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The association of cigarette smoking with the development and progression of diabetic retinopathy: based on cross-sectional survey and mendelian randomization

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Abstract

Background The relationship between cigarette smoking and diabetic retinopathy (DR) remains controversial, as existing studies have yielded inconsistent results. This study aimed to investigate the association between smoking and both the development and progression of DR.

Methods This study encompassed two complementary approaches. First, we performed a cross-sectional analysis to examine the association between smoking and DR, including its subcategories, utilizing data from the National Health and Nutrition Examination Survey. Subsequently, we implemented Mendelian randomization (MR) to explore the causal relationship between smoking and DR, as well as its specific categories, leveraging genome-wide association study data.

Results The cross-sectional study found an inverse association between smoking and DR risk across three analytical models (fully adjusted OR = 0.50, $P < 0.001$) that still persisted after propensity score matching (OR = 0.56, $P = 0.011$), and MR analysis also supported this finding (OR = 0.50, $P = 0.024$). Subgroup analyses revealed significant protective associations in males (OR = 0.41, $P < 0.001$), individuals with diabetes duration ≥ 10 years (OR = 0.43, $P = 0.011$), and those with normal clinical parameters. After categorizing DR by severity levels, smoking showed protective associations with the onset of mild and moderate-severe non-proliferative DR in the cross-sectional study, and with the onset of proliferative DR in MR analysis (OR = 0.41, $P = 0.016$). However, no association was observed between smoking and DR progression.

Conclusions Our findings suggest a protective association between smoking and DR development in specific subgroups across different DR stages, while showing no association with DR progression.

Keywords Cigarette smoking, Diabetic retinopathy, NHANES, Mendelian randomization

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Introduction

Diabetic retinopathy (DR), one of the most common microvascular complications of both type 1 and type 2 diabetes mellitus, is a leading cause of vision loss and blindness worldwide [1]. Multiple factors influence the development and progression of DR, including glycemic control, blood pressure, and serum lipid levels [2].

Cigarette smoking, an important risk factor for numerous chronic diseases and a primary global public health concern, has been implicated in compromising ocular health and function. Smoking has been shown to contribute to the onset and exacerbation of eye diseases, including age-related macular degeneration and senile cataracts [3]. Moreover, smoking impairs vascular endothelial function through enhanced oxidative stress and elevated pro-inflammatory factor production, consequently leading to insulin resistance, diabetes, and related complications such as diabetic nephropathy [4]. However, the relationship between smoking and DR remains controversial [5], with substantial heterogeneity in existing evidence. While some studies have reported a positive correlation between smoking and DR development and progression [5], others have demonstrated no significant association [6], and notably, some have suggested an inverse relationship [7]. These discrepant findings may be attributed to several limitations in previous research: inadequate control for confounding variables, absence of analysis across different DR severity stages, and insufficient investigation of potential effect modifications by demographic and clinical characteristics.

To address these issues and resolve the existing controversies, we combined the National Health and Nutrition Examination Survey (NHANES) dataset with Mendelian randomization (MR) techniques to examine the association between smoking and DR. The large sample size and robust representativeness of NHANES data, coupled with MR's use of genetic variants as instrumental variables (IVs) for smoking behavior, allow us to mitigate confounding factors in observational research. This study presents a novel approach by integrating NHANES and MR analyses to provide more reliable outcomes in investigating this relationship.

Our objective is to investigate the association between cigarette smoking and both the development and progression of DR using this dual-method approach. The findings may contribute to addressing controversies in the field, providing evidence-based guidance for clinical practice and public health interventions.

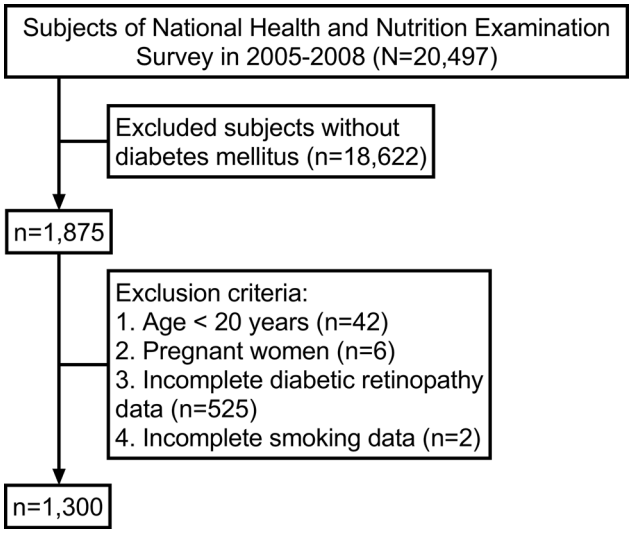

Fig. 1 Flowchart of subject selection. The selection of eligible participants from the National Health and Nutrition Examination Survey 2005–2008

Methods

Study design

This study comprised two distinct analytical approaches. First, we conducted a cross-sectional analysis using the NHANES data to examine the association between smoking and DR, with comprehensive adjustment for potential confounding factors. Subsequently, we performed MR analysis utilizing GWAS data to assess the potential causal relationship between smoking and DR.

