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ORIGINAL ARTICLE

Association between Lifestyle and Dietary Habits and Head and Neck Cancer: A Two-Sample Mendelian Randomization Study

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Abstract:

Objective: To overcome the effects of confounders and reverse causality in observational studies, a two-sample mendelian randomization method was used to explore the associations between lifestyle and dietary habits and head and neck cancer (HNC), and to provide recommendations for clinical management.

Methods: The IEU Open GWAS project website was used to obtain genome-wide association study (GWAS) data on 2 lifestyle habits (alcohol abuse and smoking) and 12 dietary habits (intake of beef, lamb, pork, fish, fresh vegetables, cereals, dried fruits, coffee, tea, cheese, micronutrients, etc.) and HNC. Single nucleotide polymorphisms (SNPs) closely and independently associated with 2 lifestyle habits and 12 dietary habits were screened as instrumental variables (IVs), and were analyzed by mendelian randomization (MR) using inverse variance weighting (IVW), MR Egger regression, and weighted multinomials (WM), respectively. Cochrane Q heterogeneity test was performed by MR-Egger method,multiple validity test by Egger-intercept method, sensitivity analysis by "leave-one-out" test.

Results: The results of the IVW method showed a positive and statistically significant causal relationship between alcohol abuse (OR=1.00, p=0.02), smoking (OR=1.00, p=0.01), and beef intake (OR=1.00, p=0.03) and HNC, respectively, in the overall population. Furthermore, these findings remained robust in various sensitivity analyses, and no evidence of heterogeneity or horizontal pleiotropy was observed.

Conclusion: The results found that alcohol abuse, smoking and beef intake might be risk factors for developing HNC, which provides further theoretical basis for the maintenance of healthy lifestyles among people at high risk of HNC.

Keywords: head and neck cancer; lifestyle; dietary habits; mendelian randomization

Introduction

Head and neck cancer (HNC) accounts for six percent of all cancers and is the sixth most common cancer in the world, the majority of HNC are squamous cell carcinomas and it accounts for approximately 1.5% of deaths due to cancer [1]. Approximately 60% of patients with squamous cell carcinoma of the head and neck are found to be at an advanced stage, and more than 65% of them have recurrence or/and metastasis [2], with a 5-year survival rate of less than 50% [3]. Epidemiologic studies have reported several potential risk factors for HNC, including smoking, alcohol consumption, HPV infection, high sodium, and gender factors; however, most of the evidence on these associations is inconclusive, and an increasing number of studies have found that the risk of developing cancer is strongly associated with lifestyle and dietary factors, which in turn suggests potential dietary triggers among HNC patients, such as heavy alcohol consumption, smoking, and a high sodium Diets that have failed to be studied in depth for the diversity and breadth of foods, thus identifying risk factors that are causally associated with HNC can help to understand its pathogenesis and provide a guiding direction for the prevention of HNC. Reverse causality, misclassification,

unobserved confounders, and other biases may largely impede causal inference of these associations in existing observational epidemiological studies, such as the possibility that smoking and alcohol consumption are overlapping behaviors.

Randomized controlled trials (RCT) are difficult to truly implement in the clinical setting due to various constraints, and observational experimental research methods have a relatively low level of confidence due to confounding factors and reverse causality, which bias the results of the study. Mendelian randomization (MR) uses genetic variants associated with exposure factors as instrumental variables that can provide causal evidence for the association between exposure factors and outcomes. Because genetic variants are randomly assigned at the time of conception, Mendelian randomization designs can minimize exposure to confounders associated with the outcome. In addition, Mendelian randomization is less affected by reverse causation because allelic randomization occurs prior to disease onset [4,5]. Although some previous studies have provided some risk factors for the development of HNC, including alcohol and tobacco use, oncology patients may also influence the intake of dietary habits, which emphasizes the potential bidirectional relationship between diet and HNC. A systematic association and a comprehensive collection of evidence on the link between diet and HNC have not yet been established. Therefore, the aim of this study was to investigate the genetic causality between lifestyle, dietary habits and head and neck malignancies using MR methods based on single nucleotide polymorphism (SNP) loci published in the GWAS database as instrumental variables (IVs).MR methods were used to explore the genetic causality between lifestyle habits, dietary habits and HNC, and sensitivity analyses were performed to further validate the reliability of the results.

