Relationship Between Genetic Prediction of Diabetes and Coronary Atherosclerosis: A Two-Sample Bidirectional Mendelian Randomization Study

Xiaoying Guo[1,2], Biaoliang Wu[1*]

[1] Department of Endocrinology, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise 533000, Guangxi, China

[2] Graduate School of Youjiang Medical University for Nationalities, Baise 533000, Guangxi, China

Abstract: Background: To explore the relationship between diabetes and coronary atherosclerosis at the genetic level by using two-sample Mendelian randomization (MR). Methods: Diabetes and coronary atherosclerosis are the exposure factors and outcomes respectively. The study employed the inverse-variance weighted method (IVW), MR-Egger regression, weighted median method (WM), simple mode, and weighted mode for bidirectional Mendelian randomization analysis, using the odds ratio (OR) to assess the causal relationship between diabetes and coronary atherosclerosis. Results: MR heterogeneity demonstrated heterogeneity in single nucleotide polymorphisms (SNPs) for each nucleotide (all P values < 0.001). Therefore, the IVW random effects model was selected. The results showed that at the gene level, there was a correlation between an increased risk of diabetes and coronary atherosclerosis (OR=1.103). Additionally, there was a correlation between an increased risk of coronary atherosclerosis and diabetes (OR=1.108). No horizontal pleiotropy of instrumental variables was found (P=0.419, 0.808). The MR analysis results remained robust after sequentially excluding SNPs. Conclusion: Diabetes and coronary atherosclerosis are mutually predisposing risk factors for each other at the gene level in the European population.

Keywords: diabetes, coronary atherosclerosis, mendelian randomization, causal inference

1. Introduction

Diabetes is one of the most common chronic diseases in the world. It not only damages the physical and mental health of patients but also exerts a heavy burden on the social economy. The diabetic population is vast, and the prevalence of the disease has been steadily increasing. From 1990 to 2017, the global incidence of diabetes increased by 102.9%[1]. With the improvement of human living standards and changes in lifestyle and dietary structure, the incidence of coronary heart disease is increasing year by year. Studies have shown that people with diabetes have a high risk of coronary heart disease[2], and the pathophysiological basis of coronary heart disease is coronary atherosclerosis. To understand the association between diabetes and coronary artery atherosclerosis, the method of Mendelian randomization (MR) is introduced.

A known genetic variation refers to the difference in DNA sequence between individuals in a population. In genetics, a trait is usually not affected by other traits, environmental exposure, or social behavior, and can be relatively stably inherited[3]. MR is a study that uses genetic variation to estimate the causal relationship between exposure and outcome. The basic idea exists in using genetic variation with a strong correlation with exposure factors as an instrumental variable to infer a causal effect between exposure factors and study outcomes. Compared to other research methods, MR has the following advantages: it can greatly eliminate reverse causality because genetic variations are present in the genes from birth and are not influenced or changed by acquired diseases[4]. Moreover, the interference of confounding factors can be effectively avoided[5]. In the present study, MR is used to study the causal relationship between diabetes and coronary atherosclerosis, to provide genetic evidence for the correlation between the two.

2. Research methods and materials

2.1 Research Design

Single nucleotide polymorphisms (SNPs) strongly associated with exposure were selected as instrumental variables (IVs), and diabetes was considered as the exposure factor and coronary atherosclerosis as the outcome variable in forward MR analysis. Reverse MR analysis took coronary atherosclerosis as the exposure factor and diabetes as the outcome variable. Causal association analysis was performed using the two-sample MR analysis method, and a pleiotropic analysis

test was performed using MR-PRESSO. After outlier SNPs were removed, the Wald ratio was used to calculate the effect value generated by a single SNP. The inverse-variance weighted (IVW), MR-Egger regression weighted, median method (WM), simple mode, and weighted mode were adopted to analyze the causal relationship between diabetes and coronary atherosclerosis, and then the heterogeneity test was performed to assess the heterogeneity of individual causal relationships. Sensitivity analysis was performed to verify the reliability of the causal association results. Furthermore, the results were visualized by drawing scatter plots, forest plots, funnel plots, and leave-one-out plots for sensitivity analysis. Three conditional hypotheses for MR were as follows: ① Correlation hypothesis: There was a strong correlation between SNP and exposure factors; ② Independence hypothesis: SNP was independent of confounding factors; ③ Exclusive hypothesis: SNP could only affect the outcome through exposure factors. See Figure 1.
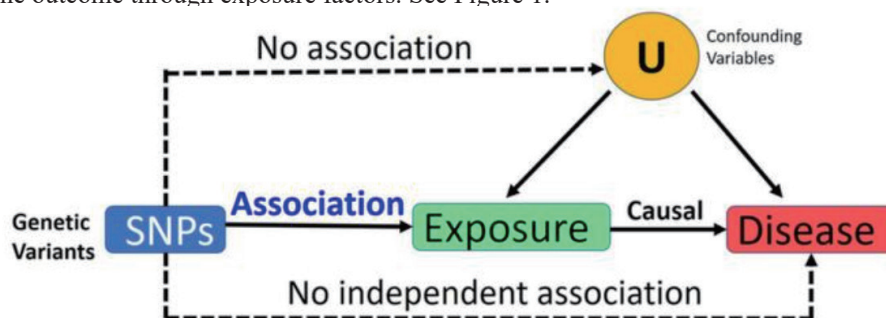

Figure 1. Two-sample MR model

2.2 Data Sources

Extracted from the website https://gwas.mrcieu.ac.uk/datasets, the study includes genome-wide association studies (GWAS) on diabetes and coronary atherosclerosis. The diabetes data was sourced from the UK Biobank, while the coronary atherosclerosis data was from the Neal Laboratory. Both datasets pertain to European populations, as detailed in Table 1.

