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Association of Immune Cell Phenotypes With Oral Cancer: A Two-Sample Mendelian Randomisation Study

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ABSTRACT

Objectives: The purpose of this study is to assess the potential causal relationship between immune cell phenotype and oral cancer using Mendelian randomisation analysis.
Methods: A two-sample Mendelian randomisation (MR) analysis using summary statistics from genome-wide association studies in European populations was conducted to explore causal relationships between immune cell phenotypes and the risk of oral cancer. Inverse-variance weighting, MR-EGGER, simple mode, weighted median, and weighted mode were applied for MR analysis. Sensitivity analyses, including the Steiger test, Cochran's Q test, Egger intercept, and leave-one-out analysis, were performed to assess the robustness of the results. Additionally, colocalisation analysis was carried out to further validate causal associations.
Results: A total of 21 immune cell phenotypes were identified as risk factors for oral cancer, while 6 immune cell phenotypes demonstrated protective effects. Sensitivity analyses indicated a lack of robustness in four causal relationships. Genetic variants at rs9469077 on chr6 might be shared between $CD28^{-}CD127^{-}CD25^{++}$CD8br AC of regulatory T cells and oral cancer.
Conclusion: This MR study provides evidence for a strong association between immune cells and oral cancer, highlighting specific immune cell phenotypes as significant risk factors for the development of oral cancer. These findings offer a foundation for future precision immunotherapy strategies for oral cancer. Further studies are required to confirm the relationship between immune cells and oral cancer risk and to elucidate the underlying mechanisms.
Clinical Relevance: This study confirms the potential relationship between specific immune cell phenotypes and oral cancer, providing theoretical support for future immunotherapy against oral cancer.

Introduction

The oral cavity is described as the anatomical area that exists between a defined coronal plane drawn from the junction of the soft and hard palate, through the tongue circumvallate papillae, to the lip mucosa. Oral cancer (OC) is a general term for malignant tumours that occur in the cavity. It is a complex and often relentless malignancy prone to local invasion and dissemination. There are seven disease susceptibilities of OC, including lip, tongue, bottom of the mouth, buccal, hard palate, soft palate, gingival, and retromolar trigone.[1] Squamous cell carcinomas are the vast majority of OC; other types such as lymphoma, sarcomas, melanoma, minor salivary gland malignancies, and malignant odontogenic tumours contribute less than 10%.[2]

OC is a highly lethal disease with a mortality rate that approaches 50%. It is the result of a combination of factors, with the high-risk ones including long-term tobacco use, alcohol use, poor oral hygiene habits, prolonged betel nut chewing, prolonged exposure to the sun or other sources of ultraviolet light, human papilloma virus infection, malnutrition, repeated irritation of the tongue and buccal mucosa by dental crowns and roots, stimulation by chronic inflammation, and more.[3,4] The standard treatment of OC involves a

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combination of approaches, depending on several factors such as the stage of the cancer, its location, and the patient's overall health. Primary treatment is surgical resection with or without postoperative adjuvant therapy,[5] supplemented by radiotherapy, chemotherapy immunotherapy, targeted therapy, cryotherapy, heating therapy, laser therapy, and other integrated treatment means.

As a critical indicator of the immune system activity, immune cell phenotypes refer to the pattern of expression of molecules on the surface, and receptor molecules that regulate processes such as cell growth, activity, and differentiation.[6] Recent developments in molecular immunology have demonstrated that immune cell lineages are constituted by distinct subpopulations capable of performing a large number of specialised functions to modulate disease progression.[7]

There is a close relationship between OC and immune cell phenotypes,[8] reflected mainly in the state and function of immune cells in OC micro-environment and their interaction with cancer cells. Immune cells have a surveillance and recognition role in OC and can kill abnormal cells, including tumour cells. Immune cells such as T-lymphocytes are responsible for recognising and removing cancer cells; for example, the proportion and activity of T-cell subsets such as $CD3^+$, $CD4^+$, and $CD8^+$ may be affected in OC. In addition to T-lymphocytes, the phenotype of other immune cells such as NK cells, B cells, dendritic cells, and so forth may change in OC.

