European Review for Medical and Pharmacological Sciences 2023; 27: 5280-5292

No causal effects between rosiglitazone and cardiovascular disease or risk factors: a Mendelian randomization study

X.-M. LI[2], Z.-J. WU[2], Z.-L. XU[2], A. LI[2], M.-Q. LIU[2], C.-G. SONG[2], K. WU[1,2]

[1]Cardiovascular Center, The First Dongguan Affiliated Hospital of Guangdong Medical University, Dongguan, China
[2]Affiliated Hospital of Guangdong Medical University, Zhanjiang, China

Xiao-Min Li and Zi-Jun Wu contributed equally to this work

Abstract. – OBJECTIVE: Although many observational studies have shown an association between rosiglitazone and cardiovascular disease (CVD) or risk factors, controversy remains. We conducted a Mendelian randomized (MR) study to explore whether rosiglitazone is causally related to CVDs and risk factors.

PATIENTS AND METHODS: Single-nucleotide polymorphisms associated with rosiglitazone at genome-wide significance were identified from a genome-wide association study of 337,159 European-ancestry individuals. Four treatments with rosiglitazone-associated single-nucleotide polymorphisms associated with a higher risk of CVDs were used as an instrumental variable (IV). Summary-level data for 7 CVDs and 7 risk factors were obtained from UK Biobank and consortia.

RESULTS: We found no causal effects of rosiglitazone, either on CVDs or risk factors. The results were consistent in sensitivity analyses using Cochran's Q test, MR-PRESSO method, leave-one-out analysis and Mendelian randomization-Egger method (MR-Egger), and no directional pleiotropy was observed. Sensitivity analyses confirmed that rosiglitazone was not significantly associated with CVDs and risk factors.

CONCLUSIONS: The findings from this MR study indicate no causal relationship between rosiglitazone and CVDs or risk factors. Hence, previous observational studies may have been biased.

Key Words:
Mendelian randomization, Genome-wide association study, Rosiglitazone, Cardiovascular disease, Risk factors.

Introduction

Cardiovascular disease (CVD) is a common disease that seriously threatens human health and is a leading cause of morbidity and mortality globally[1]. In 2019, an estimated 18.6 million people died of CVDs, of which 85% died of ischemic heart disease or stroke[2]. Rosiglitazone is a synthetic peroxisome proliferator-activated receptor γ agonist, which can exert its hypoglycemic effect by improving insulin sensitivity. Various research[3-6] in the past decade showed that rosiglitazone had beneficial effects on overall CVDs and risk factors. Studies[3-6] on type 2 diabetes (T2DM) patients with or without coronary heart disease indicated that rosiglitazone could not only improve myocardial glucose intake and utilization[3,4], but also improve cardiac systolic and diastolic function[5,6]. However, inconsistent findings[7] have been reported, and more evidence shows that rosiglitazone has adverse effects on the cardiovascular system. A meta-analysis[7] showed that the risk of myocardial infarction, heart failure, and cardiovascular mortality was significantly increased in T2DM patients treated with rosiglitazone. However, the role of rosiglitazone in heart disease is still controversial. The effects of rosiglitazone on CVDs are mostly shown in observational studies[3-7]; in these studies, mixed risk factors, reverse causality, or selection bias are inevitable.

Mendelian randomization (MR) uses single nucleotide polymorphism (SNP) as an instrumental variable (IV) to infer the causal relationships between exposure and outcome, which can overcome the influence of confounding factors and reverse causal relationship[8,9]. In MR research, confounding factors can be minimized because genetic variations are randomly assigned to individuals at birth. Similarly, reverse causality can be avoided, because the existence of diseases will not affect the genotype of individuals[10]. There-

Corresponding Author: Keng Wu, MD; e-mail: wukeng1245@hotmail.com

Table I. Genetic variants associated with rosiglitazone treatment.

SNP	Chr	Position	EA	OA	EAF	beta	SE	*p*-value	R^2	F statistic
rs187455998	1	81917480	A	G	0.008508	0.002929	0.000534	4.15E-08	0.000159	13.43434
rs138205523	7	158321203	C	G	0.032005	0.001622	0.00027	1.96E-09	0.00064	54.01021
rs117299843	12	132336077	T	C	0.006674	0.003259	0.000571	1.14E-08	0.00013	10.9901
rs144741037	16	70037510	A	G	0.012413	0.002456	0.000437	1.96E-08	0.000237	19.98889

SNP, single nucleotide polymorphism; Chr, chromosome; EA; effect allele; EAF, effect allele frequency; NEA, non-effect allele; SE, standard error.

fore, we conduct a Mendelian randomization (MR) study to explore the potential relationship between rosiglitazone and cardiovascular diseases and risk factors.

Patients and Methods

Data Sources

Summary statistic of rosiglitazone data was obtained from the UK Biobank (Table I). UK Biobank is a prospective cohort that recruited more than 500,000 men and women aged 40-96 years between 2006 and 2010, and their health is being followed in the long term. Our study did not require ethical approval because it was a reanalysis of previously collected and published data.

Seven CVDs and seven risk factors were included as outcomes in this MR study (Table II). Complete summary genome-wide association studies (GWAS) statistics for the outcomes of coronary heart disease (CHD) (UK Biobank Phenotypes Consortium, available at: https://gwas.mrcieu.ac.uk/), stroke (UK biobank consortium), venous thromboembolism (UK biobank consortium), hypertrophic cardiomyopathy (Neale Lab Consortium, available at: https://gwas.mrcieu.ac.uk/), myocardial infarction[11], fasting glucose[12,13], High-Density Lipoprotein (HDL) cholesterol (UK biobank consortium)[14,15], Low-Density Lipoprotein (LDL) cholesterol (UK biobank consortium)[15,16], total cholesterol (UK biobank consortium)[15], hypertension (MRC-IEU Consortium, available at: https://gwas.mrcieu.ac.uk/)[17], fasting insulin[12,13], T2DM (Neale Lab consortium)[18], unstable angina pectoris and coronary atherosclerosis, were obtained. Table II summarizes the numbers (including the cases and controls where relevant) included in these GWAS, the population (including ethnicity), and the sample size in the GWAS.

Table II. Detailed characteristics of GWAS associated with exposures and outcomes in the study.

Traits	Year	Data source	Race	Sample size	Cases	Controls
Rosiglitazone	2017	Neale Lab	European	337159	483	336,676
CVDs						
Major coronary heart disease	2018	UK Biobank phenotypes	European	361194	10,157	351,037
Unstable angina pectoris	2018	UK Biobank phenotypes	European	361194	3,439	357,755
Coronary atherosclerosis	2018	UK Biobank phenotypes	European	361194	14,334	346,860
Hypertrophic cardiomyopathy	2017	Neale Lab	European	337159	71	337,088
Stroke	2018	UK Biobank phenotypes	European	361194	6,146	355,048
Myocardial infarction	2021	NA	European	395795	14,825	2,680
Venous thromboembolism	2018	UK Biobank phenotypes	European	361194	4,620	356,574
Risk factors						
HDL cholesterol	2020	UK Biobank	European	403943	NA	NA
LDL cholesterol	2020	UK Biobank	European	440546	NA	NA
Total cholesterol	2020	UK Biobank	European	115078	NA	NA
Hypertension	2018	MRC-IEU	European	463010	54,358	408,652
Type 2 diabetes	2017	Neale Lab	European	337159	2,133	335,026
Fasting insulin	2021	NA	European	151013	NA	NA
Fasting glucose	2021	NA	European	200622	NA	NA

CVDs, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not available.

