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# Glioma raises periodontitis risk via CD8 upregulation on NKT cells: a Mendelian randomization study

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## Abstract

**Objectives** Gliomas, primary tumors of the central nervous system, and periodontitis, a chronic inflammatory disease impacting oral health, have both been subjects of extensive research due to their significant impact on patients' well-being. This study delves into the question of whether there is a causal relationship between glioma and periodontitis, mediated by systemic immunological changes.

**Methods** This research draws from a wealth of publicly available genetic data, including genome-wide association studies for glioma, periodontitis, and immune cell traits. A comprehensive Mendelian randomization (MR) analysis is conducted, incorporating multiple MR methods and statistical tests to assess causality and account for possible biases.

**Results** The findings indicate that individuals genetically predisposed to glioma face an increased risk of developing periodontitis. Furthermore, CD8 upregulation on NKT cells was identified as a mediator in this causal pathway, providing a partial explanation for the observed connection. This discovery aligns with clinical observations of glioma patients exhibiting a higher prevalence of poor periodontal health.

**Conclusions** This study advances our understanding of the complex interplay between glioma and systemic diseases like periodontitis. It underscores feasible implications for patient care and opens avenues for future research to explore the mechanistic underpinnings of this relationship.

**Keywords** Glioma · Glioblastoma · Periodontitis · Periodontal disease · CD8 · NKT cell · Mendelian randomization

## Abbreviations

| | |
|---|---|
| CD | Cluster of differentiation |
| CI | Confidence interval |
| LASSO | Least absolute shrinkage and selection operator |
| mRNA | Messenger ribonucleic acid |

Shanmu Jin, Ningrui Zhao and Kaiming Wang have contributed equally to this work.

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| | |
|---|---|
| NKT | Natural killer T |
| NMR | Nuclear magnetic resonance |
| OR | Odds ratio |
| PRESSO | Pleiotropy residual sum and outlier |
| rRNA | Ribosomal ribonucleic acid |
| SE | Standard error |
| Th | T helper |
| WHO | World Health Organization |

## 1 Introduction

Gliomas, the most prevalent primary tumors of the CNS with an incidence rate of 5.9 per 100,000 population, account for approximately 24% of all primary CNS tumors [1]. According to the fifth edition of the WHO classification of CNS tumors [2], gliomas are categorized into four groups, namely adult-type diffuse gliomas, pediatric-type diffuse low-grade gliomas, pediatric-type diffuse high-grade gliomas, and circumscribed astrocytic gliomas, and are stratified into grade 1 to 4 based on histological and molecular features. Despite the implementation of multimodal treatment approaches, gliomas generally have a bleak prognosis, with the most common subtype, glioblastoma (GBM), exhibiting a five-year survival rate of only 6.9% [1].

Periodontitis is a complex inflammatory disease that is triggered by subgingival dental biofilm and ultimately leads to irreversible damage to the supporting and surrounding structures of the teeth, such as the periodontal ligament and alveolar bone [3]. It is one of the most common chronic diseases in the population, with a prevalence estimated at 42.2% and 7.8% of individuals experiencing severe symptoms, including tooth loss, pain, and impaired chewing function [4].

Several studies have investigated the relationship between glioma and periodontitis. The initial study that specifically examined this connection conducted oral examinations and NMR spectroscopy analysis of salivary metabolites in both GBM patients and non-GBM controls. However, this study did not find any significant association between GBM and periodontitis [5]. Subsequently, another study focused on the association between oral microbiota and glioma grade. It discovered that the relative abundance of *Porphyromonas*, a crucial genus in the pathogenesis of periodontitis, decreased significantly in patients with high-grade glioma (HGG) compared to healthy controls, as determined through 16S rRNA gene sequencing [6]. In a more recent study, it was found that lipopolysaccharide (LPS) derived from *Porphyromonas gingivalis*, a classic periodontal pathogen, facilitated the proliferation and migration of glioma cells by activating the Akt signaling pathway. Additionally, patients with gliomas were found to have a higher prevalence of poor periodontal health compared to those with benign CNS tumors or the age-matched population from a national survey. Moreover, glioma patients with worse periodontal conditions exhibited a higher tumor proliferation index than those with better periodontal conditions [7]. A latest bioinformatics study based on mRNA expression data revealed that CXCR4, LY96, and C3 are significantly upregulated in both GBM and periodontitis, identifying them as key crosstalk genes in the comorbidity mechanisms of these conditions. These immune- and inflammation-related genes are likely regulated by the same transcription factor, FLI1, and are associated with M2 macrophage polarization [8]. Together, the results from these studies provide valuable but still limited insights into the relationship concerning glioma and periodontitis. Further exploration is needed to expand our knowledge in this field.

Glioma has long been recognized for its impact on systemic immunity [9–11], while immunological pathways play a crucial role in the development of periodontitis [12]. Given the observed lower periodontal health in glioma patients compared to the general population [7], we hypothesize that glioma may increase the susceptibility to periodontitis through immune-mediated mechanisms. Mendelian randomization (MR) emerges as a robust tool for addressing such causal inference questions in medical research, a challenge often faced by conventional study designs. It relies on genetic variations, primarily single-nucleotide polymorphisms (SNPs), as instrumental variables (IVs) to estimate causal effects, thereby mitigating bias resulting from unobserved confounding of the exposure and the outcome [13]. In this study, we employ MR to investigate the potential causal relationship between glioma and periodontitis, mediated by systemic immunological changes.

## 2 Materials and methods

### 2.1 Study design

This study used a network (or two-step) MR design [14–16], comprising a series of univariable two-sample MR analyses to assess the total effect of glioma on periodontitis, the effect of glioma on each immune trait, and the effect of each immune trait on periodontitis, as illustrated in Fig. 1.

### 2.2 Data sources

Publicly available summary-level data from existing genome-wide association studies (GWASs) were used for glioma, periodontitis, and immune cell traits. Association results for glioma risk loci were extracted from the largest GWAS meta-analysis to date [17], comprising 12,496 cases (6,191 classified as GBM and 5,819 classified as non-GBM tumors) and 18,190 controls from eight studies involving individuals of European ancestry. Summary statistics for periodontitis were obtained from the Gene-Lifestyle Interaction in the Dental Endpoints (GLIDE) consortium [18], which included 12,289 cases and 22,326 controls of European descent, with participants from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) excluded, as they were recruited from Hispanic and Latino communities in the USA. Genetic associations of 731 immunophenotypes, including 118 absolute cell counts, 389 median fluorescence intensities (MFIs) of surface antigens, 32 morphological parameters, and 192 relative counts, were retrieved from a GWAS study conducted on a cohort of 3,757 European individuals [19].

