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Causal association between Vitamin D levels and head-and-neck cancer: A Mendelian randomization study

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Abstract

Observational studies have revealed inconsistent findings regarding the association between Vitamin D levels and head-and-neck cancer (HNC). Therefore, this study aimed to examine the possible causality of Vitamin D for HNC risk using two-sample Mendelian randomization (MR) and multivariable MR (MVMR) analyses. Single-nucleotide polymorphisms closely associated with Vitamin D levels were selected from the genome-wide association research database as instrumental variables. The analytical approaches utilized included inverse variance weighted (IVW), MR-Egger, weighted median, simple mode, and weighted mode. Sensitivity analysis was performed to verify the robustness of MR findings. In addition, the causation of Vitamin D on HNC risk was assessed by controlling for the influences of smoking and alcohol intake through MVMR analysis. IVW (odds ratio = 0.9958, 95% confidence interval = 0.9934 – 0.9983, $p = 0.0007$) indicated that Vitamin D levels were negatively related to HNC risk. The weighted median yielded the same result. The results of the MVMR analysis further supported the role of Vitamin D in mitigating HNC risk. The sensitivity analysis revealed the absence of heterogeneity or pleiotropy of the instrumental variables, proving our results reliable without significant bias. This study offers genetic evidence in favor of the causality of Vitamin D for HNC risk. Increasing Vitamin D intake may help lower HNC risk.

Keywords: Head-and-neck cancer; Vitamin D; Mendelian randomization; Causality

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1. Introduction

Head-and-neck cancer (HNC) is a general term for malignant tumors of multiple sites in the upper digestive and respiratory tracts, 90% of which have squamous cell carcinoma pathology, including cancers of four anatomical sites: nasal cavity, oral cavity, pharynx, and larynx.[1] The main risk factors for HNC include smoking, alcohol consumption, and human papillomavirus infections.[1,2] According to the global cancer statistics in 2020, HNC accounted for approximately 7.4% of the total cancer population, and HNC-related deaths accounted for 5.1% of all cancer-related deaths.[3] Furthermore, the incidence of this type of cancer is steadily rising annually.[4] In addition, the outcomes for people diagnosed with HNC is typically unfavorable, as evidenced by a 5-year overall

survival rate of 40 – 50%.[5] Therefore, an in-depth study on the factors associated with HNC occurrence is important for reducing the prevalence of this disease.

Vitamin D, an essential and versatile lipid-soluble vitamin, can be obtained from solar exposure and dietary sources. 25-hydroxyvitamin D (25(OH)D), a stable Vitamin D metabolite, is acknowledged as a dependable indicator for assessing Vitamin D levels in the body.[6] Vitamin D is critical for maintaining optimal, and its deficiency is closely linked to several diseases such as cardiovascular disorders, COVID-19, bone diseases, diabetes, and autoimmune diseases.[7] In addition, considerable attention has been directed toward the relationship between vitamins and cancer incidence. Studies have shown that Vitamin D can affect particular cellular signaling pathways involved in tumor growth, consequently controlling the apoptosis, differentiation, and metastasis of tumor cells.[8,9] A meta-analysis indicated that high Vitamin D levels correlate with a decrease in cancer incidence and mortality.[10] A randomized study indicated that increasing Vitamin D levels markedly reduced cancer risk in postmenopausal women.[11] In particular, when examining the causality of Vitamin D for HNC risk, certain studies present evidence that Vitamin D decreases HNC risk.[5,12-14] However, some studies have failed to identify a noteworthy connection between the two.[15] To overcome the challenges of confounding and reverse causality prevalent in traditional observational studies and elucidate the causal association between Vitamin D and HNC risk, this study employed Mendelian randomization (MR) to investigate this potential causality more thoroughly.

Using genetic variation as an instrumental variable (IV), MR can investigate the factors that influence disease exposure.[16] This approach utilizes the random assignment of alleles during fertilization, similar to the randomization employed in randomized controlled trials.[17] Because genetic variations remain stable during an individual's life, independent of lifestyle, environmental factors, and other potential confounders, MR studies effectively mitigate confounding bias and reverse causation problems that are prevalent in traditional observational studies.[18] This approach provides reliable and accurate causal inferences for understanding disease risk factors. In addition, other MR studies have explored the association of Vitamin D with conditions such as lung disease, cardiovascular disease, and certain cancers, confirming the feasibility of this approach.[19-21] This study employed a two-sample MR approach to examine the causal link between Vitamin D levels and HNC. To ensure that our analytical results were accurate and robust, multivariate MR (MVMR) analysis was also employed as a supplementary method. This approach enabled the concurrent consideration of multiple interrelated exposures and facilitated a more refined evaluation of the direct influence of specific exposures on the outcome by controlling for confounding factors related to other exposures.[22] Using this approach, we successfully eliminated confounding due to known confounders, resulting in a more precise assessment of the causality of Vitamin D for HNC risk.