2 Materials and methods

2.1 Study Design

The study design was a two-sample MR design to investigate the causal effects of lifestyle and dietary habits on HNC (Figure 1).

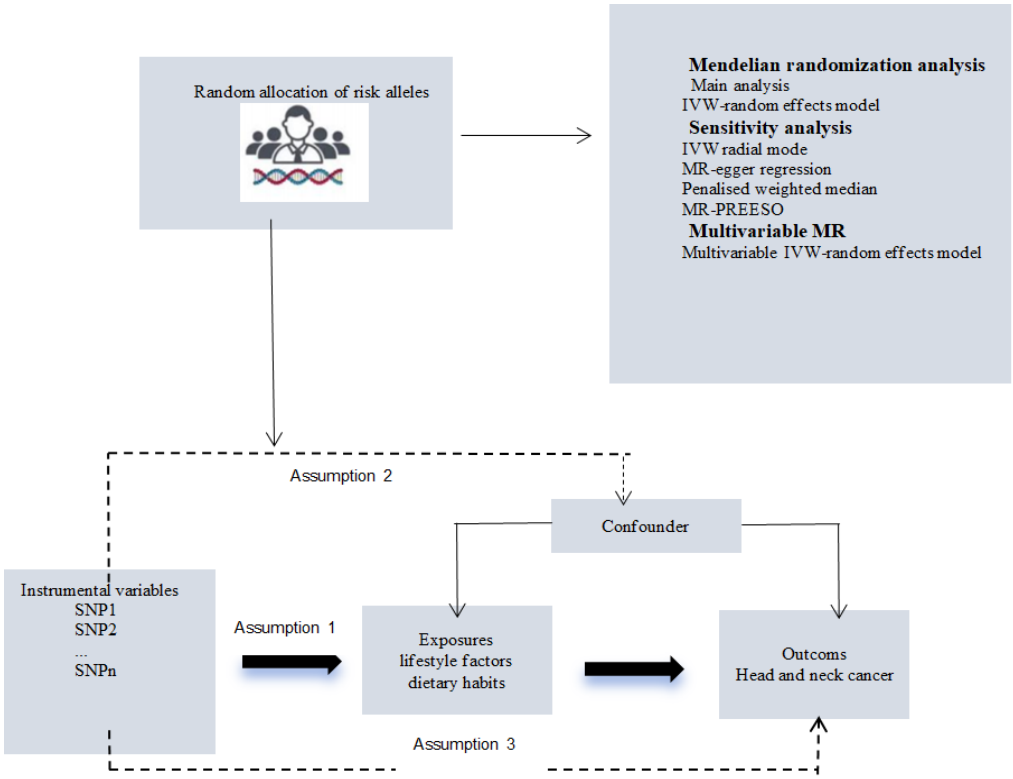

Figure.1 Overview of the research design and hypotheses for a Mendelian randomized design.The MR design was based on three core assumptions: (1) genetic instrumental variables must be strongly associated with exposure; (2) instrumental variables are independent of a variety of confounders; (3) the selected instrumental variables influence the outcome only through exposure factors.

SNPs associated with these risk factors were selected as IVs.The MR design was based on three core assumptions: (1) Genetic instrumental variables must be strongly associated with exposure; (2) IVs are independent of a variety of confounders; (3) The selected IVs influence the outcome only through exposure factors. In this study, we utilized lifestyle habits (smoking, alcohol abuse) and dietary habits (intake of beef, lamb, pork, fish, fresh vegetables, cereals, dried fruits, coffee, tea, cheese, micronutrients, etc.) as exposure factors, SNPs with significant correlation with exposure factors as IVs, and HNC were selected as the outcome variables, and causal association analysis was performed using the TwoSampleMR package in the R language. The reliability of the results was verified by Cochran Q heterogeneity test, multiple validity test and sensitivity analysis.