### 2.3 Statistical analysis

All analyses were conducted using R (version 4.2.3) within RStudio (version 2023.03.2 + 454). The MungeSumstats package (version 1.6.0) was utilized for the standardization of GWAS summary statistics. For the MR analysis, the MendelianRandomization package (version 0.7.0) and the TwoSampleMR package (version 0.5.7) were employed. The graphical presentation of the MR results was generated using the ggplot2 package (version 3.4.2).

Each two-sample analysis in the network MR was based on three core IV assumptions: (1) Relevance: the genetic variants are associated with the exposure; (2) Independence: the genetic variants are not associated with confounders; and (3) Exclusion restriction: the genetic variants influence the outcome only through the exposure [20, 21].

SNPs with $p < 5 \times 10^{-5}$ were considered statistically significant at the genome-wide level, aiming to strike a balance between a robust association with the exposure and a sufficient number of IVs [22]. Clumping thresholds were set at $r^2 < 0.1$ and a distance greater than 1000 kb to exclude SNPs with strong linkage disequilibrium (LD). For each SNP, the proportion of variance in the exposure that it explained was calculated as $R^2 = \beta^2/(\beta^2 + N \times \mathrm{SE}(\beta)^2)$, and the $F$ statistic was

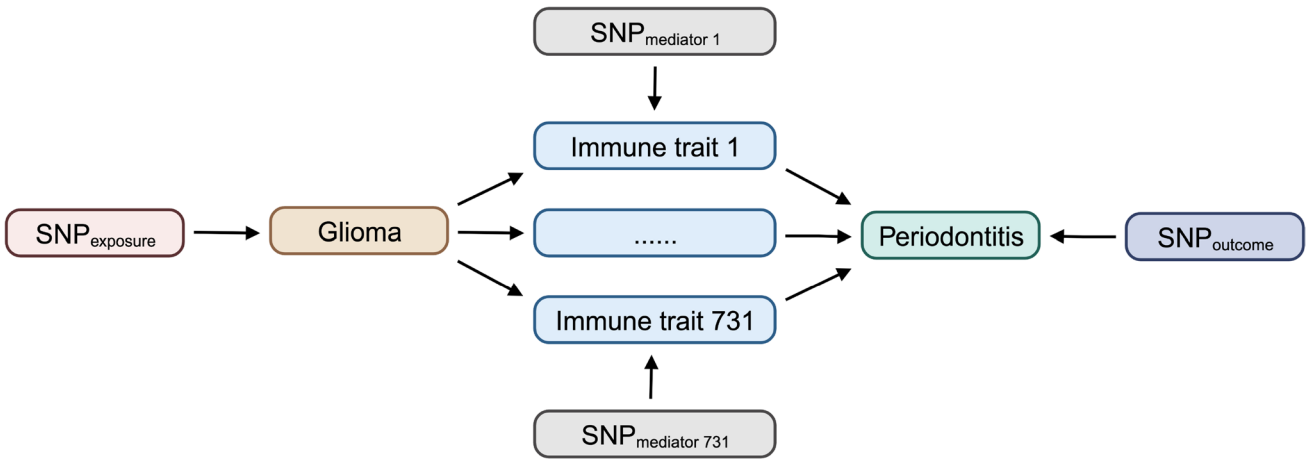


**Fig. 1** The study design of network MR, with glioma as the exposure, immune traits as the mediators, and periodontitis as the outcome. Created with BioRender.com

computed using $F=(N-2)\times R^2/(1-R^2)$, where $\beta$ represented its genetic association with the exposure, and $N$ represented the sample size (the sum of the number of cases and the number of controls) [23]. SNPs with an $F$ statistic of less than 10 were removed as weak instruments. Additionally, SNPs that showed significant genome-wide association with the outcome ($p<5\times10^{-5}$) were also eliminated.

Variants with Cook's distance exceeding 4 / $n$ (where $n$ represented the total number of instrumental SNPs) or absolute values of studentized residuals greater than two were identified as influential outliers and subsequently excluded from the MR analysis [24, 25]. The primary analysis utilized the inverse-variance weighted (IVW) method. Supplementary analyses encompassed alternative methods based on different instrumental variable assumptions, including MR-Egger [26], simple median, weighted median, simple mode, and weighted mode. MR-PRESSO [27], MR-LASSO [28], and the intercept test of MR-Egger regression were employed to detect horizontal pleiotropy. Heterogeneity in causal estimates from different variants was evaluated using Cochran's $Q$ statistic [29]. The dependence of an MR estimate on a specific variant was assessed through leave-one-out analysis. A two-sided $p$-value threshold of less than 0.05 was set for both the MR estimates and the sensitivity analyses. In light of the typical low power of MR studies, correction for multiple testing was not adopted, as a conservative approach could often be excessive [30].

## 3 Results

A total of 28 SNPs were significantly associated with glioma at the genome-wide level (Supplementary Table 1). Seven SNPs (rs2736100, rs2252586, rs4977756, rs11599775, rs498872, rs1801591, and rs6010620) were removed during the clumping process due to LD with other variants. The remaining 21 SNPs all displayed $F$ statistics greater than 10, with a mean value of 85.6. After harmonizing the alleles and effects between glioma and periodontitis, one variant (rs11196067) was excluded for being palindromic with intermediate allele frequency. No SNPs exhibited significant associations with periodontitis. Four SNPs (rs10069690, rs2235573, rs2297440, and rs55705857) with large Cook's distances or studentized residuals were identified as outliers and removed. In total, 16 SNPs were considered eligible IVs for the MR analysis. The primary IVW method demonstrated a significant association between glioma and an increased risk of periodontitis [$p=0.0002$, OR (95% CI) = 1.1328 (1.0612–1.2093)], with consistent support from other methods (Table 1 and Fig. 2A). A scatter plot of instrumental variants and regression lines of causal estimates for different MR tests were presented in Fig. 2B. Both MR-PRESSO and MR-LASSO retained all 16 IVs. The MR-Egger intercept test yielded a $p$-value of 0.19. No highly influential variants were found among the IVs in the leave-one-out analysis (Fig. 2C). The funnel plot appeared generally symmetrical (Fig. 2D). These results collectively indicated the absence of significant horizontal pleiotropy. Additionally, Cochran's $Q$ statistic did not detect heterogeneity among the eligible variants ($p=0.96$). As a result, our findings supported a causal association between glioma and periodontitis.

