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Genetic association of glycemic traits and antihyperglycemic agent target genes with the risk of lung cancer: A Mendelian randomization study

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ABSTRACT

Aims: To evaluate the potential causal effect of glycemic traits on lung cancer and investigate the impact of antihyperglycemic agent-target genes on lung cancer risk.
Methods: Genetic variants associated with glycemic traits, antihyperglycemic agent-target genes, and lung cancer were extracted from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC), expression quantitative trait loci (eQTLs), protein quantitative trait loci (pQTLs), and the International Lung Cancer Consortium (ILCCO), respectively. Mendelian randomization (MR) analyses were performed to examine the associations of glycemic traits and antihyperglycemic agent-target genes with lung cancer. Mediation analysis was conducted to explore whether overweight operated as a mediator between antihyperglycemic agents and lung cancer outcomes.
Results: Genetically determined glycated hemoglobin A1c levels were associated with squamous cell lung cancer (OR = 1.78; 95 % CI, 1.08–2.92; $p = 0.023$). The *PRKAB1* gene (the target of metformin) was associated with a lower risk of developing lung adenocarcinoma (OR = 0.85; 95 % CI, 0.76–0.96; $p = 0.006$). Further mediation analyses did not support overweight as a mediator between *PRKAB1* activation and lung adenocarcinoma.
Conclusion: Our analyses suggest an association of genetically determined abnormal glycemic traits with squamous cell lung cancer. The potential association between *PRKAB1* activation and a reduced risk of developing lung adenocarcinoma appears to be independent of the anti-obesity effects of metformin, suggesting that *PRKAB1* activation may have a direct protective effect on lung adenocarcinoma development.

1. Introduction

According to Cancer Statistics 2023, lung cancer is the leading cause of cancer death in both men and women aged 50 years and older [1]. Non-small cell lung cancer (NSCLC), including squamous cell carcinoma and adenocarcinoma, represents approximately 90 % of all lung cancers [2,3]. Although advances in earlier detection and therapeutic development have decreased the mortality of lung cancer, the 3-year survival for lung cancer patients is approximately 30 % [1]. In addition, the heterogeneity of cancer subtypes, drug resistance, and chemotherapeutic side effects hinder the effective treatment of lung cancer [4–6]. Therefore, it is necessary to identify and modify lung cancer risk factors early in high-risk populations to reduce the disease burden. In the United States, it is predicted that approximately 81 % of lung cancer deaths in 2023 will be directly caused by cigarette smoking [1]. Notably, metabolic risks, such as high body mass index (BMI), fasting glucose and glycated hemoglobin A1c (HbA1c), should not be undervalued in the prevention of lung cancer [7,8].

Previous observational studies have observed that abnormal glucose metabolism is a risk factor for lung cancer [9–12]. The UK Biobank study published in 2024, including 331,877 participants, indicated that high HbA1c concentration was associated with an increased risk of lung cancer during the 10.9-year follow-up period [7]. A study using nationally representative data from the Korean National Health Insurance

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System (KNHIS) published in 2021 reported that the highest variabilities in fasting glucose increased the risk of developing lung cancer even after adjustment for baseline fasting glucose, weight, systolic blood pressure, and total cholesterol [10]. Similarly, the results of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study published in 2017 suggested that higher fasting insulin concentrations and the presence of insulin resistance were associated with an elevated risk of lung cancer development [12]. However, most previous observational studies focused solely on the association of glycemic traits with lung cancer without distinguishing between different types of lung cancer, such as squamous cell lung cancer and lung adenocarcinoma, which have different underlying mechanisms.

Mendelian randomization (MR) has emerged as a powerful method using genetic variants as instruments to estimate causal relationships between exposures and outcomes, which can overcome the impact of potential confounding and reverse causality [13]. A recent MR study published in 2023 did not detect a significant association of fasting glucose and HbA1c with lung cancer using univariable and multivariable MR (MVMR) analyses in either East Asians or Europeans [14]. However, this study did not evaluate the influence of fasting insulin on lung cancer. Therefore, the current study aims to fill this gap by assessing the relationships of multiple glycemic traits, including fasting glucose, fasting insulin and HbA1c, with overall lung cancer as well as lung adenocarcinoma and squamous cell lung cancer.