Table 1. GWAS summary statistics of Two samples of Mendelian randomization

Variable	ID	Sample Size (cases)	Number of SNPs (pieces)	Population	Gender	Age
Diabetes	ukb-b-10753	461 578	9 851 867	Europe	Male and Female	2018
Coronary Atherosclerosis	ukb-d-I9 CORATHER	361 194	13 586 589	Europe	Male and Female	2018

2.3 Instrumental Variables

First, SNPs significantly associated with diabetes ($P<5\times10^{-8}$, $r^2<0.001$, genetic distance=10000kb) were chosen at the whole-genome level. Second, the selected SNPs were queried on the PhenoScanner v2 website to remove SNPs correlated with confounding factors. Additionally, the MR-PRESSO package in TwoSampleMR was utilized to eliminate outlier SNPs, ensuring the independence hypothesis, also known as the exclusion of SNP pleiotropy [6].

2.4 Bidirectional MR Analysis

Five regression models, namely IVW, MR-Egger, WM, simple mode, and weighted mode, were employed to analyze the causal relationship between diabetes and coronary atherosclerosis. The IVW method is the primary approach for MR analysis. IVW does not require individual-level data and can directly estimate the causal effect using summary data. The hypothesis underlying this method is that all genetic variants used as instruments are valid, with a zero overall bias [7, 8]. If P < 0.05 is indicated, there is significant heterogeneity. If heterogeneity exists, use the IVW random-effects model. If heterogeneity is not present, use the fixed-effects model. A pleiotropic analysis is conducted using the MR_pleiotropy_test function, where a P value < 0.05 indicates the presence of pleiotropy for each SNP, while P > 0.05 suggests no pleiotropy. In this study, leave-one-out plot for sensitivity analysis, scatter plot, forest plot, and funnel plot were generated to visualize the above-mentioned results using the TwoSampleMR package in R 4.3.1 software. The significance level was set at α=0.05.

3. Results

3.1 Instrumental Variables

Forward MR analysis on the association between diabetes and coronary atherosclerosis was conducted, with 71

diabetes-related SNPs as instrumental variables, and coronary atherosclerosis as the study outcome; Reverse MR analysis was performed, with 31 coronary atherosclerosis-related SNPs as instrumental variables, and diabetes as the study outcome. By individually querying relevant SNPs on the PhenoScanner v2 website, we excluded SNPs associated with confounding factors that could influence the outcome. We further used the MR-PRESSO package to remove outlier SNPs. In the end, we included 45 SNPs in the forward MR analysis and 24 SNPs in the reverse MR analysis. For detailed information, please refer to Tables 2 and 3.

Table 2. Basic information of SNPs associated with diabetes

| SNPs	EA	OA	EAF	SE	β	P
rs79687284	C	G	0.034822	0.001214	0.008655	1.00E-12
rs2793829	T	C	0.107403	0.000718	0.004475	4.60E-10
rs13414381	C	T	0.127892	0.000667	-0.00537	8.50E-16
rs2303700	C	T	0.67929	0.000482	-0.00294	1.10E-09
rs17753004	A	G	0.235314	0.000524	-0.00311	3.10E-09
rs1810534	T	C	0.479541	0.000447	0.002729	1.00E-09

Note: SNPs: single nucleotide polymorphism, EA: effect allele; OA (other alleles): non-effect allele; EAF: effect allele frequency, β: allele effect value; SE: standard error of β

Table 3. Basic information of SNPs associated with coronary atherosclerosis

SNPs	EA	OA	EAF	SE	β	P
rs11591147	T	G	0.017673	0.00171	-0.01037	1.34E-09
rs2133189	T	C	0.714278	0.0005	0.003154	2.80E-10
rs72664318 ¼¼	G	A	0.092038	0.000781	-0.0047	1.81E-09
rs77265569	T	G	0.113197	0.000721	-0.0047	7.38E-11
rs1065853	T	G	0.080299	0.00083	-0.00566	9.60E-12
rs28451064	A	G	0.13177	0.000681	0.00476	2.76E-12

Note: SNPs: single nucleotide polymorphism, EA: effect allele; OA (other alleles): non-effect allele; EAF: effect allele frequency, β: allele effect value; SE: standard error of β