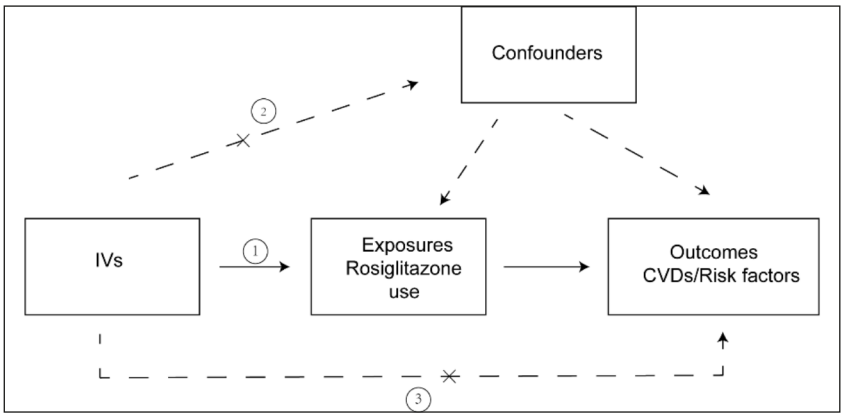

Figure 1. Schematic representation of two-sample Mendelian randomization (TSMR) analysis. Three assumptions of Mendelian randomization (MR) analysis are as follows: (1) instrumental variables (IVs) must be associated with Rosiglitazone use, (2) IVs must not be associated with confounders, (3) IVs must have an effect on cardiovascular disease (CVD)/risk factors only through Rosiglitazone use.

Study Design

An MR analysis was performed to evaluate the causal effects of rosiglitazone on CVDs and risk factors. MR analysis depends on the following assumptions: (1) there is a strong correlation between genetic variation and exposure factors (correlation hypothesis); (2) genetic variation is independent of the confounding factors that affect exposure and outcome (independence hypothesis); (3) genetic variation can only affect the outcome through exposure (exclusive hypothesis). Satisfaction of the second and third assumptions serves as a definition of independence from pleiotropy (Figure 1)[19,20].

Selection and Validation of IVs

Firstly, SNPs associated with exposure at the genome-wide significance threshold p 5×10^{-8} from a meta-analysis of GWAS were selected as instrumental variables (IVs). The corresponding linkage disequilibrium was identified. We confirmed that the SNP was in a state of linkage disequilibrium, and the independence of the SNP was realized by cutting the SNP into a 1,000 kb window ($r^2 < 0.1$)[21]. Secondly, PhenoScanner (available at: http://www.phenoscanner.medschl.cam.ac.uk/) was used to assess whether the above SNPs were related to known confounding factors (alcohol[22], smoking[23] and obesity[24]), and if so, the SNP would be excluded. Thirdly, we removed palindromic sequences in SNPs to ensure that the effects of these SNPs on exposure corresponded to the same alleles as those on the outcome. Finally, the F statistic of each SNP was calculated to test the weak IV bias in this study[25]. If the F statistic of the IV was < 10, it indicated a potential weak IV bias, and excluding this SNP was necessary to avoid its influence on the results[19].

MR Analysis

In the MR study, Inverse Variance Weighted (IVW) (random effects) method was mainly used to estimate the causal effect between exposure and outcome. Previous studies[26] had shown that the IVW method was widely used in MR research, and the test efficiency was the strongest. In order to eliminate the influence of research methods on the results and improve the accuracy, other methods such as IVW (fixed effects) method, maximum likelihood method, and penalized weighted median method were used as complementary approaches. The IVW method required that all SNPs met the three hypotheses of the IVs selection[27], especially the exclusive hypothesis and that genetic variation affected the outcome only through exposure in the study. Although known confounding SNPs were excluded as much as possible during the study, the estimation of causal effects might still be biased by gene pleiotropy caused by many unknown factors. Therefore, this study also used randomization-Egger method (MR-Egger) and the Weighted Median Estimator (WME) to test the reliability and stability of the results. MR-Egger regression method could not only test multiplicity but also correct multiplicity bias[28].

MR Sensitivity Analysis

Cochran's Q statistic was used to find the heterogeneity among SNPs. MR-Egger intercept (differs on average from zero) was used to test

whether genetic variants of CVDs have pleiotropic effects on rosiglitazone. If the result of the heterogeneity test was $p > 0.05$, it showed that there was no heterogeneity in SNPs. According to the exclusive hypothesis, it was necessary to test the causal inference between exposure and outcome. The horizontal pleiotropy was expressed by the intercept term of MR-Egger method, and the closer the intercept was to 0, the smaller the intercept was. If $p > 0.05$, it was considered that SNPs did not have horizontal pleiotropy. Therefore, there must be some unavoidable random errors in the process of IVs selection[29]. Leave-one-out analysis was performed by omitting the genetic variants one by one, and MR analysis was still conducted on the rest. The causal relationship would be credible and stable if the result of the leave-one-out analysis conformed to that of the global IVW analysis.

Statistical Analysis

To determine MR analysis of rosiglitazone on CVDs and risk factors, we conducted the IVW (random effects) method, IVW (fixed effects) method, maximum likelihood method, penalized weighted median method, MR-Egger method, and WME method[30]. MR-PRESSO was used to test the pleiotropy of rosiglitazone on CVDs and risk factors, which detected significant abnormalities in IVs in this study. If the result was $p > 0.05$, the difference was not statistically significant.

Results

IVs Selection and Validation

In total, we obtained four SNPs for the CVDs and risk factors ($r^2 < 0.1$). These IVs achieved genome-wide significance ($p < 5\times10^{-8}$) in rosiglitazone datasets (Table I). Among the four SNPs in this study, we found no association with confounding factors (alcohol consumption, smoking, and obesity). The distribution range of the F series is from 11 to 54, indicating that the causal association was less likely to be affected by the bias of weak instrumental variables. Subsequently, in the MR study, we used the MR-Egger regression intercept term to estimate exposure factors and found no horizontal pleiotropy between SNPs and CVD and risk factors (Table II).

MR Analysis and MR Sensitivity Analysis

Table III shows the association of rosiglitazone with CVDs. We found no evidence for the genetically predicted rosiglitazone on coronary heart disease (CHD) (IVW OR = 0.670, 95% CI: 0.3268-1.3717, $p = 0.273$), unstable angina pectoris (IVW OR = 0.805, 95% CI: 0.4720-1.3739, $p = 0.427$), coronary atherosclerosis (IVW OR = 1.141, 95% CI: 0.4929-2.6434, $p = 0.757$), hypertrophic cardiomyopathy (IVW OR = 0.975, 95% CI: 0.9130-1.0417, $p = 0.456$), stroke (IVW OR = 1.128, 95% CI: 0.6415-1.9844, $p = 0.675$), myocardial infarction (IVW OR = 1.973, 95% CI: 0.3097-4.5736, $p = 0.945$), venous thromboembolism (IVW OR = 0.803, 95% CI: 0.4908-1.3129, $p = 0.381$).

Table IV shows the association of rosiglitazone with risk factors. The results suggested that genetically predicted rosiglitazone was not associated with HDL cholesterol (IVW OR = 0.519, 95% CI: 0.0040-67.0145, $p = 0.792$), LDL cholesterol (IVW OR = 0.055, 95% CI: 0.0011-2.6503, $p = 0.385$), total cholesterol (IVW OR = 0.144, 95% CI: 0.0144-1.5842, $p = 0.611$), hypertension (IVW OR = 1.774, 95% CI: 0.5132-6.1331, $p = 0.365$), type 2 diabetes (IVW OR = 1.057, 95% CI: 0.7372-1.5145, $p = 0.764$), fasting insulin (IVW OR = 1.057, 95% CI: 0.7372-1.5145, $p = 0.764$), fasting glucose (IVW OR = 0.519, 95% CI: 0.1038-1.2975, $p = 0.792$). Some above associations were proved by MR-Egger, WME, IVW (fixed effects), maximum likelihood method, and penalized weighted median method.

