Original Article

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Diabetes mellitus, glycemic traits, SGLT2 inhibition, and risk of pulmonary arterial hypertension: A Mendelian randomization study

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SUMMARY This study aimed to investigate the causal role of diabetes mellitus (DM), glycemic traits, and sodium-glucose cotransporter 2 (SGLT2) inhibition in pulmonary arterial hypertension (PAH). Utilizing a two-sample two-step Mendelian randomization (MR) approach, we determined the causal influence of DM and glycemic traits (including insulin resistance, glycated hemoglobin, and fasting insulin and glucose) on the risk of PAH. Moreover, we examined the causal effects of SGLT2 inhibition on the risk of PAH. Genetic proxies for SGLT2 inhibition were identified as variants in the *SLC5A2* gene that were associated with both levels of gene expression and hemoglobin A1c. Results showed that genetically inferred DM demonstrated a causal correlation with an increased risk of PAH, exhibiting an odds ratio (OR) of 1.432, with a 95% confidence interval (CI) of 1.040-1.973, and a p-value of 0.028. The multivariate MR analysis revealed comparable outcomes after potential confounders (OR = 1.469, 95%CI = 1.021-2.115, p = 0.038). Moreover, genetically predicted SGLT2 inhibition was causally linked to a reduced risk of PAH (OR = $1.681*10^{-7}$, 95%CI = $7.059*10^{-12}$-0.004, p = 0.002). Therefore, our study identified the suggestively causal effect of DM on the risk of PAH, and SGLT2 inhibition may be a potential therapeutic target in patients with PAH.

Keywords diabetes mellitus, glycemic traits, SGLT2 inhibition, pulmonary arterial hypertension, mendelian randomization

1. Introduction

Diabetes mellitus (DM) is an increasingly common and potentially devastating medical condition, presenting a major public health challenge in the contemporary era (*1*). It is well known that DM results in a range of macro- and microvascular complications (*2*), including coronary heart disease, stroke, or retinopathy (*3*). However, the potential role of diabetes in pulmonary vasculature is underappreciated. Pulmonary arterial hypertension (PAH), as a critical pulmonary vasculature, is characterized by a chronic elevation of pulmonary arterial pressure and the remodeling of pulmonary arteries, which can ultimately lead to heart failure and mortality (*4*). Epidemiologic studies have shown that about 21% to 31% of patients with PAH have diabetes (*5,6*). Besides, patients with type II DM (T2DM) are at a higher risk of PAH independent of traditional risk factors, including smoking, congestive heart failure, coronary artery disease, and hypertension (*7*). However, all these conclusions were drawn from observational studies, which may have been biased by some known and even unknown confounders. Therefore, whether there are causal effects of DM and glycemic traits (including insulin resistance, glycated hemoglobin, fasting insulin, and glucose) on PAH remains unknown.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors represent a category of oral drugs designed to manage type 2 diabetes, with empagliflozin and dapagliflozin being among the most prominent members of this class (*8*). These agents primarily operate by inhibiting insulin resistance, thus modulating glucose metabolism (*8*). Moreover, they offer a range of additional benefits, including anti-inflammatory, antioxidant, and anti-fibrotic effects. Clinical trials examining cardiovascular outcomes of novel antidiabetic drugs, sanctioned by the United States Food and Drug Administration (FDA), have demonstrated that SGLT2 inhibitors

can significantly lower the risk of major adverse cardiovascular events (MACE), such as cardiovascular death, non-fatal stroke, or myocardial infarction (*9-13*). Furthermore, dapagliflozin and empagliflozin have been proven to reduce the likelihood of hospitalization for heart failure in patients with diabetes (*9-11,14*). Empagliflozin was initially approved for use in adult patients with type 2 diabetes in China in 2017. In 2022, the China National Medical Products Administration approved a new indication for empagliflozin in the treatment of adult patients with heart failure, regardless of the presence of diabetes, and reduced ejection fraction.

It is noteworthy that clinical studies examining patients with heart failure specifically excluded those with PAH (*9-11,14*), yet those with pulmonary hypertension (PH) due to left heart disease (PH-LHD) were not excluded from these trials. Consequently, investigators have undertaken additional basic and clinical investigations to explore the potential effects of SGLT2 inhibitors in treating PAH.

2. Research Design and Methods

2.1. Study design

The study design is depicted in Figure 1, which outlines the following steps: 1) identification of genetic variants that act as proxies for the impact of SGLT2 inhibition, 2) selection of DM and glycemic traits (including insulin resistance, glycated hemoglobin, fasting insulin, and glucose) as exposures, 3) designation of PAH as the outcome, 4) execution of a two-sample MR analysis to determine the causal effects of DM, glycemic trait, and SGLT2 inhibition on PAH, and 5) multivariable MR conditioning on potential confounders, including body mass index (BMI), smoking, heart failure (HF), and hypertension. As all the data in this study were acquired from publicly available published databases and were originally approved by their respective ethics committees, with all participants having signed informed consent, this study may be considered exempt from requiring further ethics committee approval.

2.2. Selection and validation for genetic predictors of SGLT2 inhibition

Referring to Min Xu *et al*., we used a similar approach to screen genetic variants associated with SGLT2 inhibitors (*15*). The selection of genetic variants acting as proxies for SGLT2 inhibition involved four steps, as shown in Figure 1A. Firstly, genetic variants associated with *SLC5A2* mRNA expression levels were selected using data from GTEx (*16*) and eQTLGen Consortium (*17*), along with information on the potential functional genes

