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Investigating a Causal Relationship Between Diabetes Mellitus and Oropharyngeal Cancer: A Mendelian Randomization Study

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Objective: Previous observational studies reported an association of diabetes mellitus (DM) with oropharyngeal cancer (OPC), however, the potential causality of the association between them remains unclear. ***Methods***: To explore this causal relationship in individuals of European descent, a two-sample Mendelian randomization (MR) study was conducted. A genome-wide association study (GWAS) of DM was used to represent the exposure factor (T1DM: n = 24,840; T2DM: n = 215,654), and GWAS of OPC represented the outcome (n = 3,448). ***Results***: Forty-one single nucleotide polymorphisms (SNPs) related to T1DM and fifty-four SNPs related to T2DM were identified as effective instrumental variables (IVs) in the two-sample MR analyses. In IVW estimates, neither T1DM nor T2DM significantly contributed to an increased risk of OPC [T1DM: OR 1.0322 (95% CI 0.9718, 1.0963), P = 0.3033; T2DM: OR 0.9998 (95% CI 0.9995, 1.0002), P = 0.2858]. Four other regression models produced similar results. MR-Egger regression results [Cochran's Q statistic was 47.1544 (P = 0.1466) in T1DM, and 35.5084 (P = 0.9512) in T2DM] suggested no horizontal pleiotropy between IVs and outcomes. ***Conclusion***: Our findings suggest little evidence to support the genetic role of diabetes mellitus in OPC development in the European population.

Keywords*: single nucleotide polymorphisms, diabetes mellitus, oropharyngeal cancer, Mendelian randomization, genome-wide association study*

Introduction

Oropharyngeal cancer (OPC) is a common subtype of head and neck squamous cell carcinoma (HNSCC), which includes cancers of the oral cavity and the oropharynx and is the world's sixth most common type of cancer (Ferlay *et al.*, 2019). The prognosis of OPC is poor, with a five-year survival of 15%–20% (Liu *et al.*, 2015), and its incidence varies greatly worldwide, with an increasing trend in the UK (18.8%), Australia (8.7%), Japan (21.3%), and the USA (3.7%) (Bosetti *et al.*, 2020), largely driving global incidence rates of head and neck cancer (HNC). The incidence and mortality of OPC have increased rapidly among older adults (Damgacioglu *et al.*, 2022), peaking at 50–59 years (Ghazawi *et al.*, 2020). The established risk factors include cigarette smoking and alcohol consumption (Hashibe *et al.*, 2009), as well as infection with human papilloma virus (HPV) (Ang, 2010). The notable increase in regional-stage and the concurrent recent increase in mortality renders OPC a growing public health concern and calls for urgent improvements in prevention.

Diabetes mellitus (DM), a common, complex disease that is typically divided into two major subtypes, type-1 and type-2 DM (T1DM and T2DM, respectively), along with less common types. T1DM is an endocrine disorder in which pancreatic β-cells stop producing insulin, typically due to autoimmune destruction (Syed, 2022), and the pathophysiology of T2DM includes insulin resistance and initial hyperinsulinemia, followed by a progressive decrease in the capacity of pancreatic β cells to produce insulin (Ahmad *et al.*, 2022). DM poses a substantial burden on individual and population health, and it is commonly associated with multiple types of tumors, especially liver, colorectal, and pancreatic cancer (Inoue, 2006; Sasazuki *et al.*, 2013). High levels of blood glucose (Jee, 2005; Stocks *et al.*, 2009) or glycated hemoglobin A1c (Goto *et al.*, 2016) are associated with an increased risk of cancer. From a biological perspective, these associations appear intuitive as hyperglycemia increases mitochondrial glucose oxidation, thereby promoting DNA damage through oxidative stress (Goto *et al.*, 2020). This may help identify a potential strategy to prevent some forms of cancer, considering that diabetes or high glucose levels can be prevented through lifestyle or medication adaption. Similar associations also occur between OPC and DM. A multicenter study (Spratt *et al.*, 2016) in the USA included 1,745 patients with OPC, 184 of which had DM at the time of diagnosis. Ogunsakin et al. (2018) reported that of 310 patients with squamous cell carcinoma (SCC) of the larynx or oropharynx, 54 had T2DM, and in a subgroup of oropharyngeal squamous cell carcinoma (OPSCC) patients, DM was significantly associated with tumor size (Zaoui *et al.*, 2016). DM increased the risk of HNC (Tseng *et al.*, 2014; Choi *et al.*, 2022) in men and women and T2DM was associated with HNC recurrence (Hu *et al.*, 2020). A further study examined data from 25,154 twins and found that midlife diabetes increased the risk of pharyngeal cancer in later life (Bao *et al.*, 2018). A retrospective cohort study showed that the oropharynx (24%) was the common cancer subsite in T2DM patients with HNSCC (Lee *et al.*, 2019), followed the larynx and oral cavity (Foreman *et al.*, 2017). Further, a meta-analysis showed a positive association between T2DM and OPC, when HNC was stratified by cancer type (Yan *et al.*, 2021).