Rosiglitazone on CVDs and risk factors had no heterogeneity (Tables III and IV). After MR-PRESSO and MR-Egger test, the pleiotropy test results of rosiglitazone on CVDs and risk factors-related data were all $p > 0.05$, indicating no significant outliers in the IVs of this study (Tables III and V). One-by-one elimination test of IVs showed that no single SNP had a significant impact on the robustness of the results, so the results of the MR analysis of rosiglitazone on CVDs and risk factors were stable. Funnel plots and forest plots showed that SNPs are symmetrically distributed, indicating that causal associations are unlikely to be affected by potential biases (**Supplementary Figures**).

Discussion

In the MR study, we found no significant causal relationship between rosiglitazone and CVDs or risk factors.

T2DM is a kind of metabolic syndromes characterized by elevated blood sugar. CVDs are one of the leading causes of death in diabetes patients.

Table III. Mendelian randomization estimates for the causal effect of rosiglitazone treatment on CVDs.

	MR			Heterogeneity		Pleiotropy	
Outcome Methods	**Beta**	**OR**	**95% CI**	***p*-value**	***p*-value**	**Intercept**	***p*-value**
Major coronary heart disease							
MR-Eggar	-1.128	0.324	(0.0249, 4.2006)	0.479	0.525		
weighted median	-0.357	0.700	(0.3103, 1.5779)	0.389			
IVW (RE)	-0.401	0.670	(0.3268, 1.3717)	0.273	0.654	0.002	0.621
Maximum likelihood	-0.406	0.666	(0.3227, 1.3755)	0.272			
Penalized weighted median	-0.357	0.700	(0.3065, 1.5975)	0.396			
IVW (FE)	-0.401	0.670	(0.3268, 1.3717)	0.273			
Unstable angina pectoris							
MR-Eggar	-1.262	0.283	(0.0474, 1.6905)	0.301	0.250	0.002	0.355
Weighted median	-0.076	0.927	(0.5540, 1.5513)	0.773			
IVW (RE)	-0.217	0.805	(0.4720, 1.3739)	0.427	0.191		
Maximum likelihood	-0.225	0.799	(0.5174, 1.2329)	0.310			
Penalized weighted median	-0.076	0.927	(0.7987, 1.5874)	0.782			
IVW (FE)	-0.217	0.805	(0.5267, 1.2311)	0.317			
Coronary atherosclerosis							
MR-Eggar	1.254	3.506	(0.1743, 7.5087)	0.499	0.888	-0.003	0.525
weighted median	0.187	1.205	(0.4583, 3.1698)	0.705			
IVW (RE)	0.132	1.141	(0.4929, 2.6434)	0.757	0.845		
Maximum likelihood	0.133	1.142	(0.4918, 2.1424)	0.757			
Penalized weighted median	0.187	1.205	(0.4520, 3.2136)	0.709			
IVW (FE)	0.132	1.141	(0.4929, 2.6434)	0.757			
Hypertrophic cardiomyopathy							
MR-Eggar	-0.175	0.839	(0.6632, 1.0621)	0.282	0.660	0.0003	0.323
weighted median	-0.038	0.839	(0.8883, 1.0437)	0.358			
IVW(RE)	-0.025	0.975	(0.9130, 1.0417)	0.456	0.470		
Maximum likelihood	-0.026	0.975	(0.9118, 1.0420)	0.452			
Penalized weighted median	-0.038	0.963	(0.8885, 1.0434)	0.356			
IVW (FE)	-0.025	0.975	(0.9130, 1.0417)	0.456			
Stroke							
MR-Eggar	0.996	2.707	(0.3599, 5.3686)	0.435	0.950	-0.002	0.469
weighted median	0.124	1.132	(0.5811, 2.2046)	0.716			
IVW (RE)	0.121	1.128	(0.6415, 1.9844)	0.675	0.829		
Maximum likelihood	0.121	1.129	(0.6405, 1.9906)	0.674			
Penalized weighted median	0.124	1.132	(0.6138, 2.0871)	0.692			
IVW (FE)	0.121	1.128	(0.6415, 1.9844)	0.675			
Myocardial infarction							
MR-Eggar	-1.427	0.240	(0.0412, 1.4424)	0.829	0.649	0.022	0.808
weighted median	1.495	4.460	(0.7717, 8.7714)	0.898			
IVW (RE)	0.680	1.973	(0.3097, 4.5736)	0.945	0.816		
Maximum likelihood	0.685	1.984	(0.3116, 4.6371)	9.854			
Penalized weighted median	1.495	4.460	(0.7717, 8.7714)	11.328			
IVW (FE)	0.680	1.973	(0.3097, 4.5736)	9.822			
Venous thromboembolism							
MR-Eggar	0.808	2.244	(0.3868, 13.0234)	0.463	0.615	-0.002	0.355
weighted median	-0.138	0.871	(0.4766, 1.5930)	0.655			
IVW(RE)	-0.220	0.803	(0.4908, 1.3129)	0.381			
Maximum likelihood	-0.224	0.800	(0.4859, 1.3156)	0.379			
Penalized weighted median	-0.138	0.871	(0.4793, 1.5841)	0.652			
VW (FE)	-0.220	0.803	(0.4908, 1.3129)	0.381			

MR, Mendelian randomization; OR, odds ratio; IVW (RE), inverse variance weighted (random effects); IVW (FE), inverse variance weighted (fixed effects); CI, confidence interval.

Table IV. Mendelian randomization estimates for the causal effect of rosiglitazone treatment on risk factors.

	MR			Heterogeneity		Pleiotropy	
Outcome Methods	**Beta**	**OR**	**95% CI**	***p*-value**	***p*-value**	**Intercept**	***p*-value**
HDL cholesterol							
MR-Eggar	-2.848	0.058	(9.73E-09, 3.46E+5)	0.755	0.382	-0.013	0.476
weighted median	0.309	1.362	(0.0048, 387.8615)	0.915			
IVW (RE)	-0.656	0.519	(0.0040, 67.0145)	0.792	0.444		
simple mode	0.227	1.255	(0.0007, 67.0145)	0.956			
Penalized weighted median	-1.515	0.220	(0.0031, 15.7688)	0.487			
IVW (FE)	-0.826	0.438	(0.0127, 15.0635)	0.647			
LDL cholesterol							
MR-Eggar	1.499	4.477	(0.4477, 8.9544)	0.177	0.369	-0.041	0.124
weighted median	-2.973	0.051	(0.0003, 9.9896)	0.269			
IVW (RE)	-2.907	0.055	(0.0011, 2.6503)	0.385	0.035		
Maximum likelihood	-3.109	0.045	(0.0008, 2.5810)	0.133			
Penalized weighted median	-3.006	0.049	(0.0003, 7.4264)	0.240			
IVW (FE)	-2.907	0.055	(0.0011, 2.6503)	0.142			
Total cholesterol							
MR-Eggar	-2.015	0.133	(0.0148, 1.4632)	0.503	0.730	0.031	0.558
weighted median	-0.728	0.483	(0.0966, 2.4152)	0.864			
IVW (RE)	-1.936	0.144	(0.0144, 1.5842)	0.611	0.774		
Maximum likelihood	-1.952	0.142	(0.0142, 1.5627)	0.610			
Penalized weighted median	-0.728	0.483	(0.1208, 1.9325)	0.870			
IVW (FE)	-1.936	0.144	(0.0144, 1.5842)	0.611			
Hypertension							
MR-Eggar	-0.552	0.576	(0.1420, 2.304)	0.847	0.289	0.003	0.687
weighted median	0.416	1.516	(0.3427, 6.7050)	0.584			
IVW (RE)	0.573	1.774	(0.5132, 6.1331)	0.365	0.431		
Maximum likelihood	0.586	1.796	(0.5107, 6.3170)	0.361			
Penalized weighted median	0.416	1.516	(0.3481, 6.5999)	0.579			
IVW (FE)	0.573	1.774	(0.5132, 6.1331)	0.365			
Type 2 diabetes							
MR-Eggar	-0.352	0.703	(0.1944, 2.5448)	0.645	0.839	0.001	0.584
weighted median	0.071	1.073	(0.7068, 1.6297)	0.740			
IVW (RE)	0.055	1.057	(0.7372, 1.5145)	0.764	0.857		
Maximum likelihood	0.055	1.057	(0.7366, 1.5168)	0.764			
Penalized weighted median	0.071	1.073	(0.7158, 1.6092)	0.732			
IVW (FE)	0.055	1.057	(0.7372, 1.5145)	0.764			
Fasting insulin							
MR-Eggar	-0.054	0.947	(0.4735, 1.4205)	0.996	0.839	0.0003	0.989
weighted median	-0.453	0.635	(0.2541, 1.5875)	0.885			
IVW (RE)	0.078	1.081	(0.5405, 1.6215)	0.977	0.857		
Maximum likelihood	0.078	1.081	(0.5405, 1.6215)	0.977			
Penalized weighted median	-0.453	0.635	(0.2541, 1.5875)	0.883			
IVW (FE)	0.078	1.081	(0.5405, 1.6215)	0.977			
Fasting glucose							
MR-Eggar	-2.848	0.058	(0.0292, 1.1611)	0.755	0.672	0	0.799
weighted median	0.309	1.362	(0.2724, 2.7245)	0.916			
IVW (RE)	-0.656	0.519	(0.1038, 1.2975)	0.792	0.831		
Maximum likelihood	-0.659	0.517	(0.1034, 1.2925)	0.791			
Penalized weighted median	0.309	1.362	(0.2724, 2.7245)	0.916			
IVW (FE)	-0.656	0.519	(0.1038, 1.2975)	0.792			