2.2 Sources of information

In this study, genome-wide association study data on lifestyle habits (alcoholism, smoking) and dietary habits (intake of beef, lamb, pork, fish, fresh vegetables, cereals, dried fruits, coffee, tea, cheese, micronutrients, etc.) were obtained from the IEU OpenGWAS project website, respectively (Table 1). After obtaining the data from the website using R software, in order to avoid the analysis bias caused by strong linkage disequilibrium (LD) among SNPs, the screening criteria were [6]: (1) $P < 5 \times 10\text{-}8$; (2) physical distance M > 10 000 kb between every two genes; (3) r 2 threshold of LD between genes < 0.001, and finally obtain SNPs that were independent from each other and significantly associated with lifestyle and dietary habits. The SNPs that were independent of each other and significantly associated with lifestyle and dietary habits were finally obtained as the final instrumental variables.

The data for HNC were obtained from the IEU open GWAS database at https://gwas.mrcieu.ac.uk/datasets/ieu-b-4912/, which is a GWAS dataset containing 373,122 samples, including 1106 patients with malignant brain tumors and 372,016 controls.

2.3 Statistical analyses

2.3.1 Two-sample Mendelian randomization analysis

Data extraction, data processing and data analysis in this study were done in Rstudio (version 4.3.1) using the R package "TwoSampleMR". The analysis methods used in this study were mainly the inverse variance weighting (IVW), MR-Egger regression, and weighted median (WME) methods in the TwoSampleMR package [7].IVW is the most commonly used test for calculating the weighted mean of the effect values of all the instrumental variables, and MR analyses are based on the IVW method as a reliable result.MR-Egger is the method that can be used to both perform the MR-Egger, which can be used for both MR analysis and multiple validity assessment, and is fitted using the inverse of the variance of the outcome as weights [8]. The advantage of WME is that MR analysis can be performed to obtain valid results if at least half of the valid instrumental variables are included in the analysis.

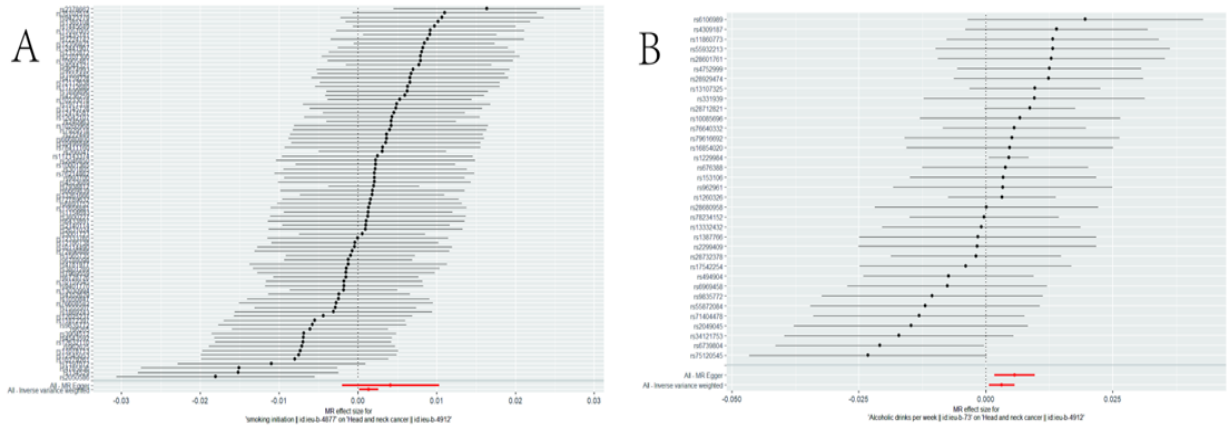

Figure. 2 Forest plot of the two-sample MR analysis.(A) Forest plot of exposure factor (smoking) on outcome (head and neck cancer).(B) Forest plot of exposure factor (alcohol abuse) on outcome (head and neck cancer)

2.3.2. Sensitivity analysis

In this study, sensitivity analyses were conducted using the Cochrane Q-test and MR-Egger to further examine heterogeneity and horizontal pleiotropy.The Cochrane Q-test was used to quantify the heterogeneity of the IVs, and if the P<0.05, heterogeneity exists; on the other hand, if the P＞0.05, there is no heterogeneity [9]. The pleiotropy test is used to verify the reliability of MR analysis results, which is often expressed by the intercept term of the MR-Egger method, and the intercept term of P＞0.05 indicates that there is no level of pleiotropy, and if pleiotropy exists, the results of the MR analysis are unreliable [10]; The sensitivity test is conducted by using the leave-one-out method [11], which is based on the principle of gradually eliminating the results of a single SNP to determine whether it is an outlier or not, and it can observe the stability of the results after the elimination of each SNP.