Given that abnormal glucose metabolism is closely associated with lung cancer risk, it prompts consideration for using antihyperglycemic agents in preventive therapeutic efficacy. However, previous observational studies provided inconsistent evidence regarding the association between antihyperglycemic agents and lung cancer [15–18]. A cohort study published in 2021, using the KNHIS database, found that metformin use in the group with diabetes had a protective effect on lung cancer incidence compared with the group without diabetes [16]. Conversely, a meta-analysis published in 2023, involving studies from 2011 to March 2021, observed that biguanide were not associated with lower risks of lung cancer [19]. Residual confounding, sample size and reverse causality may contribute to inconsistent results in previous observational studies. MR analysis has gained traction in various fields, including drug repurposing and drug target development [20–23]. However, a previous MR analysis used growth differentiation factor 15 (GDF15), which is strongly associated with metformin use, failed to establish causality between metformin use and lung cancer incidence [24]. Moreover, previous studies primarily examined the effects of individual antihyperglycemic agent, particularly metformin, on the risk of lung cancer, neglecting the associations of other types of glucose-lowering medications with lung cancer and its subtypes. Hence, a comprehensive MR study is necessary to systematically investigate the association between all types of antihyperglycemic agents and the risk of lung cancer.

2. Methods

2.1. Study design

This MR study was based on publicly available summary-level data from genome-wide association studies (GWASs), expression quantitative trait loci (eQTLs) and protein quantitative trait loci (pQTLs) studies. First, a two-sample MR analysis was performed to investigate the causal effects of glycemic traits (including fasting glucose, fasting insulin, and HbA1c) on lung cancer and its subtypes (including squamous cell lung cancer and lung adenocarcinoma). Second, summary data-based MR (SMR) analyses were conducted to explore the associations of antihyperglycemic agents with the risk of developing lung cancer. Third, antihyperglycemic agent-target genes that achieved suggestive evidence were further tested using pQTLs in blood plasma and Genotype-Tissue Expression (GTEx) project data in relevant tissues. Finally, a two-step MR study was further used to estimate whether overweight operated as a mediator between antihyperglycemic agents and lung cancer. The overall design and key assumptions of this study are shown in Fig. 1. The reporting guidelines follow the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) statement [25]. Ethical approval can be found in the original GWASs, eQTLs and pQTLs.

2.2. Genetic instrument selection

Summary-level genetic data for fasting glucose, fasting insulin and HbA1c were derived from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) involving approximately 200,000 participants of European ancestry that was released in 2021 [26]. Fasting glucose and fasting insulin analyses were adjusted for body mass index (BMI). Details of the GWASs included in our study are presented in Table 1. Genetic variants associated with fasting glucose, fasting insulin and HbA1c at the genome-wide significance level ($p < 5 \times 10^{-8}$) were selected and filtered for linkage disequilibrium (LD) coefficients (r^2) of less than 0.001 to ensure that the instrumental variables (IVs) were independent [27]. Palindromic SNPs with intermediate allele frequencies were removed [27]. Finally, radial MR was conducted to detect and remove outliers by setting a threshold for identifying outliers (0.05 in