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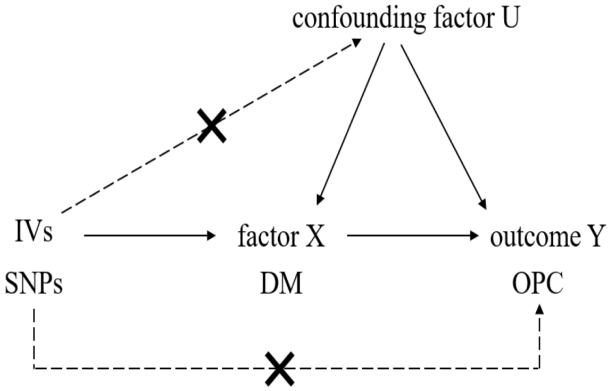
Similar results were found in studies on the association between T1DM and cancer risk, as even young adult patients with TIDM have a higher risk of cancer than those of similar age with T2DM (Kiss *et al.*, 2019). An analysis of five national cohorts of persons with T1DM (Australia, Denmark, Finland, Scotland, and Sweden) found that those with type 1 diabetes had a higher incidence of cancer of the liver, pancreas, kidney, endometrium, and ovary and a lower incidence of prostate cancer than the general population (Carstensen *et al.*, 2016). A meta-analysis of observational studies showed that patients with type 1 diabetes had an increased risk of cancer, significantly increased risk of cancers of the stomach, lung, pancreas, liver, ovary, and kidney, and a significantly decreased risk of breast cancer (Sona *et al.*, 2018). A prospective cohort study showed that women with childhood-onset type 1 diabetes in Sweden had a small but significantly elevated risk of cancer; the median age at cancer diagnosis was 28 years, and breast cancer was the most common form (Fredriksson *et al.*, 2022).

An association between DM and OPC was confirmed in multiple observational studies (Albergotti *et al.*, 2016; Zaoui *et al.*, 2016; Pleitz *et al.*, 2017), however, observational epidemiological studies are prone to reverse causality bias, detection bias, and depletion of the susceptible (Lega and Lipscombe, 2020). Therefore, alternative study designs are urgently needed to confirm whether DM is a risk factor for OPC.

Mendelian randomization (MR) in epidemiological research may provide an economical approach to address this problem. MR utilizes genetic variants that are determined before birth, and which remain constant throughout life (Vincent and Yaghootkar, 2020). Data may be obtained from large-scale genome-wide association studies (GWASs) to test potential associations between risk factors and outcomes consistent with a causal effect (Smith and Ebrahim, 2003). Genetic variants are randomly allocated at conception and are thus generally unrelated to confounders (such as environmental and self-adopted factors), which minimizes the risk of confounding (Figure 1). MR should satisfy the following three conditions: (1) there should be a strong correlation between instrumental variable (IVs, such as single nucleotide polymorphisms [SNPs] and the exposure factor X); (2) IVs should not correlate with any confounding factor U associated with exposure-outcome; (3) IVs should not affect the outcome Y unless it is possible to do so by association with exposure to X.

Whilst some epidemiological studies associate DM with OPC, reverse causality and residual confounding due to common risk factors may exist, and it remains unclear whether diabetes contributes to OPC development. Thus, we conducted an MR study to investigate the causal association between OPC and DM. Directed acyclic graphs showed the rationale for the study (Figure 1).