3.2 Forward MR analysis results of the association between diabetes and coronary atherosclerosis

The IVW and MR-Egger tests for heterogeneity showed P<0.05, indicating heterogeneity among the SNPs. Therefore, we employed the IVW random effect model. The results suggested a significant causal relationship between diabetes and coronary atherosclerosis, with an odds ratio (OR) of 1.103,[95% confidence interval (CI): 1.040~1.170; P = 0.001]. The MW method also showed a similar causal relationship:[OR, 1.062; 95% CI: 1.002~1.126, P=0.041]. However, MR-Egger regression, simple mode, and weighted mode did not reveal such a causal relationship. The OR and 95%CI of each SNP for the effect on coronary atherosclerosis calculated by the Wald ratio were shown in the forest plot (Figure 2). According to the scatter plot (Figure 3), as the severity of diabetes increased, the risk of coronary atherosclerosis also rose. This implied that diabetes was a risk factor for coronary atherosclerosis. To validate the stability of this causal relationship, the MR-Egger test was performed on the included SNPs, showing a P-value > 0.05, indicating the absence of horizontal pleiotropy. Additionally, the funnel plot was approximately symmetrical, suggesting no bias in the results (Figure 4). The included SNPs were subjected to the leave-one-out plot for sensitivity analysis (Figure 5). Specifically, the effect value of remaining SNPs on the outcome after arbitrary SNPs was removed, and no SNPs that significantly affected the result after removal were found, revealing that the result of MR analysis was reliable.