Mendelian randomisation (MR) analysis is designed to examine causal hypothesis in nonexperimental data. It is an application of instrumental variable analysis. MR typically infers the influence of biological factors on various diseases by the effect of randomly assigned genotypes on nature phenotypes.[9-11] Reverse causation is a common problem in traditional epidemiological studies; however, in MR studies, the effect of reverse causation can be ruled out and the reliability of causal inference can be improved, because genetic variation is determined before the birth of an individual; it is assigned randomly and not subject to confounding influences.[12,13] MR analysis uses the inherent characteristics of common genetic variants for relevant environmental exposures that can be altered. It has been used widely as an effective approach to exploring potential causal relationships between environmental exposures and a wide range of diseases such as different types of cancer,[14,15] diabetes,[16] cardiovascular diseases,[17,18] chronic kidney disease,[19] and other complex diseases.

MR analysis leverages genetic variation to investigate associations between traits and diseases, and it may play a crucial role in understanding the relationship between immune cell phenotypes and OC. The significance of using MR analysis in the study lies in its ability to provide insights into the underlying biological mechanisms that contribute to OC development and progression, improving the probability of identifying potential biomarkers for early detection, prognosis, and treatment response. Traditional observational or experimental studies are often subject to confounding factors and require a specific experimental setting. MR analysis can reduce potential bias by using genetic variation as an instrumental variable; it allows researchers to combine data from multiple large-scale genome-wide association studies (GWAS), which enhances the statistical efficacy and reliability of the results. Moreover, MR analysis combines the knowledge of multiple disciplines, such as genetics, oncology, and immunology, which can promote cross-collaboration in different fields, and provides a framework for dissecting the complex interplay between genetics, immune cell phenotypes, and OC.

The purpose of this article is to investigate the relationship between different immune cell phenotypes and OC, and then provide a theoretical basis for the prevention, diagnosis, treatment, and prognosis of OC, which has not been reported yet. The phenotypes of specific immune cells may be related to the prognosis of OC patients or their response to immunotherapy. Based on the expression of immune cell phenotypes in the tumour microenvironment at different stages, we can identify new immunotherapy targets and uncover unknown receptors or signalling pathways, which can help to formulate a more precise treatment plan for OC.

Methods

Data sources

Figure 1 presents the schematic diagram of the MR study aimed at exploring the causal impact of immune cell phenotypes on OC. Data on OC were sourced from the IEU Open GWAS database, focusing on individuals of European descent (https://gwas.mrcieu.ac.uk). The study included 1135 European adults diagnosed with OC, along with a control group of 2329 European participants, all derived from GWAS data (GWAS ID: ieu-b-93).[20] Summary statistics for 731 immune-related traits were accessed from the GWAS catalogue under ID ebi-a-GCST09001391 to ebi-a-GCST90002121.[21] These immune cell phenotypes were divided into several categories, including absolute cell (AC) counts (n = 118), median fluorescence intensity (MFI), which indicates surface antigen expression (n = 389), morphological parameters (MP) (n = 32), and relative cell (RC) counts (n = 192). The MFI, AC, and RC datasets included immune panels such as B cells; conventional dendritic cells (cDCs); maturation stages of T cells; monocytes; myeloid cells; T cell, B cell, natural killer cell (TBNK); and regulatory T cells (Treg). The MP feature was comprised of cDC and TBNK panel data.

Instrumental variables selection

In this MR analysis, single nucleotide polymorphisms (SNPSs) were employed as instrumental variables (IVs) to explore the causal link between immune cell phenotypes and OC. The selection of IVs was based on three core assumptions according to Davies et al.: the relevance assumption, which ensures a strong association between the IVs and immune cell traits; the independence assumption, which ensures that the IVs are not correlated with any confounding factors; and the exclusion restriction, which requires that the IVs do not directly influence immune cell phenotypes.[22] IVs were chosen at genome-wide significance ($P < .0001$) and refined under linkage disequilibrium criteria (r^2 = 0.001, window size = 10,000 kb). [23] To minimise bias from weak instruments, the F statistic was calculated for each IV. F is calculated as