SNPs = Genetic variants; DM = Diabetes Mellitus and OPC = Oro-Pharyngeal Carcinoma.
Solid arrows = permitted relationships between variables. for IVs to be a valid cause of OPC.
Dashed lines = relationships not permitted if IVs are a cause of OPC.

***Figure 1**. Directed acyclic graph of the rationale for MR study.*

Method

Genome-wide association study data of T1DM (GWAS ID: ebi-a-GCST010681), T2DM (GWAS ID: finn-b-E4_DM2), and OPC (GWAS ID: ieu-b-97) with the largest sample in recent years were obtained from the website of the IEU Open GWAS project (gwas.mrcieu.ac.uk) to generate the exposure dataset for OPC (Lesseur *et al.*, 2016). In total, 42,852 cases and 201,088 controls with European ancestry from previously published GWASs were included. The GWAS outcome datasets and T1DM cases were compiled from multiple T1DM case cohorts and control cohorts (Forgetta, 2020) that included 24,840 individuals (9,266 cases and 15,574 controls) (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST010681/). The T2DM GWAS included 32,469 cases and 183,185 controls)(https://gwas.mrcieu.ac.uk/datasets/finn-b-E4_DM2/) and the GWAS involved 3,448 individuals (1,119 cases and 2,329 controls) (https://gwas.mrcieu.ac.uk/datasets/ieu-b-96/).

We performed a two-sample MR analysis using DM-associated genetic variants as IVs.

Exposed SNPs were identified regarding associations with T1DM, T2DM, and OPC in the IEU Open GWAS. We applied several criteria for screening SNPs, i.e., genome-wide association ($P < 5\times10^{-8}$), independent inheritance ($r^2 < 0.01$), a linkage disequilibrium (LD) coefficient ($r^2 < 0.001$), and a width of the linkage disequilibrium area of 10,000 kb (Davey Smith and Hemani, 2014), but without linkage disequilibrium in the summary statistics.

The OPC-related SNPs were extracted from GWAS summary data of T1DM, T2DM; a minimum $r^2 > 0.8$ was applied, the missing SNPs were replaced with the SNPs with high linkage, and the SNPs without alternative sites were removed. The information was summarized, and SNPs ($P < 5\times10^{-8}$) directly related to T1DM or T2DM were excluded (Hartwig *et al.*, 2016).

Five regression models including MR-Egger regression, weighted median model, inverse variance weighting of random effects (IVW) model, weighted model, and simple mode were used for analysis. Cochran's Q test was also applied to determine SNP heterogeneity (Bowden *et al.*, 2018); if heterogeneity occurred, we focused on the IVW model results; the leave-one-out method was used for sensitivity analysis. The above methods were implemented using the Two Sample MR package in R software version 4.0.4, with $\alpha = 0.05$.

We generated scatter and forest plots of each outcome to demonstrate the directional effect of DM on the outcomes. We also produced leave-one-out plots and

funnel plots to roughly estimate reliability and pleiotropy. Heterogeneity of effects was assessed according to the scatter plots and Cochran's Q tests for SNP exposure and outcome associations.

Results

Single nucleotide polymorphisms related to T1DM were eliminated, leaving 41 SNPs finally included as IVs. The same was applied with 54 SNPs in T2DM (data available at https://www.cdhjournal.org/supplementary/263e46514240d764357f15e2857ad16e). The intercept term of MR-Egger regression in T1DM was 0.0202 ($P = 0.6619$), and in T2DM it was 0.0204 ($P = 0.4263$), suggesting no genetic pleiotropy between the screened SNPs and T1DM or T2DM.