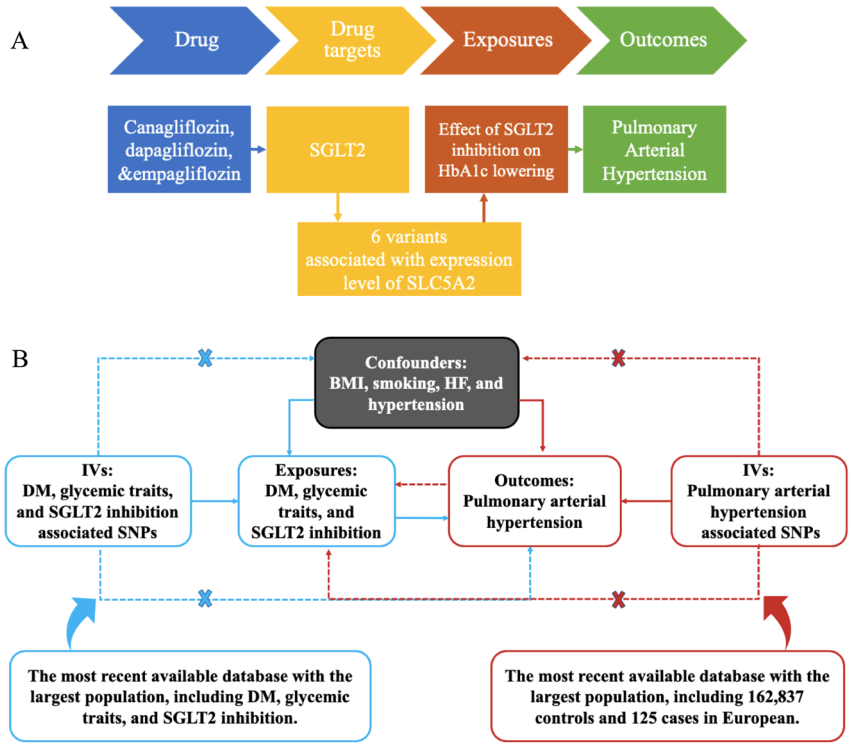

Figure 1. Study design. A: The aim of this study is to explore whether there is a causal effect of DM, glycemic traits, and SGLT2 inhibition (exposures) on PAH (outcomes). The diagram displays the selected drugs and their targets in blue, based on literature evidence. Genetic variants associated with the *SLC5A2* gene, which is the functional gene of SGLT2 inhibitors, are shown in yellow and were obtained using data from GTEx and eQTLGen. The red modules and black arrows represent the MR estimates of the effects of the variants on the HbA1c level, using data from the UK Biobank. Green modules represent selected PAH outcomes. The diagram illustrates the two-step MR models: Step 1 establishes the causal effect of DM, glycemic traits, and SGLT2 inhibition on PAH, while Step 2 establishes the causal effect of PAH on DM, glycemic traits, and SGLT2 inhibition.

of SGLT2 inhibitors. Secondly, variants showing region-wide associations with glycated hemoglobin(HbA1c) were selected using data from a subgroup of unrelated individuals with European ancestry and without diabetes in the UK Biobank (*18*). Thirdly, a genetic colocalization approach was used to verify whether *SLC5A2* and HbA1c share the same causal variant (*19*). Finally, a standard clumping process was carried out to select six genetic variants robustly associated with SGLT2 inhibition *via* HbA1c as genetic predictors for the MR analysis. The strength of the genetic predictors of each tested exposure was estimated using F statistics.

2.3. Selection of DM and glycemic traits

To achieve textual consistency and procure a sufficient quantity of single nucleotide polymorphisms (SNPs), this study has elected to utilize a GWAS significant threshold of $P < 5\times10^{6}$ as instrumental variants (IVs) associated with a given trait. First, we selected 163 variants significantly associated with T2D from a GWAS of 36,219 patients with DM and 182,573 controls (Table 1). The diagnosis of DM was determined through a range of methods, such as physician diagnosis, elevated levels of fasting glucose or HbA1c, self-reported use of DM medications, or ICD coding, either in isolation or in combination, as described in the primary GWAS articles.

Variants associated with insulin resistance were obtained from a GWAS of 37,037 individuals published in 2010 (*20*), which was the latest and largest publicly available GWAS in insulin resistance (Table 1).

Additionally, variants linked to the specific glycemic traits, including fasting insulin, fasting glucose, and HbA1c, were procured from GWAS publications authored by the Meta-Analysis of Glucose and Insulin-related Traits Consortium (MAGIC) investigators (*21*).

2.4. Selection of potential confounders

BMI, smoking, HF, and hypertension were known to be associated with both DM (*22*) and PAH (*4*) and hence, these factors could potentially confound the judgment of the causal relationship between DM and PAH. To reduce the influence of potential confounding factors on the study results, we further explored the causal association between DM and PAH by using multivariate MR analysis, after gradually adjusting for BMI, smoking, HF, and hypertension and simultaneously adjusting for the above factors.

2.5. Study outcomes

Given that MR analyses have explored the causal effects of glycemic traits, and SGLT2 inhibition on DM and recent studies have also revealed the potentially beneficial effects of DM, glycemic traits, and SGLT2 inhibition on PAH, we mainly focused our outcome on PAH. Variants associated with PAH were obtained from a GWAS of 125 cases and 162,837 controls published in 2021, including 16,380,163 SNPs in European populations.

2.6. Single nucleotide polymorphisms selection

The selection of SNPs for instrumental variables followed specific criteria. Firstly, SNPs that showed a significant correlation with our exposures with $P < 5*10^{-6}$ were chosen. Secondly, to ensure the independence of instrumental variables, the selected SNPs were clumped using the 1000 Genomes project European sample data as a reference. A clumping window of 10000 kb and a linkage disequilibrium R^2 threshold of < 0.001 were employed in this process. Lastly, the instrumental strength was evaluated using the F statistic, and weak instruments with an F statistic < 10 were excluded due to bias.

2.7. Statistical analyses

In the realm of MR studies, pleiotropy remains a central consideration, as genetic variants used as instruments may affect the outcome through pathways other than the exposure of interest. If unaccounted for, this phenomenon can introduce bias into the causal inference. Different MR methods have been developed to mitigate this, each based on distinct assumptions about the nature

Table 1. The characteristics of all enrolled traits

Exposures	Cases (N)	Controls (N)	Sample size (N)	Number of SNPs	Year
Diabetes mellitus	36,219	182,573	218,792	16,380,466	2021
Potential Confounders					
Smoking initiation	1,237	359,957	361,194	9,646,741	2018
Body mass index	/	/	51,852	/	2022
Heart failure	1,088	360,106	361,194	9,806,537	2018
Hypertension	1,237	359,957	361,194	9,646,741	2018
Glycemic traits					
Glycated hemoglobin levels	/	/	146,806	30,649,064	2021
Insulin resistance	/	/	37,037	2,435,028	2010
Fasting insulin	/	/	151,013	29,664,438	2021
Fasting glucose	/	/	200,622	31,008,728	2021

and impact of pleiotropy. The inverse variance weighted (IVW) method assumes that pleiotropic effects are balanced and average to zero, an assumption that may not hold in all scenarios.