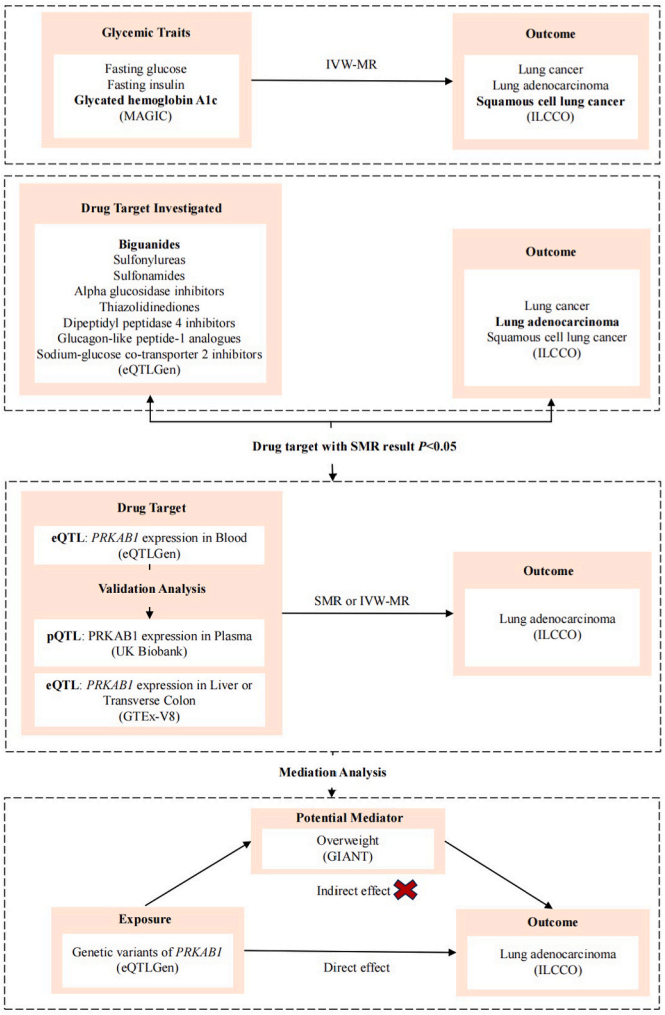

Fig. 1. Study design overview and key assumptions. MAGIC, Meta-Analyses of Glucose and Insulin-related traits Consortium; ILCCO: International Lung Cancer Consortium; GIANT, Genetic Investigation of Anthropometric Traits; MR, mendelian randomization; SMR, summary-data-based MR; IVW, inverse-variance weighted; pQTL, protein quantitative trait loci; eQTL, expression quantitative trait loci.

Table 1
Data description of contributing GWAS summary-level data.

GWASs	Resource	Sample size	Population ancestry	Data download
Glycemic Traits				
Fasting insulin	MAGIC	Number of participants: 151,013	European	https://magicinvestigators.org/downloads/
Fasting glucose	MAGIC	Number of participants: 200,622	European	https://magicinvestigators.org/downloads/
Glycated hemoglobin A1c	MAGIC	Number of participants: 146,806	European	https://magicinvestigators.org/downloads/
Lung Cancer				
Lung cancer	ILCCO	Number of cases: 11,348 Number of controls: 15,861	European	https://gwas.mrcieu.ac.uk/datasets/ieu-a-966/
Lung adenocarcinoma	ILCCO	Number of cases: 3442 Number of controls: 14,894	European	https://gwas.mrcieu.ac.uk/datasets/ieu-a-965/
Squamous cell lung cancer	ILCCO	Number of cases: 3275 Number of controls: 15,038	European	https://gwas.mrcieu.ac.uk/datasets/ieu-a-967/

GWASs: genome-wide association studies; MAGIC: Meta-Analyses of Glucose and Insulin-related traits Consortium; ILCCO: International Lung Cancer Consortium.

our model) and using modified second-order weights [28]. The remaining SNPs were used as instruments to perform two-sample MR analyses. The detailed information for genetic instruments of fasting glucose, fasting insulin and HbA1c is shown in Tables S1–S3.

Eight classes of antihyperglycemic agents were included in our study: biguanides, sulfonylureas, sulfonamides (heterocyclic), alpha-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase 4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) analogues, and sodium-glucose cotransporter 2 (SGLT2) inhibitors. We used the DrugBank database (DrugBank Online | Database for Drug and Drug Target Info) to identify the targets of these drugs (Table S4). The associations of genetically determined antihyperglycemic agents with lung cancer outcomes were assessed by using selected *cis*-eQTLs as instruments. Summary-level data for the *cis*-eQTLs were obtained from the eQTLGen Consortium (eQTLGen - *cis*-eQTLs) or GTEx-V8 (https://gtexprotal.org/). The most significant *cis*-eQTL was selected as a genetic instrument to conduct SMR analysis. Genetic variants (±200 kb of the gene location) associated with target genes at the genome-wide significance level ($p < 5 \times 10^{-8}$) were identified. They were further clumped to an LD threshold of $r^2 < 0.3$ and were selected as proxies for antihyperglycemic agent-target genes to perform a two-sample MR analysis (Table S5). For drug targets that achieved suggestive associations for the risk of developing lung cancer using eQTLGen Consortium data, we further performed a two-sample MR analysis to validate associations using selected pQTLs from UK Biobank (34,557 European participants) (https://metabolomips.org/ukbbpgwas/) (Table S6). Additionally, we conducted SMR analyses to validate associations in relevant tissues using GTEx-V8 data. Because the practicable genetic instruments for *ABCC8, KCNJ8, KCNJ1, SI,* and *AMY2A* were not found from eQTLs, seven classes of antihyperglycemic agents and twelve target genes were included in the final analyses.

2.3. Data sources for outcomes

International Lung Cancer Consortium (ILCCO) data were accessed for GWAS lung cancer (11,348 cases and 15,861 controls) and its subtypes [including squamous cell lung cancer (3275 cases and 15,038 controls) and lung adenocarcinomas (3442 cases and 14,894 controls)] [29] (Table 1).

2.4. Statistical analysis

2.4.1. Analyses of genetically determined glycemic traits and lung cancer outcomes

The inverse-variance weighted (IVW) model (≥3 SNPs) was conducted as the primary statistical method to identify the causality between genetically predicted glycemic traits and lung cancer outcomes [30]. Additionally, other statistical methods were also performed to estimate confounding by pleiotropy, including Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO), weighted median, simple mode and weighted mode. To ensure the robustness of the results, several sensitivity analyses were conducted by using Cochran's Q test based on the IVW method and MR−Egger regression. The F-statistic was calculated for each selected IV to evaluate instrument strength. If the value was below 10, it was considered indicative of a weak IV [30].