Cross-sectional study

Sample population

Data applied in this analysis were derived from the NHANES database for 2005–2008. NHANES, a national health survey conducted by the Centers for Disease Control and Prevention and the National Center for Health Statistics (NCHS), is designed to assess the health and nutritional conditions of the civilian non-institutionalized U.S. population [8]. The 2005–2008 NHANES cycles were specifically selected as they uniquely provide instrument-based DR diagnoses and severity grading, distinguishing them from other cycles which only contain questionnaire-based assessments without severity classification. From the initial 20,497 participants in NHANES 2005–2008, we excluded those without diabetes ($n=18,622$), aged < 20 years ($n=42$), pregnant women ($n=6$), and those with incomplete data on diabetic retinopathy ($n=525$) or smoking status ($n=2$). After these exclusions, 1,300 subjects were enrolled in the final analysis. The specific participant selection procedure is delineated in Fig. 1. The study was approved by the Research Ethics Review Board of the NCHS, and all participants provided written informed consent before enrollment.

Definition of diabetes and DR

Diabetes was diagnosed based on the criteria set forth by the American Diabetes Association [9] and supplemented through a self-report questionnaire. Participants

were classified as diabetic if they met any of the following conditions [9]:

1. A glycated hemoglobin (HbA_1c) level ≥ 6.5%
2. A fasting plasma glucose level ≥ 7 mmol/L
3. A 2-hour plasma glucose level > 11.1 mmol/L during an oral glucose tolerance test
4. Self-reported physician-diagnosed diabetes
5. Use of insulin or diabetes medication

DR was identified through the detection of specific indicators such as microaneurysms, hard exudates, cotton wool spots, hemorrhages, venous beading, intraretinal microvascular abnormalities, and the formation of new retinal vessels, all classified according to the severity index from the Early Treatment DR Study [1]. The extent of retinopathy in the worse eye was detected using non-mydriatic fundus photography (TRC-NW6S; Topcon, Japan). Retinopathy levels were classified into four stages: no DR, mild non-proliferative DR (M-NPDR), moderate/severe non-proliferative DR (MS-NPDR), and proliferative DR (PDR), as detailed in the NHANES Digital Grading Protocol.

Assessment of smoking

Smoking was defined based on the response to a specific question from the survey: 'Have you smoked at least 100 cigarettes in your entire life?' The binary variable categorizes responses into two distinct groups: 'Yes' and 'No.' Therefore, the smoking subgroup in this analysis refers to those who answered "yes" to this question, suggesting a substantial, though not necessarily current or continuous, experience with smoking.

Assessment of covariates

In this study, we constructed a Directed Acyclic Graph based on previous research to analyze the risk factors for DR (Figure S1) [1, 2]. The covariates considered included age, gender, race, education level, marital status, poverty income ratio (PIR), body mass index, waist circumference, systolic blood pressure, diastolic blood pressure (DBP), HbA_1c, duration of diabetes, insulin use, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol, serum uric acid, serum albumin, urinary albumin/creatinine ratio (UACR), renal failure, alcohol intake, blood C-reactive protein (CRP), blood vitamin D levels, daily energy intake, and monthly moderate-to-vigorous physical activity [1, 2]. In this study, missing data refers to unavailable values, poor quality measurements, refused answers, and "Don't know" responses. For continuous covariates, missing values were imputed with the median when the proportion of missingness was < 10%, while variables with higher missing proportions were treated by categorizing missing values as a separate category. For categorical covariates, missing values were treated as a separate category. The quantity of missingness for each covariate and its corresponding treatment are detailed in Table S1.

Statistical analysis

Following NHANES analytical protocols, stratification, sampling weights, and primary sampling units were implemented to address the complexity of the survey design. Categorical data were summarized using frequency distributions and proportions, while continuous variables were reported as mean ± standard deviation values. For univariate analyses, weighted linear regression models, Wilcoxon rank-sum tests, or chi-square tests facilitated the comparison of variables across groups.

The association of smoking with DR and its subcategories was examined using weighted logistic regression analyses, adjusting for covariates. Possible mediating effects on the relationship between smoking and DR were explored using regression models with survey design adjustments and Monte Carlo simulations for confidence interval estimation. Interaction effects between smoking and covariates on DR were investigated using logistic regression models, employing likelihood ratio tests to assess statistical significance. The propensity score matching (PSM) was used to reduce bias and adjust for potential confounding variables, matching age, gender, and race. Statistical evaluations were performed utilizing R software, version 4.3.1. A P value < 0.05, determined via a two-sided test, was deemed significant.

MR

Study design

We performed a univariable two-sample MR analysis to investigate the potential causal relationship between genetically predicted smoking behavior (trait: ever smoked) [10] and DR [11], including its subcategories background DR [12], severe NPDR [13], and PDR [14]. To establish the causal impact of the exposure on the outcome using MR analysis, three assumptions must be satisfied [15]: (1) the genetic variants are associated with ever-smoked status, (2) the variants are not associated with confounding factors, and (3) the variants influence DR only through the pathway of smoking behavior. The MR study design is summarized in Fig. 2, which provides an overview of the MR framework, including assumptions, selection criteria, and outcome details. Furthermore, we also conducted a reverse-MR analysis to preclude the possibility of reverse causality.

