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Recurrent aphthous stomatitis and neoplasms of the mouth and pharynx: a two-sample Mendelian randomization study

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Abstract

Background The association between recurrent aphthous stomatitis (RAS) and neoplasms of the mouth and pharynx (NOMAP) has been reported in some previous observational studies. However, causality is still confused. Our research aims to explore the relationship between RAS and NOMAP through a Mendelian randomization (MR) analysis and to explore whether RAS can serve as a risk factor for NOMAP to provide a reference for the clinical strategy.

Methods An exposure dataset for RAS were collected from a published study based on the UK Biobank (UKB). Outcome datasets included Genome-wide association studies (GWAS) summary statistics of NOMAP from the FinnGen datasets. The core method was inverse variance weighting (IVW). The Bonferroni correction, MR-Egger, weighted median, weighted mode, Cochcan's Q test, MR-PRESSO, and leave-one-out methods served as complementary methods.

Results We found no significant evidence of causal relationships between RAS and NOMAP. After applying the Bonferroni correction, the corrected P was equal to 0.00625 (0.05/1/8). The IVW method provided the sole evidence for RAS on Benign neoplasm of floor of mouth (BNFM) (OR = 2.509, 95% CI: 1.296–4.857, $P = 0.006$), but the subsequent MR-Egger regression method showed that this result may be due to horizontal pleiotropy ($P = 0.035$). The Cochran Q-test, MR-Egger regression, and MR-PRESSO did not reveal any heterogeneity or directional pleiotropy for the other outcomes.

Conclusions In conclusion, this is the first MR analysis to investigate the relationship between RAS and NOMAP. Our research confirmed at the genetic level that no causal association has been identified between RAS and NOMAP, therefore facilitating a logical therapeutic perspective and the development of clinical therapies for them.

Keywords Recurrent aphthous stomatitis, Neoplasms of the mouth and pharynx, Mendelian randomization, GWAS

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Background

As the third most prevalent type of cancer in the world, head and neck cancer (HANC) accounts for approximately 7.6% of all types of cancer [1]. Neoplasms of the mouth and pharynx (NOMAP), a type of HANC, can develop in the oropharynx, affect the respiratory tract, digestive tract, salivary glands, and thyroid gland, and impact the peripheral nervous system's main sensory nerves [2]. Early diagnosis and prevention are essential for NOMAP patients, since delayed diagnosis is thought to be a major contributing factor in the patients' poor 5-year survival rate [3, 4]. It is important to search for new risk factors to further tackle NOMAP.

The most prevalent disorder affecting the human oral mucosa is recurrent aphthous stomatitis (RAS) [5]. Its frequency in the general population ranges from 5–25% [6]. The connection between RAS and NOMAP is widely concerned. Hertel et al. discovered a correlation between RAS and oral squamous cell cancer [7]; Lei Qin et al. confirmed that patients with RAS and dry eye syndrome may be associated with a high risk of oral cavity cancer [8]. Rotundo LD et al. found that sores caused by inappropriate dentures were directly associated with oral cancer [9]. However, certain research continues to challenge the point above. In a national population-based study of cancer risk in Korean, a set of participants was matched by a 1:1 propensity score to create a group diagnosed with RAS for 5 years and a control group who had not yet been diagnosed, and no statistically significant differences were revealed between the two categories [10]. Additionally, in a study measuring salivary microRNA (miRNA-21, miRNA-184, and miRNA-145) as potential indicators for malignant transformation of oral mucosal lesions, RAS patients revealed no discernible difference from the normal group [11].

To date, the possible association between RAS and NOMAP has never been confirmed, so it is not listed as a potentially malignant disease or risk factor for oral mucosa [12, 13]. The majority of the studies analyzing their relationship were primarily based on observational data, which can only suggest an association and cannot establish a causal relationship between risk factors and outcomes due to confounding factors.

Mendelian randomization (MR) analysis is an emerging epidemiological method that can overcome confounding factors, reverse causality and various biases that occur in observational epidemiological studies [14]. Since genotypes are present before illness and are unaffected by environmental influences after birth, genetic variations are used as instrumental variables (IVs) in this study [15]. MR can leverage pooled data from many large-sample genome-wide association studies (GWAS) on the relationship between genetic variants for tons of traits and diseases [16]. Our research aims to explore the relationship between RAS and NOMAP through a Mendelian randomization analysis and to explore whether RAS can serve as a risk factor for NOMAP to provide a reference for the clinical strategy.