2. Materials and methods

2.1. Study design

Univariate and multivariate MR methods were employed in this study. Vitamin D levels were treated as the exposure variable, and HNC served as the outcome of interest. Single-nucleotide polymorphisms (SNPs), used as IVs, were strongly linked to Vitamin D levels. Moreover, to accurately assess the direct influence of Vitamin D on increasing HNC risk, certain adjustments were made in the MVMR analysis to account for the influence of two important factors, that is, smoking and alcohol consumption. This was performed to exclude any possible bias attributed to these factors. MR analyses must adhere to three basic assumptions (Figure 1): (1) the IVs are strongly linked to the exposures, (2) the IVs are not related to any confounders, (3) and the IVs influence the outcomes only through the exposures and are not influenced by other pathways.

2.2. Data sources

The IEU OPEAN GWAS PROJECT database provided the data for this study. The dataset for Vitamin D levels (ebi-a-GCST005367) used in the genome-wide association study (GWAS) was derived from the study by Jiang *et al.* which contained a total of 79,366 samples and 2,538,249 SNPs.[23] The HNC dataset (ieu-b-4912 was obtained from the UK Biobank, which involved 1106 patients and 372,016 controls. Data on ever-smoking (ieu-b-4858) and alcohol consumption (ieu-b-4834) were extracted from

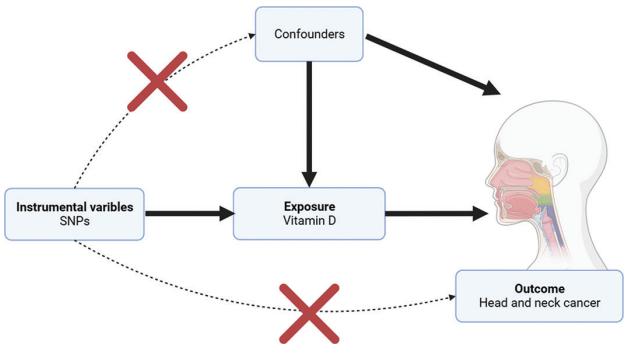


Figure 1. Core assumptions of Mendelian randomization

the within-family GWAS consortium, which includes 99,996 and 83,626 participants, respectively.[24] The GWAS datasets analyzed in this study exclusively encompassed participants of European ancestry.

2.3. IV selection

To ensure the accuracy and reliability of the MR analysis, this study employed a stringent screening process to select suitable IVs. First, SNPs exhibiting strong associations with exposure factors ($p < 5 \times 10^{-8}$) were extracted from the pooled GWAS data. To avoid potential bias that may result from linkage disequilibrium, $r^2 < 0.01$ and kb = 10000 were set. Second, the screened SNPs were further examined in the Phenoscanner database to rule out known confounders linked with the IVs. Finally, the efficacy of the IVs was evaluated using the F-statistic, and only SNPs with F > 10 were retained to address the problem of weak IVs.[25] Simultaneously, palindromic SNPs with moderate allele frequencies were eliminated to enhance robustness.

2.4. MR analysis

The primary methodology employed was the inverse variance weighted (IVW) approach to assess the possible causality of Vitamin D for HNC risk. The IVW method is based on the premise that all IVs are valid and estimates the causal effect by calculating the Wald ratio for each SNP and combining these values. This method provides the advantage of a more stable estimation of causality.[26] In addition, to evaluate the robustness of our findings, MR-Egger, weighted median, simple mode, and weighted mode were used as supplementary analytical methods.

To ensure the reliability of the MR analysis, several sensitivity analyses were performed. Cochran's Q test was performed to evaluate the heterogeneity of the IVs. In addition, the MR-Egger intercept method assesses horizontal pleiotropy, ascertaining its presence or absence through the calculated intercept term.[27] The MR pleiotropy residual sum and outlier (MR-PRESSO) analysis was employed to identify and rectify outliers in the MR analysis caused by pleiotropy.[28] Moreover, the leave-one-out analysis was employed to assess whether individual SN Ps biased the total causal effect.[29]

To account for the possible effect of ever-smoking and alcohol consumption on results and improve the precision of the causal effect, this study incorporated these two variables into the MVMR analysis, as they are recognized risk factors for HNC. This strategy aimed to minimize the potential effect of lifestyle factors on causality estimation, thereby enabling a more accurate evaluation of the direct causal relationship between Vitamin D and HNC. The IVW approach was applied in the MVMR analysis for causal effect estimation.