Five regression models were fitted in the two-sample MR analysis to detect whether diabetes was a risk factor for OPC. According to IVW estimates, neither T1DM nor T2DM significantly increased the risk of OPC (T1DM: OR 1.0322 (95% confidence interval (CI) 0.9718, 1.0963), $P = 0.3033$; T2DM: OR 0.9998 (95% CI 0.9995, 1.0002), $P = 0.2858$) (shown in Tables 1 and 2; Figures 2 and 3). Additionally, MR-Egger analysis (T1DM: OR 1.0158 (95% CI 0.9252, 1.1154), $P = 0.7436$; T2DM: OR 1.0002 (95% CI 0.9995, 1.0009), $P = 0.5424$), weighted median analysis (T1DM: OR 1.0199 (95% CI 0.9434, 1.1027), $P = 0.6201$; T2DM: OR 1.0000 (95% CI 0.9993, 1.0006), $P = 0.9887$), simple mode analysis (T1DM: OR 1.0447 (95% CI 0.8676, 1.2578), $P = 0.6472$; T2DM: OR 0.9986 (95% CI 0.9973, 0.9999), $P = 0.0308$), and weighted mode analysis (T1DM: OR 1.0146 (95% CI 0.9456, 1.0886), $P = 0.6895$; T2DM: OR 1.000 (95% CI 0.9994, 1.0007), $P = 0.8575$), also produced similar results (Tables 1 and 2; Figure 3).

MR-Egger regression was used to test for heterogeneity [Cochran's Q statistic was 47.1544 ($P = 0.1466$) for the set of 41 SNPs in T1DM, and 35.5084 ($P = 0.9512$) for the set of 54 SNPs in T2DM] suggesting no horizontal pleiotropy between IVs and outcomes. The funnel plots of T1DM and T2DM showed the basic symmetry of all included SNPs, indicating neither horizontal pleiotropy nor heterogeneity (Figure 4).

Leave-one-out sensitivity analysis suggested that, regardless of which SNP was removed, the removal would not change the findings (all lines were on the right side of 0 or crossed the invalid line and were close to the range of the total effect), which indicated that this MR result was robust (Data available at https://www.cdhjournal.org/supplementary/263e46514240d764357f15e2857ad16e).

Table 1. MR estimates of causal effects of T1DM and OPC.

Model	*SE*	*P*	*OR (95%CI)*
MR Egger	0.0477	0.7436	1.0158 (0.9252-1.1154)
Weighted median	0.0398	0.6201	1.0199 (0.9434-1.1027)
IVW	0.0308	0.3033	1.0322 (0.9718-1.0963)
Simple mode	0.0947	0.6472	1.0447 (0.8676-1.2578)
Weighted mode	0.0359	0.6895	1.0146 (0.9456-1.0886)

SE, standard error; OR, odds ratio; CI, confidence interval; MR, Mendelian randomization; IVW, inverse-variance weighted.

Table 2. MR estimates of causal effect of T2DM and OPC.

Model	*SE*	*P*	*OR (95%CI)*
MR Egger	0.0004	0.5424	1.0002 (0.9995-1.0009)
Weighted median	0.0003	0.9887	1.0000 (0.9993-1.0006)
IVW	0.0002	0.2858	0.9998 (0.9995-1.0002)
Simple mode	0.0007	0.0308	0.9986 (0.9973-0.9999)
Weighted mode	0.0003	0.8575	1.0000 (0.9994-1.0007)

SE, standard error; OR, odds ratio; CI, confidence interval; MR, Mendelian randomization; IVW, inverse-variance weighted.