We conducted a mediation analysis using a two-step MR approach, screening agnostically through the 731 immune cell traits with the same two-sample methodology used to assess the total effect. Immune traits with $p$-values less than 0.05 in the IVW regression as both outcomes and exposures were considered mediators. Among them, the MFI of CD8 on NKT cells was the only trait that met this criterion [glioma-CD8 on NKT cells: $p=0.0012$, OR (95% CI) = 1.1725 (1.0649–1.2911); CD8 on NKT cells-periodontitis: $p=0.0287$, OR (95% CI) = 1.0377 (1.0039–1.0726)]. All six MR methods consistently revealed positive correlations in both steps (Table 2; Figs. 3A and 4A). For the glioma-CD8 on NKT cells and CD8 on NKT cells-periodontitis relationships, 17 and 76 SNPs respectively qualified as instruments (Figs. 3B and 4B). Sensitivity analyses using MR-PRESSO, MR-LASSO, MR-Egger intercept test, and Cochran's $Q$ statistic revealed no evidence of horizontal pleiotropy or heterogeneity. No outlying variants were observed in leave-one-out plots (Figs. 3C and 4C) or funnel plots (Figs. 3D and 4D). Consequently, we conclude that genetically predicted higher risk of glioma leads to

**Table 1** MR estimates for the association between glioma and periodontitis

| Exposure | Outcome | Method | $\beta$ | SE | $p$ | OR | 95% CI |
|---|---|---|---|---|---|---|---|
| Glioma | Periodontitis | IVW | 0.1247 | 0.0333 | 0.0002 | 1.1328 | 1.0612–1.2093 |
| | | MR-Egger | 0.0396 | 0.0703 | 0.5821 | 1.0404 | 0.9065–1.1941 |
| | | Simple median | 0.1366 | 0.0503 | 0.0067 | 1.1464 | 1.0387–1.2653 |
| | | Weighed median | 0.1160 | 0.0452 | 0.0103 | 1.1230 | 1.0277–1.2270 |
| | | Simple mode | 0.1195 | 0.0685 | 0.1014 | 1.1270 | 0.9854–1.2888 |
| | | Weighted mode | 0.1125 | 0.0555 | 0.0608 | 1.1191 | 1.0037–1.2477 |

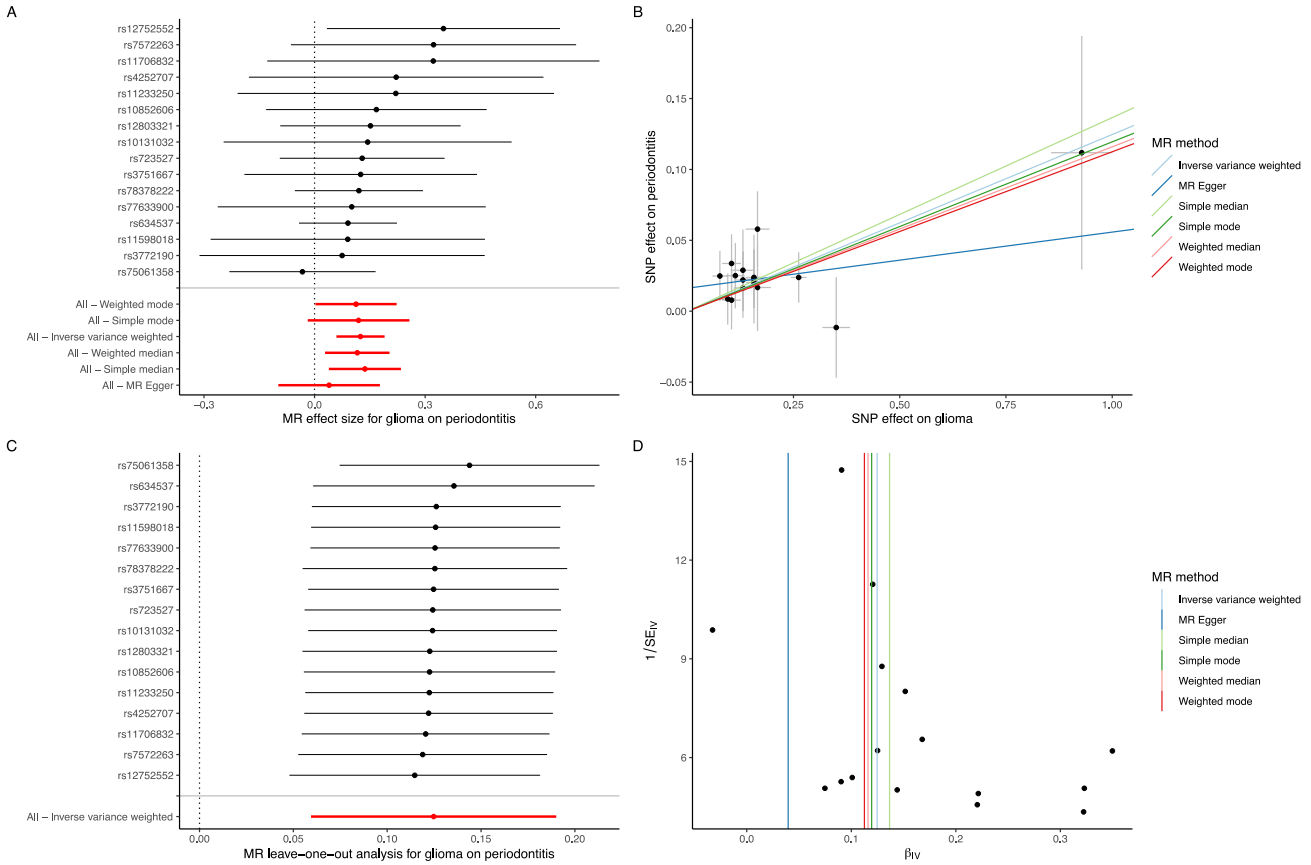


**Fig. 2** MR results supported the causal effect of glioma on periodontitis. **A** Forrest plot. **B** Scatter plot. **C** Leave-one-out plot. **D** Funnel plot

