• Meta-Analysis •

Mendelian randomization analysis of causal relationship between cheese intake and diabetic retinopathy

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Abstract

● **AIM:** To assess whether there is a possible causal link between the intake of cheese and the risk of diabetic retinopathy (DR) utilizing a two-sample Mendelian randomization (MR) analysis.

● **METHODS:** The research data were obtained from summary statistics of genome-wide association studies (GWAS). Genetic loci closely related to cheese intake were extracted as instrumental variables (IVs), and DR was the outcome variable. The data were extracted from individuals of European ethnicity. The data of cheese intake consisted of 451 486 samples with 9 851 867 single nucleotide polymorphisms (SNPs), while the DR data consisted of 206 234 samples with 16 380 446 SNPs. Sixty-one genetic loci closely related to cheese intake were selected as IVs. MR analysis was performed by inverse-variance weighted (IVW) method and MR-Egger regression respectively. The causal relationship between cheese intake and DR was evaluated using odds ratios (ORs) and 95% confidence intervals (CIs). Egger-intercept test was used to test horizontal pleiotropy and sensitivity analysis was performed by leave-one-out test.

● **RESULTS:** The *P* value of the IVW method was less than 0.05, indicating a significant negative correlation between cheese intake and DR. MR-Egger regression showed that the intercept was 0.01 with a standard error of 0.022, and a *P*-value of 0.634, indicating no evidence of horizontal pleiotropy affecting the IVs related to the exposure factors. Besides, heterogeneity tests confirmed the absence of heterogeneity, and the "leave-one-out" sensitivity analysis demonstrated that the results were stable.

● **CONCLUSION:** Cheese intake is causally negatively correlated with the occurrence of DR, and cheese intake could reduce the risk of DR.

● **KEYWORDS:** cheese intake; diabetic retinopathy; Mendelian randomization analysis

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INTRODUCTION

Dairy fats are rich in saturated fatty acids, which are theoretically linked to the risk of mortality from cerebrovascular diseases. It has been suggested that decreasing the consumption of saturated fatty acids could potentially increase the lifespan of the population[1]. However, certain research indicated that despite cheese being categorized as a full-fat dairy item, there was no evident connection, or even a reverse relationship, between cheese consumption and the incidence of cardiovascular diseases[2]. Moreover, it appears that various dairy products may play distinct roles in the onset and progression of cardiovascular conditions. A comprehensive European study known as the European Investigation into Cancer and Nutrition, which included 340 234 participants from eight different European countries, revealed no correlation between the intake of milk and type 2 diabetes, whereas there was a negative correlation between cheese consumption and the risk of diabetes[3]. The positive impact of cheese on type 2 diabetes might be due to its influence on the gut microbiota[4-5].

Diabetic retinopathy (DR) is one of the most common ocular complications of diabetes[6]. In developing countries, DR has become the leading cause of blindness[7-8]. Some of diabetic patients will develop DR, leading to vision impairment and

Table 1 Summary of the GWAS included in this study

Items	ID	Individuals (*n*)	SNPs (*n*)	Race	Gender	Year
Cheese intake	ukb-b-1489	451486	9851867	European	Male and female	2018
DR	finn-b-DM_BCKGRND_RETINA	206234	16380446	European	Male and female	2021

GWAS: Genome-wide association studies; DR: Diabetic retinopathy; SNPs: Single nucleotide polymorphisms.

seriously affecting quality of life[9]. In recent years, due to the increase of the world's population and the extension of the average life expectancy, the incidence of diabetes has gradually been increased, resulting in an upward trend of the blindness rate caused by DR[10]. Based on research conducted in the US, the occurrence of DR in people with type 2 diabetes is estimated to be between 28.5% and 40.3%. Furthermore, it is projected that between 4.4% and 8.2% of these patients will experience vision impairment[11]. A large cohort study, involving 8122 participants among the working-aged Australian population with diabetes, showed that consumption of cheese could lower the likelihood of DR progression. However, the observational study had a limited sample size and was influenced by bias and confounding factors[12].