3 Results

3.1 SNP Selection

The analyses in this study included 2 lifestyle habits and 12 dietary habits, with the number of SNPs for each ranging from 17 to 94 (Table 1).

Table 1 Exposure factors GWAS data

data set	data set number	Sample size (cases)	Number of SNPs	crowds
Alcoholic drinks	ieu-b-73	335394	35	European
Smoking	ieu-b-4877	607291	93	European
Meat intak	ukb-b-6324	461981	23	European
Beef intake	ukb-b-2862	461053	17	European
Fish intake	ukb-b- 17627	460880	11	European
Pork intake	ukb-b-5640	460162	14	European
Mutton intak	ukb-b- 14179	460006	32	European
Vegetable intake	ukb-b-8089	448651	17	European
Fresh fruit intake	ukb-b-3881	446462	55	European
Tea intake	ukb-b-6066	447485	41	European
Coffee intake	ukb-b-5237	428860	40	European
Cheese intake	ukb-b- 1489	451486	65	European
Cereal intake	ukb-b- 15926	441640	43	European
Dried fruit intake	ukb-b-16576	421764	43	European

Detailed information on the IVs for the 14 lifestyle and dietary habits is listed in Supplementary Table S1.Exposure factor SNPs after removal of linkage disequilibrium Obtained after harmonization of processed data with matched SNPs from the GWAS database for HNCr The number of SNPs ranged from 9-93 (Supplementary Table S), which in turn validated the association hypothesis of MR assumption 1.

3.2 Causal effect in the main analysis

The results of IVW method showed that among the 2 lifestyle habits, (OR=1.00, 95% CI: 1.00-1.00, p=0.01) for the smoking population, (OR=1.00, 95% CI: 1.00-1.00, p=0.02)for the alcoholic population(Table 2A-B).It is suggested that the smoking and drinking habits have a positive causal relationship with HNC in the whole population.The OR values, 95%CI, and p-values calculated by WME and MR-Egger regression for the populations with alcohol consumption and head and neck cancer are as follows: (WME: OR=1.00, 95% CI: 1.00-1.00, p=0.01), (MR-Egger: OR=1.00, 95% CI: 1.00-1.00, p=0.00). For the populations with smoking and head and neck cancer, the values are: (WME: OR=1.00, 95% CI: 1.00-1.00, p=0.02), (MR-Egger: OR=1.00, 95% CI: 1.00-1.01, p=0.19).(Table 2).

Table 2 Two-sample Mendelian randomization shows the effect of lifestyle and dietary habits on head and neck cancer