In this study, statistical analyses were performed using the "TwoSampleMR" and "MRPRESSO" packages in R 4.3.2, with $p < 0.05$ indicating statistical significance.

3. Results

3.1. Genetic instruments

This study involved screening of eight SNPs as IVs to investigate the potential causality of Vitamin D for HNC (Table 1). Among them, two SNPs (rs11940196 and rs10745742) were missing in the genetic association database for HNC. In addition, one SNP (rs8018720) was excluded due to the presence of palindromic sequences. In addition, the F-statistic of all SNPs included in the MR analysis was >10, showing a low risk of weak IV bias.

3.2. Two-sample MR analysis

The IVW method, used as the main analytical approach, suggested a possible causal relationship between Vitamin D levels and HNC risk, indicating that higher Vitamin D levels may decrease the HNC risk (odds ratio [OR] = 0.9958, 95% confidence interval [CI] = 0.9934 – 0.9983, $p = 0.0007$). The results from the weighted median analysis further support the conclusion that Vitamin D lowers the HNC risk. Although the MR-Egger, weighted mode, and simple model analyses did not yield significant results, the direction of their results was consistent with that of the IVW analysis. The results are displayed in Figures 2-4.

Cochran's Q test was utilized to evaluate heterogeneity, revealing the lack of notable heterogeneity in both MR-Egger (Cochran Q = 4.5369, $p = 0.6044$) and IVW (Cochran Q = 6.0413, $p = 0.5349$) analyses. The MR-Egger intercept indicated the lack of significant evidence of horizontal pleiotropy (Egger intercept = –0.00012, $p = 0.2659$), and the MR-PRESSO analysis did not detect any substantial outliers ($p = 0.6050$). In addition, the leave-one-out analysis, which involved sequentially excluding each SNP to evaluate the effect of the remaining SNPs, revealed that no single SNP significantly influence the overall results (Figure 5).

3.3. Multivariate MR analysis

After adjustment for ever-smoking alone, a significant negative association between Vitamin D levels and HNC risk was observed (OR = 0.9959, 95% CI = 0.9937 – 0.9982, $p = 0.0004$). Similarly, this causality was confirmed when only alcohol consumption was considered (OR = 0. 9952, 95% CI = 0.9926 – 0.9979, $p = 0.0004$). When both ever-smoking and alcohol consumption were considered (OR = 0.9956, 95% CI = 0.9933 – 0.9979, $p = 0.0002$),

Table 1. Summary of information on instrumental variables for two-sample MR

SNP	Chr	Pos	Effect allele	Other allele	*p*	SE	F
rs10741657	11	14914878	G	A	2.05E-46	0.0022	195.9951
rs12785878	11	71167449	T	G	3.81E-62	0.0022	272.2431
rs16848425	4	73515825	T	C	1.01E-08	0.004	32.48918
rs17216707	20	52732362	C	T	8.14E-23	0.0027	94.87964
rs17767445	4	72745267	A	G	2.30E-32	0.0033	136.8157
rs2597193	11	14505962	A	G	6.26E-17	0.0022	72.24818
rs3755967	4	72609398	T	C	1.00E-200	0.0023	1504.053
rs928493	20	52700529	A	G	3.98E-08	0.0023	29.06543

Abbreviations: Chr: Chromosome; F: F-statistic; *p*: *p* value for the genetic association; Pos: Position; SE: Standard error; SNP: Single-nucleotide polymorphism; MR: Mendelian randomization.

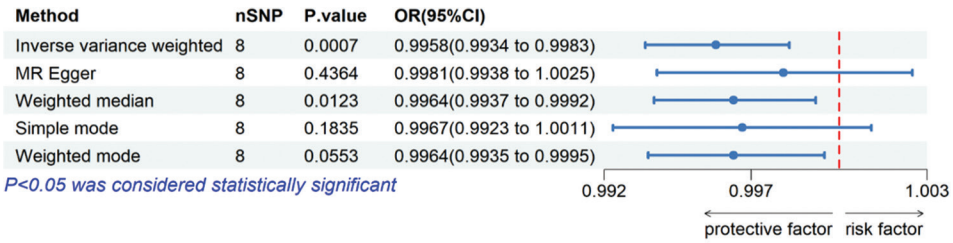


Figure 2. Results of Mendelian randomization analysis between Vitamin D levels and HNC
Abbreviations: OR: Odds ratio; CI: Confidence interval; HNC: Head-and-neck cancer.