Genetic instrument selection

Genetic instruments for smoking exposure (trait: ever smoked) were derived from a large-scale GWAS of

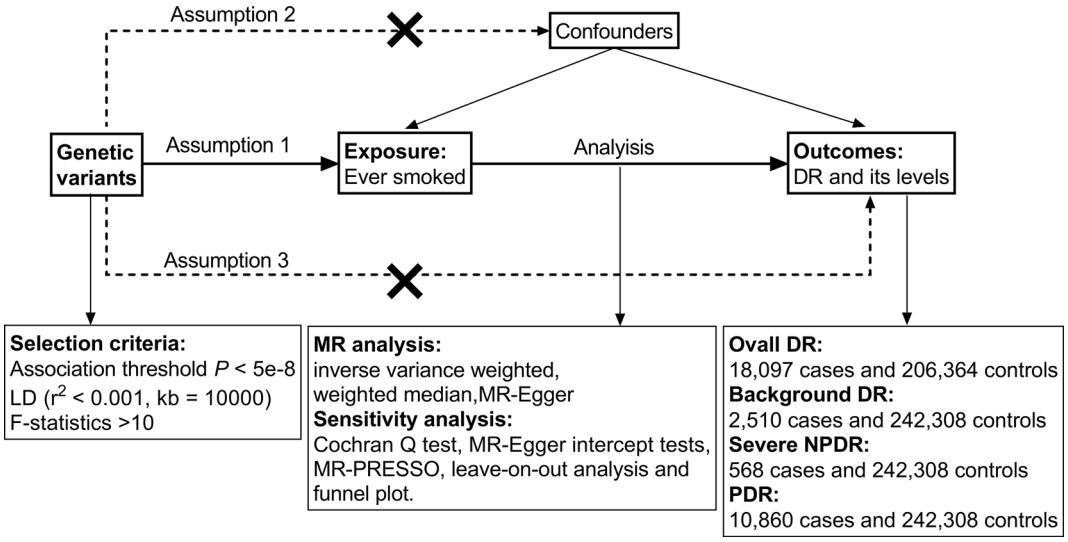

Fig. 2 Principles of Mendelian randomization and assumptions. Abbreviations: DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; MR, Mendelian randomization. Assumption 1: exposure is robustly associated with genetic variants; Assumption 2: confounders are not associated with genetic variants; Assumption 3: genetic variants should influence the outcomes only mediated by the exposure of interest

336,067 individuals of European ancestry from the UK Biobank dataset. This analysis, accessed through the OPEN GWAS database (dataset identifier: ukb-a-236), was originally performed by the Neale Lab in 2017, covering 202,585 cases and 133,482 controls, with a total of 10,894,596 single nucleotide polymorphisms (SNPs). We selected genetic variants using the following criteria: First, SNPs exhibiting genome-wide significant associations ($P<5\times10^{-8}$) with smoking behavior were identified as IVs. Second, to ensure independence, SNPs in linkage disequilibrium were filtered out (r^2 threshold <0.001 within a 10,000 kb window), retaining only the remaining uncorrelated SNPs matched to the outcome data. Third, we computed F-statistics for each SNP to quantify its explanatory power for the exposure, omitting weak instruments (F-statistic <10). The SNPs employed as IVs in this study are documented in Table S2.

Outcome data

The study utilized summary-level GWAS dataset for DR (12,584 cases and 202,082 controls) [11] and its subcategories: background DR (2,510 cases and 242,308 controls) [12], severe NPDR (568 cases and 242,308 controls) [13], and PDR (10,860 cases and 242,308 controls) [14]. The details of the data sets are listed in Table S3.

Statistical analysis

The inverse-variance weighted (IVW) method was employed as the primary analysis due to its highest statistical power, although it is susceptible to horizontal pleiotropy. To address potential pleiotropic effects, we utilized the weighted median (WM) technique and MR-Egger regression as complementary analytical strategies. Sensitivity analyses were performed. Cochran's Q test was used to examine the heterogeneity in effect sizes across the genetic IVs. The intercept from the MR-Egger regression was evaluated to assess potential horizontal pleiotropy. We also performed leave-one-out (LOO) analyses to determine if any single SNP unduly influenced the MR estimate. Funnel plots and MR-PRESSO were used to detect bias and outliers among the SNPs. Reverse MR analysis was performed to examine the possibility of reverse causation between smoking and DR outcomes. All statistical analyses were done using the "TwoSampleMR" and "MR-PRESSO" packages in R software, version 4.3.1. Statistical significance was defined as $P<0.05$ in two-sided tests.

Results

Cross-sectional study

Baseline characteristics by DR status

Participants were stratified into two groups based on their DR status: the DR and DR-free groups (Table 1). Compared with the DR-free group, the DR group showed the following characteristics: higher proportion of non-Hispanic Blacks, lower PIR, lower DBP, higher HbA_1c levels, higher proportion with diabetes duration ≥ 10 years, higher insulin use, lower serum albumin levels, higher UACR, higher prevalence of renal failure, lower vitamin D levels, and lower smoking prevalence (all $P<0.05$). However, no significant differences were found in other demographic and basic health parameters such as age, gender, and BMI ($P>0.05$).