Exposure	SNPs	Inverse Variance Weighted method OR(95% CI) p	Weighted Median OR(95% CI) p	MR-Egger method OR(95% CI) p	Cochrane's Q test Q P		Pleiotropy Intercept	p
Alcoholic drinks	35	1.00（1.00-1.00） **0.01**	1.00（1.00-1.00） **0.01**	1.00（1.00-1.00） **0.00**	36.54	0.35	-6.53	0.12
Smoking	93	1.00（1.00-1.00） **0.02**	1.00（1.00-1.00） **0.02**	1.00（1.00-1.01） **0.19**	93.36	0.25	-0.73	0.37
Meat intak	23	1.00（0.99-1.00） 0.71	1.00（0.99-1.00）0.71	0.99（0.97-1.01） 0.36	13.37	0.81	0.00	0.31
Beef intake	17	1.00（1.00-1.01） **0.03**	1.00（1.00-1.01） **0.03**	1.01（0.97-1.05） **0.47**	19.41	0.15	-7.74	0.74
Fish intake	9	0.99（0.98-1.00） 0.16	0.99（0.98-1.00）0.16	0.98（0.95-1.02）0.51	5.20	0.73	8.17	0.70
Pork intake	11	1.00（0.99-1.00） 0.98	1.00（0.99-1.00）0.98	1.03（0.96-1.10）0.36	6.23	0.79	-0.00	0.36
Mutton intak	30	1.00（0.99-1.00） 0.88	1.00（0.99-1.00）0.88	0.98（0.96-1.00）0.23	25.96	0.57	0.00	0.23
Vegetable intake	16	0.99（0.98-1.00） 0.07	0.99（0.98-1.00）0.07	1.04（0.97-1.13）0.24	15.37	0.42	-0.00	0.17
Fresh fruit intake	49	0.99（0.99-1.00） 0.41	0.99（0.99-1.00）0.41	1.01（0.99-1.02）0.18	46.23	0.46	-0.00	0.10
Tea intake	36	1.00（0.99-1.00） 0.54	1.00（0.99-1.00）0.54	1.00（1.00-1.01）0.42	42.76	0.17	-3.62	0.54
Coffee intake	39	1.00（1.00-1.00） 0.67	1.00（1.00-1.00）0.67	1.00（1.00-1.00）0.45	28.61	0.83	-5.10	0.29
Cheese intake	60	1.00（1.00-1.00） 0.79	1.00（1.00-1.00）0.79	1.00（0.99-1.00）0.47	65.14	0.24	5.84	0.45
Cereal intake	40	1.00（1.00-1.00） 0.86	1.00（1.00-1.00）0.86	0.99（0.98-1.01）0.79	38.53	0.31	2.62	0.82
Dried fruit intake	38	1.00（0.99-1.00） 0.24	1.00（0.99-1.00）0.24	1.99（0.98-1.01）0.97	38.80	0.47	-3.28	0.75

The effect values and range of the intervals obtained by MR-Egger regression and WME and IVW tended to converge, with statistically significant p-values, and the three algorithms yielded the same direction of the causal effect (Figure 3), which all showed that alcohol abuse and smoking were the risk factors for HNC. The effect values and range of intervals obtained from the MR-Egger regression and WME and IVW tended to converge, with statistically significant p-values, and the three algorithms yielded a consistent direction of the causal effect (Fig. 3A-B), which showed that alcohol abuse and smoking may be the risk factors for HNC.

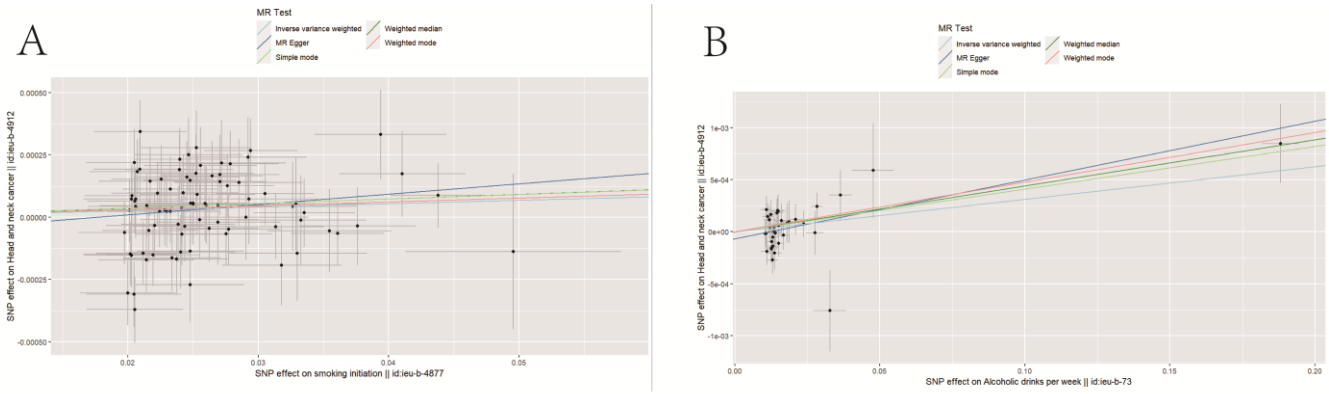

Figure. 3 Scatter plot of the two-sample MR analysis.(A) Scatterplot of exposure factor (smoking) on outcome (head and neck cancer).(B) Scatterplot of exposure factor (alcohol abuse) on outcome (head and neck cancer)