Mendelian randomization (MR) analysis is an approach proposed by Professor Katan in 1986 and applied to epidemiological observation. Genetic variations serve as instrumental variables (IVs) to investigate the potential causal links between exposure and outcome factors[13]. The exposure and outcome factors are analogous to random allocation to treatment and control groups in a randomized controlled trial. Genetic variations, which are not affected by external factors and are established at birth, can significantly mitigate the impact of confounding variables and the issue of reverse causality in research analysis[14]. The objective of the study was to investigate the link between the intake of cheese and DR based on a broad dataset from a genome-wide association study (GWAS), employing single nucleotide polymorphism (SNP) sites as IVs for genetic variation as well as MR analysis.

MATERIALS AND METHODS

Ethical Approval This study was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (No.2024-E382-01).

Research Design This study utilized cheese intake as the exposure factor and SNPs significantly associated with DR as IVs. DR was designated as the outcome variable for this study. The causal association analysis was performed using the Two Sample MR package within RStudio. The reliability of the results was confirmed through heterogeneity test, pleiotropy test, as well as sensitivity analysis.

Data Sources The data for cheese intake and DR utilized in this study were sourced from the website https://gwas.mrcieu.ac.uk/datasets. The dataset for cheese intake, identified as ukb-b-1489, was derived from GWAS statistical outcomes published in 2018. This dataset encompassed a substantial sample size of 451 486 individuals and included 9 851 867 SNPs. The data for DR (finn-b-DM_BCKGRND_RETINA) were obtained from statistical results of GWAS published in 2021, including a sample size of 206 234 individuals, with 2026 cases and 204 208 controls. The dataset contained a total of 16 380 446 SNPs. Both the cheese intake and DR samples were from European populations, comprising both males and females. Table 1 showed the summary of the GWAS included in this study.

Instrumental Variables To prevent analysis bias that could arise from strong linkage disequilibrium (LD) among SNPs, the following criteria were applied for the selection of SNPs: 1) $P<5\times10^{-8}$; 2) a physical distance of more than 10 000 kb between each pair of genes; 3) an LD threshold of $r^2<0.001$ and the F value >10 between genes. After applying these stringent criteria, a set of independent SNPs that demonstrated a significant association with cheese intake was identified. These SNPs were then used as the final IVs in the study. Additionally, to confirm that these SNPs were not linked to any possible confounding factors, a search was performed in the PhenoScanner database (http://www.phenoscanner.medschl.cam.ac.uk).

Mendelian Randomization Analysis It mainly involved inverse-variance weighted (IVW), MR-Egger regression, as well as weighted median (WME) using the Two Sample MR package. The IVW method is the most commonly used method for detection, calculating the weighted average of the effect estimates from all IVs. MR analysis relies on the IVW method to obtain reliable results. In addition, MR-Egger can not only perform MR Analysis, but also be applied to pleiotropy evaluation. It fits the model utilizing the reciprocal of the outcome variance as weights. The advantage of WME is that it can produce valid results in MR analysis even when at least half of the IVs are effective.

Sensitivity Analysis A heterogeneity test was performed to assess the variability among individual IVs. A P-value exceeding 0.05 suggested that there was no significant heterogeneity. The Cochran Q test is a standard method for evaluating heterogeneity. A P-value below 0.05 would signal the presence of heterogeneity, whereas a P-value above 0.05 would suggest its absence. A pleiotropy test was also conducted to ascertain the validity of the MR analysis results, often represented by the intercept term of the MR-Egger

regression. If the *P*-value for the intercept term was greater than 0.05, it indicated the absence of horizontal pleiotropy, suggesting that the MR analysis results were likely reliable. Conversely, if there was evidence of pleiotropy, it would cast doubt on the reliability of the MR analysis findings[15]. Sensitivity analysis was carried out using the "leave-one-out" approach to test the sensitivity of the results. The principle was to gradually remove individual SNPs and examining the stability of the results to identify potential outliers.

RESULTS

Situation of Instrumental Variables In this research, the consumption of cheese was designated as the exposure factor. Utilizing RStudio, we filtered SNPs that met genome-wide significance levels based on established screening criteria. As a result, 61 SNPs were identified to serve as IVs. The F-statistics for these SNPs exceeded a value of 10, which suggests that there was no significant weak instrument bias in our study, thereby validating the robustness of our findings. Through two-sample MR, the effects of each SNP locus on the outcome were determined (Figure 1).

Mendelian Randomization Analysis of Cheese Intake on Diabetic Retinopathy MR analysis examining the relationship between cheese consumption and DR utilized IVW, MR-Egger regression, as well as WME methods, all of which were accessible within the Two Sample MR software package. Table 2 showed the results of MR analysis. The odds ratio (OR) values for the three methods were 0.448 [95% confidence interval (CI): 0.247-0.813] for IVW, 0.244 (95%CI: 0.019-3.139) for MR-Egger, and 0.347 (95%CI: 0.148-0.816) for WME, respectively.