Table 1 Baseline characteristics of participants by Diabetic Retinopathy Status among Diabetes, NHANES 2005–2008

Characteristics	DR-free (*N* = 915)	DR (*N* = 385)	*p*-value[a]
Age, mean ± SD (years)	60.90 ± 11.45	62.67 ± 11.60	0.072
Male, NO. (%)	454 (47.2%)	201 (53.0%)	0.150
Race, NO. (%)			0.003
Non-Hispanic White	418 (70.3%)	137 (61.9%)	
Non-Hispanic Black	226 (13.3%)	131 (21.8%)	
Mexican American	173 (7.2%)	79 (9.2%)	
Other	98 (9.2%)	38 (7.2%)	
Education below high school, NO. (%)	602 (56.9%)	260 (60.0%)	0.400
Married/Partner, NO. (%)	565 (67.4%)	231 (63.6%)	0.300
PIR, mean ± SD	2.92 ± 1.58	2.61 ± 1.48	0.027
SBP, mean ± SD (mmHg)	132.17 ± 19.62	135.34 ± 22.91	0.13
DBP, mean ± SD (mmHg)	71.03 ± 14.04	66.72 ± 14.47	< 0.001
BMI, mean ± SD (kg/m^2)	32.44 ± 7.28	32.17 ± 6.77	0.700
Waist circumference, mean ± SD (cm)	109.63 ± 16.00	108.08 ± 15.58	0.200
HbA1c(%), mean ± SD	7.07 ± 1.62	7.74 ± 1.78	< 0.001
Diabetes duration (≥ 10 years), NO. (%)	136 (12.4%)	196 (49.8%)	< 0.001
Use of insulin, NO. (Yes %)	83 (8.8%)	138 (42.6%)	< 0.001
Alcohol intake, NO. (Yes %)	188 (19.4%)	90 (22.5%)	0.300
Triglyceride (< 150 mg/dL %), NO. (%)	259 (29.6%)	127 (35.4%)	0.089
Total cholesterol, mean ± SD (mg/dL)	191.26 ± 47.47	185.91 ± 47.63	0.062
HDL-c, mean ± SD (mg/dL))	48.49 ± 14.76	49.68 ± 13.31	0.047
LDL-c (< 100 mg/dL %), NO. (%)	222 (26.6%)	100 (27.7%)	0.600
Serum albumin, mean ± SD (g/dL)	4.15 ± 0.32	4.08 ± 0.35	0.018
serum uric acid, mean ± SD (mg/dL)	5.86 ± 1.52	5.73 ± 1.72	0.100
UACR, mean ± SD (mg/g)	69.15 ± 357.23	260.66 ± 1,059.79	< 0.001
Renal failure, NO. (Yes %)	52 (4.9%)	38 (9.1%)	0.017
C-reactive protein, mean ± SD (mg/L)	6.74 ± 11.11	5.56 ± 8.69	0.200
Vitamin D, mean ± SD (nmol/L)	57.42 ± 20.29	54.73 ± 20.12	0.047
Energy intake, mean ± SD (kcal)	1,897.7 ± 821.3	1,844.1 ± 817.7	0.300
MVPA, NO. (Yes %)	442 (50.9%)	161 (48.5%)	0.500
Smoking, NO. (Yes %)	517 (55.1%)	185 (46.1%)	0.023

Abbreviations: NHANES, National Health and Nutrition Examination Surveys; DR, diabetic retinopathy; N/NO, sample size; PIR: poverty income ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index (calculated as weight in kilograms divided by height in square meters); HbA1c, hemoglobin A1c; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; UACR, urinary albumin/creatinine ratio; MVPA, moderate-vigorous physical activity. Data are represented as mean±standard deviation or median (Q1-Q3) or unweighted-n (%). [a] P value were calculated using weighted linear regression analyses or wilcoxon rank sum test for continuous variables and the weighted chi-square test for categorical variables

Further stratification by DR severity (M-NPDR, MS-NPDR, and PDR) revealed that with increasing severity of DR, diastolic blood pressure decreased, while HbA_1c levels, duration of diabetes, insulin use rate, and UACR increased (Table S4).

Baseline characteristics categorized by smoking status

The analysis stratified by smoking status showed that smokers had a higher proportion of males and higher levels of CRP and UACR, but lower PIR, DR prevalence, and total cholesterol levels compared with non-smokers (Table S5).

Associations between smoking and DR incidence

We assessed multicollinearity among the included variables before analyzing the association between smoking and DR. The results showed that no variable exhibited a variance inflation factor > 10 or a tolerance < 0.1 (Table S6).