**Table 2** MR estimates for the associations of glioma-CD8 on NKT cells and CD8 on NKT cells-periodontitis

| Exposure | Outcome | Method | *β* | SE | *p* | OR | 95% CI |
|---|---|---|---|---|---|---|---|
| Glioma | CD8 on NKT cells | IVW | 0.1592 | 0.0491 | 0.0012 | 1.1725 | 1.0649–1.2911 |
| | | MR-Egger | 0.1215 | 0.0936 | 0.2138 | 1.1292 | 0.9399–1.3567 |
| | | Simple median | 0.1083 | 0.0815 | 0.1839 | 1.1144 | 0.9499–1.3074 |
| | | Weighed median | 0.1129 | 0.0709 | 0.1112 | 1.1196 | 0.9743–1.2865 |
| | | Simple mode | 0.0762 | 0.1091 | 0.4950 | 1.0792 | 0.8714–1.3364 |
| | | Weighted mode | 0.1037 | 0.0798 | 0.2120 | 1.1093 | 0.9487–1.2970 |
| CD8 on NKT cells | Periodontitis | IVW | 0.0370 | 0.0169 | 0.0287 | 1.0377 | 1.0039–1.0726 |
| | | MR-Egger | 0.0081 | 0.0303 | 0.7898 | 1.0082 | 0.9499–1.0699 |
| | | Simple median | 0.0353 | 0.0273 | 0.1959 | 1.0360 | 0.9820–1.0929 |
| | | Weighed median | 0.0046 | 0.0284 | 0.8714 | 1.0046 | 0.9502–1.0621 |
| | | Simple mode | 0.0269 | 0.0466 | 0.5657 | 1.0272 | 0.9376–1.1255 |
| | | Weighted mode | 0.0124 | 0.0282 | 0.6616 | 1.0125 | 0.9580–1.0700 |

an increase in CD8 on NKT cells, subsequently elevating the susceptibility to periodontitis. The mediated proportion of the total effect, calculated using the delta method [15], was determined to be 4.72% with a 95% CI ranging from 0.37% to 10.78%. Subtype analyses in GBM and non-GBM tumors did not uncover concordant relationships. The overall causal effect was found to be non-significant in GBMs [$p = 0.0968$, OR (95% CI) = 1.0443 (0.9922–1.0990)]. In non-GBM gliomas, despite a significant and consistent direct effect in the IVW regression [$p = 0.0101$, OR (95% CI) = 1.0674 (1.0157–1.1217)], mediation analysis did not identify any eligible mediators.

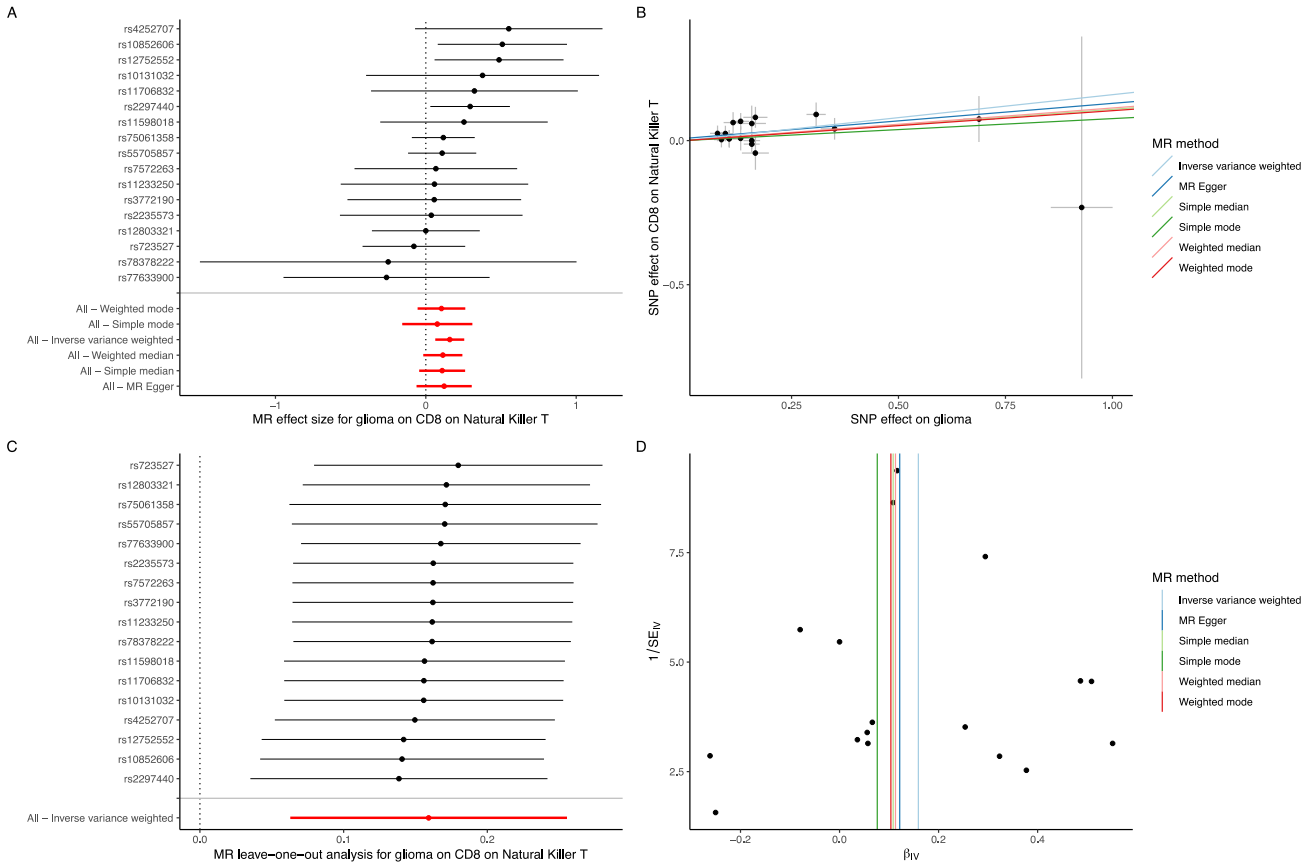


**Fig. 3** MR results supported the causal relationship between glioma and CD8 on NKT cells. **A** Forrest plot. **B** Scatter plot. **C** Leave-one-out plot. **D** Funnel plot

## 4 Discussion

In this study, we unveil a casual association between glioma and periodontitis. A mediation analysis using a network MR approach revealed that an increase in CD8 on NKT cells could partially account for the relationship between these two diseases. Our findings shed light on the explanation for the higher incidence of poor periodontal health in glioma patients, as previously reported [7]. Moreover, these results contribute to a deeper understanding of the connection between glioma and systemic conditions.