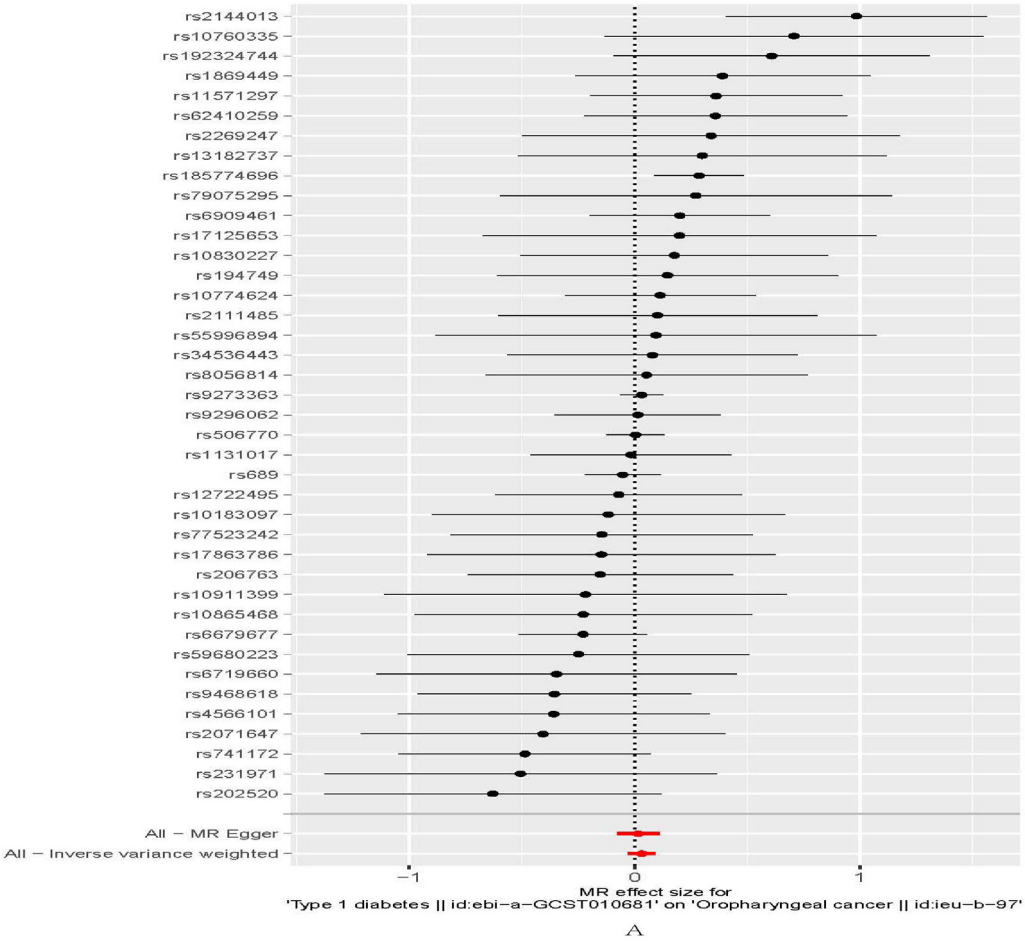
Discussion

Previous observational studies have associated T1DM and T2DM with the development of oropharyngeal cancer. We thus used various recent large datasets from the website of IEU Open GWAS project and MR methods to verify the causal relationship between DM and OPC. Taken together, the results did not support a causal association between these two diseases.

A substantial amount of epidemiological evidence indicates that diabetes increases the risk of many types of cancer and affects the long-term efficacy of cancer treatment. The American Oncology Association and the American Diabetes Association divided the possible links between cancer and diabetes into three categories: (a) unmodifiable risk factors (age, sex, and ethnicity), (b) modifiable risk factors (including obesity, physical activity, smoking, and alcohol consumption), and (c) biological links between diabetes and cancer (for example, hyperinsulinemia, hyperglycemia, insulin resistance, and chronic inflammation) (Gallagher and LeRoith, 2011). According to site-specific cancer grouping, previous meta-analyses showed that the relative risks were highest (approximately two-fold or higher) for cancer of the pancreas, liver, and endometrium, and moderate (approximately 1.2-1.5-fold) for cancer of the colon and rectum, breast, and bladder (Tsilidis *et al.*, 2015; Pearson-Stuttard *et al.*, 2018).

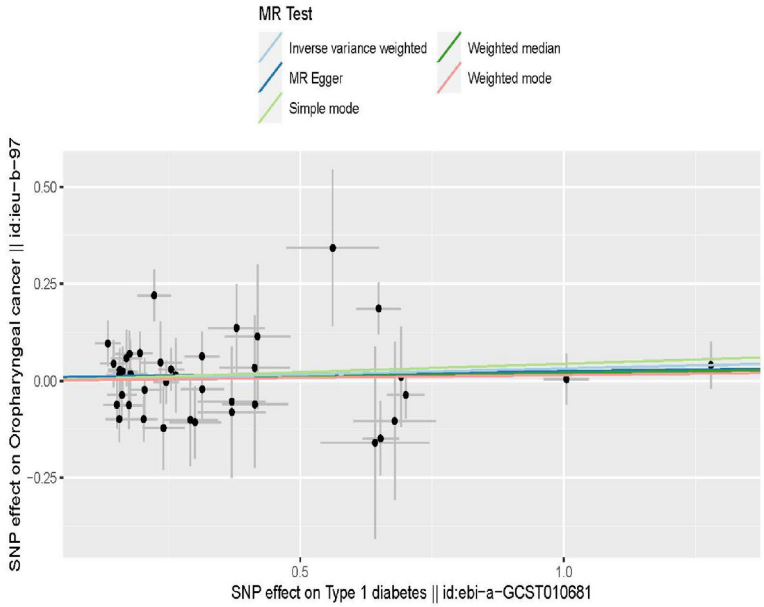
Randomized controlled trials are considered the gold standard to determine causality and strong statistical associations may be observed between an exposure and outcome, however, it remains uncertain whether all confounders of the association have been identified, measured, and appropriately adjusted for (Bowden and Holmes, 2019). To minimize the influence of bias and residual confounders in observational studies, MR can be applied with GWAS data to assess the causality in putative exposure–outcome pathways (Burgess *et al.*, 2015), utilizing the random allocation of genotypes at conception, which renders genotypes independent of potential confounders and also avoids reverse causation (Smith and Ebrahim, 2003).

