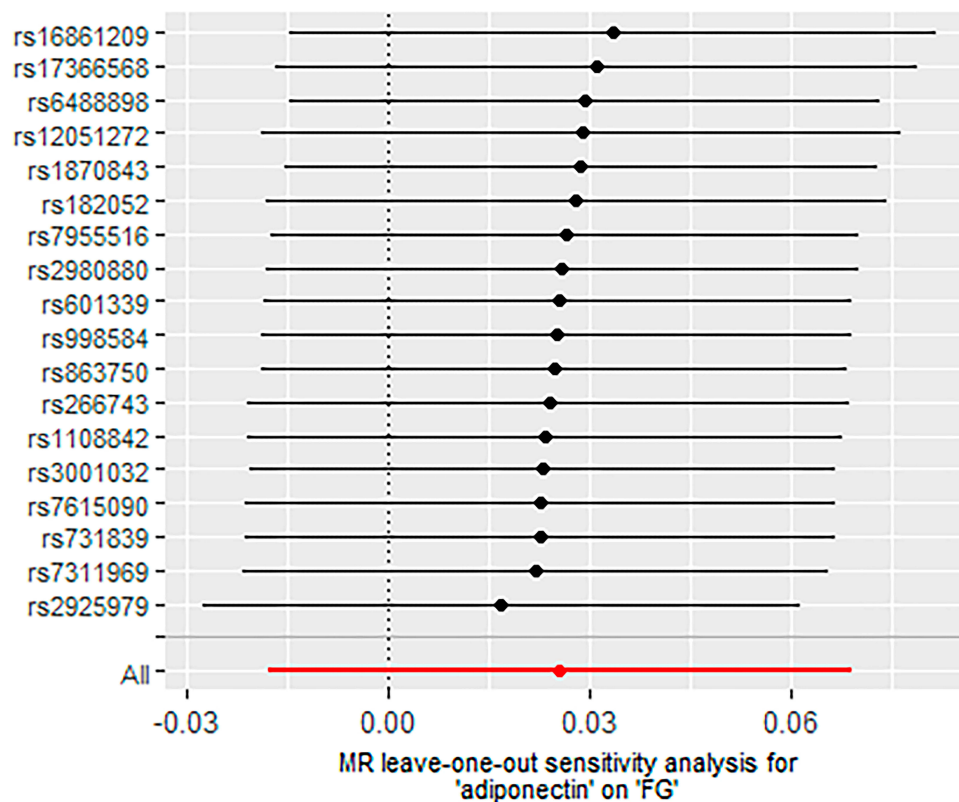


Figure 8 The forest plot of leave-one-out analysis depicting the relationship between adiponectin and FG without removing the pleiotropic IVs.

Notes: Leave-one-out analysis depicted adiponectin-to-FG MR results (IVW method) by sequentially re-evaluating the causal estimate after discarding one IV at a time, which helped determine whether the overall effect was driven by one specific genetic variant. The black point was the causal effect estimate of adiponectin and FG after discarding one IV, and the black line indicated the 95% CI of the estimate. The red point was the causal effect estimate of adiponectin and FG with the 18 valid SNPs using the IVW methods, and the red lines indicated the 95% CI of the estimate.

Abbreviations: SNPs, single-nucleotide polymorphisms; Egger, MR Egger regression; IVW, Inverse variance weighting; FG, fasting glucose; CI, confidence interval.

before was likely mediated by pleiotropy. No causal effect, heterogeneity, and directional pleiotropy existed between adiponectin and FG before and after removing the pleiotropic SNPs, and leave-one-out analysis was symmetrical (Figure 8), which indicated obesity maybe is not the confounding factor. No causal effect existed between adiponectin and T2DM before and after removing the pleiotropic SNPs, but the heterogeneity still existed after removed the ten pleiotropic SNPs, which is more likely at the price of excessive proxies.

Our research did not provide evidence that decreasing circulating adiponectin levels, which were regulated by genetic factors, increased the risk of T2DM or glucose homeostasis. No causal effects were determined among adiponectin and T2DM, HOMA- β , HOMA-IR, FI with the power of 100%, 100%, 100%, 84%, respectively (Table S5), which was consistent with the recent study.⁵² Any pharmaceutical and lifestyle interventions designed to alter adiponectin levels would not improve glucose homeostasis or prevent T2DM.⁵¹ The inconsistency of the estimates from different

methods indicated that the genome-wide significant variants were not all valid IVs, and the causal conclusion based on these variants would be unreliable.⁵³ Nevertheless, our results were consistent, and our IVs were valid. The findings suggested that circulating adiponectin concentration was more likely to be an epiphenomenon in the context of T2DM and glucose homeostasis than a key determinant, which was also consistent with the previous study.⁵⁴

Our study exhibits several strengths. Firstly, we had larger samples than similar studies before. Secondly, we considered ethnicity into account, ie, the summary data of adiponectin, T2DM, and glucose homeostasis all came from the same ethnicity (European). The calculation of LD was also conducted on a special website using the European descent of 1000 Genomes Project. Furthermore, to the best of our knowledge, no existing MR analyses have investigated the causal relationship between adiponectin and HOMA- β , FG, FI. Our research is the first to investigate the causal relationship between them by the MR approach and a variety of sensitivity analyses. Lastly, the numbers of IVs

were comprehensive, including liberal and conservative SNPs. Conservative SNPs were less likely to go against the MR assumption that the instrument should not affect the outcome other than the exposure of interest.

However, our research has its shortcomings. First, adiponectin exists as high-, medium-, and low-molecular-weight isoforms in this research, namely total circulating adiponectin, which may have distinct functions. Second, we cannot account for complex feedback loops and rule out the possibility that the causal-free estimates among adiponectin and T2DM, glucose homeostasis were caused by age or sex. Although we did our best to manage the pleiotropic or confounding factors, the residual pleiotropic or confounding factors may remain.²⁷ Moreover, only eight SNPs of the 162 SNPs significantly associated with adiponectin were used as IVs, although limiting the numbers of IVs used in the MR study contributes to relieving the biases from weak instruments. Further, the MR analysis of adiponectin and T2DM was conducted with excessive proxies, which may produce unreliable results.⁵⁵ Lastly, most IVs used in the MR research do not significantly associate with adiponectin in Asians, which is probable a lack of the related research, so performing further study on adiponectin in Asians is needed. And the subjects referred to in our study are European ancestry only, which helps minimize the likelihood of ethnicity bias, so performing MR analyses in other populations is expected in the future.

Conclusion

In all, our MR study revealed that adiponectin had no causal effect on T2DM and glucose homeostasis and the associations among them in observational studies may be due to confounding factors.

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Disclosure

The authors report no conflicts of interest in this work.

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