NKT cells are CD1d-restricted, lipid antigen-reactive T lymphocytes that modulate cell-mediated immune responses in a broad spectrum of diseases, including cancer, infection, allergy, autoimmunity, allograft rejection, and graft-versus-host disease [31]. Three subsets of NKT cells have been described in humans: CD4+CD8- (CD4+), CD4-CD8+(CD8+), and CD4-CD8- (double negative, DN) [32]. CD4+NKT cells mainly produce Th2-like cytokines such as IL-4, whereas the CD4- (CD8+ and DN) subpopulations predominantly produce Th1-like cytokines like IFN-γ [33]. The peripheral ontogeny of human NKT cells follows a linear differentiation model. CD4- NKT cells display lower clonogenic potential but greater functionality compared to CD4+NKT cells, indicating a more differentiated phenotype. The expression of CD8 on CD4- NKT cells is reversible and arises as a consequence of in vivo activation rather than programmed differentiation [34]. Gene expression patterns of CD8 NKT cells differ significantly from other NKT cell subsets, with the upregulation of genes such as CD16, NKG2C, NKG2D, CX3CR1, CCL7, STAT4 and STAT5B, suggesting a Th1-biased cytotoxic effector cell phenotype [35].

A significant proportion of tumor-infiltrating lymphocytes (TILs) in GBM are CD4 single-positive CD3+CD56+NKT cells that produces Th2 cytokines such as IL-4 and IL-13, which are likely involved in the suppression of cytotoxic T lymphocytes (CTLs) within the GBM microenvironment. In contrast, the vast majority of CD3+CD56+NKT cells found in the periphery blood of GBM patients are CD8 single-positive [36]. This observation leads to the speculation that glioma may

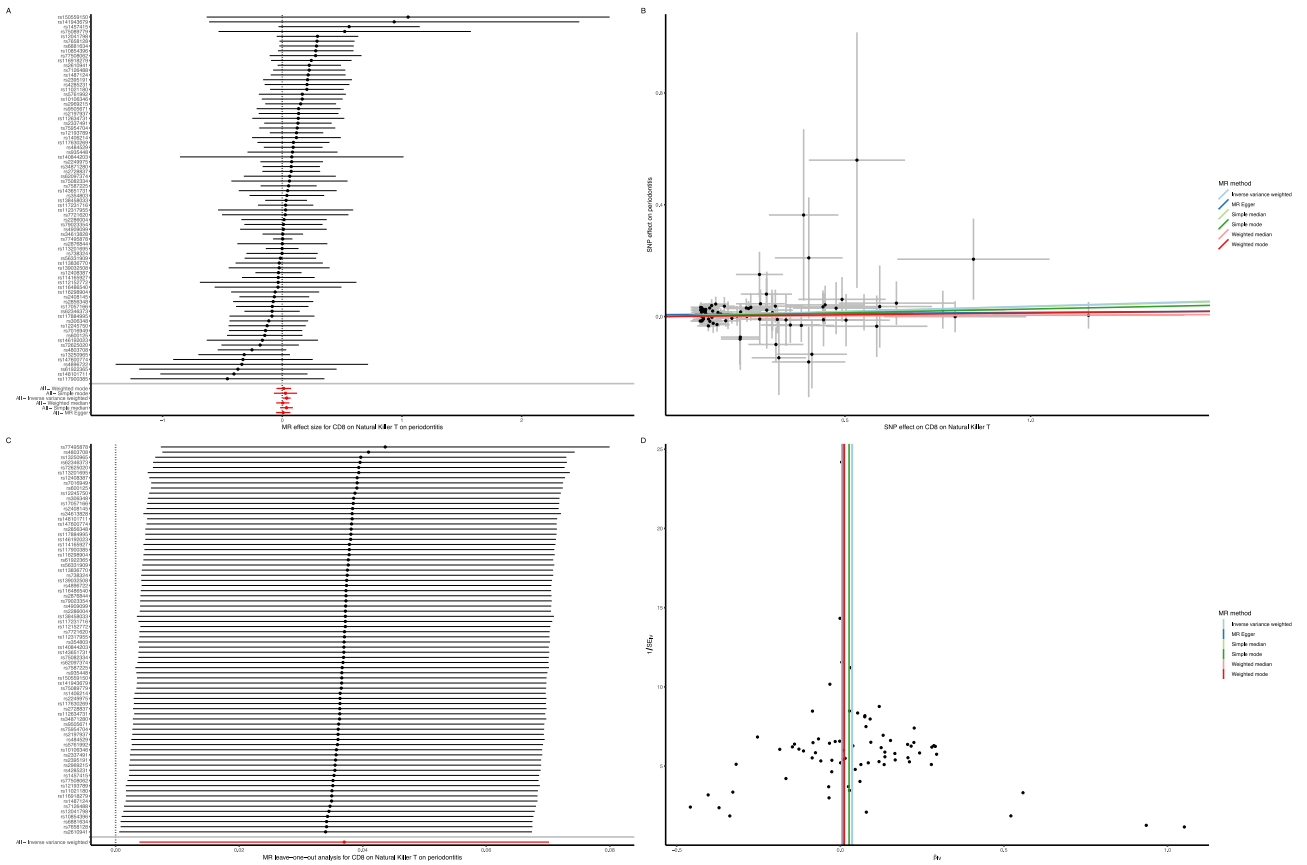

**Fig. 4** MR results supported the causal relationship between CD8 on NKT cells and periodontitis. **A** Forrest plot. **B** Scatter plot. **C** Leave-one-out plot. **D** Funnel plot

stimulate the increase of peripheral CD8+NKT cells while selectively recruiting immunosuppressive CD4+NKT cells into the tumor in the retention of immunopromoting CD8+NKT cells in the circulation, contributing to the establishment of a cold tumor microenvironment.