Smoking was consistently associated with reduced DR risk across three analytical models (Table 2), with the strongest association observed in the fully adjusted model (OR = 0.50, $P < 0.001$). Subgroup analyses revealed significant protective associations in the following categories: (1) demographic characteristics: aged 45–64 years (OR = 0.49, $P = 0.008$), males (OR = 0.41, $P < 0.001$), non-Hispanic Whites (OR = 0.47, $P = 0.005$); (2) clinical parameters: DBP < 90 mmHg (OR = 0.55, $P = 0.003$), HbA_1c levels < 7% (OR = 0.43, $P = 0.001$), diabetes duration ≥ 10 years (OR = 0.43, $P = 0.011$), without renal failure (OR = 0.51, $P < 0.001$); and (3) biochemical indicators: HDL-c ≥ 40 mg/dL (OR = 0.56, $P = 0.008$), serum albumin ≥ 3.5 g/dL (OR = 0.56, $P = 0.002$), UACR < 30 mg/g (OR = 0.49, $P = 0.002$), vitamin D ≥ 50 nmol/L (OR = 0.48,

Table 2 Associations between smoking and risk of diabetic retinopathy among diabetes

	Model 1 **OR (95% CI), *P***	**Model 2** **OR (95% CI), *P***	**Model 3** **OR (95% CI), *P***
Smoking	Reference	Reference	Reference
no	0.70 (0.51, 0.95) 0.024	0.65 (0.47, 0.90) 0.009	0.50 (0.34, 0.73) < 0.001
yes			
Stratified by age			
20–44 years	0.34 (0.09, 1.35) 0.131	0.40 (0.09, 1.82) 0.243	0.31 (0.03, 3.36) 0.342
45–64 years	0.69 (0.44, 1.09) 0.115	0.67 (0.42, 1.07) 0.095	0.49 (0.29, 0.83) 0.008
≥ 65 years	0.78 (0.50, 1.21) 0.264	0.77 (0.48, 1.22) 0.266	0.71 (0.42, 1.19) 0.190
Stratified by gender			
male	0.58 (0.37, 0.92) 0.021	0.56 (0.36, 0.89) 0.015	0.41 (0.25, 0.67) < 0.001
female	0.77 (0.50, 1.20) 0.251	0.88 (0.56, 1.38) 0.579	0.78 (0.47, 1.29) 0.333
Stratified by race			
Non-Hispanic White	0.67 (0.43, 1.04) 0.078	0.61 (0.39, 0.97) 0.036	0.47 (0.28, 0.79) 0.005
Non-Hispanic Black	0.70 (0.44, 1.13) 0.146	0.70 (0.44, 1.13) 0.150	0.68 (0.40, 1.16) 0.160
Mexican American	0.70 (0.38, 1.28) 0.243	0.60 (0.32, 1.13) 0.114	0.52 (0.25, 1.06) 0.073
Other	0.82 (0.27, 2.50) 0.725	0.80 (0.26, 2.53) 0.710	0.50 (0.13, 1.97) 0.323
Stratified by PIR			
< 2	0.72 (0.46, 1.10) 0.131	0.70 (0.44, 1.12) 0.139	0.59 (0.36, 0.96) 0.033
≥ 2	0.66 (0.41, 1.06) 0.088	0.59 (0.36, 0.96) 0.033	0.50 (0.28, 0.89) 0.020
Stratified by DBP			
< 90 mmHg	0.72 (0.51, 1.01) 0.057	0.68 (0.47, 0.96) 0.031	0.55 (0.37, 0.82) 0.003
≥ 90 mmHg	0.31 (0.10, 1.03) 0.060	0.29 (0.08, 1.05) 0.063	0.30 (0.08, 1.13) 0.080
Stratified by HbA1c(%)			
< 7	0.63 (0.40, 0.99) 0.048	0.55 (0.34, 0.88) 0.014	0.43 (0.26, 0.72) 0.001
≥ 7	0.73 (0.45, 1.16) 0.185	0.72 (0.44, 1.16) 0.180	0.72 (0.43, 1.21) 0.212
Stratified by diabetes duration			
< 5 years	1.19 (0.54, 2.64) 0.663	0.91 (0.37, 2.24) 0.835	1.02 (0.43, 2.43) 0.969
5–9 years	0.72 (0.34, 1.52) 0.386	0.68 (0.33, 1.44) 0.317	0.70 (0.31, 1.60) 0.397
≥ 10 years	0.63 (0.35, 1.15) 0.132	0.57 (0.32, 1.03) 0.063	0.43 (0.22, 0.82) 0.011
Stratified by Insulin usage			
no	0.70 (0.48, 1.01) 0.055	0.68 (0.46, 0.99) 0.047	0.62 (0.42, 0.92) 0.019
yes	0.51 (0.24, 1.07) 0.074	0.47 (0.23, 0.98) 0.046	0.38 (0.17, 0.85) 0.020
Stratified by renal failure			
no	0.66 (0.48, 0.92) 0.014	0.64 (0.46, 0.90) 0.010	0.51 (0.35, 0.74) < 0.001
yes	1.03 (0.33, 3.23) 0.962	0.71 (0.19, 2.65) 0.616	0.69 (0.14, 3.34) 0.649
Stratified by HDL-c			
< 40 mg/dL	0.75 (0.38, 1.46) 0.392	0.69 (0.35, 1.37) 0.292	0.57 (0.27, 1.17) 0.126
≥ 40 mg/dL	0.71 (0.50, 1.03) 0.073	0.66 (0.45, 0.97) 0.033	0.56 (0.37, 0.86) 0.008
Stratified by Serum albumin			
< 3.5 g/dL	1.05 (0.16, 6.83) 0.963	1.42 (0.27, 7.46) 0.685	0.39 (0.03, 4.55) 0.460
≥ 3.5 g/dL	0.68 (0.49, 0.94) 0.021	0.63 (0.45, 0.88) 0.007	0.56 (0.38, 0.81) 0.002
Stratified by UACR			
<30 mg/g	0.58 (0.39, 0.88) 0.009	0.55 (0.36, 0.84) 0.005	0.49 (0.31, 0.77) 0.002
30–300 mg/g	0.75 (0.41, 1.39) 0.363	0.80 (0.42, 1.51) 0.489	0.73 (0.38, 1.40) 0.350
> 300 mg/g	0.69 (0.23, 2.11) 0.521	0.52 (0.19, 1.43) 0.210	0.80 (0.24, 2.66) 0.721
Stratified by Vitamin D			
< 50 nmol/L	0.78 (0.48, 1.27) 0.318	0.82 (0.50, 1.34) 0.420	0.57 (0.33, 0.98) 0.043
≥ 50 nmol/L	0.60 (0.38, 0.96) 0.033	0.52 (0.32, 0.84) 0.007	0.48 (0.28, 0.82) 0.008