The results of the IVW method showed a statistically significant (OR=1.00, 95% CI: 1.00-1.01, p=0.03) for the beef intake population out of the 12 dietary habits (Table 2) suggesting that there is a positive causal association between the beef intake and HNC risk (WME: OR=1.01, 95% CI: 1.00-1.02; MR-Egger: OR=1.01, 95% CI: 0.97-1.05).(Fig. 4A). Although the p-value of MR-Egger was >0.05, the way of assessing the MR of the two samples was mainly IVW, and the effect values and range of the intervals obtained by the three methods of calculation tended to be consistent, and the direction of the causal effect was the same (Figure 4B)

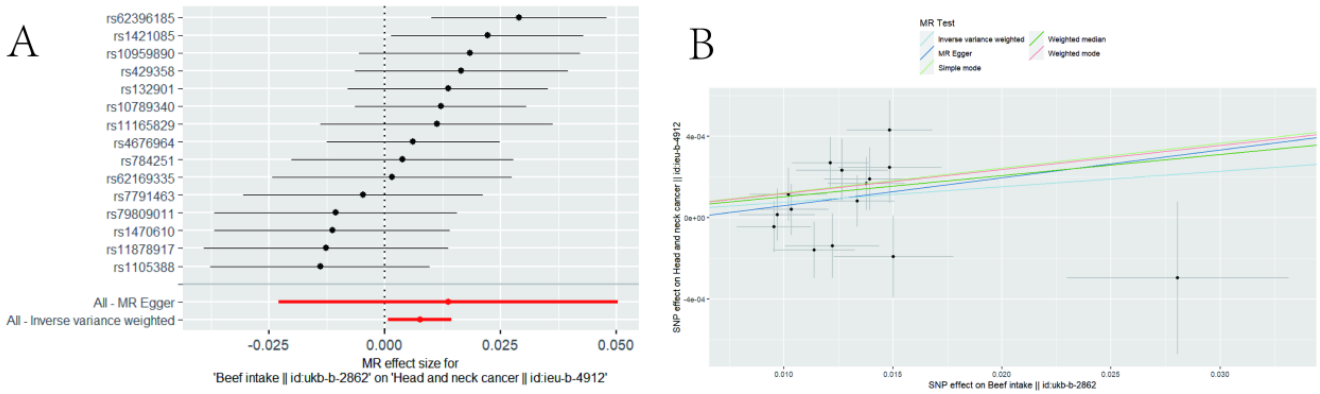

Figure. 4 (A)Forest plot of exposure factors (beef intake population) on outcome (head and neck cancer).(B)Scatterplot of exposure factors (beef intake population) on outcome ((head and neck cancer)

3.3 Heterogeneity test

The CochraneQ test was used in this study to quantify the heterogeneity of the IVs, and in Table 2 it shows that in the alcohol drinking population (CochraneQ test result p=0.35), in the smoking population(CochraneQ test result p=0.25), and in the beef intake population (CochraneQ test result p=0.15),the heterogeneity was not evident.

3.4 Reliability evaluation

3.4.1 Multiple validity test

Table 2 shows the MR-Egger method regression intercept for the alcohol drinking population as intercept=-6.53, p=0.12. Similarly, the MR-Egger method regression intercept for the smoking population is intercept=-0.73, p=0.37, and for the beef intake population intercept=-7.74, p=0.74. All p-values are greater than 0.05, indicating that the causal effect analysis was not multivariate and supporting the hypothesis 2 exclusivity.

3.4.2 Sensitivity analysis

The results of the "leave-one-out" method for alcoholics, smokers, and beef consumers (Fig. 5A-C)