In periodontitis gingival tissues, the number of infiltrating CD3+CD56+NKT cells is significantly higher than healthy specimens [37]. CXCL16 released by gingival fibroblasts in diseased periodontal tissues attracts NKT cells which normally express CXCR6. IFN-γ secreted by NKT cells further stimulates the production of CXCL16, increasingly shifting the Th1/Th2 balance in the Th1 direction, thus causing inflammation and periodontal destruction [38]. Systemic administration of α-galactosylceramide (αGC), an NKT cell agonist, promoted *Porphyromonas gingivalis*-induced alveolar bone resorption, while this bone destruction was markedly suppressed in CD1d$^{-/-}$ mice, suggesting a pivotal role for NKT cells in the pathogenesis of periodontitis [39]. Indeed, NKT cells favor the increase of RANKL expression, the key factor responsible for osteoclastogenesis and osteoclast-mediated bone resorption [40].

Given the preceding information, we propose a model to elucidate the findings derived from our MR analysis (Fig. 5), assuming that the increase in average CD8 expression on NKT cells corresponds to an increase in the proportion of CD8+NKT cells. In this model, glioma serves as the trigger for the transformation of DN NKT cells into CD8+NKT cells within the peripheral blood. Subsequently, these CD8+NKT cells migrate in a chemotactic manner into periodontal tissues affected by pathogenic microbes such as *Porphyromonas gingivalis*. In a CD1d-dependent manner, they produce pro-inflammatory Th1-like cytokines that activate osteoclasts, ultimately leading to periodontal bone destruction.

Despite being productive, our analysis does have limitations. Firstly, confounding may have been introduced into the mediation analysis by population stratification [13]. The GWAS of 731 immune traits was conducted in Sardinians, who demonstrate certain differences in genetic backgrounds compared to general Europeans [19]. However, since a comprehensive GWAS on immune cell characteristics has not been carried out in the general European population, we were limited to working with the currently available genetic instruments. Secondly, although network MR could be used to detect the presence or absence of a causal pathway between the exposure, mediator, and outcome in cases involving

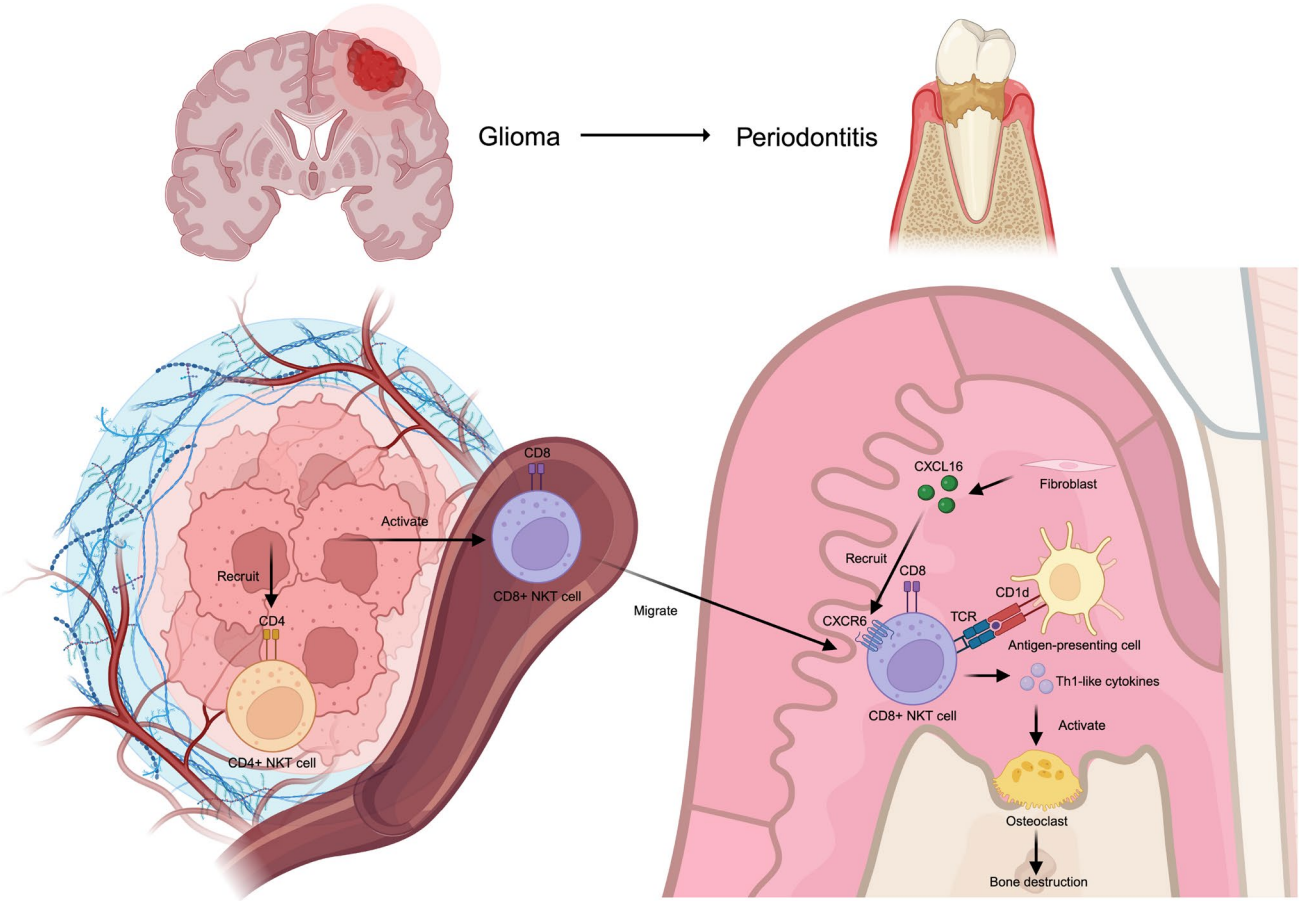


**Fig. 5** Mechanistic model for the causal effect of glioma on periodontitis through elevation of CD8 on NKT cells. CD8 + NKT cells stimulated by glioma chemotactically migrate into periodontal tissues, secrete Th-1 cytokines in a CD1d-dependent manner, and cause bone destruction via osteoclast activation. Created with BioRender.com

binary variables, it may not give precise estimates of the direct and indirect effects. Furthermore, the estimates of mediated and total effects are difficult to completely avoid other known biases in MR, including those arising from weak instruments and pleiotropy [16, 41]. Lastly, our study may have left meaningful associations undetected due to inadequate statistical power. This is particularly relevant in the context of two-step MR [14, 41] and glioma subtype analyses [42–44], considering that the full extent of the causal effect remains largely unexplained except for the upregulation of CD8 on NKT cells and that effect estimates are inconsistent in GBM and non-GBM tumors.