MR, Mendelian randomization; OR, odds ratio; IVW (RE), inverse variance weighted (random effects); IVW (FE), inverse variance weighted (fixed effects); CI, confidence interval.

Table V. DMR-PRESSO estimates the causal effects of rosiglitazone treatment on CVDs and risk factors.

Outcomes	Number of SNPs	Effect	MR p-value	MR-PRESSO Global test *p*-value
CVDs				
Major coronary heart disease	4	-0.401	0.233	0.684
Unstable angina pectoris	4	-0.217	0.485	0.261
Coronary atherosclerosis	4	0.132	0.596	0.844
Hypertrophic cardiomyopathy	4	-0.025	0.476	0.529
Myocardial infarction	4	0.680	0.909	0.827
Stroke	4	0.121	0.497	0.833
Venous thromboembolism	4	-0.220	0.400	0.526
Risk factors				
HDL cholesterol	4	-0.826	0.661	0.514
LDL cholesterol	4	-2.907	0.449	0.089
Hypertension	4	0.573	0.414	0.492
type 2 diabetes	4	0.055	0.595	0.840
Fasting insulin	4	0.078	0.920	0.983
Fasting glucose	4	-0.656	0.659	0.844
Total cholesterol	4	-1.936	0.465	0.777

CVDs, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not available.

T2DM is characterized by insulin resistance and impaired glucose tolerance[31]. Rosiglitazone is a thiazolidinedione drug, which can significantly enhance the sensitivity of target tissue to insulin. Rosiglitazone can protect pancreatic β-cell function and improve insulin resistance. It can be used alone or in combination with biguanides, sulfonylureas, or insulin. It is currently the most widely used insulin sensitizer in the treatment of T2DM[32]. In recent years, clinical trials[36,38] have shown that rosiglitazone has the strongest long-term ability to control blood glucose compared with glibenclamide and metformin in T2DM patients.

In addition, rosiglitazone also has the effect of improving cardiovascular disease risk factors. *In vivo*, rosiglitazone could reduce the infarct size of the rat model of ischemia/reperfusion (I/R) injury and improve the myocardial contractile dysfunction induced by I/R[33-35]. In obese rat model, rosiglitazone could reduce systolic blood pressure and improve systolic function; it could also reduce blood glucose, triglyceride, free fatty acid levels, and enhance myocardial glucose oxidation of ischemic myocardium[36]. Some *in vitro* studies[37] found that rosiglitazone treatment enhanced the antioxidant stress capacity of rat cardiomyocytes and played a protective role in the heart. In addition, a clinical study[38] reported that rosiglitazone treatment could improve coronary atherosclerosis in diabetes patients by reducing the pulse wave speed. To sum up, rosiglitazone has potential cardiovascular protection, which can improve blood vessels, blood pressure, blood lipids and some common cardiovascular disease risk factors.

However, in recent years, more and more evidence[7,39] has shown that rosiglitazone has adverse effects on the cardiovascular system. For example, in 2003, Lygate et al[39] reported for the first time that rosiglitazone would not change the remodeling of rats after myocardial infarction but would increase the mortality. A meta-analysis[7] showed that T2DM patients had a significantly increased risk of myocardial infarction, heart failure, and cardiovascular mortality after rosiglitazone. At present, the research on the cardiovascular safety of rosiglitazone remains controversial.

An *in vitro* experiment showed that the expression of the antioxidant enzyme heme oxygenase-1 was up-regulated in rosiglitazone-treated rat cardiomyocytes, and rosiglitazone exerted its antioxidant stress effect to protect cardiac function[37]. On the contrary, when isolated vascular smooth muscle cells were treated with rosiglitazone, caspase-3 activity was increased through the extracellular signal-regulated kinase 1/2 pathway, which led to apoptosis[40]. *In vivo*, intravenous administration of rosiglitazone significantly improved left ventricular systolic function in I/R rats[34,41]. However, other studies[39] have shown that after 8 weeks of oral administration of rosiglitazone in rats with non-diabetic myocardial infarction, there is no significant change

in myocardial infarction size and left ventricular hypertrophy, but this led to an increase in mortality after Imax R injury. These results suggest that rosiglitazone has no protective effect on the heart. A clinical study[38] on patients with T2DM showed that taking rosiglitazone for 12 weeks can not only alleviate insulin resistance but also reduce pulse velocity, thus preventing arteriosclerosis. Similarly, a meta-analysis study[42] showed that patients treated with rosiglitazone for more than 12 months had a significantly increased risk of myocardial infarction and heart failure. The controversy about the cardiovascular effect of rosiglitazone may be due to the following two reasons: (1) differences in results due to different species of drug metabolism, different experimental models, different drug delivery methods, and different intervals of drug treatment (2) differences in clinical characteristics of patients[36].

The randomized controlled trial (RCT) is the most powerful method to prove the pathogenic hypothesis in epidemiological studies. However, the research design of RCT is complex and expensive. Therefore, it is difficult to implement. MR can skillfully make up for the shortcomings of traditional epidemiological studies that are susceptible to interference from confounding factors and reverse causality when inferring the causes of complex diseases, and provide a new idea for epidemiological studies[43]. Since the genotype of offspring is randomly inherited from parents, it is a very reliable method to use SNP as a genetic variable tool to infer the causal relationship. In order to determine the cardiovascular benefits or risks of rosiglitazone, we analyzed the correlation between rosiglitazone and CVDs and risk factors through large-scale GWAS for the first time. The study finds that there is no causal relationship between rosiglitazone and CVDs and risk factors. Therefore, we should reconsider the potential use of rosiglitazone in preventing cardiovascular disease and further verify it in a randomized controlled trial.

Study Limitations

(1) Ethnic factors can affect the study at the gene level. In this study, GWAS data used are mainly from European populations, which to some extent undermines the universality of the study results to other populations. Follow-up studies are needed[44]. (2) The number of cases of some CVDs and risk factors is minimal, which leads to low estimation accuracy. (3) The four SNPs in this study may not be able to replace all exposure factors, which affects the accuracy of MR analysis results.

Conclusions

Using MR analysis, we found that rosiglitazone is not causally associated with CVDs and risk factors. However, additional clinical and basic studies are needed to confirm our results further.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

Not applicable.

Funding

This work was supported by grants from the National Natural Science Foundation of China (8167021020).