2.4.2. Analyses of drug target gene expression and lung cancer outcomes

The SMR approach was conducted as the main MR analysis to investigate the association between genetically determined antihyperglycemic agents and lung cancer outcomes. The SMR method can be used to evaluate the effects of gene expression on complex traits using summary data from eQTLs and GWASs [31]. The heterogeneity in dependent instruments (HEIDI) test was performed to determine whether the observed association between gene expression and outcome was due to a linkage scenario, and a p value of less than 0.05 for the HEIDI test indicates that the association may be due to linkage. Second, to validate the robustness of the suggestive results, we additionally performed the IVW model and four other models. Cochran's Q statistic, MR−Egger and F-statistic were conducted to evaluate heterogeneity, pleiotropy and weak instrument bias, respectively. For drug targets that reached suggestive evidence for the risk of developing lung cancer in both SMR and IVW-MR analyses, we used pQTLs and GTEx-V8 data for the relevant tissues to validate the associations.

2.4.3. Mediation analysis

For suggestive associations between antihyperglycemic agents and lung cancer outcomes, a two-step MR study was applied to evaluate whether overweight operated as a mediator. The first step was to identify the causal association of genetically proxied antihyperglycemic agent-target genes with overweight. The second step was to evaluate the causal effect of overweight on lung cancer based on an univariable MR approach. The total effect was then divided into a direct effect (the effect of antihyperglycemic agents on lung cancer independent of being overweight) and an indirect effect (the effect of being overweight on lung cancer). The GWASs for overweight (93,015 cases and 65,840 controls) were obtained from the Genetic Investigation of Anthropometric Traits (GIANT) consortium (GIANT consortium data files - Giant Consortium (broadinstitute.org). The control individuals had a BMI <25 kg/m^2, and overweight was defined as a BMI ≥25 kg/m^2 [32]. Genetic variants associated with overweight at the genome-wide significance level were identified and clumped to an LD threshold of $r^2 < 0.001$ to perform MR analysis. The IVW model was the primary statistical method. Furthermore, to validate the mediation effect of overweight, SMR analyses were used to evaluate the causality between genetically predicted use of antiobesity drugs and lung cancer outcomes. Gene targets of antiobesity drugs were also identified using the DrugBank database (Table S7).

Bonferroni correction was used to adjust for multiple testing. For the association of glycemic traits with lung cancer outcomes, strong

evidence was defined as $p < 0.0056$ (3 exposures and 3 outcomes), and suggestive evidence was defined as $0.0056 \leq p < 0.05$. For the association between antihyperglycemic agents and lung cancer outcomes, strong evidence was defined as $p < 0.0014$ (12 exposures and 3 outcomes), and suggestive evidence was defined as $0.0014 \leq p < 0.05$. For the association of antiobesity drugs with lung cancer, strong evidence was defined as $p < 0.0036$ (14 exposures and 1 outcome), and suggestive evidence was defined as $0.0036 \leq p < 0.05$. For the other analyses, an observed two-sided $p < 0.05$ was considered indicative of statistical significance. Statistical analyses were performed using the TwoSampleMR (version 0.5.6), MR-PRESSO (version 1.0), and RadialMR (version 1.0) packages in R (version 4.2.1) and SMR software (version 1.3.1) (SMR | Yang Lab (westlake.edu.cn)).

3. Results

3.1. Causal effects of glycemic traits on lung cancer outcomes

Among fasting insulin, fasting glucose, and HbA1c, we found that only genetically determined HbA1c levels were suggestively associated with an increased risk of squamous cell lung cancer in the IVW-MR results [odds ratio (OR) = 1.78; 95 % confidence interval (CI), 1.08–2.92; $p = 0.023$] (Fig. 2 and Table S8). A nonsignificant association was found between either fasting insulin or fasting glucose and lung cancer outcomes. The results of the MR-PRESSO method were consistent with the IVW-MR results (Table S8). In sensitivity analyses, F-statistics for all IVs were over 10, avoiding the existence of weak instrumental bias (Tables S1–S3). Heterogeneity and pleiotropy were not observed with Cochran's Q test (all $p > 0.05$) or the MR−Egger intercept test (all p of intercept >0.05) (Table S8).