On the other hand, methods such as MR-Egger allow for the detection and correction of unbalanced pleiotropy by introducing an intercept term, effectively adjusting the estimate if pleiotropic effects are directional. The Weighted Median approach provides a robust median-based estimate as long as at least 50% of the information comes from valid instruments. Furthermore, advanced methods like MR pleiotropy residual sum and outlier (MR-PRESSO) detect and correct for pleiotropic outliers, and the recently developed multivariable MR framework was used to adjust for multiple pleiotropic pathways simultaneously by including multiple confounders. In addition, the Maximum Likelihood Method is employed as an alternative analytical strategy to the IVW approach, particularly in scenarios where the association between the IVs and the exposure is weak, or when there is an overlap of samples within the study (*23*). We employed a multi-step methodology to ascertain the most appropriate and efficacious MR analytical approach for our study. Initially, the best-fitting model was determined by considering heterogeneity through the use of the goodness-of-fit heterogeneity statistic method (*24*). Concurrently, the F statistic was utilized to appraise the strength of the association between IVs and the outcomes of interest, ensuring the selection of robust IVs. Lastly, we leveraged PhenoScanner, an expansive repository containing detailed genotypic and phenotypic association data, to discern whether the chosen SNPs were linked to any potential risk factors, which allowed us to exclude SNPs that exhibited associations with possible confounding factors, thus refining our instrumental variable selection.

A stable causal association was only established if the sensitivity analyses yielded similar results to the IVW method. The leave-one-out analysis was used to evaluate whether a variant drove the correlation between exposure and outcome by removing a single SNP each time. In addition to utilizing PhenoScanner, our analytical approach also incorporated the mr_pleiotropy_test to further ascertain the presence of horizontal pleiotropy. The Cochrane Q statistic was used to evaluate heterogeneity. Should one or more SNPs exert a significant influence on the outcomes, it becomes imperative to exclude these SNPs and reiterate the analysis to arrive at a definitive conclusion (*25-27*). R (4.0.3) and TwoSampleMR version 0.5.6 packages were used for all statistical analyses. The Bonferroni correction was applied in our analysis to account for multiple comparisons, thereby maintaining control over the family-wise error rate by adjusting the significance threshold in accordance with the number of tests conducted. Within the framework of the current MR analysis, a p-value of less than 0.008 (0.05 divided by 6) was deemed significant, whereas a p-value greater than 0.008 but less than 0.05 was indicative of a suggestive association.

3. Results

3.1. Details of the GWAS datasets and selected SNPs for the exposures

As is shown in Figure 1, we explored the causal effect of DM, SGLT2 inhibition, and glycemic traits on the risk of PAH and estimated the verse causal effects of PAH on DM, SGLT2 inhibition, and glycemic traits. The detailed characteristics of all enrolled traits are shown in Table 1. A total of 163 SNPs were significantly associated with DM, with an F value of 21-659 (Table S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*), and 6 SNPs were significantly associated with SGLT2 inhibition (with an F value of 13-64 in Table 2), suggesting all of those SNPs were strong instrumental variants. Similarly, 140 SNPs with glycated hemoglobin (F values of 14-1391 in Table S2, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*), 8 SNPs with insulin resistance (F values of 20-28 in Table S3, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*), 88 SNPs with fasting insulin (F values of 13-173 in Table S4, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*), and 120 SNPs with fasting glucose (F values of 14-1650 in Table S5, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*) were enrolled in the present MR analysis of the causal associations between

Table 2. Genetic variants selected for the Instrument Variables of SGLT2 inhibition

rs ID	Chr.	Position	EA	OA	Beta	Se	P	EAF	Included
rs4488457	16	31659189	T	G	-0.013	0.003	2.90E-07	0.712	Yes
rs8057326	16	31524123	T	C	-0.008	0.002	2.80E-04	0.523	Yes
rs11865835	16	31509816	T	C	-0.011	0.003	1.34E-05	0.248	Yes
rs9930811	16	31400360	A	G	-0.016	0.002	8.96E-12	0.365	No*
rs34497199	16	31551332	C	T	-0.012	0.002	5.98E-07	0.475	Yes
rs35445454	16	31699326	C	T	-0.013	0.002	1.24E-07	0.344	Yes

EA, Effect allele; OA, Other alleles. EAF, Effect allele frequency. *rs9930811 was excluded because this SNP showed significant heterogeneity in the leave-one analysis.

glycemic traits and PAH. In the potential confounders, 50 SNPs with BMI (F values of 20-173 in Table S6, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*), 228 SNPs with smoking (F values of 20-145 in Table S7, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*), 19 SNPs with HF (F values of 20-31 in Table S8, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*), and 13 SNPs with hypertension (F values of 21-29 in Table S9, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*) were included in the causal estimation between potential confounders and PAH.

DM, Diabetes mellitus. BMI, Body mass index. HF, Heart failure. SGLT2, Sodium-glucose cotransporter 2. PAH, Pulmonary arterial hypertension.