Authors' Contributions

X.-M. Li conceived the project, made a statistical analysis, and wrote the manuscript. Z.-J. Wu and Z.-L. Xu helped to write the manuscript and performed a quality assessment. A. Li, M.-Q. Liu and C.-G. Song helped to revise the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

Not applicable.

References

1) Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, Boehme AK, Buxton AE, Carson AP, Commodore-Mensah Y, Elkind MSV, Evenson KR, Eze-Nliam C, Ferguson JF, Generoso G, Ho JE, Kalani R, Khan SS, Kissela BM, Knutson KL, Levine DA, Lewis TT, Liu J, Loop MS, Ma J, Mussolino ME, Navaneethan SD, Perak AM, Poudel R, Rezk-Hanna M, Roth GA, Schroeder EB, Shah SH, Thacker EL, VanWagner LB, Virani SS, Voecks JH, Wang NY, Yaffe K, Martin SS. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. Circulation 2022; 145: e153-e639.

2) Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton AZ, Benjamin EJ, Benziger CP, Bonny A, Brauer M, Brodmann M, Cahill TJ, Carapetis J, Catapano AL, Chugh SS, Cooper LT, Coresh J, Criqui

M, DeCleene N, Eagle KA, Emmons-Bell S, Feigin VL, Fernandez-Sola J, Fowkes G, Gakidou E, Grundy SM, He FJ, Howard G, Hu F, Inker L, Karthikeyan G, Kassebaum N, Koroshetz W, Lavie C, Lloyd-Jones D, Lu HS, Mirijello A, Temesgen AM, Mokdad A, Moran AE, Muntner P, Narula J, Neal B, Ntsekhe M, Moraes de Oliveira G, Otto C, Owolabi M, Pratt M, Rajagopalan S, Reitsma M, Ribeiro ALP, Rigotti N, Rodgers A, Sable C, Shakil S, Sliwa-Hahnle K, Stark B, Sundstrom J, Timpel P, Tleyjeh IM, Valgimigli M, Vos T, Whelton PK, Yacoub M, Zuhlke L, Murray C, Fuster V, Group, G-N-JGBoCDW. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol 2020; 76: 2982-3021.

3) Lautamaki R, Airaksinen KE, Seppanen M, Toikka J, Luotolahti M, Ball E, Borra R, Harkonen R, Iozzo P, Stewart M, Knuuti J, Nuutila P. Rosiglitazone improves myocardial glucose uptake in patients with type 2 diabetes and coronary artery disease: a 16-week randomized, double-blind, placebo-controlled study. Diabetes 2005; 54: 2787-2794.

4) Investigators DT, Gerstein HC, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, Hanefeld M, Hoogwerf B, Laakso M, Mohan V, Shaw J, Zinman B, Holman RR. Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. Lancet 2006; 368: 1096-1105.

5) Nilsson PM, Hedblad B, Donaldson J, Berglund G. Rosiglitazone reduces office and diastolic ambulatory blood pressure following 1-year treatment in non-diabetic subjects with insulin resistance. Blood Press 2007; 16: 95-100.

6) Reynolds LR, Konz EC, Frederich RC, Anderson JW. Rosiglitazone amplifies the benefits of lifestyle intervention measures in long-standing type 2 diabetes mellitus. Diabetes Obes Metab 2002; 4: 270-275.

7) Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007; 356: 2457-2471.

8) Ebrahim S, Davey Smith G. Mendelian randomization: can genetic epidemiology help redress the failures of observational epidemiology? Hum Genet 2008; 123: 15-33.

9) Thomas DC, Conti DV. Commentary: the concept of 'Mendelian Randomization'. Int J Epidemiol 2004; 33: 21-25.

10) Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 2014; 23: R89-R98.

11) Hartiala JA, Han Y, Jia Q, Hilser JR, Huang P, Gukasyan J, Schwartzman WS, Cai Z, Biswas S, Trégouët DA, Smith NL; INVENT Consortium; CHARGE Consortium Hemostasis Working Group; GENIUS-CHD Consortium; Seldin M, Pan C, Mehrabian M, Lusis AJ, Bazeley P, Sun YV, Liu C, Quyyumi AA, Scholz M, Thiery J, Delgado GE, Kleber ME, März W, Howe LJ, Asselbergs FW, van Vugt M, Vlachojannis GJ, Patel RS, Lyytikäinen LP, Kähönen M, Lehtimäki T, Nieminen TVM, Kuukasjärvi P, Laurikka JO, Chang X, Heng CK, Jiang R, Kraus WE, Hauser ER, Ferguson JF, Reilly MP, Ito K, Koyama S, Kamatani Y, Komuro I; Biobank Japan; Stolze LK, Romanoski CE, Khan MD, Turner AW, Miller CL, Aherrahrou R, Civelek M, Ma L, Björkegren JLM, Kumar SR, Tang WHW, Hazen SL, Allayee H. Genome-wide analysis identifies novel susceptibility loci for myocardial infarction. Eur Heart J 2021; 42: 919-933.

12) Chen J, Spracklen CN, Marenne G, Varshney A, Corbin LJ, Luan J, Willems SM, Wu Y, Zhang X, Horikoshi M, Boutin TS, Mägi R, Waage J, Li-Gao R, Chan KHK, Yao J, Anasanti MD, Chu AY, Claringbould A, Heikkinen J, Hong J, Hottenga JJ, Huo S, Kaakinen MA, Louie T, März W, Moreno-Macias H, Ndungu A, Nelson SC, Nolte IM, North KE, Raulerson CK, Ray D, Rohde R, Rybin D, Schurmann C, Sim X, Southam L, Stewart ID, Wang CA, Wang Y, Wu P, Zhang W, Ahluwalia TS, Appel EVR, Bielak LF, Brody JA, Burtt NP, Cabrera CP, Cade BE, Chai JF, Chai X, Chang LC, Chen CH, Chen BH, Chitrala KN, Chiu YF, de Haan HG, Delgado GE, Demirkan A, Duan Q, Engmann J, Fatumo SA, Gayán J, Giulianini F, Gong JH, Gustafsson S, Hai Y, Hartwig FP, He J, Heianza Y, Huang T, Huerta-Chagoya A, Hwang MY, Jensen RA, Kawaguchi T, Kentistou KA, Kim YJ, Kleber ME, Kooner IK, Lai S, Lange LA, Langefeld CD, Lauzon M, Li M, Ligthart S, Liu J, Loh M, Long J, Lyssenko V, Mangino M, Marzi C, Montasser ME, Nag A, Nakatochi M, Noce D, Noordam R, Pistis G, Preuss M, Raffield L, Rasmussen-Torvik LJ, Rich SS, Robertson NR, Rueedi R, Ryan K, Sanna S, Saxena R, Schraut KE, Sennblad B, Setoh K, Smith AV, Sparsø T, Strawbridge RJ, Takeuchi F, Tan J, Trompet S, van den Akker E, van der Most PJ, Verweij N, Vogel M, Wang H, Wang C, Wang N, Warren HR, Wen W, Wilsgaard T, Wong A, Wood AR, Xie T, Zafarmand MH, Zhao JH, Zhao W, Amin N, Arzumanyan Z, Astrup A, Bakker SJL, Baldassarre D, Beekman M, Bergman RN, Bertoni A, Blüher M, Bonnycastle LL, Bornstein SR, Bowden DW, Cai Q, Campbell A, Campbell H, Chang YC, de Geus EJC, Dehghan A, Du S, Eiriksdottir G, Farmaki AE, Frånberg M, Fuchsberger C, Gao Y, Gjesing AP, Goel A, Han S, Hartman CA, Herder C, Hicks AA, Hsieh CH, Hsueh WA, Ichihara S, Igase M, Ikram MA, Johnson WC, Jørgensen ME, Joshi PK, Kalyani RR, Kandeel FR, Katsuya T, Khor CC, Kiess W, Kolcic I, Kuulasmaa T, Kuusisto J, Läll K, Lam K, Lawlor DA, Lee NR, Lemaitre RN, Li H; Lifelines Cohort Study; Lin SY, Lindström J, Linneberg A, Liu J, Lorenzo C, Matsubara T, Matsuda F, Mingrone G, Mooijaart S, Moon S, Nabika T, Nadkarni GN, Nadler JL, Nelis M, Neville MJ, Norris JM, Ohyagi Y, Peters A, Peyser PA, Polasek O, Qi Q, Raven D, Reilly DF, Reiner A, Rivideneira F, Roll K, Rudan I, Sabanayagam C, Sandow K, Sattar N, Schürmann A, Shi J, Stringham HM, Taylor KD, Teslovich