Methods

Study design

An MR analysis was performed using single-nucleotide polymorphisms (SNPs) as the IVs, and three key assumptions must be satisfied [17]: (1) IVs are closely related to exposure; (2) IVs are independent of the confounding factors between exposure and outcome; (3) IVs do not directly affect the outcome but only influence the outcome through exposure. As shown in Fig. 1, SNPs worked as IVs to explore the causal relationship between exposure and outcome.

The flow chart of the study is shown Fig. 2, using SNPs associated with RAS and NOMAP to study the casual relationship between them.

Data sources

We gathered an exposure dataset for RAS from a published study that identified immune regulatory loci associated with mouth ulcers [18]. The data collected were based on the UK Biobank (UKB), in which all participants completed a baseline questionnaire in which they were all questioned about their oral health condition, and "oral ulcer (yes/no)" was defined as having experienced an oral ulcer during the previous year [18]. In this GWAS study (47079 cases and 413927 controls), the heritability was estimated to be 8.2% (95% CI: 6.4%, 9.9%), and 97 genetic variants were identified. The underlying population is British.

Outcomes datasets included GWAS summary statistics of malignant neoplasm of oral cavity (MNOC), malignant neoplasm of hypopharynx (MNH), benign neoplasm of floor of mouth (BNFM), benign neoplasm of hypopharynx (BNH), benign neoplasm of the mouth and pharynx (BNMP), benign neoplasm of other parts of oropharynx (BNOPO), benign neoplasm of other and unspecified parts of mouth (BNOUPM) and benign neoplasm of unspecified pharynx (BNUP) (Table 1). These data were all derived from the R9 module of the FinnGen database. Because all outcomes were diagnosed before being selected for genotyping in the database, we could not obtain definitive diagnostic criteria. The underlying population is Finnish.

No further ethnic permission was required because all the data utilized had already been made available in public databases. Additionally, there was no sample overlap between the exposure and outcome among the participants in the research.

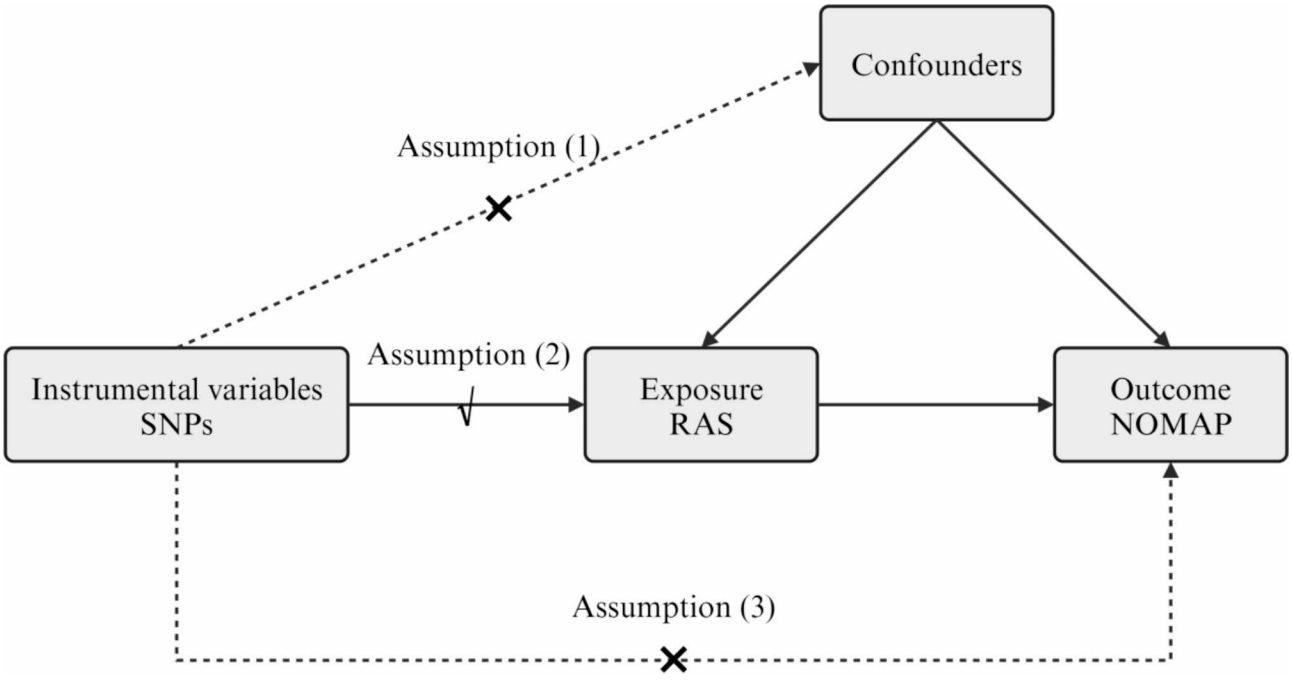

Fig. 1 SNPs worked as IVs to explore the causal relationship between exposure (RAS) and outcome (NOMAP)