Abbreviations: OR, odds ratio; 95% CI, 95% confidence intenval; PIR: poverty income ratio; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL-c, high-density lipoprotein cholesterol; UACR, urinary albumin/creatinine ratio

Mode 1 = Non-adiusted mode

Mode 2 = Mode 1 + age, gender, and race were adusted

Model 3 = Mode 2 + PIR, DBP, diabetes duration, HbA1c(%), use of insulin, HDL-c, serum Vitamin-D, serum albumin, UACR, and renal failure

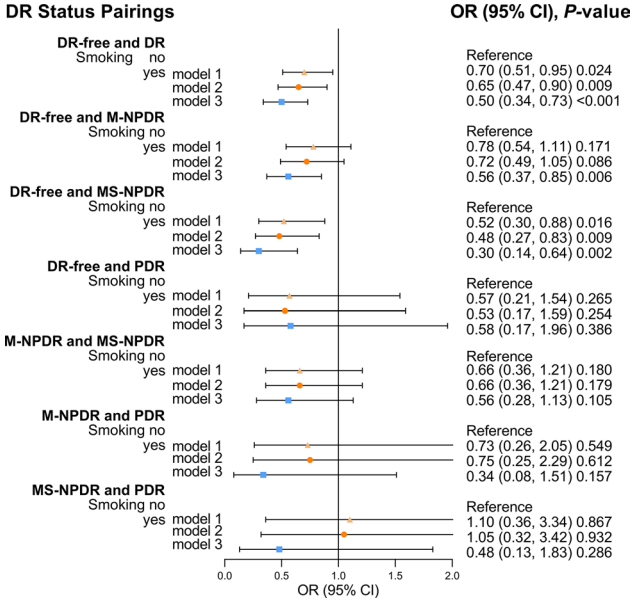

Fig. 3 Forest Plot of the Association between Smoking and Diabetic Retinopathy Incidence and Severity. Abbreviations: DR, diabetic retinopathy; OR, odds rato; 95% CI, 95% confidence intenval; M-NPDR: mild non-proliferative diabetic retinopathy; MS-NPDR: moderate/severe non-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy. Mode 1 = Non-adiusted mode. Mode 2 = Mode 1 + age, gender, and race were adusted. Model 3 = Mode 2 + poverty income ratio, diastolic blood pressure, diabetes duration, HbA1c(%), use of insulin, high-density lipoprotein cholesterol, serum Vitamin-D, serum albumin, urinary albumin/creatinine ratio, and renal failure. For each paired analysis, the reference category is the non-diseased or milder condition

P=0.008). Post-PSM (Table S7), the protective association persisted in the fully adjusted model (OR=0.56, P=0.011).

The subgroup analysis was not adjusted for the stratification variable itself.

Interaction effects between smoking and covariates on DR risk

Analysis of interaction effects revealed significant interactions between smoking and several covariates (Table S8): non-Hispanic Black race (coefficient=0.657, P=0.016), DBP (coefficient = -0.025, P=0.027), diabetes duration ≥ 10 years (coefficient=1.046, P<0.001), and insulin use (coefficient=1.396 for "yes," P<0.001).

Mediating variables in the smoking-DR relationship

Based on multivariable logistic regression results (Table S9), we examined potential mediating variables (Table S10). Mediation analysis did not identify any significant mediation effects concerning DBP (coefficient=0.031, P=0.923), HbA_1c (coefficient=0.063, P=0.373), or insulin use (coefficient = -0.100, P=0.386).

Associations of smoking with severity and progression of DR

As shown in Fig. 3, we evaluated the effect of smoking on DR severity or progression by comparing different combined subgroups. In the fully adjusted model, smoking was associated with reduced risk in DR-free versus M-NPDR subgroups (OR=0.56, P=0.006) and DR-free versus MS-NPDR subgroups (OR=0.30, P=0.002). However, no significant associations were identified in the combined analyses for DR-free versus PDR, M-NPDR versus MS-NPDR, M-NPDR versus PDR, or MS-NPDR versus PDR subgroups.