3.3 Reverse MR analysis results of an association between coronary atherosclerosis and diabetes

The heterogeneity test indicated P<0.05, suggesting heterogeneity among various SNPs. Therefore, we employed the IVW random-effect model. The results revealed a significant causal relationship between coronary atherosclerosis and diabetes:[OR: 1.108, 95% CI: 1.022-1.201, P=0.013]. The MW method and weighted mode yielded similar results:[OR: 1.092. 95% CI: 1.012~1.177, P = 0.023,[OR: 1.115, 95% CI: 1.032~1.206, P = 0.012]. The effect values of specific SNP were visualized by forest plot (Figure 2). The scatter plot (Figure 3) showed that coronary atherosclerosis was a risk factor for diabetes. P>0.05 in MR-Egger pleiotropy test indicated that there was no horizontal pleiotropy for each SNP, and the funnel plot was basically symmetrical left and right, indicating that the causal association was less likely to be affected by potential bias (Figure 4). The included SNPs was subjected to leave-one-out plot for sensitivity analysis (Figure 5). SNPs that significantly affected the results after removal were not found, reflecting that the results of the MR analysis were reliable.
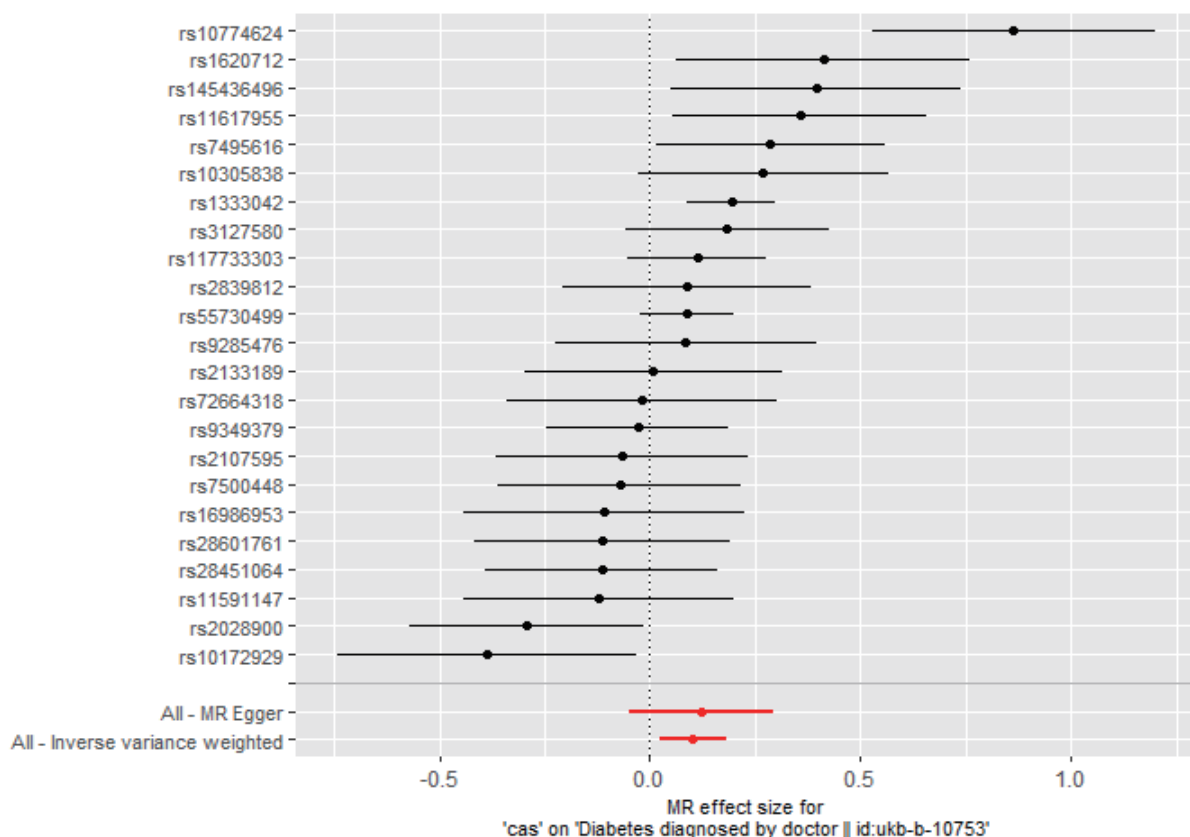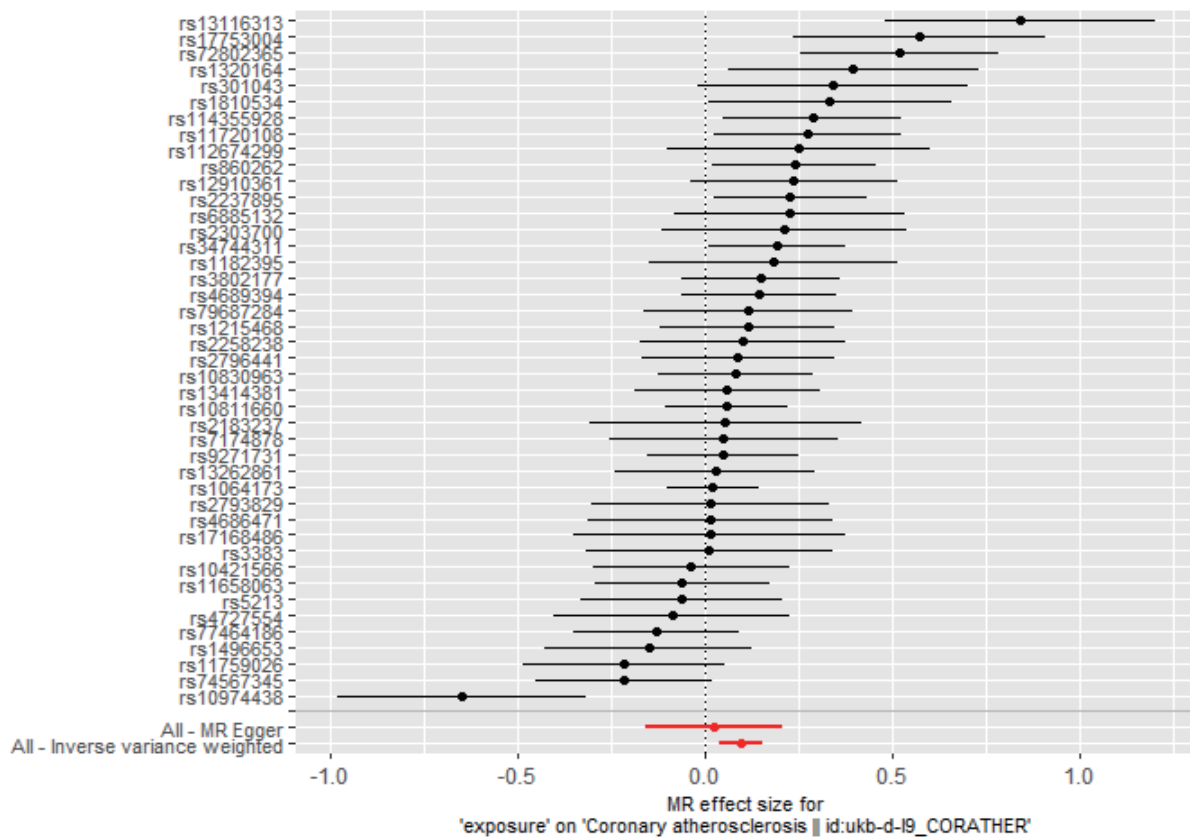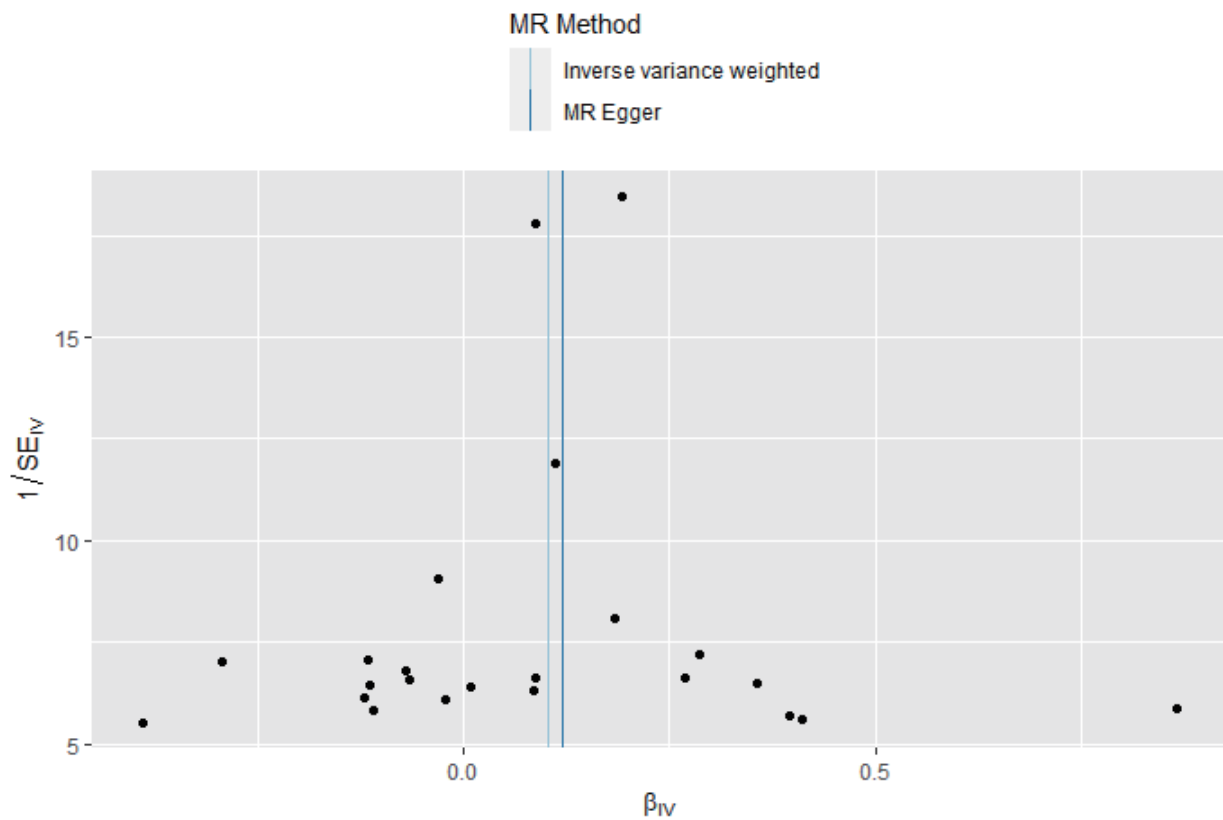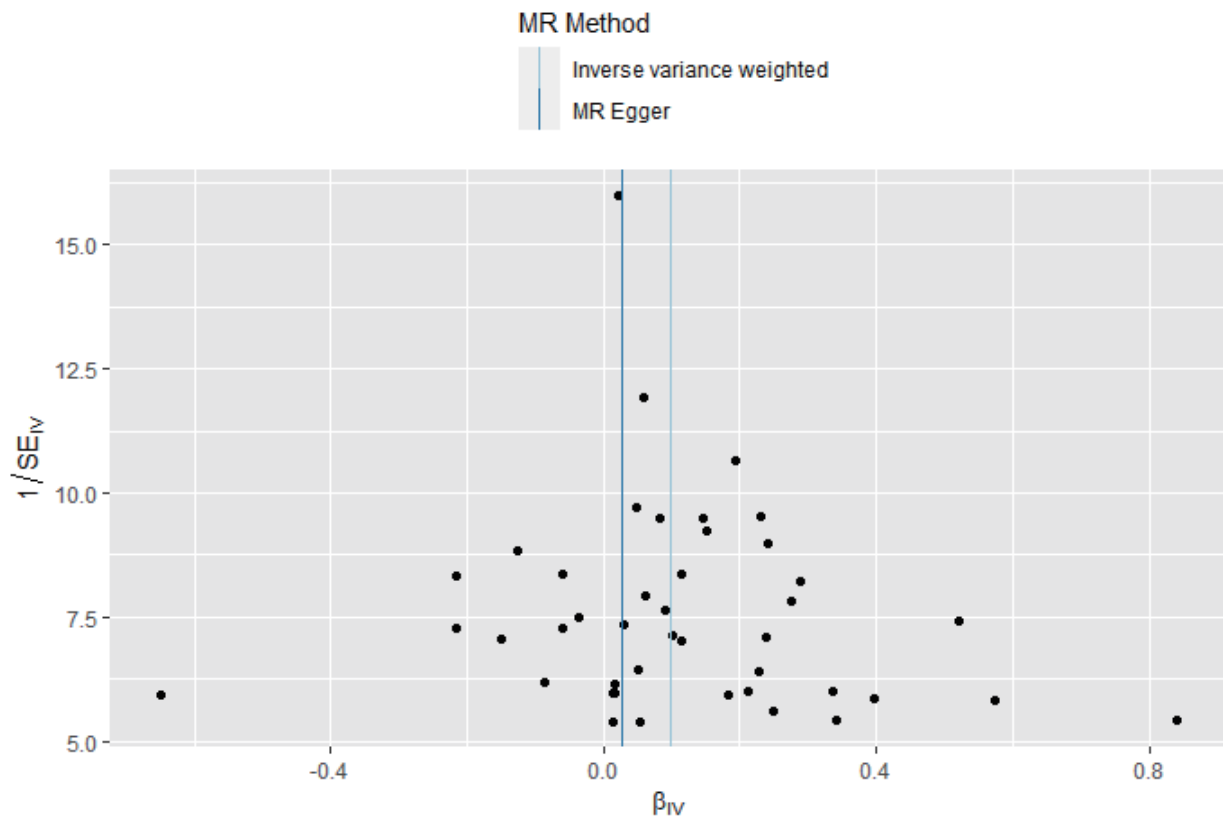
Note: The left side represents the forward analysis result of MR, and the right side stands for the reverse analysis result of MR