MR analysis revealed that consuming cheese might lower the risk of developing DR. The respective *P*-values for these tests were 0.008, 0.284, and 0.015. The IVW result, being below 0.05, suggests a statistically significant association. The β values obtained from each analysis method were consistent in direction, with the results from IVW method serving as the benchmark. The MR analysis outcomes indicated a potential causal link between cheese consumption and a reduced risk of DR (Figure 2).

Results of Stability Analysis In this study, stringent criteria were applied for the selection of IVs, ensuring that the participants were of the same species. Therefore, the likelihood of false-negative results was quite low. To ensure the reliability of the aforementioned findings, a heterogeneity test was conducted. The *Q*-values and *QP*-values for IVW and MR-Egger were 60.45 (0.460) and 60.211 (0.432), respectively, both of which exceeded 0.05. This suggests that there was no significant heterogeneity present. The results were graphically represented, and the funnel plot indicated that the study findings were free from bias. These visual representations are detailed in Figure 3.

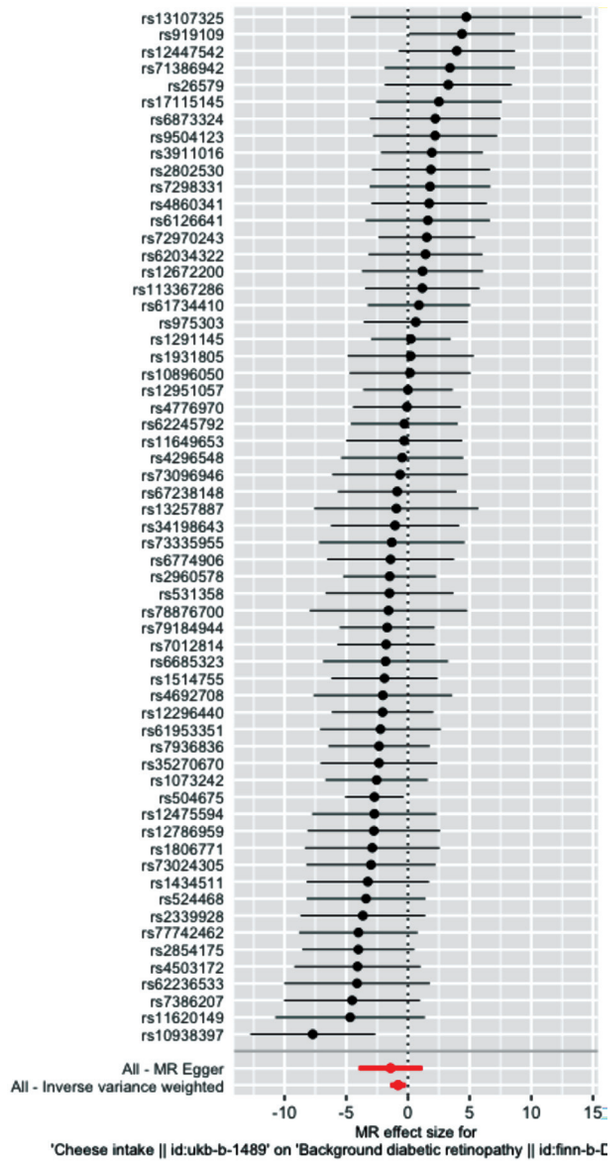

Figure 1 Forest plot of the two-samples MR analysis MR: Mendelian randomization.

Table 2 Result of MR analysis

Methods	*B* value	SE	OR (95%CI)	*P*
IVW	-0.803	0.304	0.448 (0.247~0.813)	0.008
MR-Egger	-1.409	1.302	0.244 (0.019~3.139)	0.284
WME	-1.057	0.436	0.347 (0.148~0.816)	0.015

MR: Mendelian randomization; IVW: Inverse-variance weighted; WME: Weighted median; SE: Standard error; OR: Odds ratio; CI: Confidence interval.