TM, Thuesen B, Timmers PRHJ, Tremoli E, Tsai MY, Uitterlinden A, van Dam RM, van Heemst D, van Hylckama Vlieg A, van Vliet-Ostaptchouk JV, Vangipurapu J, Vestergaard H, Wang T, Willems van Dijk K, Zemunik T, Abecasis GR, Adair LS, Aguilar-Salinas CA, Alarcón-Riquelme ME, An P, Aviles-Santa L, Becker DM, Beilin LJ, Bergmann S, Bisgaard H, Black C, Boehnke M, Boerwinkle E, Böhm BO, Bønnelykke K, Boomsma DI, Bottinger EP, Buchanan TA, Canouil M, Caulfield MJ, Chambers JC, Chasman DI, Chen YI, Cheng CY, Collins FS, Correa A, Cucca F, de Silva HJ, Dedoussis G, Elmståhl S, Evans MK, Ferrannini E, Ferrucci L, Florez JC, Franks PW, Frayling TM, Froguel P, Gigante B, Goodarzi MO, Gordon-Larsen P, Grallert H, Grarup N, Grimsgaard S, Groop L, Gudnason V, Guo X, Hamsten A, Hansen T, Hayward C, Heckbert SR, Horta BL, Huang W, Ingelsson E, James PS, Jarvelin MR, Jonas JB, Jukema JW, Kaleebu P, Kaplan R, Kardia SLR, Kato N, Keinanen-Kiukaanniemi SM, Kim BJ, Kivimaki M, Koistinen HA, Kooner JS, Körner A, Kovacs P, Kuh D, Kumari M, Kutalik Z, Laakso M, Lakka TA, Launer LJ, Leander K, Li H, Lin X, Lind L, Lindgren C, Liu S, Loos RJF, Magnusson PKE, Mahajan A, Metspalu A, Mook-Kanamori DO, Mori TA, Munroe PB, Njølstad I, O'Connell JR, Oldehinkel AJ, Ong KK, Padmanabhan S, Palmer CNA, Palmer ND, Pedersen O, Pennell CE, Porteous DJ, Pramstaller PP, Province MA, Psaty BM, Qi L, Raffel LJ, Rauramaa R, Redline S, Ridker PM, Rosendaal FR, Saaristo TE, Sandhu M, Saramies J, Schneiderman N, Schwarz P, Scott LJ, Selvin E, Sever P, Shu XO, Slagboom PE, Small KS, Smith BH, Snieder H, Sofer T, Sørensen TIA, Spector TD, Stanton A, Steves CJ, Stumvoll M, Sun L, Tabara Y, Tai ES, Timpson NJ, Tönjes A, Tuomilehto J, Tusie T, Uusitupa M, van der Harst P, van Duijn C, Vitart V, Vollenweider P, Vrijkotte TGM, Wagenknecht LE, Walker M, Wang YX, Wareham NJ, Watanabe RM, Watkins H, Wei WB, Wickremasinghe AR, Willemsen G, Wilson JF, Wong TY, Wu JY, Xiang AH, Yanek LR, Yengo L, Yokota M, Zeggini E, Zheng W, Zonderman AB, Rotter JI, Gloyn AL, McCarthy MI, Dupuis J, Meigs JB, Scott RA, Prokopenko I, Leong A, Liu CT, Parker SCJ, Mohlke KL, Langenberg C, Wheeler E, Morris AP, Barroso I; Meta-Analysis of Glucose and Insulin-related Traits Consortium (MAGIC). The trans-ancestral genomic architecture of glycemic traits. Nat Genet 2021; 53: 840-860.

13) Manning AK, Hivert MF, Scott RA, Grimsby JL, Bouatia-Naji N, Chen H, Rybin D, Liu CT, Bielak LF, Prokopenko I, Amin N, Barnes D, Cadby G, Hottenga JJ, Ingelsson E, Jackson AU, Johnson T, Kanoni S, Ladenvall C, Lagou V, Lahti J, Lecoeur C, Liu Y, Martinez-Larrad MT, Montasser ME, Navarro P, Perry JR, Rasmussen-Torvik LJ, Salo P, Sattar N, Shungin D, Strawbridge RJ, Tanaka T, van Duijn CM, An P, de Andrade M, Andrews JS, Aspelund T, Atalay M, Aulchenko Y, Balkau B, Bandinelli S, Beckmann JS, Beilby JP, Bellis C, Bergman RN, Blangero J, Boban M, Boehnke M, Boerwinkle E, Bonnycastle LL, Boomsma DI, Borecki IB, Böttcher Y, Bouchard C, Brunner E, Budimir D, Campbell H, Carlson O, Chines PS, Clarke R, Collins FS, Corbatón-Anchuelo A, Couper D, de Faire U, Dedoussis GV, Deloukas P, Dimitriou M, Egan JM, Eiriksdottir G, Erdos MR, Eriksson JG, Eury E, Ferrucci L, Ford I, Forouhi NG, Fox CS, Franzosi MG, Franks PW, Frayling TM, Froguel P, Galan P, de Geus E, Gigante B, Glazer NL, Goel A, Groop L, Gudnason V, Hallmans G, Hamsten A, Hansson O, Harris TB, Hayward C, Heath S, Hercberg S, Hicks AA, Hingorani A, Hofman A, Hui J, Hung J, Jarvelin MR, Jhun MA, Johnson PC, Jukema JW, Jula A, Kao WH, Kaprio J, Kardia SL, Keinanen-Kiukaanniemi S, Kivimaki M, Kolcic I, Kovacs P, Kumari M, Kuusisto J, Kyvik KO, Laakso M, Lakka T, Lannfelt L, Lathrop GM, Launer LJ, Leander K, Li G, Lind L, Lindstrom J, Lobbens S, Loos RJ, Luan J, Lyssenko V, Mägi R, Magnusson PK, Marmot M, Meneton P, Mohlke KL, Mooser V, Morken MA, Miljkovic I, Narisu N, O'Connell J, Ong KK, Oostra BA, Palmer LJ, Palotie A, Pankow JS, Peden JF, Pedersen NL, Pehlic M, Peltonen L, Penninx B, Pericic M, Perola M, Perusse L, Peyser PA, Polasek O, Pramstaller PP, Province MA, Räikkönen K, Rauramaa R, Rehnberg E, Rice K, Rotter JI, Rudan I, Ruokonen A, Saaristo T, Sabater-Lleal M, Salomaa V, Savage DB, Saxena R, Schwarz P, Seedorf U, Sennblad B, Serrano-Rios M, Shuldiner AR, Sijbrands EJ, Siscovick DS, Smit JH, Small KS, Smith NL, Smith AV, Stančáková A, Stirrups K, Stumvoll M, Sun YV, Swift AJ, Tönjes A, Tuomilehto J, Trompet S, Uitterlinden AG, Uusitupa M, Vikström M, Vitart V, Vohl MC, Voight BF, Vollenweider P, Waeber G, Waterworth DM, Watkins H, Wheeler E, Widen E, Wild SH, Willems SM, Willemsen G, Wilson JF, Witteman JC, Wright AF, Yaghootkar H, Zelenika D, Zemunik T, Zgaga L; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium; Multiple Tissue Human Expression Resource (MUTHER) Consortium; Wareham NJ, McCarthy MI, Barroso I, Watanabe RM, Florez JC, Dupuis J, Meigs JB, Langenberg C. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet 2012; 44: 659-669.