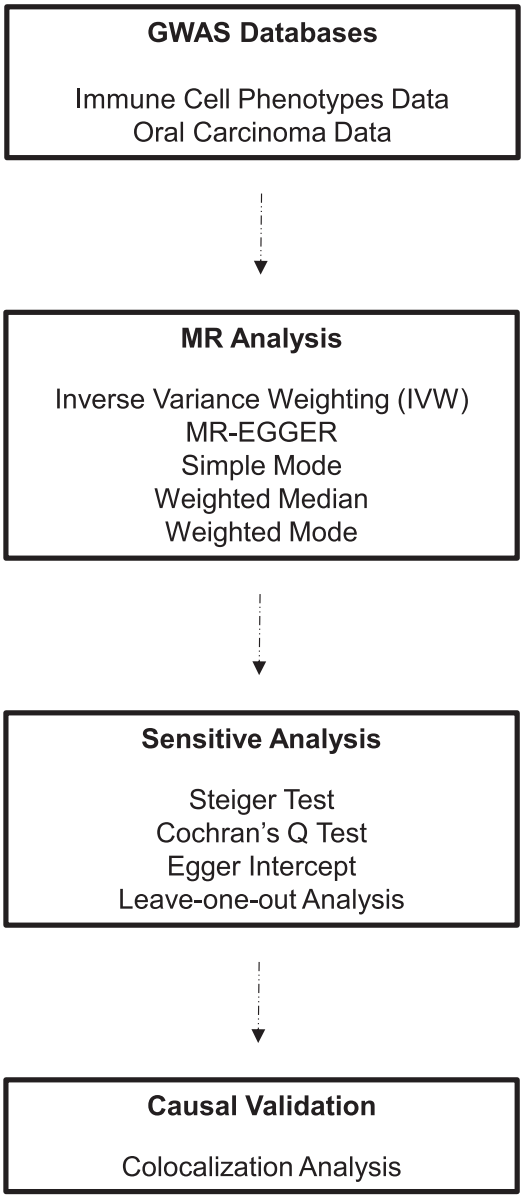


Fig. 1 – Study design.

$F = (N - 2)^*(R^2/(1 - R^2))$. N represents the sample size of the exposure database, while R^2 represents explained variance of each SNP in the exposure. R^2 is calculated as $R^2 = (\beta/se)^2/(N - 2 + (\beta/se)^2)$ using get_r_from_bsen in the Two Sample MR package (version 0.6.3), while β equals the coefficient of each allele. Only instruments with $F > 10$ were retained for the MR analysis.

Statistical analysis

The causal relationship between immune cells and OC was evaluated using R software (version 4.3.1) and the Two Sample MR (version 0.6.3). Five different MR methods were employed, including inverse variance weighting (IVW), MR-EGGER, the simple mode method, the weighted median method, and the weighted mode method, with IVW results serving as the gold standard. The sensitivity analysis included the Steiger test, Cochran's Q test, Egger intercept, and leave-one-out analysis. To reduce the risk of instability caused by potential bidirectional causality, the Steiger test was applied to confirm the direction of causality for each exposure–outcome pair. A causal relationship between the exposure and outcome was considered significant with IVW $P < .05$. Cochran's Q test was used to assess heterogeneity, while the Egger intercept evaluated the presence of horizontal pleiotropy. Additionally, leave-one-out analysis was performed to test the robustness of the findings through sensitivity analysis. All sensitivity analyses were considered significant with $P < .05$.

Colocalisation analysis

We further validated the positive causal relationships between immune cells and OCs using colocalisation analysis. Following the approach of Wei-Ming Su et al., colocalisation analysis was conducted for significant MR findings, focusing on SNPSs located within 1000 kb of the leading SNP of immune cell phenotypes and oral cancer GWAS summary data. This analysis was performed using the R package COLOC (version 5.2.028) with the following prior probabilities: $P1 = 1 \times 10^{-4}$, $P2 = 1 \times 10^{-4}$, and $P12 = 1 \times 10^{-5}$. The COLOC package evaluates five hypotheses, quantified using posterior probabilities (PP) as follows: PPH0, no association with either trait; PPH1, association with gene expression but not AD risk; PPH2, association with AD risk but not gene expression; PPH3, association with both AD risk and gene expression, though driven by distinct causal variants; and PPH4, association with both AD risk and gene expression, with a shared causal variant. To ensure sufficient confidence in the colocalisation results, only the casual relationships with PPH3 + PPH4 ≥ 0.8 were considered significant, given the limited statistical power available for colocalisation.[24] The results of the colocalisation analyses point to possible shared genetic mechanism behind causal relationships, indicating the existence of common genetic variants linking specific immune cells to OC.

Results

Instrumental Variable Result

A total of 18,728 instrumental variables (IVs) were selected for the analysis, each meeting the strength requirement with an F statistic ≥ 19, ensuring their reliability. This high F statistic across all instruments confirms the robustness of the IVs and minimises the risk of weak instrument bias, providing strong support for the validity of the MR analysis.