3.2. Causal effects of antihyperglycemic agents on the risks of lung cancer outcomes

A total of 165, 1362, 764, 101, 232, 134, 331, 186, 1127, 166, 1019, and 13 *cis*-eQTLs were selected from eQTLGen for the antihyperglycemic agent-target genes *DPP4, ETFDH, GAA, GANAB, GANC, GLP1R, KCNJ11, MGAM, PPARG, PRKAA1, PRKAB1, and SLC5A2,* respectively. The SMR analyses using the most significant *cis*-eQTL in blood as a proxy of exposure found that *PRKAB1* of metformin was the only drug target suggestively associated with a lower risk of developing lung adenocarcinoma (OR = 0.85; 95 % CI, 0.76–0.96; $p = 0.006$) (Fig. 3 and Table S9). Other genetic instruments of drug targets did not show causal effects on lung cancer outcomes (Tables S9–S11). In sensitivity analyses, the F-statistics for selected *cis*-eQTLs were >30. The HEIDI test indicated that no associations were due to linkage ($p > 0.05$) (Tables S9–S11).

The IVW-MR analysis using 36 selected significant *cis*-eQTLs and 4 pQTLs also provided suggestive evidence for the effect of *PRKAB1* expression on lung adenocarcinoma (OR = 0.91; 95 % CI, 0.85–0.98; $p = 0.009$) and (OR = 0.72; 95 % CI, 0.56–0.93; $p = 0.012$), which was consistent with the SMR result (Tables S12–S13). In sensitivity analyses, weak instrumental bias, pleiotropy and heterogeneity were not found with F-statistics, the MR−Egger intercept test (all p of intercept >0.05) or Cochran's Q test (all $p > 0.05$) (Tables S5–6 and Tables S12–S13).

Furthermore, genetic variants related to *PRKAB1* expression in liver and transverse colon tissues were applied as IVs for further validation using SMR analyses. Seven *cis*-eQTLs were identified from GTEx-V8 data for the *PRKAB1* gene in the liver and transverse colon. The results from the SMR analyses found a causal relationship between higher expression of the *PRKAB1* gene and a lower risk of developing lung adenocarcinoma in both the liver (OR = 0.88; 95 % CI, 0.80–0.97; $p = 0.010$) and transverse colon (OR = 0.69; 95 % CI, 0.52–0.92; $p = 0.010$) (Table S14). In sensitivity analyses, the F-statistic for rs11064881 was >50. The HEIDI test showed that no associations were due to linkage ($p > 0.05$) (Table S14).

3.3. Mediation analysis

The two-step MR analysis did not provide evidence that overweight was a mediator between metformin use and lung adenocarcinoma. The results of IVW-MR analyses showed that genetically determined *PRKAB1* expression was not associated with overweight (OR = 0.98, 95 % CI, 0.93–1.03, $p = 0.465$), and overweight was not associated with lung adenocarcinoma risk (OR = 0.91, 95 % CI, 0.74–1.10, $p = 0.327$)