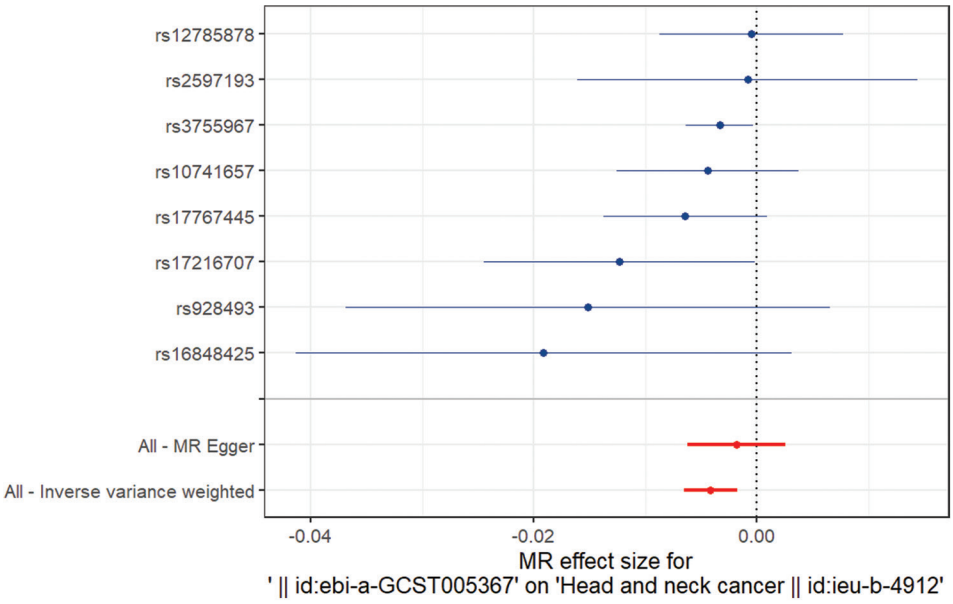


Figure 3. Forest plot for the causal relationship between Vitamin D levels and head-and-neck cancer

the potential role of Vitamin D in reducing HNC risk was further emphasized (Figure 6). This finding provides strong genetic evidence for the importance of Vitamin D in HNC prevention.

4. Discussion

This study explored the possible causality of Vitamin D for HNC risk using MR analysis. The analysis revealed that Vitamin D levels were negatively related to HNC