Figure 2. Forest plot of the causal association between diabetes and coronary atherosclerosis
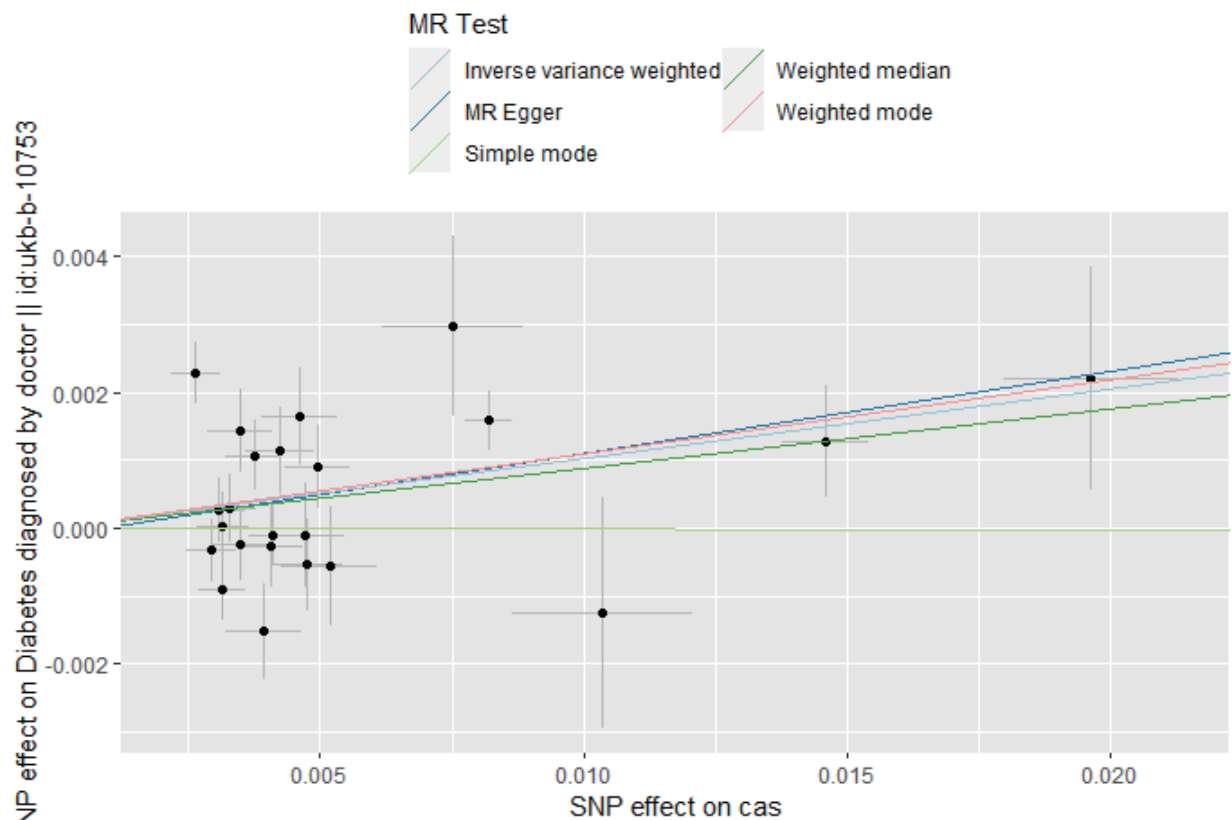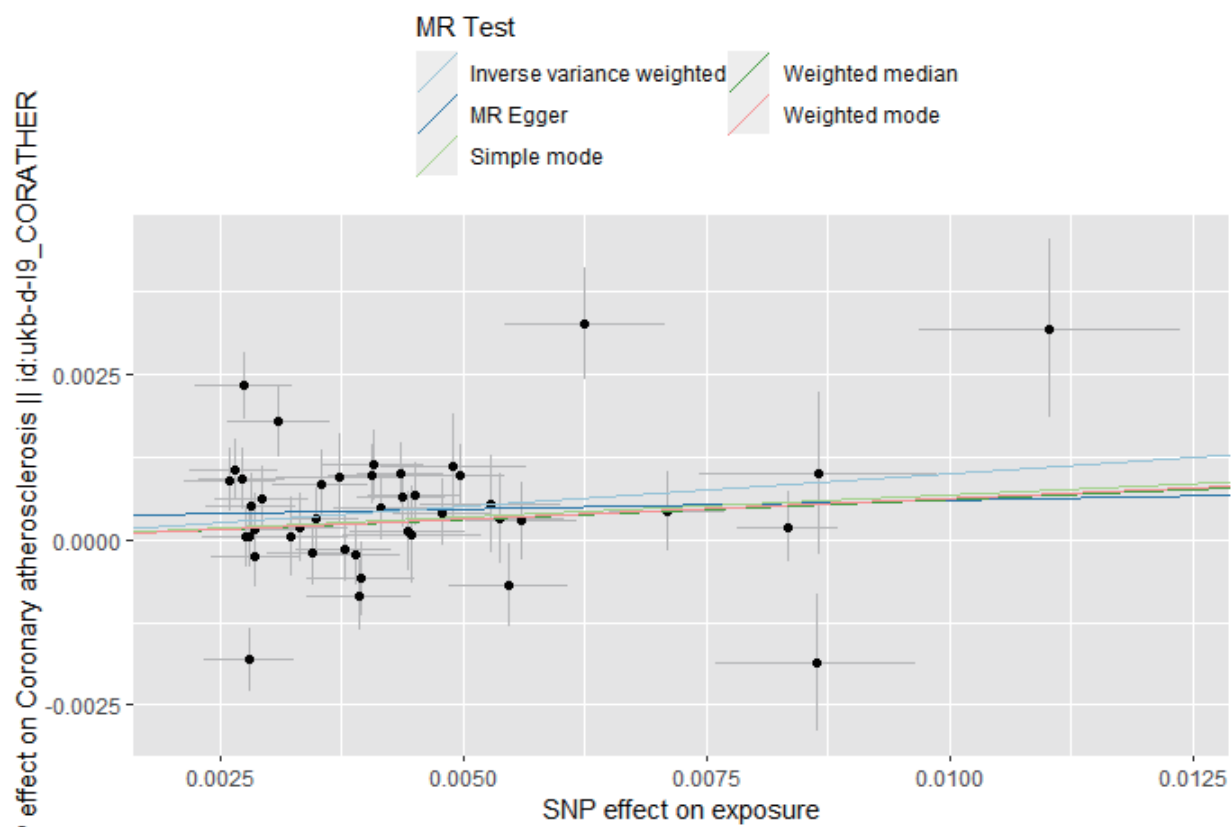