Selection of IVs

We performed a rigorous approach to find suitable SNPs as IVs using the GWAS summary data obtained. First, SNPs must have a genome-wide significance level of P value$<5\times10^{-8}$ and be substantially related to exposure. Second, we carried out the clumping technique with R2=0.05 and window size=10,000 kb as the cut-off parameters to prevent biased findings caused by linkage disequilibrium (LD). Third, by using the exposure data obtained in the above steps, we extracted the outcome data and then removed the SNPs substantially associated with the outcome (P value$<5\times10^{-5}$). Fourth, allele inconsistent palindromic and ambiguous SNPs were eliminated in the exposure and outcome datasets to preserve consistency between the effects alleles on exposure and outcome. Finally, confounding variables associated with genetic variation were screened out using the Phenoscanner database (http://www.phenoscanner.medschl.cam.ac.uk/).

Additionally, we evaluated the F-statistic of each SNP independently and chose SNPs with F-statistics larger than 10 as IVs to prevent weak instrumental variable bias and better support the main hypothesis (1) [19]. Following the aforementioned filtering stages, these carefully chosen SNPS were utilized as the final IV for the succeeding MR analysis.

Statistical analyses

To assess the causal relationship between RAS and NOMAP, the following methods were utilized in this study: inverse variance weighting (IVW), MR-Egger, weighted median, and weighted mode. IVW was the main analysis method, which is a meta-analysis technique that combines the causal estimate of Wald for each IV to offer an overall assessment of the impact of exposure on the outcome and is sensitive to pleiotropy [20]. If the number of SNPs exceeds four or the causal estimate shows heterogeneity, the random-effect IVW is recommended; otherwise, the fixed-effect IVW should be utilized [21]. However, studies indicate that in the absence of additional heterogeneity, the results of random-effect IVW and fixed-effect IVW will be identical [22]. This study employed random-effect IVW. To offer reliable causal estimates under various hypotheses, MR-Egger, weight median, and weighted mode were utilized as supplementary methods. Based on the assumption of instrument strength independent of direct effects (InSIDE), MR-Egger performs weighted linear regressions to produce consistent causal effect estimates [23]. However, the accuracy of MR-Egger is relatively poor and susceptible to peripheral genetic variation [24]. By comparing the weighted median of the ratio instrumental variable estimates, the weighted median offers low type I error estimates of effects without the requirement for InSIDE validation [25]. Finally, weighted mode, which yielded a lower type I error rate and less bias than the primary approach, was used to examine the overall causal influence of a large number of genetic instruments [26]. We applied the Bonferroni correction (corrected P=0.05/number of exposures/number of outcomes) to adjust the test level and address the issue of multiple testing.