Previous studies have shown that DM promotes the development of some cancers, whereas different results were obtained when MR was used to examine the causal relationship between DM and these cancers. MR, which is essentially a genetic analogue of the RCT (Barroso and McCarthy, 2019), uses genetics to assess how key environmental and lifestyle factors (e.g. T2DM) influence complex diseases (e.g. cancer). Our results support the assumption that DM (T1DM or T2DM) *per se* may not be the responsible factor underlying the reported positive association of diabetes with OPC. Five analysis methods



Black dots and bars indicate the causal estimate and 95% CI using each SNP. Red dots and bars indicate the overall estimate and 95% CI meta-analyzed in IVW.

Figure 2. *Forest plot of the association of T1DM and T2DM with OPC.*



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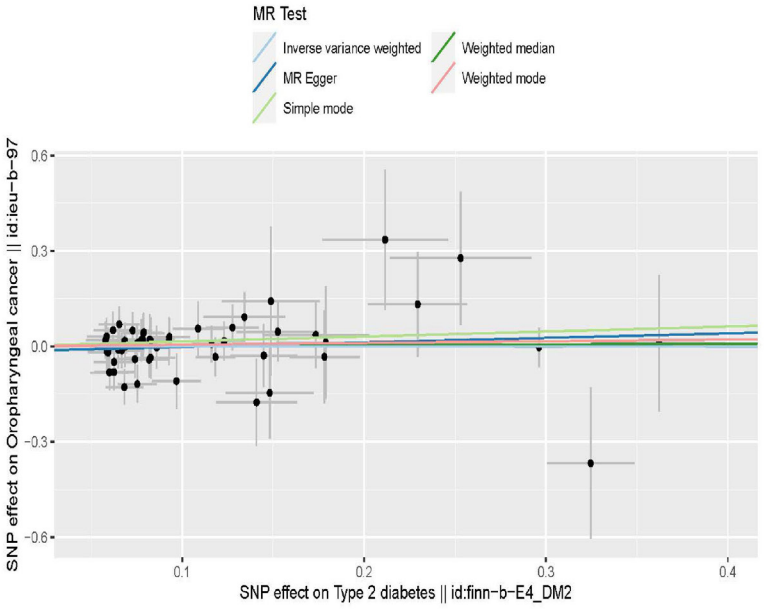

B

The slopes of the solid lines denote the magnitudes of the associations estimated from the MR analyses.

Figure 3. *Scatter plot of the effects of genetic variants on T1DM, T2DM, and OPC.*

(IVW, MR-Egger regression, weighted median model, weighted model, and simple mode) were used for our MR Analysis, producing consistent results, and none of them supported the causal relationship between DM and OPC. After excluding potential pleiotropic SNPs, the results of the three MR analyses remained consistent, confirming the robustness of the various analytical methods and results. To test the reliability of the conclusions obtained by the MR analysis, 'leave one out' sensitivity analysis excluded the possibility of individual SNPs driving causal results; further, the MR-Egger method can be used to assess the unbalanced pleiotropic effects and the causal effect of exposure on outcome. Our results are in general agreement with those reported in earlier MR studies. An analysis among 10,536 subcohort subjects and 3,541 incident cancer cases in a Japanese population produced no strong evidence supporting the associations between DM and the risks of total and site-specific cancer such as colon cancer, pancreatic cancer, or liver cancer (Goto *et al.*, 2020). A different study produced the same result with regard to T2DM and pancreatic, endometrial, renal cell, and ovarian cancer (Vincent and Yaghootkar, 2020). These studies and our findings indicate little evidence to sustain the genetic role of DM in OPC development. Conventional regression analyses may have over-estimated the true association, possibly due to uncontrolled confounding by common risk factors or reverse causation.