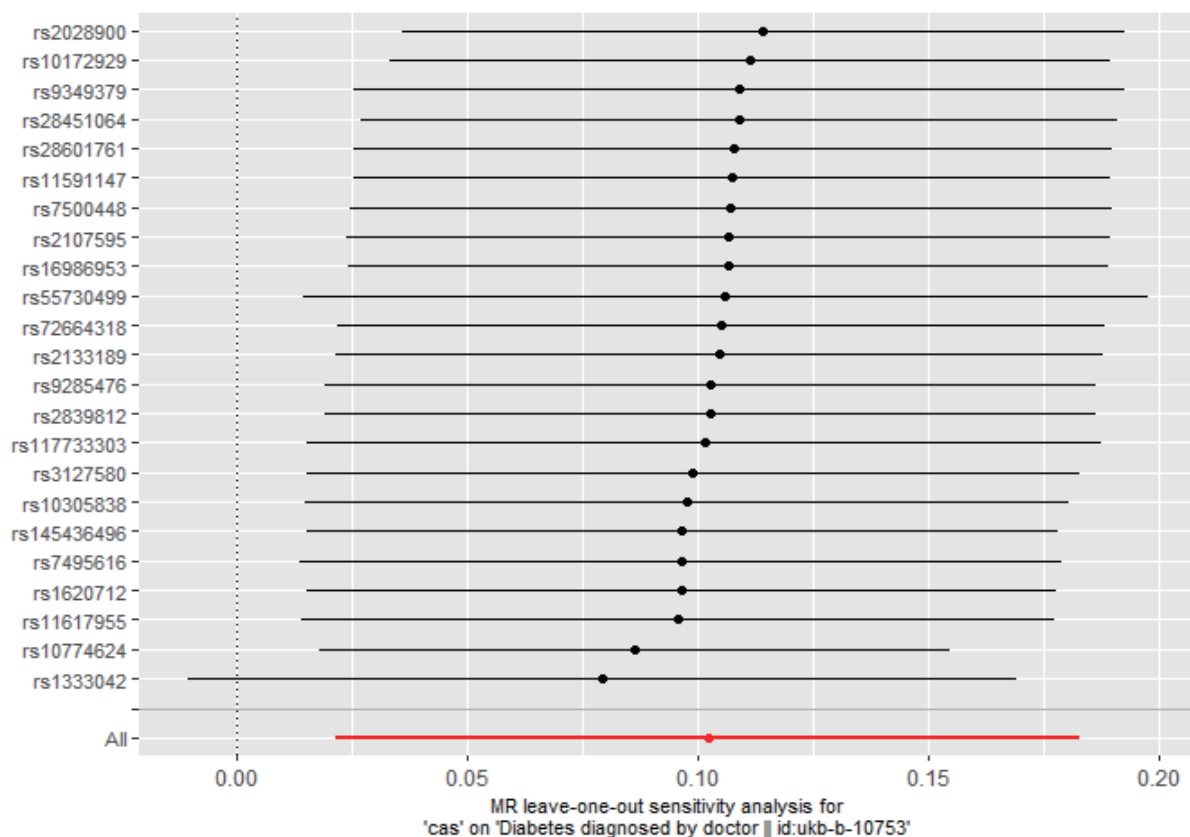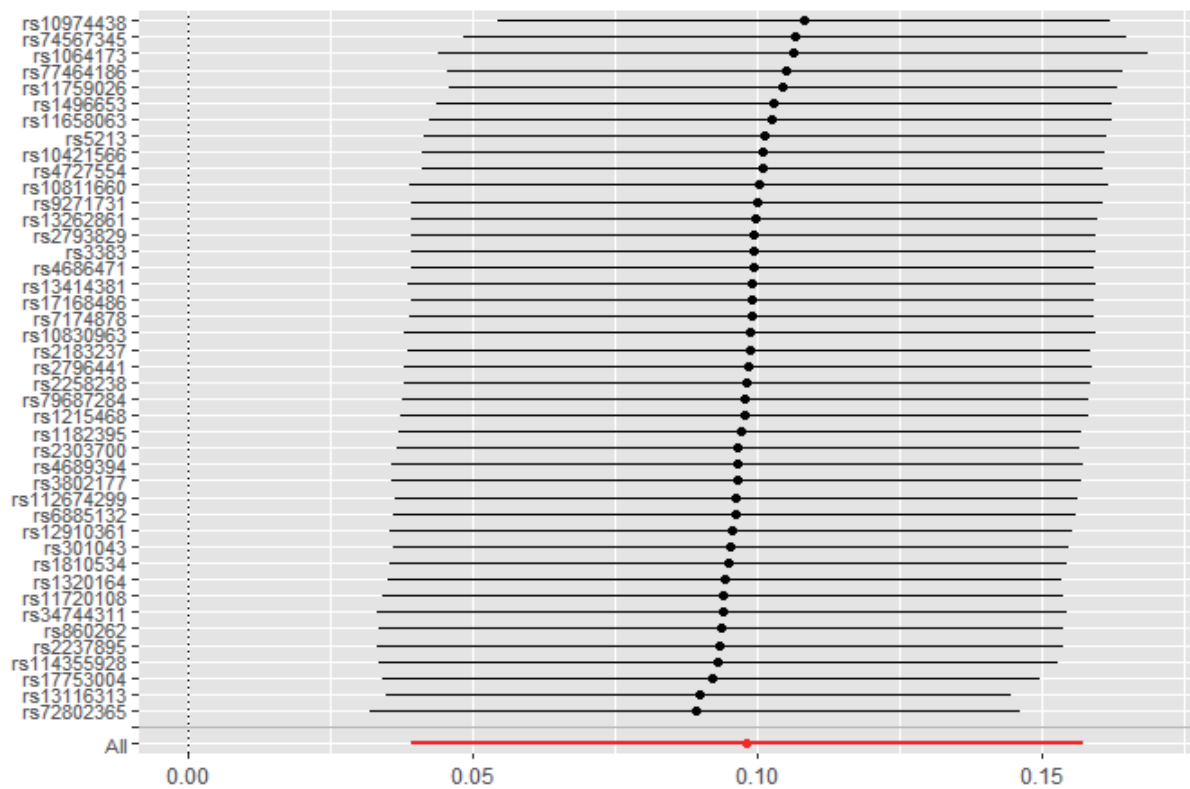
Note: The left side represents the forward analysis result of MR, and the right side stands for the reverse analysis result of MR