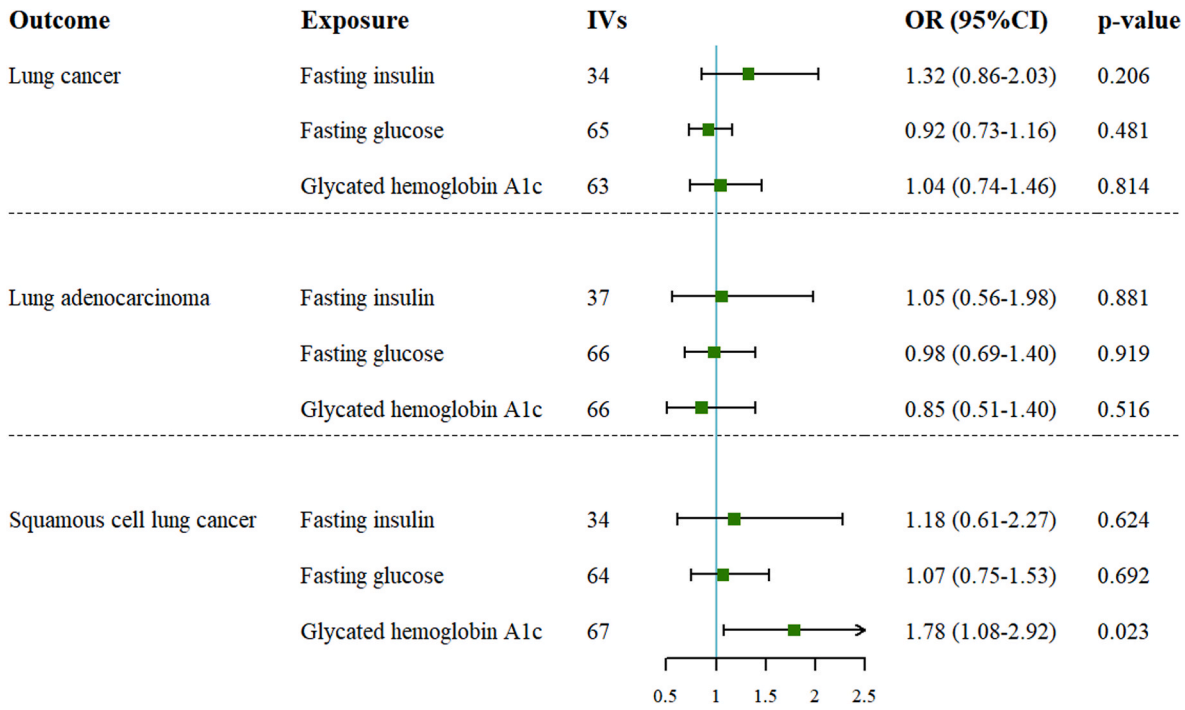

Fig. 2. IVW-MR analyses for association of glycemic traits with the risks of lung cancer outcomes. IVW, Inverse-variance-weighted; IVs: instrumental variables; MR, Mendelian randomization; OR, odds ratio; CI, confidence interval.

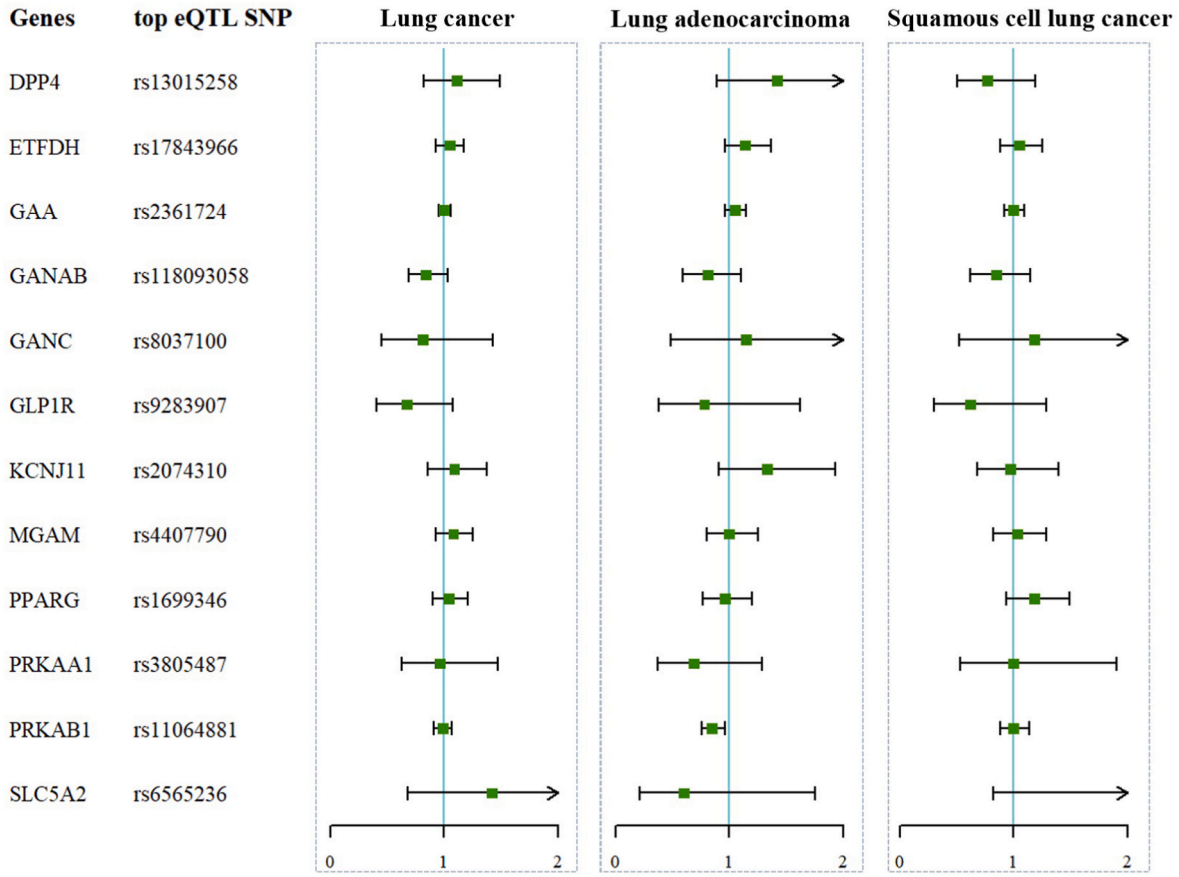

Fig. 3. SMR analyses for associations of expression of antihyperglycemic agent target genes with the risk of lung cancer outcomes. SMR, Summary-data-based Mendelian randomization; eQTL, expression quantitative trait loci.