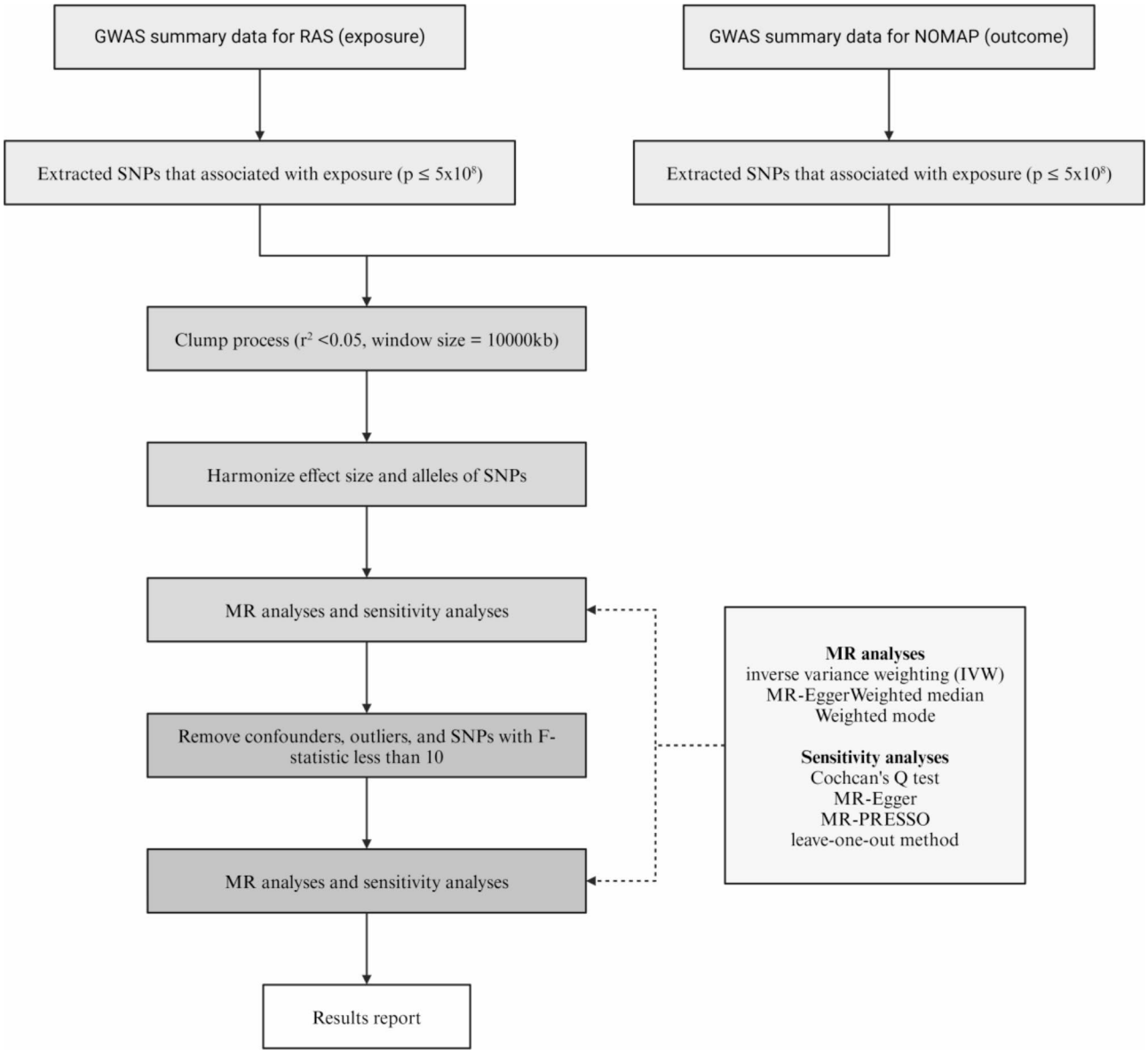

Fig. 2 The flow chart of this study

Table 1 Overview of the GWAS consortiums utilized for each disease

Category	Disease	Sample size	Cases	Controls	Data sourse	GWAS ID
Exposure	RAS	461006	47079	413927	UKB	6149_1
Outcome	MNH	287225	88	287137	FinnGen	C3_HYPOPHARYNX_EXALLC
	MNOC	287916	779	287137	FinnGen	C3_ORALCAVITY_EXALLC
	BNFM	377277	91	377186	FinnGen	CD2_BENIGN_FLOOROFMOUTH
	BNH	377277	117	377160	FinnGen	CD2_BENIGN_HYPOPHARYNX
	BNMP	377277	3216	374061	FinnGen	CD2_BENIGN_MOUTH_PHARYNX
	BNOPO	377277	225	377052	FinnGen	CD2_BENIGN_OROPHA_OTHER
	BNOUPM	377277	920	376357	FinnGen	CD2_BENIGN_MOUTH_NOS
	BNUP	377277	182	377095	FinnGen	CD2_BENIGN_PHARYNX_NOS

In addition, Cochcan's Q test was used to determine if there was heterogeneity in the instrumental variables utilized in the analysis for the IVW and MR-Egger [27, 28]. Sensitivity analyses using MR-Egger regression and MR-PRESSO were performed to assess horizontal pleiotropy between the diseases [29].

The stability and dependability of the findings were further examined using the leave-one-out method. To determine each SNP's impact on illnesses, it is recommended to exclude each linked SNP in turn before calculating the cumulative impact of the SNPs that remain.

The R (version 4.3.0) software's 'TwoSampleMR' and 'MRPRESSO' packages were used for all statistical analyses.