3.2. Causal estimation of DM on PAH

In the context of IVW analysis, genetically predicted DM demonstrated a causal association with a 43% elevated risk of PAH, denoted by an odds ratio (OR) of 1.432, and a 95% confidence interval (CI) of 1.040-1.973, with a p-value of 0.028 ($0.008 < p < 0.05$, Figure 2). Nevertheless, statistical significance was not reached in MR Egger's sensitivity analysis (OR = 1.059, 95%CI = 0.619-1.812, $p = 0.863$, Figure 2A) nor in the Weighted Median analysis (OR = 0.945, 95% CI = 0.497-1.798, $p = 0.829$, Figure 2A). The causal estimation between DM and PAH was observed to have no significant pleiotropy (egger intercept = 0.039, $p = 0.146$) nor heterogeneity (Q = 173.804, $p = 0.232$ in the IVW MR heterogeneity test, the scatter plot of Figure 3A, the forest plot of each SNP in Figure S1 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=182*), and the leave-one-out analysis in Figure S2, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=183*), implying that the IVW causal estimation was more reliable. Besides, the results of MR-PRESSO revealed similar results (OR = 1.432, 95%CI = 1.040-1.973, $p = 0.029$) as IVW and no outlier-corrected results due to there being no significant heterogeneity and pleiotropy. The results of the Maximum Likelihood Method (OR = 1.436, 95%CI = 1.037-1.988, $p = 0.030$) were also similar to that of IVW due to the strong association between the IVs and the exposure (all F statistics > 10, Table S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*) and no overlap of samples within two samples. Therefore, DM was causally associated with a 43% higher risk of PAH, which can also be found in the Radial IVW plot (Figure 4).

In the multivariate MR analysis, comparable results were observed after adjusting for smoking (OR = 1.440, 95%CI = 1.017-2.040, $p = 0.039$), BMI (OR = 1.412, 95%CI = 0.972-2.051, $p = 0.069$), HF (OR = 1.397, 95%CI = 1.011-1.931, $p = 0.043$), hypertension (OR = 1.400, 95%CI = 1.007-1.946, $p = 0.045$), and all potential confounders (OR = 1.469, 95%CI = 1.021-2.115, $p = 0.038$, Figure 2). However, it is noteworthy that MR results were no longer significant after adjusting for BMI, suggesting that further investigation is required to determine whether DM increases the risk of PAH through BMI.

3.3. Causal estimation of glycemic traits and SGLT2 inhibition on PAH

Furthermore, we investigated the potential causal association between glycemic traits and PAH, but our IVW analysis and all sensitivity analyses revealed no significant direct causal relationship between them (all $p > 0.05$, Table 3).

Building upon our investigation of DM and glycemic traits, we proceeded to explore the causal relationship between SGLT2 inhibitors and PAH. When we

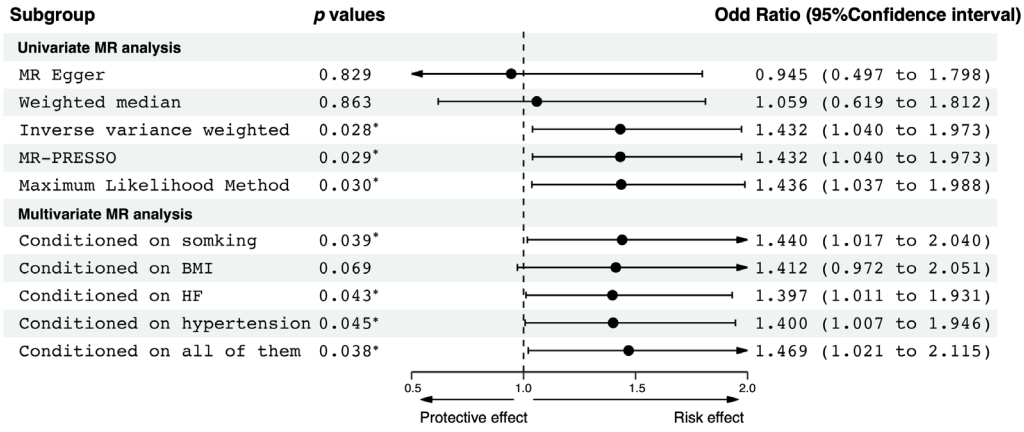

Figure 2. The forest plot for the causal role of diabetes mellitus on PAH. The causal effect of diabetes mellitus on the risk of PAH in different MR analyses and the causal effect of diabetes mellitus on the risk of PAH in the multivariate MR analysis. $^{*}p < 0.05$. The p for Bonferroni adjustment is 0.008 (0.05 divided by 6). MR, Mendelian randomization. PAH, Pulmonary arterial hypertension. MR-PRESSO, Mendelian randomization Pleiotropy RESidual Sum and Outlier.

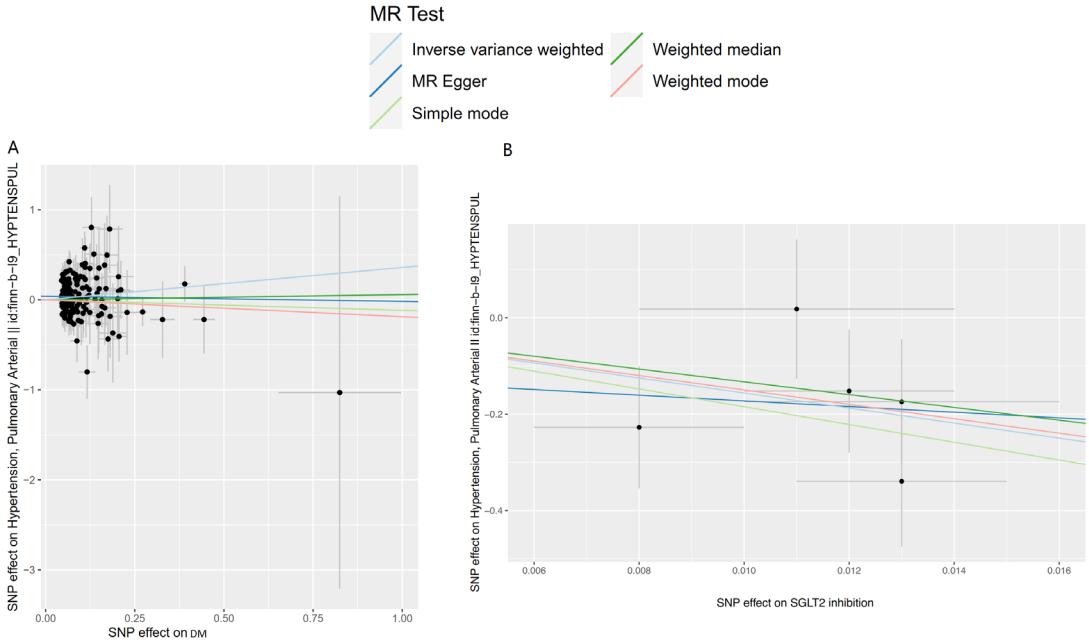

Figure 3. The scatter plot for the causal estimation of diabetes mellitus and SGLT2 inhabitation on PAH. A. the scatter plot for the causal estimation of diabetes mellitus on PAH and B. the scatter plot for the causal estimation of SGLT2 inhabitation on PAH. SGLT2, Sodium-glucose cotransporter 2. PAH, Pulmonary arterial hypertension.