(Tables S15–S16). In sensitivity analyses, no heterogeneity or pleiotropy was observed using Cochran's Q (all $p > 0.05$) or the MR−Egger intercept test (all p of intercept >0.05) (Tables S15–S16). The F-statistics for all IVs were over 20, indicating strong instrumental variables (Tables S17–18).

Furthermore, three classes of antiobesity drugs were included: centrally acting antiobesity products, peripherally acting antiobesity products, and other antiobesity drugs. A total of 67, 37, 53, 614, 1588, 750, 434, 312, 2, 676, 87, 288, 34 and 609 *cis*-eQTLs were identified from eQTLGen for the antiobesity drug target genes *SCN3A, SCN5A, ADRB2, CNR1, CA2, SIGMAR1, CACNB2, ADRB1, CACNA1C, CACNB3, SCN8A, CA4, SCN4A, and GRIK1*, respectively, and the most significant *cis*-eQTL SNP was selected as a genetic instrument for the target gene (Table S19). The SMR analyses identified no association of antiobesity drug target gene expression levels with lung adenocarcinoma risk. In sensitivity analysis, the HEIDI test indicated that observed associations were not due to a linkage ($p > 0.05$), except for expression of the gene *ADRB1* ($p = 0.005$). The F-statistics for the IVs were over 30 (Table S19).

4. Discussion

In this comprehensive MR study, we found a suggestive association between HbA1c levels and the risk of squamous cell lung cancer. Additionally, upregulated expression of *PRKAB1* (the target of metformin) in blood was suggestively associated with a decreased risk of developing lung adenocarcinoma, which was validated using pQTLs in plasma and eQTLs both in the liver and transverse colon. Mediation analysis indicated that overweight did not participate in the mediating pathway from *PRKAB1* activation to lung adenocarcinoma incidence.

Two previous studies estimated the causal association between glycemic traits and lung cancer using MR analyses. *Liu* et al. [14] did not detect a significant association of fasting glucose and HbA1c with lung cancer. However, the influence of fasting insulin on lung cancer was not evaluated in this study. According to *Ding* et al. [33], a causal relationship was also not provided between glycemic traits and lung cancer, including HbA1c, fasting glucose, and fasting insulin. However, their analysis was limited by a small dataset of only 2485 cases, which lacked information on lung cancer subtypes. Consistent with previous findings, we could not provide strong evidence to support significantly positive associations of glycemic traits with lung cancer. However, we found that the genetically determined HbA1c level was suggestively associated with an increased risk of squamous cell lung cancer. This observation aligns with a study from the UK Biobank, which also found an association between high HbA1c levels and squamous cell lung cancer after adjusting for potential confounders [7]. The HbA1c level represents a long-term state of blood glucose [34], while fasting glucose and fasting insulin are highly influenced by other factors, which might explain the observed inconsistency for the three glycemic traits with squamous cell lung cancer [11,35]. The observed association suggests that individuals with genetically higher HbA1c levels, indicative of elevated average blood glucose over time, may have an increased risk of developing squamous cell lung cancer.

It has been proposed that metformin may have an antitumoral function [36]. An extensive cohort study found that patients with diabetes taking metformin had a significantly reduced risk of developing lung cancer compared with patients not using metformin in the unadjusted model [hazard ratio (HR) = 0.49, 95 % CI, 0.32–0.44], while the protective effect on lung cancer incidence was not significant after adjustment (HR = 0.70, 95 % CI, 0.43–1.15) [18]. Another Korean study with 12.86 years of median follow-up found that metformin use could