Results of Sensitivity Analysis A leave-one-out sensitivity analysis was performed to assess the impact of each single SNP locus on the overall causal relationship. The results showed that after systematically removing each SNP, the effect estimates derived from the IVW for the remaining SNPs remained stable, without significant variation, and were

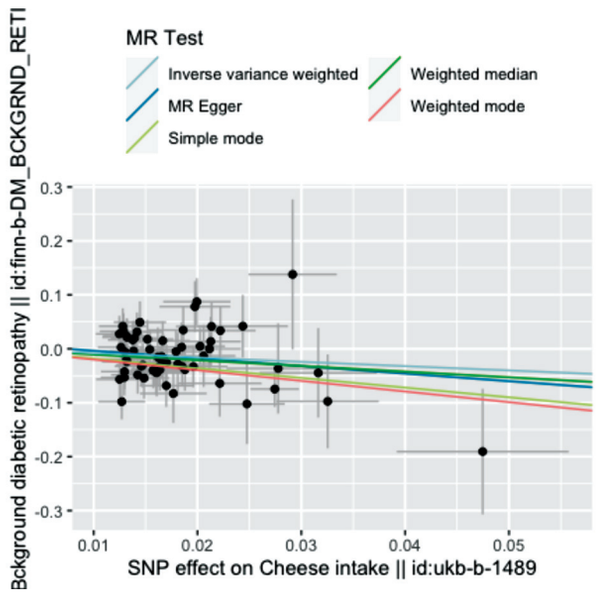

Figure 2 Scatter plot of the two-samples MR analysis SNP: Single nucleotide polymorphism; MR: Mendelian randomization.

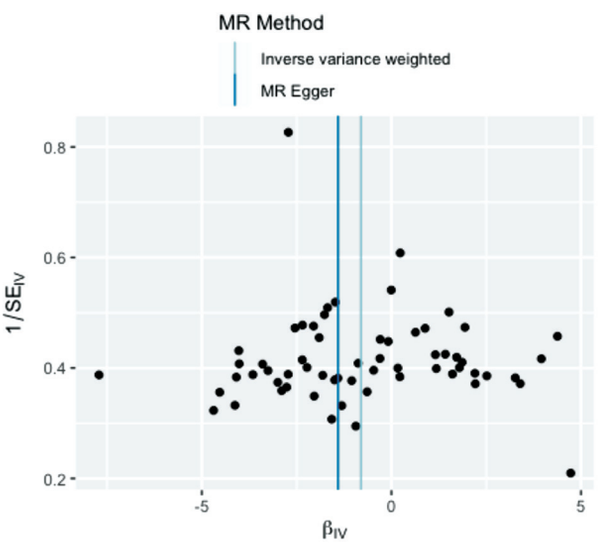

Figure 3 Funnel plot of the two-samples MR analysis MR: Mendelian randomization.

consistently near the position of the red dot in the graphical representation. Additionally, the *P*-values associated with these findings were all above 0.05, indicating that none of the SNPs exerted a significant influence on the IVs' outcomes. This indicated that the outcomes derived from the IVW method were both stable and reliable. The visual representation of these results can be found in Figure 4.

DISCUSSION

The findings from the MR analysis suggested that consuming cheese was associated with a reduced risk of developing DR. This has significant implications for the clinical understanding of the etiology, diagnosis, treatment, and prevention of DR. The pathogenesis of DR is mainly the disorder of glucose metabolism, leading to retinal microcirculation disorder, which leads to vascular occlusion, local ischemic necrosis and nerve