These associations persisted after PSM (Table S11), with significant protective effects remaining in DR-free versus M-NPDR (OR=0.60, P=0.035) and DR-free versus MS-NPDR comparisons (OR=0.42, P=0.034), while other comparisons showed no significant associations.

MR study

Causal effects of smoking on DR and its subcategories

In the IVW model (Table 3), genetically predicted smoking was inversely associated with overall DR risk (OR=0.50, P=0.024), a finding corroborated by the WM model with a stronger association (OR=0.36, P=0.015). When analyzing the association between smoking and various subcategories of DR, the IVW model revealed that genetically predicted smoking was inversely associated with PDR risk (OR=0.41, P=0.016). However, no significant associations were observed for background DR (OR=1.19, P=0.816) or severe NPDR (OR=0.15, P=0.267) under the IVW model. Scatter plots detailing these relationships are provided in Figure S2, and forest plots summarizing the magnitude of the MR effects of smoking on DR and its subcategories can be found in Figure S3.

Table 3 Associations of genetically predicted smoking with DR and its levels by mendelian randomization analysis

Outcomes	Inverse variance weighted OR (95% CI), *P*	MR Egger OR (95% CI), *P*	Weighted median OR (95% CI), *P*
Overall DR	0.50(0.27, 0.91) 0.024	0.11(0.01, 1.71) 0.124	0.36(0.16, 0.82) 0.015
Background DR	1.19(0.27, 5.23) 0.816	0.56(0, 526.31) 0.869	1.86(0.20, 17.4) 0.588
Severe NPDR	0.15(0.01, 4.29) 0.267	56.33(0, 2708.51) 0.611	0.17(0.00, 12.66) 0.424
PDR	0.41(0.20, 0.85) 0.016	0.29(0.01, 8.14) 0.473	0.42(0.15, 1.15) 0.091

Abbreviations: OR, odds rato; 95% CI, 95% confidence intenval; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy

Sensitivity analysis

LOO analyses of the above MR results are detailed in Figure S4, where no significant strong SNP effects were detected. The sensitivity analysis revealed neither horizontal pleiotropy (P-intercept > 0.05) nor heterogeneity (P-Q > 0.05) among the chosen genetic instruments, as reported in Table S12. Furthermore, in the MR-PRESSO global test, all P values were above 0.05. The funnel plots indicated that variations in effect size around the central estimates were predominantly symmetric, suggesting no evidence of bias (Figure S5). Additionally, there was no identified reverse causality between smoking and DR or its subcategories (Table S13).

Discussion

This study aimed to investigate the association between smoking and both the development and progression of DR, utilizing a novel combination of NHANES database analysis and MR analysis. The analysis of cross-sectional data revealed that smoking was associated with a reduced risk of DR development, an association that remained robust after PSM. This protective association was further substantiated by MR analysis. However, no significant association was observed between smoking and DR progression.

Previous studies have reported inconsistent findings regarding the relationship between cigarette smoking and DR incidence. In type 1 diabetes, studies have consistently demonstrated that smoking raises DR risk [5, 7], potentially through mechanisms involving decreased retinal blood flow and impaired vascular reactivity [16]. However, the relationship in type 2 diabetes (T2DM) remains inconclusive, with studies showing positive, null, and even inverse associations with DR occurrence [7, 17, 18]. Our findings support the inverse association perspective. This is not entirely unexpected, as smoking, despite its well-documented adverse health effects, may exhibit protective effects in certain conditions, including Sjögren's syndrome, Parkinson's disease, silicosis, schizophrenia-related cognitive deficits, and ulcerative colitis [18–22]. Additionally, a study has shown that smoking suppresses the expression of pro-inflammatory cytokines, chemokines, and angiogenic factors in periodontitis patients, resulting in reduced gingival inflammation and angiogenesis [24].

Stratification by DR severity revealed varying associations. Cross-sectional analysis demonstrated a protective association of smoking with M-NPDR and MS-NPDR. However, this protection was not observed in advanced PDR, possibly due to the severe pathological changes at this stage that may override any potential protective effects of smoking [1]. Conversely, MR analysis revealed a contrasting outcome, showing protection against PDR onset only. This stage-specific effect suggests that smoking-related genetic variations may be involved in PDR development, while their effects might be overshadowed by other risk factors in NPDR. Notably, no association was observed between smoking and DR progression, consistent with previous finding [6].