Figure 3. Scatter plot

Note: The left side represents the forward analysis result of MR, and the right side stands for the reverse analysis result of MR

Figure 4. Funnel plot

Note: The left side represents the forward analysis result of MR, and the right side stands for the reverse analysis result of MR

Figure 5. Forest plot of "leave-one-out" sensitivity analysis

4. Discussion

This study employed Mendelian randomization with two independent samples, utilizing GWAS data to explore the causal relationship between diabetes and coronary artery atherosclerosis. The findings indicate that diabetes serves as a risk factor for coronary artery atherosclerosis, and conversely, coronary artery atherosclerosis may also contribute to the onset of diabetes.

It has been gradually recognized that diabetes is a risk factor for coronary atherosclerosis, and the specific mechanism is still being explored. Multiple studies have compared the coronary artery conditions of diabetic and non-diabetic patients through coronary angiography and found that diabetes is positively correlated with subclinical coronary atherosclerosis[9-11]. That is to say, compared with non-diabetic patients, diabetic patients have a higher risk of developing coronary artery disease (CAD) and are more likely to develop coronary heart disease. This is consistent with the results of our present study. In terms of the pathophysiological mechanisms through which diabetes exacerbates coronary atherosclerosis, Yahagi K et al. proposed that factors driving coronary atherosclerosis in diabetes include oxidative stress, endothelial dysfunction, alterations in mineral metabolism, increased production of inflammatory cytokines, and the release of osteoprogenitor cells from the bone marrow into the circulation[12]. Additionally, research has revealed that high blood glucose induces NLRP3 inflammasome activation in endothelial cells, thereby contributing to atherosclerosis[13].

Our study has provided genetic support for diabetes as a risk factor for coronary atherosclerosis and found that coronary atherosclerosis was related to the occurrence of diabetes. According to Colaiori et al.'s study[14], among 570 patients with coronary atherosclerosis, the risk of progressing to T2DM was found to be 8 times higher for those with moderate to severe coronary stenosis compared to those with mild stenosis. This suggests that the severity of coronary atherosclerosis serves as a strong predictor for the development of T2DM. However, the mechanisms by which diabetes leads to atherosclerosis remain unclear. Fu et al [15] obtained transcriptomic datasets of T2DM and atherosclerosis from the GEO database. The analysis revealed a potential shared pathological mechanism between the two conditions. Specifically, the CD4 and PLEK genes were identified as key players in both T2DM and atherosclerosis, presenting novel diagnostic and therapeutic targets for AS and T2DM. This process involves chronic inflammation and immune response.

This study has certain limitations. Firstly, the study population only consisted of Europeans, so the findings cannot be extrapolated to populations of other ethnicities or regions to determine if a similar causal relationship exists. Secondly, MR analysis only offers genetic evidence, and further validation is needed to elucidate the specific underlying mechanisms and causal relationships.

Acknowledgments

Author Information: Xiaoying Guo (1993-), female, Hebei, graduate student, main research direction: Basic and Clinical Diabetes.

*Corresponding Author: Biaoliang Wu (1978-), male, Guangxi, doctoral supervisor, professor, main research direction: Basic and Clinical Diabetes.

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