Results

Descriptive data

There were 54 SNPs and they were all exactly identical for each outcome. No SNPs were found to be associated with confounders. Each SNP's F-statistic was larger than 10, and the average F value for all SNPs was 922.851, indicating that weak instrumental variable bias was less likely (details can be found in Additional file 1: Table S1).

Main results

Figures 3 and 4 show a list of the MR estimates for the various methods used to evaluate the causative impact of RAS on NOMAP. The MR-PRESSO outlier test did not identify any outlier SNPs. After applying the Bonferroni correction, the corrected P was equal to 0.00625 (0.05/1/8). We found no significant evidence of causal relationships between RAS and NOMAP. The IVW method provided the sole evidence for RAS on BNFM (OR=2.509, 95% CI: 1.296–4.857, P=0.006). But according to the MR-Egger, weighted median, and weighted mode, the effect estimates of RAS on BNFM were 0.680 (95% CI: 0.176–2.621, P=0.577), 1.467 (95% CI: 0.534–4.028, P=0.457), and 1.203 (95% CI: 0.349–4.152, P=0.771), respectively. In sensitivity analysis, the MR-Egger regression method showed that there was horizontal pleiotropy between RAS and BNFM (P=0.035), suggesting a high possibility of false discovery (Table 2). For the other outcomes, the Cochran Q-test, MR-Egger regression, and MR-PRESSO did not reveal any heterogeneity or directional pleiotropy (Additional file 1: Table S2). No abnormal SNP was found by the leave-one-out method, indicating the robustness of the results (Additional file 1: Figure S1).

Discussion

This is the first study to investigate the causal relationship between RAS and NOMAP using MR analysis. Our results suggested that RAS was not significantly causally related to NOMAP. Among all the causal analyses, only BNFM was found to be related to RAS by the IVW method, but it may be due to horizontal pleiotropy, as the subsequent MR-Egger regression analysis suggested. The existence of horizontal pleiotropy shows that genetic variation might influence a different trait through several distinct routes, contradicting assumptions (2) and (3) of MR analysis, rendering the causal impact estimates derived inaccurate [23]. For the other outcomes, the Cochran Q-test, MR-Egger regression, and MR-PRESSO did not reveal any heterogeneity or directional pleiotropy.

As research on the relationship between inflammation and tumors has become more in-depth, many types of tumors have been found to be associated with chronic inflammation. Such as Crohn's disease, ulcerative colitis, and inflammatory bowel disease, which may progress to colorectal cancer, and bronchitis, which may result in lung cancer [30, 31]. On the other hand, research indicates that while those who have inflammatory bowel illness have a higher risk of acquiring colorectal cancer, only around 2% of those who have the condition had inflammatory bowel disease prior to the cancer emerging [32]. There is no exact evidence for the inevitable connection between the inflammation and tumors, as certain damage can be allowed to continue via DNA repair mechanisms, which can lead to mutagenesis but not always malignancy [33].

Inflammation was initially emphasized for its important role in defense against pathogens and for its contribution to tissue repair, regeneration, and remodeling [34]. Subtle forms of inflammation can play a crucial role in regulating tissue homeostasis [35]. The normal inflammatory response typically initiates when an infection or injury damages epithelial tissue, triggering the activation of myeloid cells [36]. These cells then produce inflammatory cytokines, activate innate and adaptive immunity, eliminate pathogens, and stimulate the proliferation of epithelial cells to close the barrier dysfunction that leads to pathogen translocation and repair other injuries caused by the stimulus [36]. As a result of the concerted effort, the damaged epithelial tissue returned to its normal homeostatic state [37]. However, if the original disruption of epithelial homeostasis is triggered by an oncogenic event, immunity will not repair the damage, and increased inflammation and cytokine-driven proliferation will promote tumour development rather than restore normal epithelial homeostasis [37].