In discussing the potential mechanisms behind smoking's protective effect against the onset of DR, the following points may be considered: (1) Vascular Mechanisms: Diabetic smokers exhibit wider retinal microvascular diameters [25], yet demonstrate lower vessel length density [26], reduced retinal blood flow, and decreased blood velocity [16] compared with non-smoking diabetic individuals. These changes make the retinas of smokers more susceptible to ischemic and hypoxic conditions. Under prolonged exposure to insufficient blood and oxygen supply, the retina becomes increasingly tolerant to ischemia and hypoxia, leading paradoxically to a reduced propensity for the development of DR among diabetic patients who smoke [27]. Additionally, smoking-associated hypotension may contribute to decreased DR risk [7]. (2) Inflammatory and Immunomodulatory Mechanisms: The anti-inflammatory effects of smoking may attenuate DR-related inflammatory pathways. Changes in the immune environment of smokers, particularly decreased CD4+T cell activity and altered cytokine profiles [19], may reduce retinal inflammatory responses and DR incidence, similar to smoking's effects in Sjögren's syndrome and ulcerative colitis [19, 23]. (3) Neuroprotective Effects: Tobacco components, particularly nicotine, may exert neuroprotective effects, similar to those observed in Parkinson's disease [20]. These effects could protect the retina's neural components, potentially influencing DR development. (4) Lifestyle Choices and Behavioral Changes: Smoking-associated lifestyle factors, including stress reduction and dietary habits, may indirectly influence DR occurrence, analogous to alcohol consumption's effects on diabetes [4]. Furthermore, post-diagnosis behavior changes and improved adherence to diabetes management may reduce DR risk [4].

Beyond these mechanistic considerations, our subgroup analyses revealed important demographic variations in smoking's protective effects. With regard to gender differences, the protective effect was observed only in males, not in females. This discrepancy might result from the distinct impacts of smoking on biochemical and hematological parameters between men and women [28]. Estrogens play crucial roles in regulating immune responses and anti-inflammatory actions that protect against diabetic complications, such as retinopathy. However, smoking disrupts estrogen signaling pathways, particularly in females, potentially weakening estrogen's protective effects, which may explain the lack of observed protection in female smokers against DR [29]. Moreover, smoking-induced increase in hepatic

clearance rates reduces estrogen levels, potentially diminishing its role in maintaining vascular health, as seen in T2DM where the burden of smoking on coronary disease incidence is greater in females than in males [30, 31].

Age-stratified analyses further revealed distinct patterns in smoking's association with DR, with protective effects present only in middle-aged individuals, not in the elderly. This observation may be attributed to the decreased adaptability of older individuals to physiological stressors [32], which prevents them from developing tolerance to smoking-induced ischemic and hypoxic conditions. Additionally, the protective effect was only observed in individuals with normal clinical parameters, possibly due to their better metabolic regulation and compensatory abilities [32]. These characteristics may enable better adaptation to smoking-induced retinal ischemia and hypoxia, thereby reducing DR risk. Furthermore, diabetes duration influenced the protective effect, as this protection was only observed in individuals with diabetes duration of ten years or more, possibly due to the higher baseline DR incidence in this group [1], making it easier to observe any factors that reduce its risk.

This study has several notable strengths and limitations. The major strength lies in its use of two distinct analysis methods, the NHANES database analysis and MR analysis. The NHANES database features standardized data collection protocols, while providing us with a large sample size and comprehensive clinical information, enabling reliable analyses with broad population representation. MR analysis reduces potential confounding factors and biases related to reverse causation inherent in observational studies, thereby enhancing the reliability of our findings.

However, several limitations must also be recognized. First, due to the lack of differentiation between Type 1 and Type 2 diabetes in the original public data, this study could not distinguish between different types of DR. Second, this research examined only binary smoking status (≥100 lifetime cigarettes), without assessing the impacts of smoking intensity, duration, or patterns on DR risk. Third, the cross-sectional study data were collected through interviews with participants, potentially introducing recall bias. In addition, the study populations were restricted to specific ethnic groups, with NHANES data representing the US population and MR analysis based on genetic instruments derived from European ancestry. Finally, while MR can alleviate some confounding biases, it cannot eliminate residual confounding from unmeasured factors such as lifestyle and genetic background. These limitations may affect the generalisability of our findings to other populations and settings.

Given the complexity and variability of our findings, future research should focus on longitudinal cohort studies to better elucidate the causal relationships and temporal dynamics between cigarette smoking and different severities of DR. Additionally, mechanistic studies are warranted to explore the underlying biological pathways and molecular mechanisms behind the potential protective effects of smoking on DR.

Conclusion

Our findings suggest a protective association between smoking and DR development across different DR stages, but not in DR progression. However, the protective effect was present only in specific demographic and clinical subgroups, indicating that the impact of smoking on DR is complex and potentially influenced by various individual factors. Despite these findings, given the known health risks associated with smoking, these results do not advocate for smoking as a preventative measure against DR onset. Importantly, these observations suggest potential overlapping molecular pathways between DR pathogenesis and smoking-induced retinal changes, providing potential insights for DR mechanism investigation and therapeutic target identification.

Supplementary Information

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Supplementary Material 1

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Author contributions

Bin Wang: Data curation, Writing - Original draft preparation, Writing - Reviewing and Editing; ZaiHong Chen: Supervision; Huafeng Ma: Conceptualization; Hui Li: Methodology, Software, Visualization, Investigation, Validation. All authors read and approved the final manuscript.

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Data availability

Summary statistics of genome-wide association studies (GWAS) and data from the National Health and Nutrition Examination Survey (NHANES) used in this study are publicly available, which can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Declarations

Ethics approval and consent to participate

Since the data adopted in this study were all publicly available data from the Finngen database, UK Biobank, ieu Open Gwas Project and NHANES, all data related studies were approved by their respective ethical review committees and received written informed consent from patients. Therefore, this study does not need additional ethics approval.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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