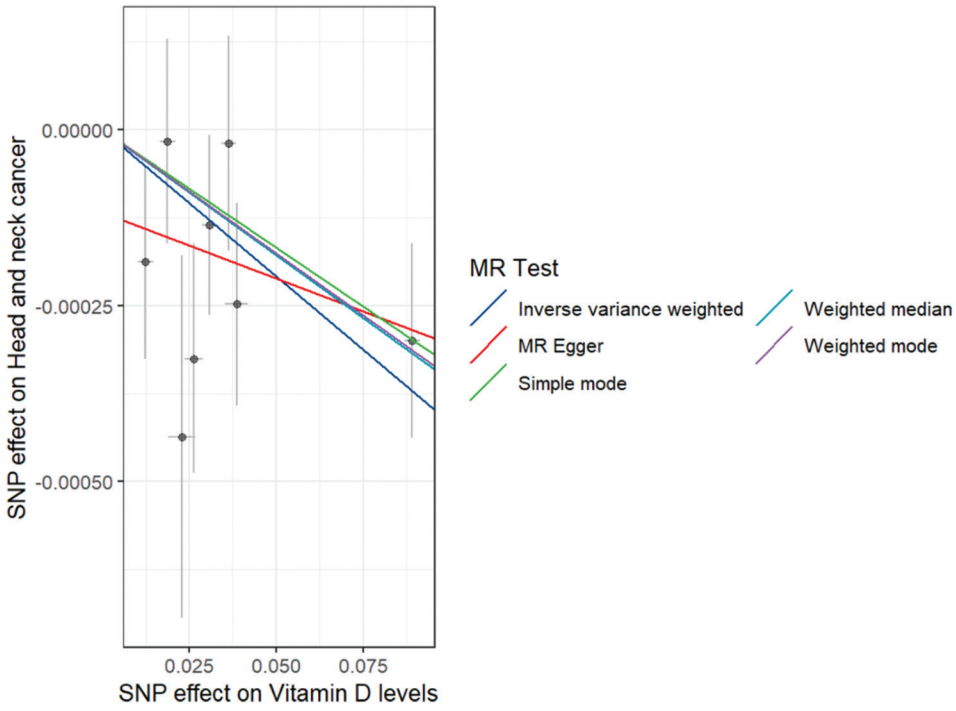


Figure 4. Scatterplot for the causal relationship between Vitamin D levels and head-and-neck cancer

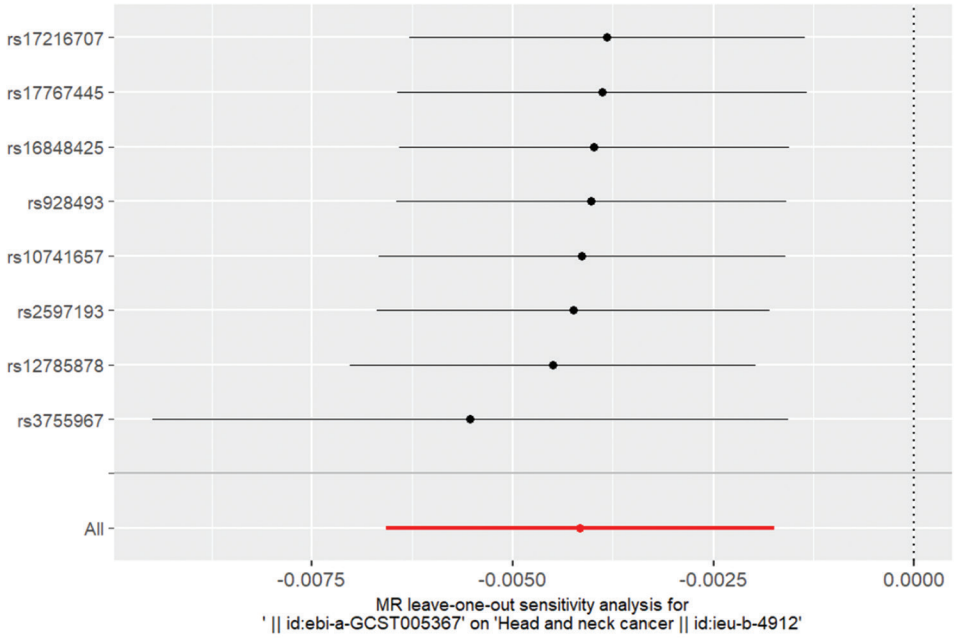


Figure 5. Leave-one-out analysis for the association of Vitamin D levels with head-and-neck cancer

risk. Furthermore, MVMR analysis revealed that this causal relationship was independent of the effects of ever-smoking and alcohol consumption.

Previous studies on whether Vitamin D levels affect HNC occurrences have revealed inconsistent results.[5,12-15] A nested case–control study was conducted among male smokers in Finland to examine the relationship between blood 25(OH)D levels and HNC risk in the context of alpha-tocopherol and beta-carotene. The study included 340 HNC cases and 340 controls and used conditional logistic