Therefore, identification of the causative factor is the key to determining whether the inflammation is carcinogenic or not. The etiology and pathophysiology of RAS are quite complex. It has been proven to be influenced by a variety of variables, including nutritional inadequacy, microbial flora imbalance, stress, unhealthy lifestyle choices, medicines, allergens, psychological issues, anemia, immunological disorders, genetic predisposition,

Outcome	N SNP	Method	OR(95% CI)	OR	P value
MNH	54	IVW	0.84 (0.43 to 1.66)	0.844	0.623
		MR Egger	0.54 (0.13 to 2.24)	0.535	0.396
		Weighted median	0.69 (0.24 to 1.92)	0.686	0.474
		Weighted mode	0.91 (0.25 to 3.34)	0.909	0.887
MNOC	54	IVW	0.94 (0.75 to 1.17)	0.936	0.559
		MR Egger	0.89 (0.56 to 1.41)	0.886	0.614
		Weighted median	0.80 (0.56 to 1.15)	0.801	0.227
		Weighted mode	0.81 (0.55 to 1.19)	0.806	0.280
BNFM	54	IVW	2.51 (1.30 to 4.86)	2.509	0.006
		MR Egger	0.68 (0.18 to 2.62)	0.680	0.577
		Weighted median	1.47 (0.53 to 4.03)	1.467	0.457
		Weighted mode	1.20 (0.35 to 4.15)	1.203	0.771
BNH	54	IVW	1.34 (0.69 to 2.60)	1.342	0.383
		MR Egger	0.58 (0.15 to 2.26)	0.579	0.436
		Weighted median	1.25 (0.49 to 3.18)	1.253	0.635
		Weighted mode	0.97 (0.37 to 2.57)	0.970	0.952
BNMP	54	IVW	0.96 (0.86 to 1.08)	0.964	0.514
		MR Egger	0.88 (0.70 to 1.10)	0.879	0.275
		Weighted median	0.91 (0.77 to 1.07)	0.912	0.273
		Weighted mode	0.91 (0.74 to 1.10)	0.906	0.325
BNOPO	54	IVW	0.84 (0.56 to 1.27)	0.842	0.415
		MR Egger	0.91 (0.38 to 2.15)	0.908	0.828
		Weighted median	0.85 (0.44 to 1.65)	0.854	0.640
		Weighted mode	0.87 (0.41 to 1.85)	0.870	0.719
BNOUPM	54	IVW	0.96 (0.78 to 1.18)	0.960	0.699
		MR Egger	1.08 (0.70 to 1.66)	1.080	0.725
		Weighted median	0.96 (0.70 to 1.32)	0.963	0.816
		Weighted mode	1.06 (0.74 to 1.52)	1.061	0.749
BNUP	54	IVW	0.78 (0.48 to 1.29)	0.784	0.338
		MR Egger	0.66 (0.23 to 1.87)	0.659	0.438
		Weighted median	0.83 (0.41 to 1.67)	0.829	0.601
		Weighted mode	0.76 (0.30 to 1.96)	0.763	0.577

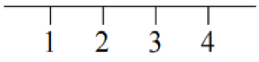

Fig. 3 The MR estimates for the various methods used to evaluate the causative impact of RAS on NOMAP. N SNP, number of Single-nucleotide polymorphisms