Although there are differences in the pathogenesis of T1DM and T2DM, hazard ratios for site-specific cancers in persons with T1DM were similar to those observed among persons with T2DM, suggesting a potential common mechanism among persons with T1DM and T2DM. Perhaps a combination of multiple factors may explain the link between DM and OPC. However, the observed association

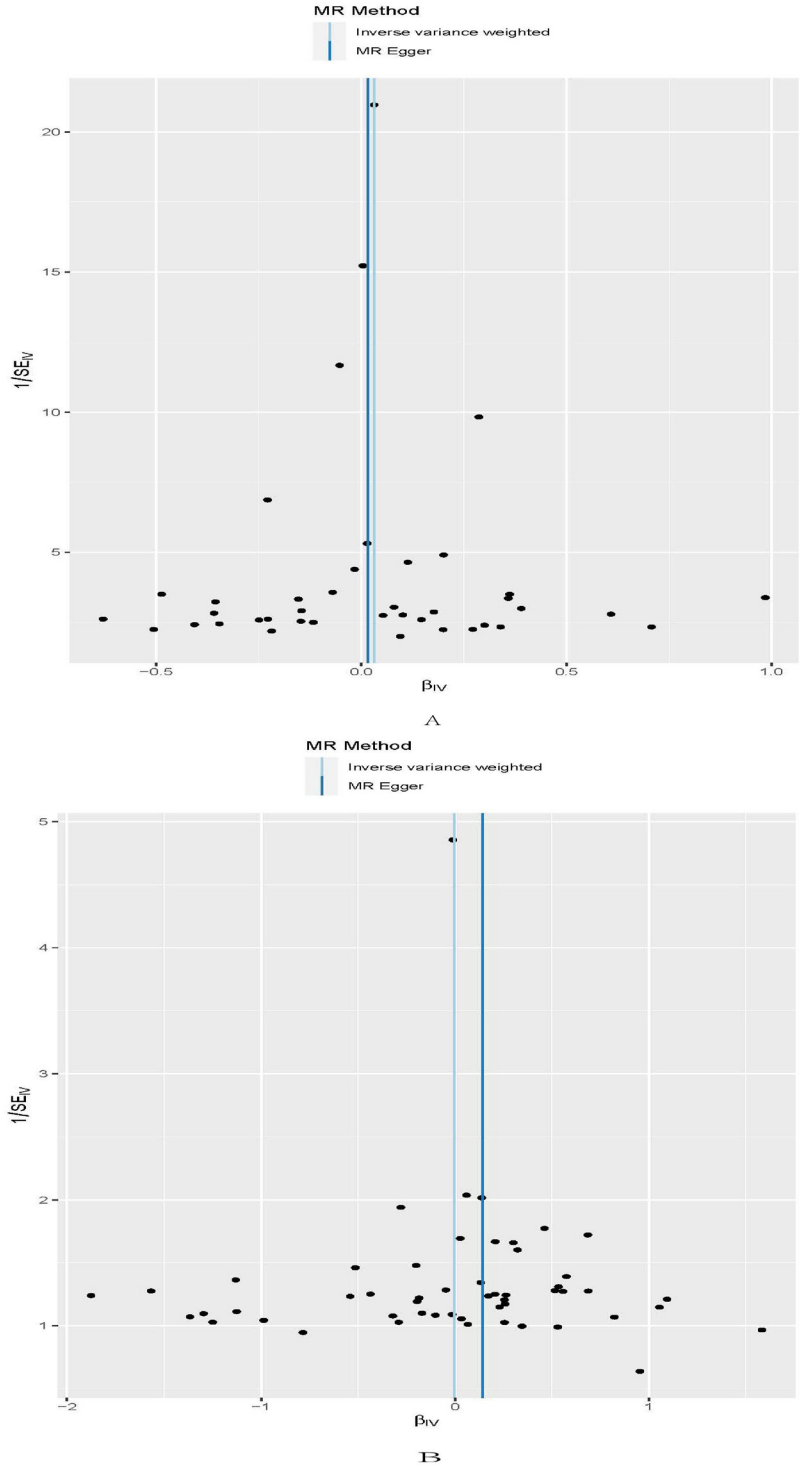

Figure 4. *Funnel plot of the association of T1DM and T2DM with OPC.*

between T1DM/T2DM and cancer may be confounding, i.e., owing to common risk factors (Tsilidis *et al.*, 2015), including advanced age, poor diet and lifestyle, and environmental factors that are associated with diabetes and HNSCC (Tanaka *et al.*, 2017). Further, biological alterations caused by DM, including inflammation, insulin resistance, hyperinsulinemia, and hyperglycemia, but not genetic aspects, may also promote the initiation and progression of tumors (Hong *et al.*, 2021). Hyperglycemia is a hallmark of T1DM and T2DM, and most cancer cells are highly dependent on glucose, thus high glucose concentrations can promote the growth of cancer cells (Dang, 2012) and the survival of bacteria, which renders diabetic patients susceptible to infection, and high-risk HPV 16/18 subtype infection is one of the key independent pathogenic factors for OPC (Gillison, 2000). DM is also associated with greater intracellular oxidative stress (Muniraj, 2012), which inactivates insulin receptors and is carcinogenic. Furthermore, the insulin receptor, IGF receptor, and IGFs, hyperinsulinemia and other factors also play important roles in tumorigenesis, which strongly implicated in cancer progression and modulate cell survival and proliferation, migration, angiogenesis and metastasis (Kerr and Baxter, 2022), further increasing the risk of some cancers associated with DM (de Kort *et al.*, 2019).

The DM and OPC datasets that we used were the most abundant publicly available GWAS data source, to our knowledge, however, the study population was of European

ancestry, which helped reduce the bias attributable to population stratification but also reduced population stratification and cannot represent other ancestry. Further, we did not perform causal analysis stratified by gender or age.