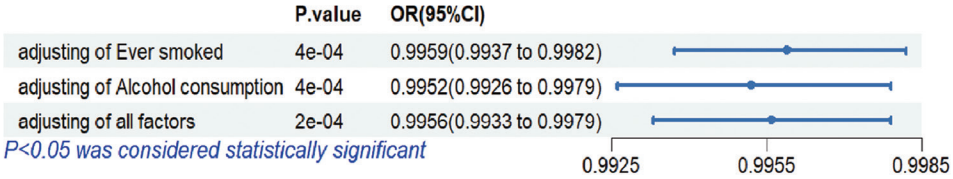


Figure 6. Results of the multivariable Mendelian randomization analysis

regression models adjusted for potential confounders such as age, body mass index, smoking volume, and alcohol consumption. The results indicated the lack of significant associations between serum 25(OH)D levels and the risk of either overall HNC or specific subtypes (e.g., oral cavity, pharynx, and larynx cancers).[15] However, a meta-analysis of 16 studies revealed that higher Vitamin D intake was associated with a reduced incidence of HNC.[5] This meta-analysis showed that individuals with higher serum 25(OH)D levels had a 32% lower incidence of HNC than those with lower serum 25(OH)D levels. In addition, patients with higher 25(OH)D levels had significantly higher 5-year survival rates than those with lower 25(OH) D levels. The Copenhagen City Heart Study, a prospective investigation involving 9791 cancer-free participants at baseline, revealed that diminished Vitamin D levels markedly increased the incidence of HNC.[13] Similarly, a study based on the European Prospective Investigation into Cancer and Nutrition reported that higher Vitamin D levels were strongly linked to a lower HNC risk. In particular, a doubling in Vitamin D concentration corresponded to approximately a 30% reduction in HNC risk.[12] Given these conflicting findings, a more thorough examination of the interaction between Vitamin D levels and HNC risk is required. Vitamin D supplementation may reduce HNC risk, as shown in this study, and provides new genetic insights into this problem. In a prospective cohort study that measured 25(OH)D levels in the Finnish HNC population before therapy commenced, 25(OH)D levels were <50 nmoL/L in 65% of the patients,[30] which may indicate a connection between Vitamin D and HNC.

Evidence suggests that Vitamin D is involved in cancer regulation by influencing aspects such as cell proliferation, angiogenesis, immune function, and cell metabolism.[9,31,32] An *in vitro* study investigating the effects of Vitamin D on HNC cells revealed that Vitamin D could inhibit cancer cell proliferation, make them prone to apoptosis, and increase their sensitivity to chemotherapeutic drugs.[33] Similarly, in a hamster buccal pouch model, Vitamin D inhibited tumorigenesis and growth.[34] In patients with head-and-neck squamous cell carcinoma (HNSCC), severe Vitamin D deficiency was related to altered levels of immune cell infiltration in and around tumors and impaired immune function. When supplemented with Vitamin D, patients' natural killer cells expressed significantly enhanced cytotoxic activity.[35] In addition, the infiltration of activated CD4+CD69+ T cells and regulatory Foxp3+CD4+ T cells in HNSCC tissue was related to a better prognosis.[36] In patients with HNC and high Vitamin D levels, higher CD+4 cell counts in the tumor and peritumoral mesenchyme were strongly associated with longer overall survival.[35] This finding indicates that Vitamin D significantly regulates the immune response to tumors. Vitamin D upregulates antioxidant pathways and limits oxidative DNA damage, thereby exerting antitumor effects.[37] Moreover, the Vitamin D metabolite can directly control the expression of multiple genes that produce proteins involved in repairing DNA damage and programmed cell death.[8] This mechanism provides protection against cancer development. Chronic inflammatory responses are crucial in tumorigenesis,[38] and Vitamin D may protect against carcinogenesis by inhibiting prostaglandin synthesis; inhibiting the p38 MAPK-mediated proinflammatory signaling pathway, or the nuclear factor kappa B signaling pathway; and regulating the coaction between immune cells and cancer cells to exert anti-inflammatory effects.[39] Moreover, Vitamin D induces tumor cell apoptosis by stimulating the endogenous apoptotic pathway and can change the autophagic mode of cancer cells from cell survival to cell death.[39,40]

This study had several strengths. First, the MR analysis mitigated the disruptions of irrelevant factors and reverse causality prevalent in traditional observational research. Second, the data used in this study were sourced from the GWAS database, which focuses on European populations, effectively addressing population heterogeneity. MVMR analysis was also employed to control for potential confounders, guaranteeing the validity of the results. Finally, various analysis methods were used, and sensitivity analysis was performed for validation.

However, this study had some limitations. First, data were derived from a European population, which could restrict the applicability of the findings to other populations. Second, despite performing sensitivity analyses, the possibility of pleiotropy cannot be entirely excluded, particularly due to biological pleiotropy. Finally,

although a causal link between Vitamin D levels and HNC risk was established in this study, data limitations hindered a deeper investigation into the relationship.

5. Conclusions

Overall, this study identified the causality of Vitamin D for HNC risk using MR methods. Vitamin D supplementation may reduce the HNC risk, providing fresh perspectives on cancer prevention and therapy.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Xiaosong Ru
Formal analysis: Xiaosong Ru, Ziying Su
Investigation: Ziying Su
Methodology: Xiaosong Ru
Supervision: Yong Guo
Writing–original draft: Xiaosong Ru
Writing–review & editing: Ziying Su, Yong Guo

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All GWAS datasets utilized in this study can be accessed through online databases. (https://gwas.mrcieu.ac.uk/).

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