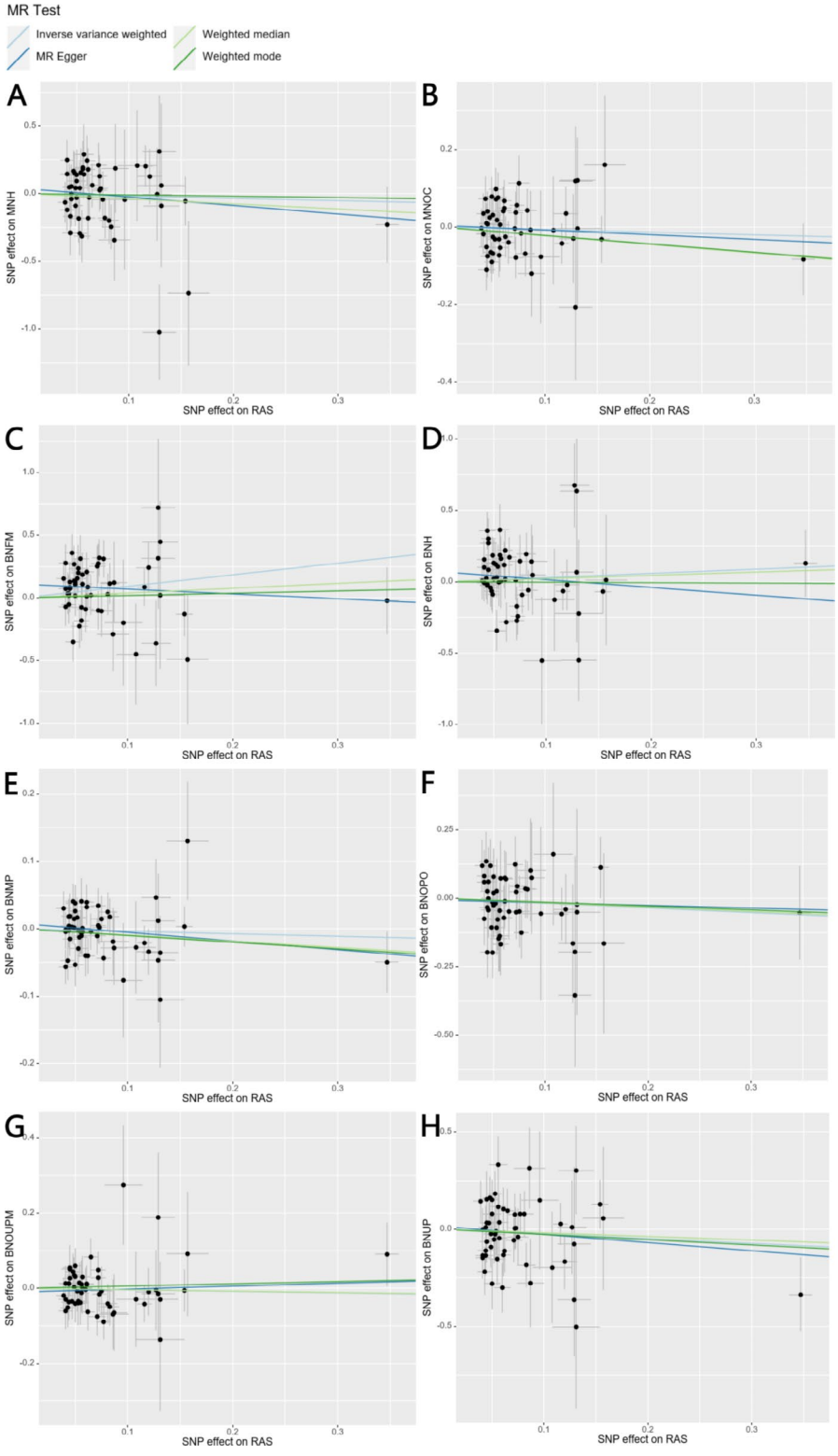

Fig. 4 Scatter plot of the causal effect of recurrent aphthous stomatitis (RAS) on neoplasms of the mouth and pharynx. (**A**) MNH, malignant neoplasm of hypopharynx; (**B**) MNOC, malignant neoplasm of oral cavity; (**C**) BNFM, benign neoplasm of floor of mouth; (**D**) BNH, benign neoplasm of hypopharynx; (**E**) BNMP, benign neoplasm of the mouth and pharynx; (**F**) BNOPO, benign neoplasm of other parts of oropharynx; (**G**) BNOUPM, benign neoplasm of other and unspecified parts of mouth; (**H**) BNUP, benign neoplasm of unspecified pharynx. The causal relationship between RAS and NOMAP was assessed using inverse variance weighting (IVW), MR-Egger, weighted median, and weighted mode. Estimates of the causal effects for each approach are shown by the slope of the line

Table 2 Pleiotropy test of the instrumental variables for RAS on NOMAP

Exposure	Outcome	Egger_intercept	SE	P vaule	MR-PRESSO global Test	P vaule
RAS	MNH	0.038	0.053	0.482	57.811	0.385
	MNOC	0.004	0.017	0.797	48.983	0.692
	BNFM	0.109	0.050	0.035	57.542	0.376
	BNH	0.070	0.051	0.174	73.202	0.057
	BNMP	0.008	0.009	0.372	46.127	0.805
	BNOPO	-0.006	0.032	0.844	36.246	0.973
	BNOUPM	-0.010	0.016	0.542	35.114	0.985
	BNUP	0.014	0.039	0.711	64.517	0.178

etc [5]. While certain causative factors such as tobacco, alcohol, and betel nuts, identified in our research, are recognised risk factors for NOMAP linked to the development of RAS, the majority of other causative factors have not been definitively proven to be carcinogenic [38–40]. If there is no other oncogenic event, the RAS is unlikely to be a carcinogenic inflammation.

The ambiguity surrounding the etiology of RAS, the vulnerability of observational studies to confounding factors, reverse causality, and various biases, along with the challenges of conducting experimental studies, complicate the potential of traditional research methodologies to establish a causal relationship between RAS and NOMAP.