Causal Relationship Between Immune Cell and OC

SNPSs for all selected immune cell phenotypes are listed in Supplementary Tables S1 and S2. The results of the preliminary MR analysis of the 731 immune cell phenotypes and the risk of OC are presented in Figure 2 and Supplementary Table S3. A total of 27 immune cell phenotypes were significantly associated with OC at a significance threshold of $P < .05$. The statistical results and visualised forest plots are

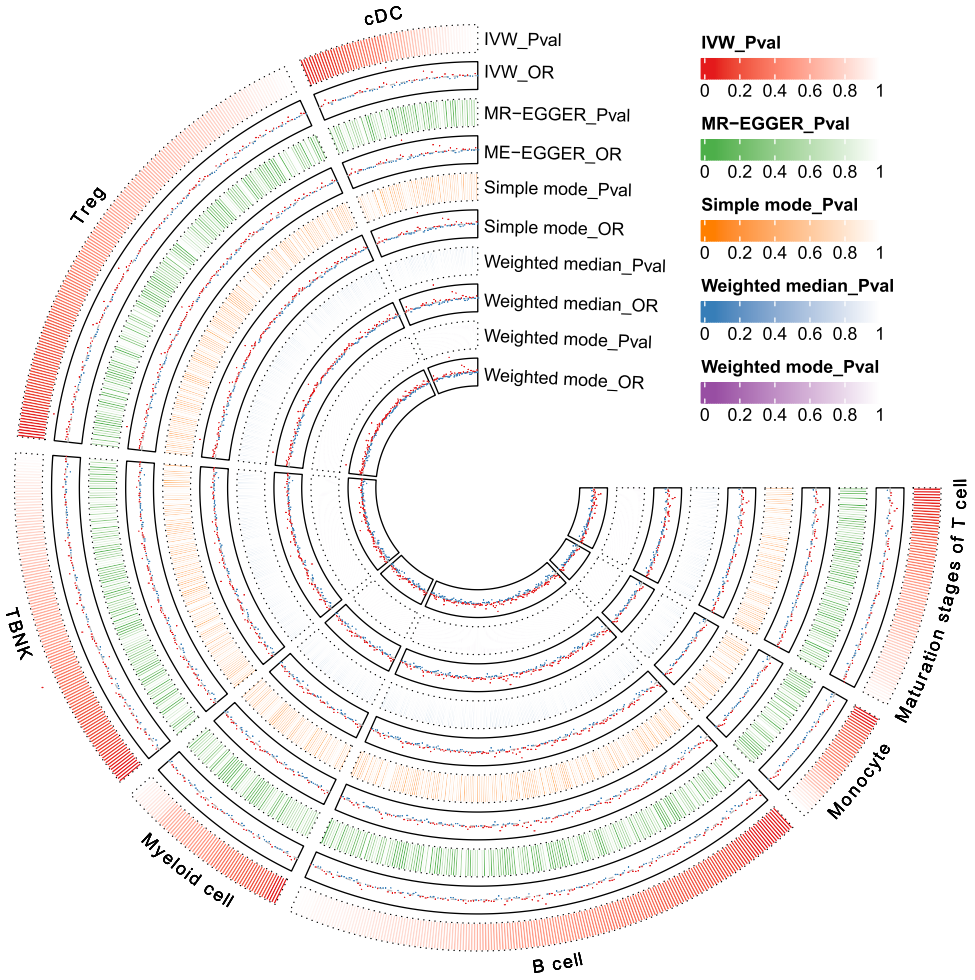
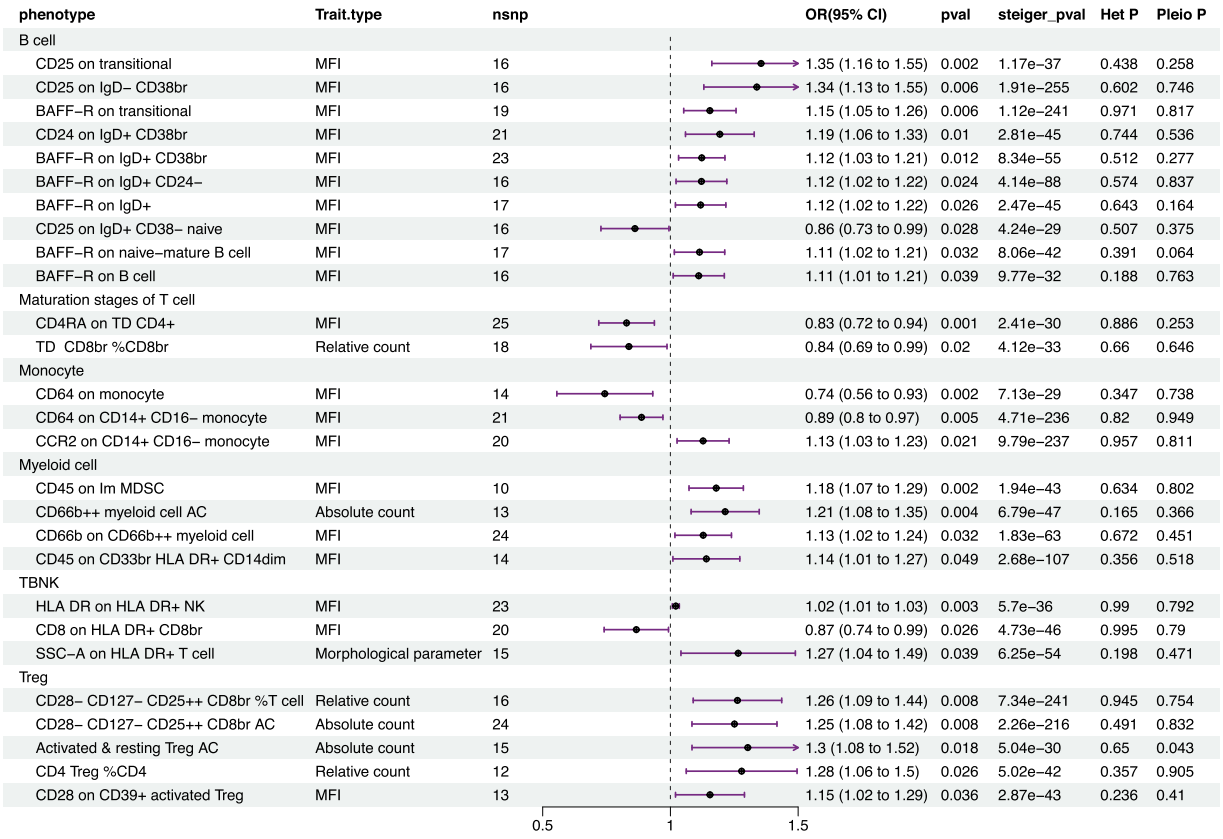