not decrease lung cancer risk in either male (HR = 1.29, 95 % CI, 0.98–1.69) or female patients with diabetes (HR = 0.66, 95 % CI, 0.37–1.18) in the fully adjusted model [17]. However, observational studies on whether metformin could affect lung adenocarcinoma and squamous cell lung cancer are limited. Our study suggested that genetically determined *PRKAB1* is associated with a decreased risk of developing lung adenocarcinoma. In general, metformin plays a significant role in anti-non-small cell lung cancer cell activity through the liver kinase B1 (LKB1)-5′-adenosine monophosphate-activated protein kinase (AMPK) pathway [37,38]. Metformin inhibits its signaling [39] and growth [40] by directly activating AMPK via LKB1. Mammalian target of rapamycin (mTOR) is the downstream target of the LKB1-AMPK pathway, which is an important target of metformin in tumor inhibition [37]. AMPK and its homologs appear to exist in heterotrimers consisting of catalytic α subunits and regulatory β and γ subunits [41], which are encoded by the *PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2 and PRKAG3* genes [42]. AMPK activation plays an important role in inhibiting the proliferation and metastasis of cancer cells. Induction of AMPK-mediated caspase-dependent mitochondrial apoptotic conditions can selectively inhibit the proliferation of hepatocellular cancer cells, and activation of AMPK and inhibition of ERK-signaling pathway can reduce the metastasis potential of melanoma A375 cells [6]. In our study, only genetic variants of *PRKAB1* were suggestively associated with a decreased risk of developing lung adenocarcinoma. Metformin acts on the liver and intestines to lower blood glucose levels by decreasing glucose production and increasing glucose absorption and utilization [43]. Consistent results between *PRKAB1* expression and lung adenocarcinoma risk were provided in the liver and transverse colon using SMR analyses. The AMPK-HOXB9-KRAS axis was proposed in 2022 and revealed a mechanism for metformin inhibition of lung adenocarcinoma [44]. HOXB9, a significant transcription factor, is closely linked to adverse outcomes in individuals diagnosed with lung adenocarcinoma [44]. In both mice and lung adenocarcinoma cells, AMPK mediates HOXB9 T133 phosphorylation and downregulates the level of HOXB9, ultimately controls lung adenocarcinoma progression [44].

Previous studies suggested that the antitumor effects of metformin may depend on BMI [45]. To test this hypothesis, a two-step MR approach was conducted to explore whether overweight operated as a mediator from metformin use to lung cancer incidence. In this study, the lack of causal association between overweight and lung adenocarcinoma incidence suggests that higher *PRKAB1* expression reduces the risk of developing lung adenocarcinoma through other mechanisms. A prior investigation into the influence of common genetic variants in the genes *PRKAA2*, *PRKAB1*, and *PRKAB2* on type 2 diabetes and related traits suggested that the impact of these genes on BMI is either minimal or non-existent [46].

Based on our findings, HbA1c levels could be considered as one of the risk assessment indicators for the development of squamous cell lung cancer. For individuals with genetic variants associated with high HbA1c levels, more frequent screening or closer monitoring may be warranted to detect potential lung cancer early. Additionally, our study suggests potential avenues for developing novel targeted therapies or interventions aimed at modulating AMPK activity for lung adenocarcinoma prevention or treatment.

The main strength of this study was that we used genetic variants to proxy antihyperglycemic agent exposure based on eQTLs and validated the association in related tissues, thus overcoming limitations from observational studies. Second, we attempted to analyze the mediating role of overweight between metformin use and lung cancer risk using a two-step MR analysis. Third, radial MR and several sensitivity analyses were conducted to exclude potential outliers and examine the robustness of the results.

4.1. Limitations

However, this study had several potential limitations. First, genetic variants evaluate the effect of lifelong changes in glycemic traits and antihyperglycemic agents on lung cancer risk, and the magnitude of the effect may be different from the short-term effects of clinical interventions. Second, because of the unavailability of data on *ABCC8, KCNJ8, KCNJ1, SI* and *AMY2A* from the eQTLGen Consortium, we could not investigate the association between sulfonamide use and lung cancer outcomes. Finally, the GWAS, eQTLs and pQTLs data for this study were based on the European population, and our findings might not apply to populations with other ancestries.

5. Conclusions

In summary, this MR analysis revealed that genetically determined HbA1c levels were suggestively associated with a higher risk of squamous cell lung cancer. Additionally, the activation of *PRKAB1* was associated with a lower risk of lung adenocarcinoma, suggesting that targeting *PRKAB1* could potentially prevent lung adenocarcinoma. Moreover, the anti-tumor effect of *PRKAB1* in lung adenocarcinoma may be independent of its anti-obesity effect.

Ethics approval and consent to participate

The ethical approval and consent to participate can be found in the original GWASs, eQTLs and pQTLs.

Consent for publication

Not applicable.

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Authors' contributions

Deqiang Zheng, Jianguang Ji, and Wen Sun designed the research question; Wen Sun, Xiaoyu Zhang did the analyses; Wen Sun, Xiaoyu Zhang, Ning Li, Yan He, Jianguang Ji, and Deqiang Zheng drafted and revised the manuscript; Deqiang Zheng is responsible for the decision to submit the manuscript; Ning Li verified the analysis. All authors read and approved the final manuscript.

Data availability

The source of data presented in this study are shown in Table 1.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2024.103048.

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