In summary, this study represents a pioneering effort in establishing a causal link between glioma and extracranial inflammatory diseases, specifically periodontitis, in part through systemic immune alterations. Future research endeavors should prioritize investigating the periodontal health of glioma patients within prospective cohorts of larger sizes, validating periodontitis susceptibility in the presence of glioma using orthotopic tumor models, and providing mechanistic insights into this phenomenon through experimental evidences at the cellular and molecular levels. Unraveling the genetic links between glioma and periodontitis also carries direct clinical implications. Identifying the genetic predisposition to periodontitis could facilitate innovative management strategies for individuals with glioma. Incorporating regular periodontal assessments and treatments into standard care protocols for glioma patients offers the potential to disrupt the vicious circle in which glioma predisposes periodontitis while periodontal pathogens like *Porphyromonas gingivalis* promote glioma progression through pathogenic factors such as LPS. This proactive approach may not only enhance oral health but also improve the quality of life and survival outcomes for glioma patients.

**Author contribution** S.J., N.Z., and K.W. designed the workflow, analyzed the data, prepared the figures and tables, and drafted the manuscript. X.W., Y.W., and W.M conceptualized the study, provided resources, and critically revised the manuscript. All authors have approved the final version of the article.

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**Data availability** GWAS summary statistics for glioma and periodontitis are available at https://static-content.springer.com/esm/art%3A10.1038%2Fng.3823/MediaObjects/41588_2017_BFng3823_MOESM54_ESM.xlsx and https://data.bris.ac.uk/datasets/tar/2j2rqgzedxlq02oqbb4vmycnc2.zip, respectively. GWAS summary statistics for 731 immune-related traits are accessible in the OpenGWAS database (https://gwas.mrcieu.ac.uk) with accession numbers from ebi-a-GCST90001391 to ebi-a-GCST90002121. The R code used for the MR analysis in this study has been deposited and will be maintained in the following GitHub repository: https://github.com/jinshanmu/glioma_and_periodontitis.

## Declarations

**Ethics approval and consent to participate** Not applicable.

**Informed consent** Not applicable.

**Competing interests** The authors declare no competing interests.



## References

1. Ostrom QT, Price M, Neff C, Cioffi G, Waite KA, Kruchko C, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2015–2019. Neuro Oncol. 2022;24(Suppl 5):v1–95. https://doi.org/10.1093/neuonc/noac202.
2. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol. 2021;23(8):1231–51. https://doi.org/10.1093/neuonc/noab106.
3. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15(1):30–44. https://doi.org/10.1038/nri3785.
4. Eke PI, Borgnakke WS, Genco RJ. Recent epidemiologic trends in periodontitis in the USA. Periodontol 2000. 2020;82(1):257–67. https://doi.org/10.1111/prd.12323.
5. Garcia-Villaescusa A, Morales-Tatay JM, Monleon-Salvado D, Gonzalez-Darder JM, Bellot-Arcis C, Montiel-Company JM, et al. Using NMR in saliva to identify possible biomarkers of glioblastoma and chronic periodontitis. PLoS ONE. 2018;13(2): e0188710. https://doi.org/10.1371/journal.pone.0188710.
6. Wen Y, Feng L, Wang H, Zhou H, Li Q, Zhang W, et al. Association between oral microbiota and human brain glioma grade: a case-control study. Front Microbiol. 2021;12: 746568. https://doi.org/10.3389/fmicb.2021.746568.
7. Gao Z, Weng X, Yu D, Pan Z, Zhao M, Cheng B, et al. *Porphyromonas gingivalis*-derived lipopolysaccharide promotes glioma cell proliferation and migration via activating AKT signaling pathways. Cells. 2022. https://doi.org/10.3390/cells11244088.
8. Huang JH, Chen Y, Kang YB, Yao ZJ, Song JH. The potential crosstalk genes and molecular mechanisms between glioblastoma and periodontitis. Sci Rep. 2024;14(1):5970. https://doi.org/10.1038/s41598-024-56577-2.
9. Waziri A. Glioblastoma-derived mechanisms of systemic immunosuppression. Neurosurg Clin N Am. 2010;21(1):31–42. https://doi.org/10.1016/j.nec.2009.08.005.
10. Grabowski MM, Sankey EW, Ryan KJ, Chongsathidkiet P, Lorrey SJ, Wilkinson DS, et al. Immune suppression in gliomas. J Neurooncol. 2021;151(1):3–12. https://doi.org/10.1007/s11060-020-03483-y.
11. Lorrey SJ, Waibl Polania J, Wachsmuth LP, Hoyt-Miggelbrink A, Tritz ZP, Edwards R, et al. Systemic immune derangements are shared across various CNS pathologies and reflect novel mechanisms of immune privilege. Neurooncol Adv. 2023;5(1):vdad035. https://doi.org/10.1093/noajnl/vdad035.
12. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000. 2014;64(1):57–80. https://doi.org/10.1111/prd.12002.
13. Sanderson E, Glymour MM, Holmes MV, Kang H, Morrison J, Munafo MR, et al. Mendelian randomization. Nat Rev Methods Primers. 2022. https://doi.org/10.1038/s43586-021-00092-5.
14. Relton CL, Davey SG. Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. Int J Epidemiol. 2012;41(1):161–76. https://doi.org/10.1093/ije/dyr233.
15. Burgess S, Daniel RM, Butterworth AS, Thompson SG, Consortium EP-I. Network Mendelian randomization: using genetic variants as instrumental variables to investigate mediation in causal pathways. Int J Epidemiol. 2015;44(2):484–95. https://doi.org/10.1093/ije/dyu176.