14) Richardson TG, Sanderson E, Palmer TM, Ala-Korpela M, Ference BA, Davey Smith G, Holmes MV. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomization analysis. PLoS Med 2020; 17: e1003062.

15) Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog HM, Do R, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkilä K, Hyppönen E, Isaacs A, Jackson AU, Johansson Å, Johnson T, Kaakinen M, Kettunen J, Kleber ME, Li X, Luan J, Lyytikäinen LP, Magnusson PKE, Mangino M,

Mihailov E, Montasser ME, Müller-Nurasyid M, Nolte IM, O'Connell JR, Palmer CD, Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C, Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van den Herik EG, Voight BF, Volcik KA, Waite LL, Wong A, Wu Y, Zhang W, Absher D, Asiki G, Barroso I, Been LF, Bolton JL, Bonnycastle LL, Brambilla P, Burnett MS, Cesana G, Dimitriou M, Doney ASF, Döring A, Elliott P, Epstein SE, Ingi Eyjolfsson G, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Illig T, Jones MR, Kaleebu P, Kastelein JJP, Khaw KT, Kim E, Klopp N, Komulainen P, Kumari M, Langenberg C, Lehtimäki T, Lin SY, Lindström J, Loos RJF, Mach F, McArdle WL, Meisinger C, Mitchell BD, Müller G, Nagaraja R, Narisu N, Nieminen TVM, Nsubuga RN, Olafsson I, Ong KK, Palotie A, Papamarkou T, Pomilla C, Pouta A, Rader DJ, Reilly MP, Ridker PM, Rivadeneira F, Rudan I, Ruokonen A, Samani N, Scharnagl H, Seeley J, Silander K, Stančáková A, Stirrups K, Swift AJ, Tiret L, Uitterlinden AG, van Pelt LJ, Vedantam S, Wainwright N, Wijmenga C, Wild SH, Willemsen G, Wilsgaard T, Wilson JF, Young EH, Zhao JH, Adair LS, Arveiler D, Assimes TL, Bandinelli S, Bennett F, Bochud M, Boehm BO, Boomsma DI, Borecki IB, Bornstein SR, Bovet P, Burnier M, Campbell H, Chakravarti A, Chambers JC, Chen YI, Collins FS, Cooper RS, Danesh J, Dedoussis G, de Faire U, Feranil AB, Ferrières J, Ferrucci L, Freimer NB, Gieger C, Groop LC, Gudnason V, Gyllensten U, Hamsten A, Harris TB, Hingorani A, Hirschhorn JN, Hofman A, Hovingh GK, Hsiung CA, Humphries SE, Hunt SC, Hveem K, Iribarren C, Järvelin MR, Jula A, Kähönen M, Kaprio J, Kesäniemi A, Kivimaki M, Kooner JS, Koudstaal PJ, Krauss RM, Kuh D, Kuusisto J, Kyvik KO, Laakso M, Lakka TA, Lind L, Lindgren CM, Martin NG, März W, McCarthy MI, McKenzie CA, Meneton P, Metspalu A, Moilanen L, Morris AD, Munroe PB, Njølstad I, Pedersen NL, Power C, Pramstaller PP, Price JF, Psaty BM, Quertermous T, Rauramaa R, Saleheen D, Salomaa V, Sanghera DK, Saramies J, Schwarz PEH, Sheu WH, Shuldiner AR, Siegbahn A, Spector TD, Stefansson K, Strachan DP, Tayo BO, Tremoli E, Tuomilehto J, Uusitupa M, van Duijn CM, Vollenweider P, Wallentin L, Wareham NJ, Whitfield JB, Wolffenbuttel BHR, Ordovas JM, Boerwinkle E, Palmer CNA, Thorsteinsdottir U, Chasman DI, Rotter JI, Franks PW, Ripatti S, Cupples LA, Sandhu MS, Rich SS, Boehnke M, Deloukas P, Kathiresan S, Mohlke KL, Ingelsson E, Abecasis GR; Global Lipids Genetics Consortium. Discovery and refinement of loci associated with lipid levels. Nat Genet 2013; 45: 1274-1283.

16) Markle H, Tellier A. Inference of coevolutionary dynamics and parameters from host and parasite polymorphism data of repeated experiments. PLoS Comput Biol 2020; 16: e1007668.

17) Lo CCW, Lo ACQ, Leow SH, Fisher G, Corker B, Batho O, Morris B, Chowaniec M, Vladutiu CJ, Fraser A, Oliver-Williams C. Future Cardiovascular Disease Risk for Women With Gestational Hypertension: A Systematic Review and Meta-Analysis. J Am Heart Assoc 2020; 9: e013991.

18) Scott RA, Scott LJ, Mägi R, Marullo L, Gaulton KJ, Kaakinen M, Pervjakova N, Pers TH, Johnson AD, Eicher JD, Jackson AU, Ferreira T, Lee Y, Ma C, Steinthorsdottir V, Thorleifsson G, Qi L, Van Zuydam NR, Mahajan A, Chen H, Almgren P, Voight BF, Grallert H, Müller-Nurasyid M, Ried JS, Rayner NW, Robertson N, Karssen LC, van Leeuwen EM, Willems SM, Fuchsberger C, Kwan P, Teslovich TM, Chanda P, Li M, Lu Y, Dina C, Thuillier D, Yengo L, Jiang L, Sparso T, Kestler HA, Chheda H, Eisele L, Gustafsson S, Frånberg M, Strawbridge RJ, Benediktsson R, Hreidarsson AB, Kong A, Sigurðsson G, Kerrison ND, Luan J, Liang L, Meitinger T, Roden M, Thorand B, Esko T, Mihailov E, Fox C, Liu CT, Rybin D, Isomaa B, Lyssenko V, Tuomi T, Couper DJ, Pankow JS, Grarup N, Have CT, Jørgensen ME, Jørgensen T, Linneberg A, Cornelis MC, van Dam RM, Hunter DJ, Kraft P, Sun Q, Edkins S, Owen KR, Perry JRB, Wood AR, Zeggini E, Tajes-Fernandes J, Abecasis GR, Bonnycastle LL, Chines PS, Stringham HM, Koistinen HA, Kinnunen L, Sennblad B, Mühleisen TW, Nöthen MM, Pechlivanis S, Baldassarre D, Gertow K, Humphries SE, Tremoli E, Klopp N, Meyer J, Steinbach G, Wennauer R, Eriksson JG, Männistö S, Peltonen L, Tikkanen E, Charpentier G, Eury E, Lobbens S, Gigante B, Leander K, McLeod O, Bottinger EP, Gottesman O, Ruderfer D, Blüher M, Kovacs P, Tonjes A, Maruthur NM, Scapoli C, Erbel R, Jöckel KH, Moebus S, de Faire U, Hamsten A, Stumvoll M, Deloukas P, Donnelly PJ, Frayling TM, Hattersley AT, Ripatti S, Salomaa V, Pedersen NL, Boehm BO, Bergman RN, Collins FS, Mohlke KL, Tuomilehto J, Hansen T, Pedersen O, Barroso I, Lannfelt L, Ingelsson E, Lind L, Lindgren CM, Cauchi S, Froguel P, Loos RJF, Balkau B, Boeing H, Franks PW, Barricarte Gurrea A, Palli D, van der Schouw YT, Altshuler D, Groop LC, Langenberg C, Wareham NJ, Sijbrands E, van Duijn CM, Florez JC, Meigs JB, Boerwinkle E, Gieger C, Strauch K, Metspalu A, Morris AD, Palmer CNA, Hu FB, Thorsteinsdottir U, Stefansson K, Dupuis J, Morris AP, Boehnke M, McCarthy MI, Prokopenko I; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. An Expanded Genome-Wide Association Study of Type 2 Diabetes in Europeans. Diabetes 2017; 66: 2888-2902.

19) Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008; 27: 1133-1163.

20) Verduijn M, Siegerink B, Jager KJ, Zoccali C, Dekker FW. Mendelian randomization: use of genetics to enable causal inference in observational studies. Nephrol Dial Transplant 2010; 25: 1394-1398.

21) He J, Luo X, Xin H, Lai Q, Zhou Y, Bai Y. The Effects of Fatty Acids on Inflammatory Bowel Disease: A Two-Sample Mendelian Randomization Study. Nutrients 2022; 14: 2883.

22) Djousse L, Lee IM, Buring JE, Gaziano JM. Alcohol consumption and risk of cardiovascular

disease and death in women: potential mediating mechanisms. Circulation 2009; 120: 237-244.

23) Yang JJ, Yu D, Shu XO, Wen W, Rahman S, Abe S, Saito E, Gupta PC, He J, Tsugane S, Gao YT, Yuan JM, Koh WP, Sadakane A, Tomata Y, Tsuji I, Sugawara Y, Matsuo K, Ahn YO, Park SK, Chen Y, Inoue M, Kang D, Zheng W; remaining authors are listed at the end of the paper. Reduction in total and major cause-specific mortality from tobacco smoking cessation: a pooled analysis of 16 population-based cohort studies in Asia. Int J Epidemiol 2022; 50: 2070-2081.
24) Xue R, Li Q, Geng Y, Wang H, Wang F, Zhang S. Abdominal obesity and risk of CVD: a dose-response meta-analysis of thirty-one prospective studies. Br J Nutr 2021; 126: 1420-1430.
25) Palmer TM, Lawlor DA, Harbord RM, Sheehan NA, Tobias JH, Timpson NJ, Davey Smith G, Sterne JA. Using multiple genetic variants as instrumental variables for modifiable risk factors. Stat Methods Med Res 2012; 21: 223-242.
26) Allen NE, Sudlow C, Peakman T, Collins R. Biobank, UK, UK biobank data: come and get it. Sci Transl Med 2014; 6: 224ed4.
27) Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013; 37: 658-665.
28) Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015; 44: 512-525.
29) Mokry LE, Ross S, Timpson NJ, Sawcer S, Davey Smith G, Richards JB. Obesity and Multiple Sclerosis: A Mendelian Randomization Study. PLoS Med 2016; 13: e1002053.
30) Guo M, Feng T, Liu M, Hua Z, Ma Y, Cai JP, Li XJ. Causal roles of daytime sleepiness in cardiometabolic diseases and osteoporosis. Eur Rev Med Pharmacol Sci 2022; 26: 2755-2764.
31) Jay MA, Ren J. Peroxisome proliferator-activated receptor (PPAR) in metabolic syndrome and type 2 diabetes mellitus. Curr Diabetes Rev 2007; 3: 33-39.
32) Day C. Thiazolidinediones: a new class of antidiabetic drugs. Diabet Med 1999; 16: 179-192.
33) Yue TL, Bao W, Gu JL, Cui J, Tao L, Ma XL, Ohlstein EH, Jucker BM. Rosiglitazone treatment in Zucker diabetic Fatty rats is associated with ameliorated cardiac insulin resistance and protection from ischemia/reperfusion-induced myocardial injury. Diabetes 2005; 54: 554-562.
34) Yue TL, Chen J, Bao W, Narayanan PK, Bril A, Jiang W, Lysko PG, Gu JL, Boyce R, Zimmerman DM, Hart TK, Buckingham RE, Ohlstein EH. In vivo myocardial protection from ischemia/reperfusion injury by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. Circulation 2001; 104: 2588-2594.
35) Gonon AT, Bulhak A, Labruto F, Sjoquist PO, Pernow J. Cardioprotection mediated by rosiglitazone, a peroxisome proliferator-activated receptor gamma ligand, in relation to nitric oxide. Basic Res Cardiol 2007; 102: 80-89.
36) Palee S, Chattipakorn S, Phrommintikul A, Chattipakorn N. PPARgamma activator, rosiglitazone: Is it beneficial or harmful to the cardiovascular system? World J Cardiol 2011; 3: 144-152.
37) Mersmann J, Tran N, Zacharowski PA, Grotemeyer D, Zacharowski K. Rosiglitazone is cardioprotective in a murine model of myocardial I/R. Shock 2008; 30: 64-68.
38) Yu J, Jin N, Wang G, Zhang F, Mao J, Wang X. Peroxisome proliferator-activated receptor gamma agonist improves arterial stiffness in patients with type 2 diabetes mellitus and coronary artery disease. Metabolism 2007; 56: 1396-1401.
39) Lygate CA, Hulbert K, Monfared M, Cole MA, Clarke K, Neubauer S. The PPARgamma-activator rosiglitazone does not alter remodeling but increases mortality in rats post-myocardial infarction. Cardiovasc Res 2003; 58: 632-637.
40) Gouni-Berthold I, Berthold HK, Weber AA, Ko Y, Seul C, Vetter H, Sachinidis A. Troglitazone and rosiglitazone induce apoptosis of vascular smooth muscle cells through an extracellular signal-regulated kinase-independent pathway. Naunyn Schmiedebergs Arch Pharmacol 2001; 363: 215-221.
41) Geng DF, Wu W, Jin DM, Wang JF, Wu YM. Effect of peroxisome proliferator-activated receptor gamma ligand. Rosiglitazone on left ventricular remodeling in rats with myocardial infarction. Int J Cardiol 2006; 113: 86-91.
42) Singh S, Loke YK, Furberg CD. Long-term risk of cardiovascular events with rosiglitazone: a meta-analysis. JAMA 2007; 298: 1189-1195.
43) Badsha MB, Fu AQ. Learning Causal Biological Networks With the Principle of Mendelian Randomization. Front Genet 2019; 10: 460.
44) Seyed Khoei N, Jenab M, Murphy N, Banbury BL, Carreras-Torres R, Viallon V, Kühn T, Bueno-de-Mesquita B, Aleksandrova K, Cross AJ, Weiderpass E, Stepien M, Bulmer A, Tjønneland A, Boutron-Ruault MC, Severi G, Carbonnel F, Katzke V, Boeing H, Bergmann MM, Trichopoulou A, Karakatsani A, Martimianaki G, Palli D, Tagliabue G, Panico S, Tumino R, Sacerdote C, Skeie G, Merino S, Bonet C, Rodríguez-Barranco M, Gil L, Chirlaque MD, Ardanaz E, Myte R, Hultdin J, Perez-Cornago A, Aune D, Tsilidis KK, Albanes D, Baron JA, Berndt SI, Bézieau S, Brenner H, Campbell PT, Casey G, Chan AT, Chang-Claude J, Chanock SJ, Cotterchio M, Gallinger S, Gruber SB, Haile RW, Hampe J, Hoffmeister M, Hopper JL, Hsu L, Huyghe JR, Jenkins MA, Joshi AD, Kampman E, Larsson SC, Le Marchand L, Li CI, Li L, Lindblom A, Lindor NM, Martín V, Moreno V, Newcomb PA, Offit K, Ogino S, Parfrey PS, Pharoah PDP, Rennert G, Sakoda LC, Schafmayer C, Schmit SL, Schoen RE, Slattery ML, Thibodeau SN, Ulrich CM, van Duijnhoven FJB, Weigl K, Weinstein

SJ, White E, Wolk A, Woods MO, Wu AH, Zhang X, Ferrari P, Anton G, Peters A, Peters U, Gunter MJ, Wagner KH, Freisling H. Circulating bilirubin levels and risk of colorectal cancer: serological and Mendelian randomization analyses. BMC Med 2020; 18: 229.