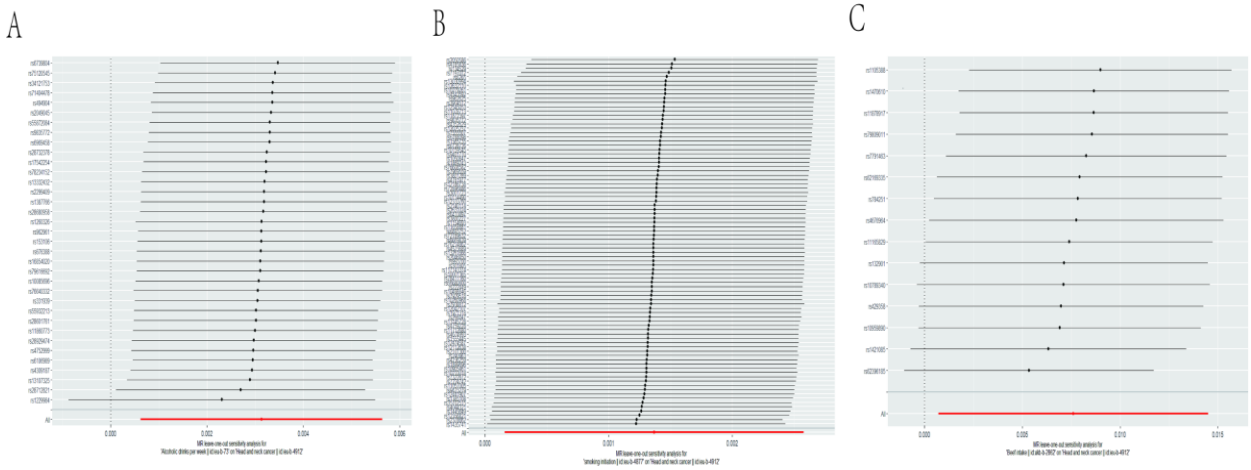

Figure. 5 Leave-one-out" sensitivity analysis.(A)Alcoholism SNPs-by-exclusion test sensitivity analysis results.(B)Smoking SNPs-by-exclusion test sensitivity analysis results.(C)Beef intake population SNPs-by-exclusion test sensitivity analysis results

Showed that after removing individual SNPs one by one, the results of the remaining SNPs were similar to the results of the IVW analyses that included all SNPs, and they were all on the right side of the null line, which indicated that there were no SNPs that had a fundamental effect on the results. The results of this study are robust in that no matter which SNPs are excluded, they do not have a large impact on the results. The funnel plot shows that when a single SNP is used as an

instrumental variable, it represents a roughly symmetrical distribution of causal effect points, which represents a low likelihood of being affected by bias (Figs.6A-C)

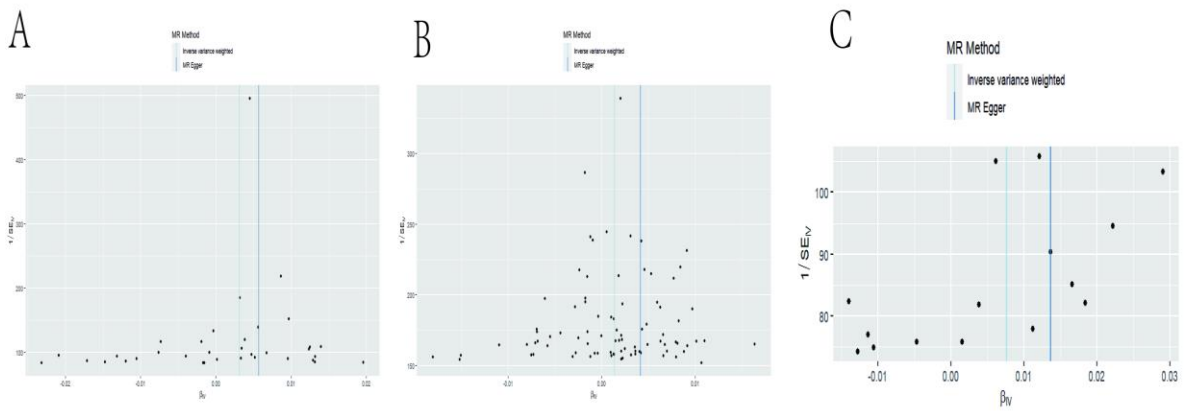

Figure. 6 (A)Funnel plot of MR results for two samples of Alcoholism.(B)Funnel plot of MR results for both samples of smoking.(C) Funnel plot of MR results for two samples of the beef intake population

Discussion

In this study, the association between lifestyle and dietary habits and HNC was verified using two-sample MR analysis, and the three methods of analysis, IVW method, MR-Egger regression, and WM, showed that there was a causal relationship between lifestyle and dietary habits and HNC, and the causal effect remained stable.