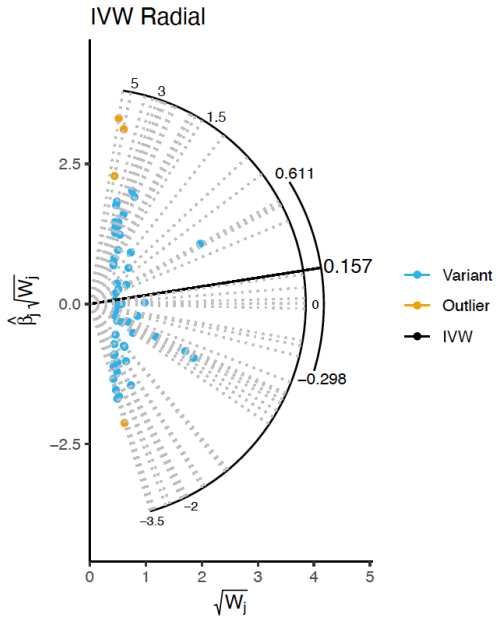

Figure 4. The plots of Radial IVW in exploring the causal associations between diabetes mellitus and pulmonary arterial hypertension.

enrolled all 6 SNPs in the MR analysis, no significant causal association was observed in the IVW analysis (Table S10, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=184*). However, Figure 5A illustrates that upon the exclusion of rs9930811, the forest plots depicting the effects on PAH remained entirely on the left side of the zero line, without crossing it. Conversely, in the leave-one-out analysis for all other SNPs, the forest plots for PAH effects did intersect with the zero line. This pattern suggests that rs9930811 may exhibit heterogeneity in comparison to the other SNPs assessed (Figure 5A).

Therefore, in the formal analysis, we excluded rs9930811, resulting in the inclusion of a total of 5 SNPs in the MR analysis. In the IVW analysis, genetically predicted SGLT2 inhibition was causally associated with a lower risk of PAH (OR = $1.681*10^{-7}$, 95%CI = $7.059*10^{-12}$-0.004, $p = 0.002 < 0.008$, Table 3). In the sensitivity analysis, both MR Egger and Weighted median revealed similar estimations (Table 3). The leave-one-out analysis (Figure 5B), scatter plot (Figure 3B), forest plot (Figure 6A), funnel plot (Figure 6B), and the MR heterogeneity test revealed no significant heterogeneity. Both MR-PRESSO (OR = $1.432*10^{-7}$, 95%CI = $6.222*10^{-12}$-0.003, $p = 0.037 < 0.050$) and the Maximum Likelihood Method (OR = $6.908*10^{-8}$, 95%CI = $9.810*10^{-13}$-0.005, $p = 0.004 < 0.008$) also yielded results consistent with those obtained *via* the IVW analysis in determining this causality. Besides, no pleiotropy was observed in the MR pleiotropy test, suggesting that the results of IVW analysis are robust and reliable.

3.4. Causal estimation of PAH on the risk of DM and glycemic traits

In further analysis, we explored the causal association between PAH and the risk of DM or the levels of glycemic traits. As is shown in Figure 7, no causal associations were observed in the estimation between PAH and the risk of DM or the levels of glycemic traits (all $p > 0.05$).

Table 3. The causal estimation of glycemic traits and SGLT2 inhibition on PAH

Exposure	method	Nsnp	OR (95%CI)	*p* values
Insulin resistance	MR Egger	8	0(0-859.940)	0.283
	Weighted median	8	0.016(0-4.655)	0.147
	Inverse variance weighted	8	0.026(0-2.529)	0.118
	MR-PRESSO	8	0.026(0.002-0.431)	0.038*
	Maximum Likelihood Method	8	0.026(0-2.743)	0.125
Glycated hemoglobin	MR Egger	141	49.577(1.199-2050.744)	0.042*
	Weighted median	141	1.028(0.034-31.423)	0.987
	Inverse variance weighted	141	0.591(0.077-4.548)	0.614
	MR-PRESSO	141	0.591(0.077-4.548)	0.614
	Maximum Likelihood Method	141	0.587(0.075-4.609)	0.613
Fasting insulin	MR Egger	88	1.215(0.001-1061.085)	0.948
	Weighted median	88	0.31(0.009-10.772)	0.515
	Inverse variance weighted	88	0.458(0.046-4.607)	0.508
	MR-PRESSO	88	0.458(0.046-4.607)	0.509
	Maximum Likelihood Method	88	0.461(0.044-4.803)	0.517
Fasting glucose	MR Egger	120	12.796(1.044-156.863)	0.049*
	Weighted median	120	1.633(0.144-18.535)	0.698
	Inverse variance weighted	120	1.323(0.317-5.525)	0.701
	MR-PRESSO	120	1.282(0.308-5.334)	0.733
	Maximum Likelihood Method	120	1.283(0.305-5.396)	0.734
SGLT2 inhibition	MR Egger	5	$0.003(0\text{-}4.108*10^{25})$	0.870
	Weighted median	5	1.681e-07 (0-1.148)	0.052
	Inverse variance weighted	5	$1.681*10^{-7}(7.059*10^{-12}\text{-}0.004)$	0.002*#
	MR-PRESSO	5	$1.432*10^{-7}(6.222*10^{-12}\text{-}0.003)$	0.037*
	Maximum Likelihood Method	5	$6.908*10^{-8}(9.810*10^{-13}\text{-}0.005)$	0.004#

Glycemic traits, including insulin resistance, glycated hemoglobin levels, fasting insulin, and fasting glucose. PAH, pulmonary arterial hypertension. OR, odd ratio. CI: Confidence interval. SGLT2: Sodium-glucose cotransporter 2. *$p < 0.05$. #$p < 0.008$ (0.05 divided by 6 in the Bonferroni adjustment). MR-PRESSO, Mendelian randomization Pleiotropy RESidual Sum and Outlier.