In contrast to observational studies, our MR analysis suggests that the genetic mechanisms responsible for DM may not play major roles in OPC development, suggesting that previous associations between DM and OPC are possibly confounded by potential biases or due to reverse causation, which can address the ambiguity in this aspect of clinical observation research. Further large-scale prospective studies are warranted to replicate our findings. Simultaneously, from a public health perspective, it is certainly important to control common risk factors for both diseases, considering that DM and cancer share a number of established modifiable risk factors. Future research efforts should focus on fully identifying the pathogenesis of OPC, reduce modifiable cancer risk factors, and promote targeted clinical and social prevention strategies to reduce the development of OPC.

In conclusion, we found little evidence for a causal role of genetically predicted DM in the risk of OPC in a large, well-powered study, indicating that previous associations between DM and OPC are possibly confounded by potential biases or due to reverse causation.

Author Contributions

YXH, LLJ and RCT conceived and designed the experiments. YXH, LLJ and JXL wrote the manuscript. JXS, YXH, LLJ, YJX and FFM collected data. YXH, LLJ, JXL and RCT analyzed the data. All authors reviewed the manuscript.

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Institutional Review Board Statement

This study complied with all relevant ethical regulations, as the individual studies had previously obtained relevant ethical approval by studies from local boards as described in the included GWAS.

Informed Consent Statement

The individual studies had previously obtained participant consent by studies as described in the included GWAS.

Data Availability Statement

The T1DM summary data (GWAS ID: ebi-a-GCST010681) are available at https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST010681/, T2DM summary data (GWAS ID:finn-b-E4_DM2) are available at https://gwas.mrcieu.ac.uk/datasets/finn-b-E4_DM2/, and OPC summary data (GWAS ID: ieu-b-97) are available at https://gwas.mrcieu.ac.uk/datasets/ieu-b-96/. All original GWAS data were obtained from the website of the IEU Open GWAS project. All data that support the conclusions of this manuscript are included within the article.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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