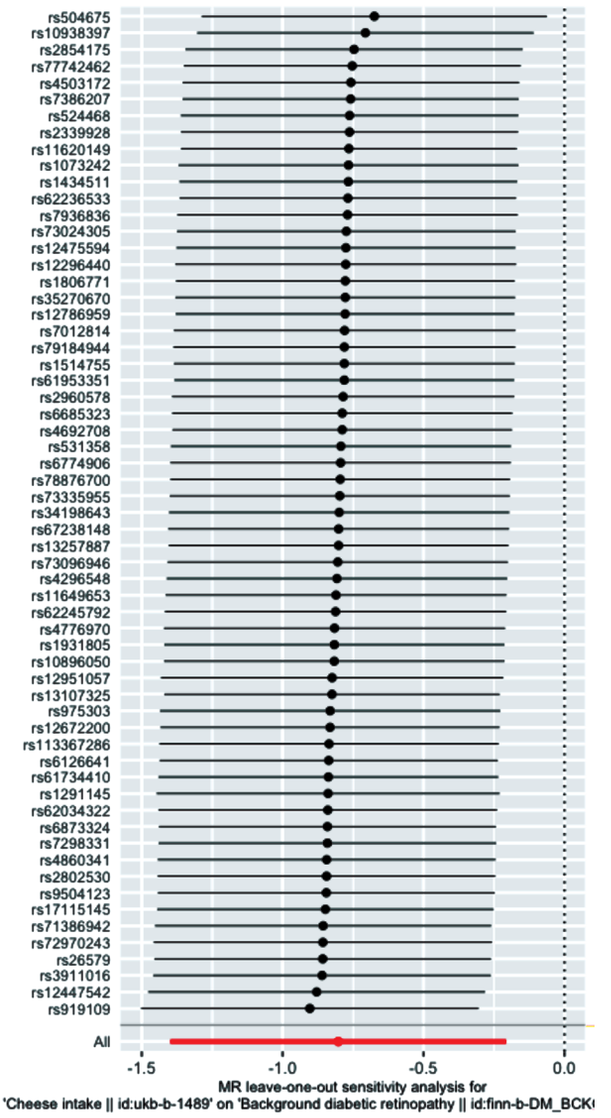

Figure 4 Result of "leave-one-out" analysis.

cell apoptosis. Although the exact mechanisms are not fully elucidated, studies have found numerous inflammatory factors involved. Nuclear factor-kappaB could regulate the expression of various inflammatory factors, including intercellular cell adhesion molecule, tumor necrosis factor-alpha (TNF-α), as well as cyclooxygenase-2 (COX-2). Activation of these inflammatory factors can cause cell apoptosis and endothelial cell injury, which is one of the reasons for vascular lesions associated with DR[16-18]. In addition, studies have shown a significant increase in angiopoietins-2 (Ang-2) levels in the retina of patients with DR, suggesting Ang-2 as a major regulatory factor in retinal vascular proliferation and inflammation[19]. TNF-α is one of the most important inflammatory factors, which is distributed on the surface of vascular endothelial cells and plays an important role in many aspects such as cell apoptosis, inflammatory response and immune mediation[20]. The study has found that the link between the TNF-α and the increased expression and activation of transcription factor runt-related transcription factor 1.

Runt-related transcription factor 1 is significantly involved in the progression of proliferative diabetic retinopathy[21]. Gao *et al*[22] found that the consumption of cheese could lower the likelihood of developing type 2 diabetes. Guo *et al*[23] further investigated and found that cheese intake was negatively correlated with cardiovascular disease. Cheese can reduce the risk of DR, and the potential mechanisms are as follows. Cheese is a rich source of protein, including casein, whey protein, and lactalbumin, potentially mitigating the inflammatory responses associated with chronic diseases in animal models[24]. Studies have also found that proteins in dairy products have anti-inflammatory effects[25]. One potential mechanism that cheese reduces the risk of DR by reducing the inflammatory response through its protein richness.

In this study, we used the publicly available GWAS data, specifically selecting a population of European ethnicity. This choice was made to minimize the potential bias in the results that could arise from ethnic variability. Heterogeneity tests were conducted, and the QP-values for both the IVW and MR-Egger methods exceeded 0.05, suggesting that there was no evidence of heterogeneity within the data obtained from these analyses. The multi-pleiotropy test showed that $P>0.05$, suggesting that the results were not pleiotropy. Sensitivity analysis using the "leave-one-out" approach confirmed the stability of the findings. This investigation delved into the correlation between the consumption of cheese and DR. With cheese intake considered as the exposure factor and DR as the outcome factor, the causality between these two was examined through MR analysis, suggesting that consuming cheese might lower the likelihood of developing DR.

There were certain limitations in the present study. First, the research was confined to European populations, indicating it could not establish whether genetic variations exist among different ethnic groups, countries, or regions. Second, the absence of comprehensive clinical data precluded the execution of subgroup analyses, which would have been necessary to ascertain specific causal relationships.

In conclusion, this study considered cheese intake as the exposure factor, selected SNPs with significant correlation as the tool variable and analyzed by IVW, MR-Egger regression and WME methods. Sensitivity analysis found no pleiotropy and heterogeneity, suggesting that the consumption of cheese may reduce the risk of DR. Nevertheless, due to the genetic variations exist among different ethnicities, countries, and regions, further studies on different populations are needed in the future.

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