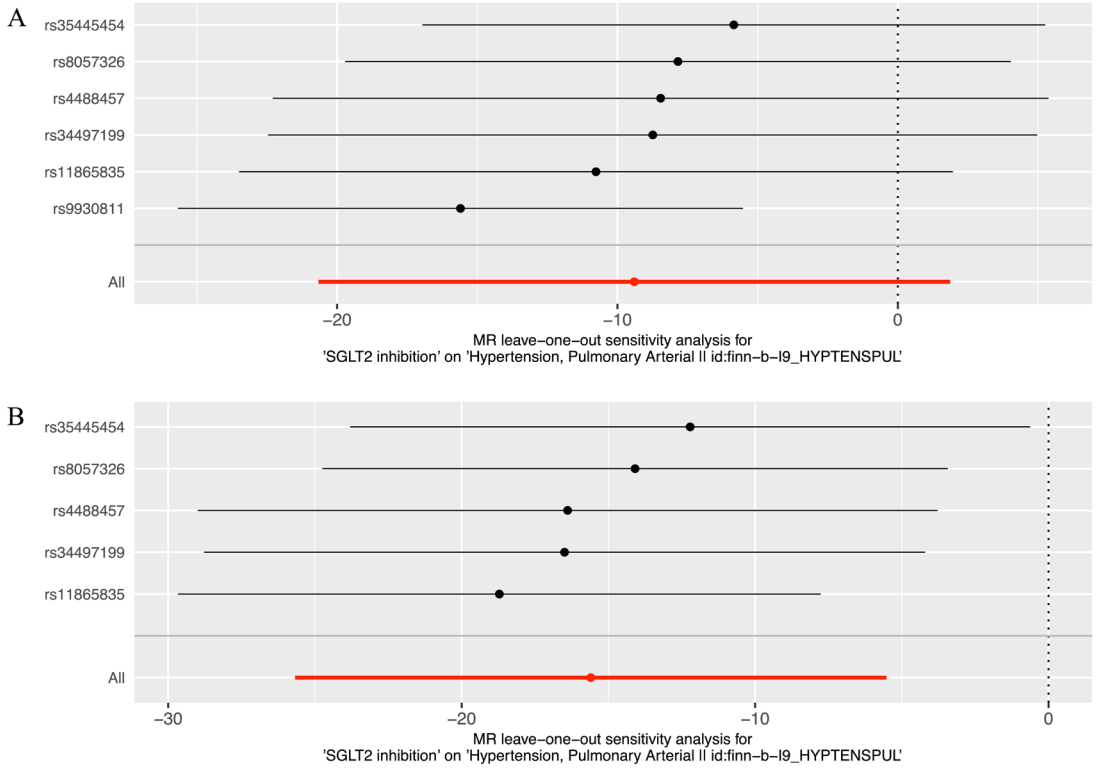

Figure 5. Mendelian randomization leave-one-out sensitivity analysis for SGLT2 inhibition on pulmonary arterial hypertension. A. The leave-one-out sensitivity analysis of all 6 variants showed significant heterogeneity when leaving the rs9930811. B. When rs9930811 was excluded, sensitivity analysis was performed again and there was no significant heterogeneity between SNPs.

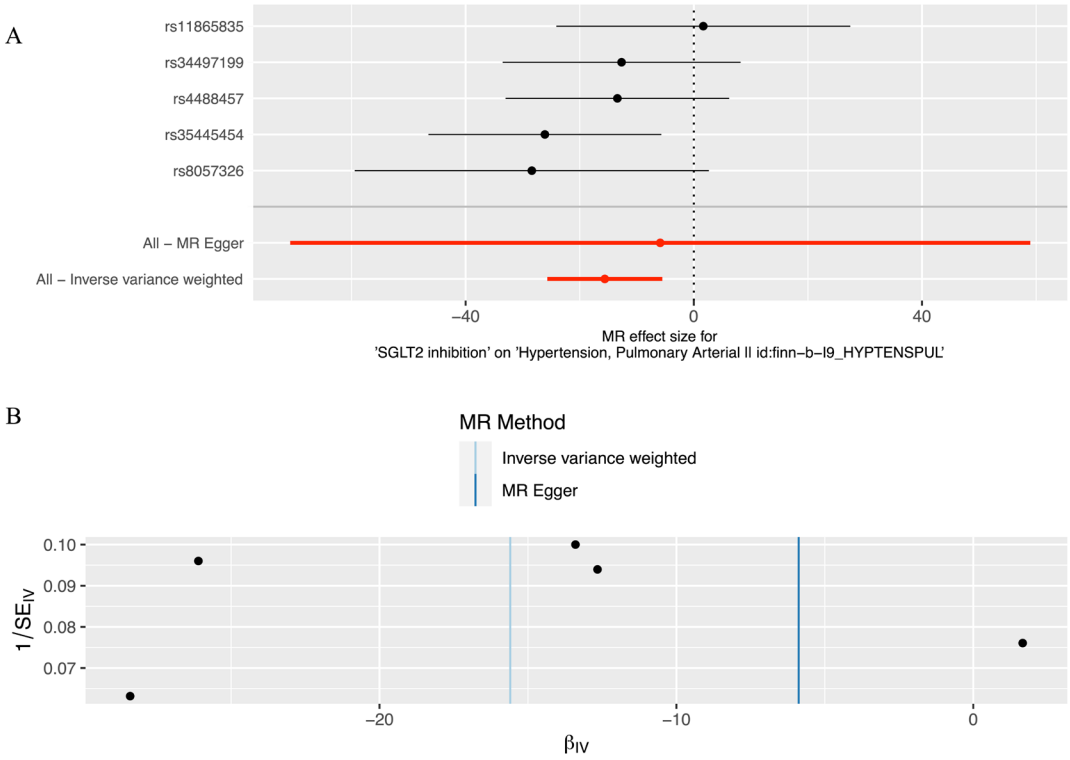

Figure 6. The sensitivity analysis for the causal association between SGLT2 inhibition and the risk of PAH. A. the forest plot for the causal estimation of SGLT2 inhibition on PAH, B. the funnel plots for the causal effect of SGLT2 inhibition on PAH. SGLT2, Sodium-glucose cotransporter 2. PAH, Pulmonary arterial hypertension.