Fig. 2 – Heat map of all exposures (immune cell phenotypes) and outcomes (OC).

shown in Figure 3. Ten phenotypes in the B-cell group, two phenotypes in the maturation stages of the T-cell group, three phenotypes in the monocyte group, four phenotypes in the myeloid cell group, three phenotypes in the TBNK group, and five phenotypes in the Treg group were respectively included. The MR analysis identified immune cell phenotypes with both positive and negative causal relationships associated with OC.

Twenty-one immune cell phenotypes were identified as significantly risk factors, demonstrating a positive causal association with OC progression. (Figure 4). Among the B-cell phenotypes, CD25 on transitional (OR = 1.35, 95% CI: 1.16 −1.55, P = .002), CD25 on IgD$^+$ CD38br (OR = 1.34, 95% CI: 1.13 −1.55, P = .006), BAFF-R on transitional (OR = 1.15, 95% CI: 1.05 −1.26, P = .006), CD24 on IgD$^+$ CD38br (OR = 1.19, 95% CI: 1.06 −1.33, P = .01), BAFF-R on IgD$^+$ CD38br (OR = 1.12, 95% CI: 1.03 −1.21, P = .012), BAFF-R on IgD$^+$ CD24$^-$ (OR = 1.12, 95% CI: 1.02 −1.22, P = .024), BAFF-R on IgD$^+$ (OR = 1.12, 95% CI: 1.02−1.22, P = .026), BAFF-R on naive-mature B cell (HR = 1.11, 95% CI: 1.02−1.21, P = .032), and BAFF-R on B cell (OR = 1.11, 95% CI: 1.01−1.21, P = .039) were associated with increased risk, highlighting the importance of B-cell–related pathways in oral tumour development. In other immune cell types, multiple phenotypes were significantly associated with increased carcinoma risk. These include CCR2 on CD14$^+$ CD16− monocyte of monocytes (OR = 1.13, 95% CI: 1.03−1.23, P = .021), CD45 on Im MDSC of myeloid cells (OR = 1.18, 95% CI: 1.07 −1.29, P = .002), CD66b^{++} myeloid cell AC of myeloid cells (OR = 1.21, 95% CI: 1.08−1.35, P = .004), CD66b on CD66b^{++} myeloid cell of myeloid cells (OR = 1.13, 95% CI: 1.02−1.24, P = .032), CD45 on CD33br HLA DR$^+$ CD14dim of myeloid cells (OR = 1.14, 95% CI: 1.01−1.27, P = .049), HLA DR on HLA DR$^+$ NK of TBNK (OR = 1.02, 95% CI: 1.01−1.03, P = .003), SSC−A on HLA DR$^+$ T cell of TBNK (OR = 1.27, 95% CI: 1.04−1.49, P = .039), CD28− CD127− CD25^{++} CD8br %T cell of Treg (OR = 1.26, 95% CI: 1.09−1.44, P = .008), CD28− CD127− CD25^{++} CD8br AC of Treg (OR = 1.25, 95% CI: 1.08−1.42, P = .008), activated and resting Treg AC of Treg (OR = 1.3, 95% CI: 1.08−1.52, P = .018), CD4 Treg %CD4 of Treg (OR = 1.28, 95% CI: 1.06−1.5, P = .026), and CD28 on CD39$^+$ activated Treg of Treg (OR = 1.15, 95% CI: 1.02 −1.29, P = .036).