16. Sanderson E. Multivariable Mendelian randomization and mediation. Cold Spring Harb Perspect Med. 2021. https://doi.org/10.1101/cshperspect.a038984.
17. Melin BS, Barnholtz-Sloan JS, Wrensch MR, Johansen C, Il'yasova D, Kinnersley B, et al. Genome-wide association study of glioma subtypes identifies specific differences in genetic susceptibility to glioblastoma and non-glioblastoma tumors. Nat Genet. 2017;49(5):789–94. https://doi.org/10.1038/ng.3823.
18. Shungin D, Haworth S, Divaris K, Agler CS, Kamatani Y, Keun Lee M, et al. Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data. Nat Commun. 2019;10(1):2773. https://doi.org/10.1038/s41467-019-10630-1.
19. Orru V, Steri M, Sidore C, Marongiu M, Serra V, Olla S, et al. Complex genetic signatures in immune cells underlie autoimmunity and inform therapy. Nat Genet. 2020;52(10):1036–45. https://doi.org/10.1038/s41588-020-0684-4.
20. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. JAMA. 2021;326(16):1614–21. https://doi.org/10.1001/jama.2021.18236.
21. Skrivankova VW, Richmond RC, Woolf BAR, Davies NM, Swanson SA, VanderWeele TJ, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomisation (STROBE-MR): explanation and elaboration. BMJ. 2021;375: n2233. https://doi.org/10.1136/bmj.n2233.
22. Baumeister SE, Alayash Z, Baurecht H, Reckelkamm SL, Kocher T, Holtfreter B, et al. Cannabis use and the risk of periodontitis: a two-sample Mendelian randomization study. J Clin Periodontol. 2022;49(7):654–61. https://doi.org/10.1111/jcpe.13632.
23. Luo S, Li W, Li Q, Zhang M, Wang X, Wu S, et al. Causal effects of gut microbiota on the risk of periodontitis: a two-sample Mendelian randomization study. Front Cell Infect Microbiol. 2023;13:1160993. https://doi.org/10.3389/fcimb.2023.1160993.
24. Altman N, Krzywinski M. Analyzing outliers: Influential or nuisance? Nat Methods. 2016;13(4):281–2. https://doi.org/10.1038/nmeth.3812.
25. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32(5):377–89. https://doi.org/10.1007/s10654-017-0255-x.
26. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–25. https://doi.org/10.1093/ije/dyv080.
27. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018;50(5):693–8. https://doi.org/10.1038/s41588-018-0099-7.
28. Rees JMB, Wood AM, Dudbridge F, Burgess S. Robust methods in Mendelian randomization via penalization of heterogeneous causal estimates. PLoS ONE. 2019;14(9): e0222362. https://doi.org/10.1371/journal.pone.0222362.
29. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. Stat Med. 2015;34(21):2926–40. https://doi.org/10.1002/sim.6522.
30. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing Mendelian randomization investigations: update for summer 2023. Wellcome Open Res. 2019;4:186. https://doi.org/10.12688/wellcomeopenres.15555.3.
31. Godfrey DI, Stankovic S, Baxter AG. Raising the NKT cell family. Nat Immunol. 2010;11(3):197–206. https://doi.org/10.1038/ni.1841.
32. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: What's in a name? Nat Rev Immunol. 2004;4(3):231–7. https://doi.org/10.1038/nri1309.
33. Lin H, Nieda M, Nicol AJ. Differential proliferative response of NKT cell subpopulations to in vitro stimulation in presence of different cytokines. Eur J Immunol. 2004;34(10):2664–71. https://doi.org/10.1002/eji.200324834.
34. Liu J, Hill BJ, Darko S, Song K, Quigley MF, Asher TE, et al. The peripheral differentiation of human natural killer T cells. Immunol Cell Biol. 2019;97(6):586–96. https://doi.org/10.1111/imcb.12248.
35. Lin H, Nieda M, Hutton JF, Rozenkov V, Nicol AJ. Comparative gene expression analysis of NKT cell subpopulations. J Leukoc Biol. 2006;80(1):164–73. https://doi.org/10.1189/jlb.0705421.
36. Waziri A, Killory B, Ogden AT 3rd, Canoll P, Anderson RC, Kent SC, et al. Preferential in situ CD4+CD56+ T cell activation and expansion within human glioblastoma. J Immunol. 2008;180(11):7673–80. https://doi.org/10.4049/jimmunol.180.11.7673.
37. Muthukuru M. Technical advance: decreased helper T cells and increased natural killer cells in chronic periodontitis analyzed by a novel method for isolating resident lymphocytes. J Leukoc Biol. 2012;92(3):683–92. https://doi.org/10.1189/jlb.0212103.
38. Hosokawa Y, Hosokawa I, Ozaki K, Nakae H, Matsuo T. CXC chemokine ligand 16 in periodontal diseases: expression in diseased tissues and production by cytokine-stimulated human gingival fibroblasts. Clin Exp Immunol. 2007;149(1):146–54. https://doi.org/10.1111/j.1365-2249.2007.03398.x.
39. Aoki-Nonaka Y, Nakajima T, Miyauchi S, Miyazawa H, Yamada H, Domon H, et al. Natural killer T cells mediate alveolar bone resorption and a systemic inflammatory response in response to oral infection of mice with *Porphyromonas gingivalis*. J Periodontal Res. 2014;49(1):69–76. https://doi.org/10.1111/jre.12080.
40. Tilkeridis K, Kiziridis G, Ververidis A, Papoutselis M, Kotsianidis I, Kitsikidou G, et al. Immunoporosis: a new role for invariant natural killer T (NKT) cells through overexpression of nuclear factor-kappaB ligand (RANKL). Med Sci Monit. 2019;25:2151–8. https://doi.org/10.12659/MSM.912119.
41. Carter AR, Sanderson E, Hammerton G, Richmond RC, Davey Smith G, Heron J, et al. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. Eur J Epidemiol. 2021;36(5):465–78. https://doi.org/10.1007/s10654-021-00757-1.
42. Saunders CN, Cornish AJ, Kinnersley B, Law PJ, Claus EB, Il'yasova D, et al. Lack of association between modifiable exposures and glioma risk: a Mendelian randomization analysis. Neuro Oncol. 2020;22(2):207–15. https://doi.org/10.1093/neuonc/noz209.
43. Howell AE, Robinson JW, Wootton RE, McAleenan A, Tsavachidis S, Ostrom QT, et al. Testing for causality between systematically identified risk factors and glioma: a Mendelian randomization study. BMC Cancer. 2020;20(1):508. https://doi.org/10.1186/s12885-020-06967-2.
44. Saunders CN, Cornish AJ, Kinnersley B, Law PJ, Houlston RS, Collaborators. Searching for causal relationships of glioma: a phenome-wide Mendelian randomisation study. Br J Cancer. 2021;124(2):447–54. https://doi.org/10.1038/s41416-020-01083-1.