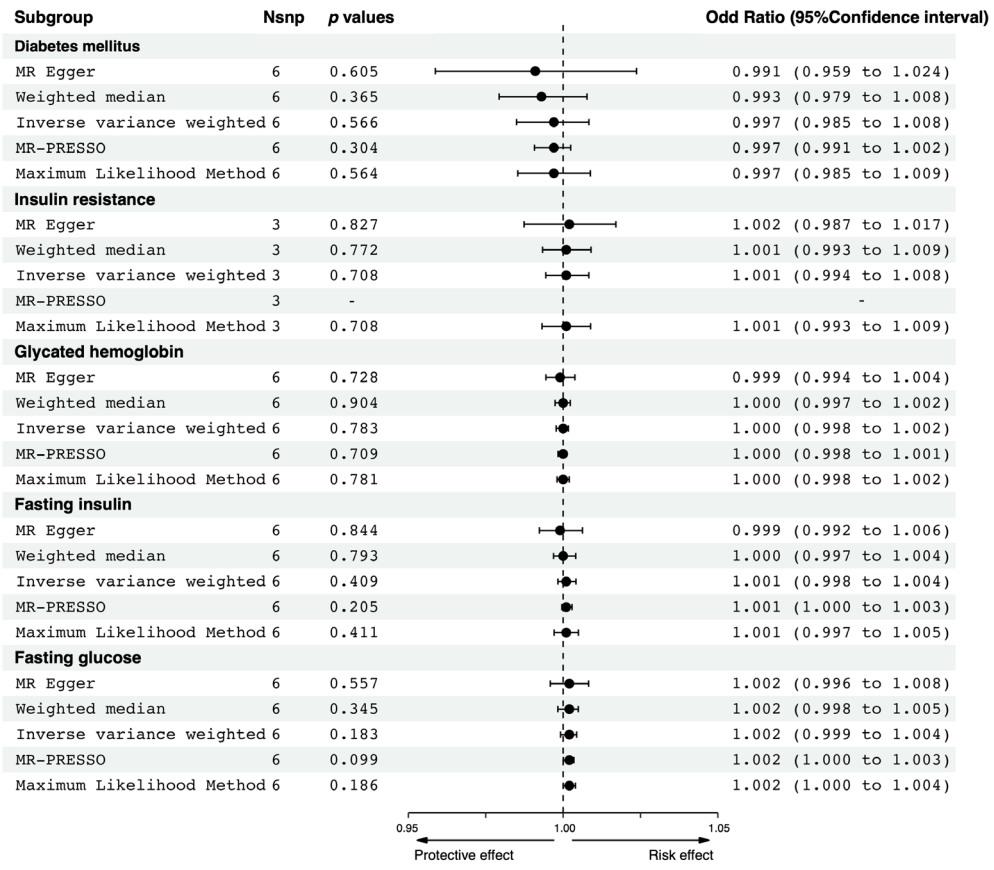

Figure 7. The MR analysis for the causal effect of PAH on the risk of DM and the levels of glycemic traits. PAH, Pulmonary arterial hypertension. MR, Mendelian randomization. The *p* for Bonferroni adjustment is 0.008 (0.05 divided by 6).

4. Discussion

In the present MR study, we found potential evidence of the causal roles of genetically predicted DM and SGLT2 inhibition in the risk of PAH. For the association between DM and PAH, the relationship attenuated when we included BMI in the analyses, suggesting that the causal effects, if real, might be partially mediated by BMI. We did not find any association between genetic determinants of IR, fasting insulin, fasting glucose, or HbA1c and PAH. These findings shed additional light on the relationships between DM, SGLT2 inhibition and the risk of PAH, supporting the potential implication of SGLT2 inhibition in patients with DM to prevent the development of PAH, even in PAH without DM to treat them.

Diabetes was a common comorbidity in patients with PAH, accounting for 21% to 31% (*5,6*). Besides, patients with chronic obstructive pulmonary disease (COPD) and diabetes exhibit more severe PH than patients with COPD alone (*28*). All of these observational studies suggested potential causal associations between DM and PAH. However, many known or unknown confounders may bias the conclusion of observational studies. Therefore, the causal association between DM and PAH remains unknown. As far as I am concerned, this is the first study to systematically investigate the causal effect of DM, glycemic traits, and SGLT2 inhibition on the risk of PAH.

This MR study revealed that patients with DM were causally associated with a 43% higher risk of PAH, which was consistent with observational studies (*5,6*). Besides its impact on morbidity, DM can exacerbate the severity of PAH and adversely affect the prognosis of patients (*6,28*) . Although managed similarly, survival in patients with DM was significantly worse than those with PAH alone and persisted as such even after statistical adjustment (*6*). Animal studies have demonstrated that diabetes contributes to PH not only by inducing left-heart dysfunction but also through direct effects of hyperglycemia on the pulmonary vasculature, which may also elucidate why patients suffering from COPD and diabetes exhibit more severe PH than patients with COPD alone (*28*). Notably, the causal association between DM and PAH was not significant after adjusting the BMI. However, the role of obesity in PAH has not yet reached a unified consensus. A cohort study of adults with PAH from the Pulmonary Hypertension Association Registry revealed that patients with overweight or obese had a worse health-related quality of life despite better transplant-free survival compared with normal-weight patients (*29,30*). Therefore, the relationship between BMI and PAH and whether the causal effect of DM on the risk of PAH was mediated by BMI needs further investigation.