In contrast, six immune cell phenotypes displayed protective effects against OC (Figure 5), including CD25 on IgD$^+$ CD38$^-$ naive stages of B cells (OR = 0.86, 95% CI: 0.73−0.99, P = .028), CD4RA on TD CD4$^+$ of maturation stages of T cells (OR = 0.83, 95% CI: 0.72−0.94, P = .001), TD CD8br %CD8br of maturation stages of T cells (OR = 0.84, 95% CI: 0.69−0.99, P = .02), CD64 on the monocyte of monocytes (OR = 0.74, 95%



phenotype	Trait.type	nsnp	OR(95% CI)	pval	steiger_pval	Het P	Pleio P
B cell							
CD25 on transitional	MFI	16	1.35 (1.16 to 1.55)	0.002	1.17e−37	0.438	0.258
CD25 on IgD− CD38br	MFI	16	1.34 (1.13 to 1.55)	0.006	1.91e−255	0.602	0.746
BAFF−R on transitional	MFI	19	1.15 (1.05 to 1.26)	0.006	1.12e−241	0.971	0.817
CD24 on IgD+ CD38br	MFI	21	1.19 (1.06 to 1.33)	0.01	2.81e−45	0.744	0.536
BAFF−R on IgD+ CD38br	MFI	23	1.12 (1.03 to 1.21)	0.012	8.34e−55	0.512	0.277
BAFF−R on IgD+ CD24−	MFI	16	1.12 (1.02 to 1.22)	0.024	4.14e−88	0.574	0.837
BAFF−R on IgD+	MFI	17	1.12 (1.02 to 1.22)	0.026	2.47e−45	0.643	0.164
CD25 on IgD+ CD38− naive	MFI	16	0.86 (0.73 to 0.99)	0.028	4.24e−29	0.507	0.375
BAFF−R on naive−mature B cell	MFI	17	1.11 (1.02 to 1.21)	0.032	8.06e−42	0.391	0.064
BAFF−R on B cell	MFI	16	1.11 (1.01 to 1.21)	0.039	9.77e−32	0.188	0.763
Maturation stages of T cell							
CD4RA on TD CD4+	MFI	25	0.83 (0.72 to 0.94)	0.001	2.41e−30	0.886	0.253
TD CD8br %CD8br	Relative count	18	0.84 (0.69 to 0.99)	0.02	4.12e−33	0.66	0.646
Monocyte							
CD64 on monocyte	MFI	14	0.74 (0.56 to 0.93)	0.002	7.13e−29	0.347	0.738
CD64 on CD14+ CD16− monocyte	MFI	21	0.89 (0.8 to 0.97)	0.005	4.71e−236	0.82	0.949
CCR2 on CD14+ CD16− monocyte	MFI	20	1.13 (1.03 to 1.23)	0.021	9.79e−237	0.957	0.811
Myeloid cell							
CD45 on Im MDSC	MFI	10	1.18 (1.07 to 1.29)	0.002	1.94e−43	0.634	0.802
CD66b++ myeloid cell AC	Absolute count	13	1.21 (1.08 to 1.35)	0.004	6.79e−47	0.165	0.366
CD66b on CD66b++ myeloid cell	MFI	24	1.13 (1.02 to 1.24)	0.032	1.83e−63	0.672	0.451
CD45 on CD33br HLA DR+ CD14dim	MFI	14	1.14 (1.01 to 1.27)	0.049	2.68e−107	0.356	0.518
TBNK							
HLA DR on HLA DR+ NK	MFI	23	1.02 (1.01 to 1.03)	0.003	5.7e−36	0.99	0.792
CD8 on HLA DR+ CD8br	MFI	20	0.87 (0.74 to 0.99)	0.026	4.73e−46	0.995	0.79
SSC−A on HLA DR+ T cell	Morphological parameter	15	1.27 (1.04 to 1.49)	0.039	6.25e−54	0.198	0.471
Treg							
CD28− CD127− CD25++ CD8br %T cell	Relative count	16	1.26 (1.09 to 1.44)	0.008	7.34e−241	0.945	0.754
CD28− CD127− CD25++ CD8br AC	Absolute count	24	1.25 (1.08 to 1.42)	0.008	2.26e−216	0.491	0.832
Activated & resting Treg AC	Absolute count	15	1.3 (1.08 to 1.52)	0.018	5.04e−30	0.65	0.043
CD4 Treg %CD4	Relative count	12	1.28 (1.06 to 1.5)	0.026	5.02e−42	0.357	0.905
CD28 on CD39+ activated Treg	MFI	13	1.15 (1.02 to 1.29)	0.036	2.87e−43	0.236	0.41