The basic idea is to use genetic factors that are strongly correlated with exposure as instrumental variables to evaluate the causal relationship between exposure and outcome [12]. A large body of previous literature points to alcohol abuse and attraction as possible risk factors for HNC, but it is difficult to prove that alcohol abuse or smoking is a major cause of HNC. For example, having an oncological disease may make people more likely to adopt these habits in order to reduce pain or discomfort caused by the disease (an effect known as reverse causation). This association may also be the result of confounding factors, such as alcoholism, which is often associated with unhealthy lifestyles and nutritional deficiencies [13]. In addition, many people who smoke also drink alcohol and vice versa, making it difficult to determine whether one or both behaviors are responsible for the disease. In this study, two-sample MR using genetic variants as IVs for causal association analysis between exposure and outcome was used to investigate at the genetic level whether people with genetic variants that predispose them to be smokers or drinkers are at greater risk of developing HNC using this statistical method. This reduces the likelihood of confounding and reverse causation because genetic variants between exist from birth and are not influenced by external factors. In this study, genetic variants were used as IVs to analyze the causal relationship between alcohol and tobacco use and the development of HNC using a two-sample MR method, and the results of the study showed a positive causal relationship between alcohol and tobacco use and HNC.

The International Agency for Research on Cancer (WHO-IARC) classified red meat and processed meat as probable human carcinogens [14].These conclusions are mainly based on studies on colorectal cancer, but scientific evidence for other cancer sites is still limited [15,16]. One of the types of red meat includes the meat of mammals such as cow, sheep, deer, donkey, horse, pig, and rabbit, which is commonly referred to as red meat.Two meta-analyses have indicated that high consumption of beef may be positively associated with breast cancer risk [17,18]. Red meat contains high levels of heme iron, which may initiate carcinogenesis through a variety of mechanisms, including the production of genotoxic free radicals or through lipid peroxidation [19]. The results of the present study provide evidence to support the genetically predicted positive association between beef intake and HNC risk.

There are some limitations in this study: Firstly, the GWAS database from which the data were

extracted was for European populations, which may limit the application of the findings to other ethnic populations, and therefore further studies in other populations are still needed. And based on the research design of large samples, obtaining more instrumental variables can improve the reliability of the results, which can be followed up to carry out more relevant studies in Asian populations. Secondly,the MR-Egger regression method for gene pleiotropy in this study has some limitations. The MR-Egger method can only detect directional gene pleiotropy, and if there is balanced pleiotropy, i.e., all the SNPs have different directions of pleiotropy and they cancel each other out, then the method will not be able to detect them, which will lead to the bias due to the gene pleiotropy [20].Thirdly,the selection of exposures and outcomes in two-sample MR analysis is different from traditional single-sample MR analysis, which often comes from two different databases. However, it is difficult to guarantee that the two databases are completely different.There is a possibility that some population data may appear in two GWAS databases simultaneously, leading to an overlap of samples and resulting in weak instrumental variable bias, similar to that in single-sample MR analysis [21]. Finally, MR analyses can only explore causal relationships between exposure factors and outcomes, and cannot investigate specific biological mechanisms.

In conclusion, this study utilized 2 lifestyle habits and 12 dietary habits as exposure factors, selected significantly correlated SNPs asIVs, and analyzed them using IVW, MR-Egger regression, and WM, and the sensitivity analyses found no pleiotropy or heterogeneity, and the results showed a causal relationship between alcoholism, smoking, and beef ingestors and the development of HNC, and the results of the study showed positive causality between alcoholism, smoking, and beef ingestors and HNC.

Data availability statement

The data utilized in this study originated from publicly available databases. This data can be found here: All GWAS data used in this study are available in the IEU open GWAS project (https://gwas.mrcieu.ac.uk/).

Ethics statement

As the data utilized in this study originated from publicly available databases and did not involve individual patients, it falls under the exemption from ethical review and the requirement for patient informed consent. Because all GWAS data used in this study are available in the IEU open GWAS project (https://gwas.mrcieu.ac.uk/).

Author Contributions

The study was designed by changya Li and Houyu Zhao. Statistical analyses were performed by changya Li, and Xianlu Zhuo. The manuscript was written by changya Li. All authors contributed to the interpretation of data and commented on the manuscript. All authors read and approved the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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