Our work utilized the MR method and confirmed at the genetic level that no causal association between RAS and NOMAP was identified, contributing to addressing them dialectically and the formulation of clinical strategies for them.

There are some major strengths in our MR analysis. First, in contrast to observational research, the inclusion of genetic variations as IVs lessens the possibility of common confounding variables and reverse causation. Second, in order to reliably research the causal relationship between RAS and NOMAP, we employed SNP-exposure and SNP-outcome estimations from studies with the greatest sample sizes to date (varying from 287916 to 461106 individuals). Third, the GWAS dataset we used was based primarily on populations of European ancestry, which minimized the effect of population stratification [41]. Fourth, our conclusion is based on a comprehensive analysis involving eight oropharyngeal tumor characteristics using a variety of causal estimation models, heterogeneity tests, and sensitivity tests, which can effectively reduce the occurrence of various biases and ensure the validity and stability of the results.

The limitations of our study are as follows. First, RAS in the UKB was diagnosed from a questionnaire instead of a medical evaluation. This restriction is essential due to the unavailability of clinical oral examination data and the brief, intermittent occurrence of mouth ulcers, which frequently renders them invisible during clinical examinations even to those afflicted [18]. As with any data obtained from questionnaires, this may result in some misclassification, exemplified by our study's inability to differentiate between various types of mouth ulcers. Nevertheless, we anticipate that our results primarily represent the causal assoiation between RAS and NOMAP, as various groups have corroborated the majority of variations from UKB, including three variants specific to RAS, the predominant form of oral ulcer [42]. Second, we could not completely eliminate the effects of horizontal pleiotropy, despite implementing a comprehensive series of measures to identify and mitigate aberrant variations. This is probably attributable to the intricate biological functions of numerous genetic variants. Third, due to the scarcity of GWAS data for non-white populations and the potential for population stratification arising from the amalgamation of GWAS data across multiple races, our analysis only used data from white individuals, excluding data from other racial groups [41]. When applying our results to populations outside Europe, care must be taken, since numerous environmental factors may significantly impact RAS and NOMAP. Fourth, we did not take into account sex-specific effects, and the incidence of RAS and NOMAP may differ by sex due to hormonal changes. Finally, to corroborate the findings, more substantial sample numbers and more sophisticated techniques are required.

Conclusion

In conclusion, this is the first MR analysis to investigate the relationship between RAS and NOMAP. Our research confirmed at the genetic level that no causal association has been identified between RAS and NOMAP, therefore facilitating a logical therapeutic perspective and the development of clinical therapies for them.

Abbreviations

NOMAP Neoplasms of the Mouth and Pharynx
RAS Recurrent Aphthous Stomatitis

MR	Mendelian Randomization
IVs	Instrumental Variables
GWAS	Genome-Wide Association Studies
SNPs	Single-Nucleotide Polymorphisms
UKB	UK Biobank
MNOC	Malignant Neoplasm of Oral Cavity
MNH	Malignant Neoplasm of Hypopharynx
BNFM	Benign Neoplasm of Floor of Mouth
BNH	Benign Neoplasm of Hypopharynx
BNMP	Benign Neoplasm of the Mouth and Pharynx
BNOPO	Benign Neoplasm of Other Parts of Oropharynx
BNOUPM	Benign Neoplasm of Other and Unspecified Parts of Mouth
BNUP	Benign Neoplasm of Unspecified Pharynx
IVW	Inverse Variance Weighting
InSIDE	Instrument Strength Independent of Direct Effects

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-13140-6.

Supplementary Material 1

Supplementary Material 2

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Author contributions
Conceptualization: Y.Y. and C.Y. Methodology: Y.Y. and Z.C. Data analysis: Y.Y. Manuscript drafting: Y.Y. and J.Z. Draft review and editing: Y.Y., J.Z., C.Y. and Z.C. The final submitted version was reviewed and approved by each author.

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Data availability
On the website http://www.nealelab.is/uk-biobank, we may obtain the UKB summary data. The website https://www.finngen.fi/fi allows users to obtain FinnGen's summary data. The R (version 4.3.0) software's 'TwoSampleMR' and 'MRPRESSO' packages were used for all statistical analyses. The mRnd website (https://shiny.cnsgenomics.com/mRnd/) was used to assess statistical power.

Declarations

Ethics approval and consent to participate
Summary statistics from the publicly accessible genome-wide association study (GWAS) were used in our research. No new information was gathered, and no additional ethical clearance was needed.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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