Similarly, IR has also been associated with PAH in observational studies (*31*). Evidence suggests that IR is more prevalent among females with PAH compared to the general population, suggesting that insulin resistance may serve as a novel risk factor or disease modifier that could impact the survival of patients with PAH (*31*). Besides, studies have suggested that ET-1 antagonists may exert some of their effects on the pulmonary vasculature *via* IR pathways (*32,33*). Therefore, we also explored the causal effect of glycemic traits on the risk of PAH. Since glycemic traits are continuous variables, positive results were not observed as in DM, indicating that a single standard deviation change in glycemic traits (such as IR, fasting insulin, fasting glucose, and HbA1c) is not significant enough to increase the risk of PAH. However, DM is a categorical variable and has been found to significantly increase the risk of PAH.

SGLT2 inhibitors are antihyperglycemic agents that have been approved for use in patients with diabetes to lower blood glucose levels and reduce cardiovascular risk. In this study, we also found a causal effect of SGLT2 inhibitors on a lower risk of PAH, implying that SGLT2 inhibitors may contribute to reducing the risk of PAH. An *in vitro* analysis of pulmonary and coronary arteries in diabetic mice revealed that inhibition of the SGLT enzyme with either a nonspecific SGLT inhibitor or the SGLT2-specific inhibitor canagliflozin resulted in targeted dilation of pulmonary arteries (*34*). Biswajit Chowdhury *et al*. investigated the potential therapeutic impact of empagliflozin on rats with PAH by constructing a rat model of monocrotaline (MCT)-induced PAH (*35*). The results demonstrated that treatment with empagliflozin could considerably reduce mean pulmonary artery pressure and right ventricular systolic pressure in the MCT-induced PAH rats regarding hemodynamics (*35*). Pathologically, it could decrease pulmonary vascular remodeling, right ventricular hypertrophy, and fibrosis, thereby significantly decreasing the mortality rate of the PAH rats (*35*).

In addition to fundamental research, clinical investigators have delved into the impact of empagliflozin on pulmonary arterial stress. In Japan, a randomized, controlled, open-label trial was conducted to assess the effects of dapagliflozin treatment on the development of exercise-induced PH (post-exercise echocardiographic right ventricular (RV) systolic pressure (RVSP) of 50 mmHg) in 78 patients with type 2 diabetes, hypertension, and/or stable ischemia with normal EF at baseline. The trial excluded patients with advanced heart failure (New York Heart Association class 3 or 4 or any previous hospitalization for heart failure) or patients with resting RVSP >50 mmHg. An investigator-initiated, randomized, multicenter, double-blind, placebo-controlled trial named EMBRACE-HF (Empagliflozin assessed by measuring the effect on hemodynamics in patients with heart failure) (*11*) demonstrated that empagliflozin (10 mg/day) significantly reduced PA diastolic blood pressure using an implanted pulmonary artery (PA) pressure transducer (CardioMEMS), with effects beginning at Week 1 and amplifying over time. This result was consistent with PA systolic pressure and PA mean pressure. Additionally, a study by Klara Kirschbaum *et al*. further confirmed the

reliability and repeatability of the above study findings through retrospective analysis of 17 patients with heart failure or diabetes (*9*). After ten weeks of SGLT2 inhibitor treatment (empagliflozin or dapagliflozin), PA systolic blood pressure (-3.59 ± 1.55 mmHg; P = 0.034), mean pressure (-3.06 ± 1.22 mmHg; P = 0.014), and diastolic blood pressure (-2.65 ± 0.98mmHg; P = 0.008), and the change in PAP were already present after three weeks of treatment and increased over time. It is noteworthy that the use of diuretics and other heart failure medications did not change significantly during the observation period. Although these three human-based clinical studies have some methodological limitations, they suggest that SGLT2 inhibitors may be effective in addressing myocardial dysfunction and pulmonary vascular disease in patients with PAH and PH-LHD.

Both fundamental and clinical studies corroborate the potential of SGLT2 inhibitors in reducing PA pressure. However, the aforementioned clinical trials were conducted in patients with heart failure or diabetes, and there is a dearth of effective data regarding the direct application of SGLT2 inhibitors in patients with PAH. Therefore, this study was designed to evaluate the causal effect of SGLT-2 inhibitors in PAH patients using MR. The conclusion of this MR study is in line with what was drawn from the aforementioned clinical and fundamental studies, indicating that SGLT-2 inhibitors are expected to emerge as a new treatment alternative for PAH patients and offer new insights for research into the mechanisms of PAH.

MR is a widely used method in epidemiological research to investigate the causal relationship between exposure and outcome by exploiting genetic variants as instrumental variables. While MR has several advantages over traditional observational studies, such as reducing confounding and reverse causation, it also has limitations. One of the main limitations of MR analysis is the assumption that genetic variants used as IVs only affect the outcome through the exposure of interest and not through other pathways. Violation of this assumption, known as pleiotropy, can result in biased estimates of the causal effect. In this study, we have conducted several sensitivity analyses to confirm no pleiotropy exists. Additionally, in our investigation, both MR Egger's sensitivity analysis and the weighted median method indicated a trend congruent with the IVW analysis, yet they fell short of achieving statistical significance. Consequently, we employed a variety of methodologies to ascertain the most appropriate MR analysis technique, ultimately determining that IVW was the superior one. Furthermore, the results obtained from IVW were comparable to those derived from MR-PRESSO and the Maximum Likelihood Method, which bolsters the credibility of our findings. The sample size and the strength of the genetic instruments used are very important in the MR analysis. Therefore, the sample size of included trials may be a limiting factor. Future studies should include larger sample sizes and use the application of novel MR methodologies that are emerging in the field to further validate our conclusions. Lastly, it is notable that this MR was finished based on European populations and whether the conclusion can be generalized to other populations needs further study (*36*).

In summary, this is the first study to systematically explore the causal associations between DM, glycemic traits, SGLT2 inhibition, and the risk of PAH. Our results suggested that DM is suggestively associated with PAH, and SGLT2 inhibition may be a potential therapeutic target in patients with PAH.

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