Fig. 3 – Forest plot depicting the causal relationships of immune cell phenotypes on OC by inverse−variance−weighted (IVW) method. Results of sensitivity analysis employed including Steiger test, Cochran's Q test, and Egger intercept are displayed.

CI: 0.56–0.93, P = .002), CD64 on $CD14^+$ $CD16^-$ monocyte of monocytes (OR = 0.89, 95% CI: 0.8–0.97, P = .005), and CD8 on HLA DR^+ CD8br of TBNK (OR = 0.87, 95% CI: 0.74–0.99, P = .026), indicating their potential role in antitumour immunity.

Sensitive analysis

To verify the reliability and robustness of the results, we performed the Steiger test to screen for 27 immune cell phenotypes causally associated with OC. As illustrated in the forest plot (Figure 3), the results of the Steiger test confirmed the correct directionality of the associations. The highly significant Steiger P values (most <1e-30) for multiple immune cell phenotypes validate that these immune markers are likely to influence the development of cervical carcinoma rather than being consequences of the disease. This strengthens the inference that immune dysregulation plays a primary role in cervical carcinoma progression. In addition, the Cochran's Q test indicated no significant heterogeneity (P > .05), suggesting that the causal estimates were consistent across the instrumental variables used in the study (Supplementary Table S3). However, one of the results from the Egger intercept showed a P value of .043, indicating the possibility of weak directional pleiotropy for this specific association between activated and resting Treg AC and OC (Supplementary Table S4). While most associations remained free from pleiotropy, this borderline result underscores the importance of cautious interpretation for that specific finding. Meanwhile, the leave-one-out analysis revealed that some individual SNPSs, including CD24 on IgD^+ CD38br of B cells, CD64 on $CD14^+$ $CD16^-$ monocyte of monocytes, and CCR2 on CD14+ CD16- monocyte of monocytes, exerted a noticeable influence on specific causal estimates, indicating that not all results are entirely stable (Figure 6). While the overall pattern of associations remained consistent, certain immune phenotypes showed variability when individual SNPSs were removed, suggesting the need for cautious interpretation of those associations. These results highlight the importance of considering potential outliers and suggest further validation of these associations through independent replication studies.

Validation of causal relationships

Through colocalisation analysis of 27 significant causal relationships, we found the possibility of shared causal genetic variants between OC and CD28- CD127- $CD25^{++}$ CD8br AC of Treg at rs9469077 on chr6 (PPH3 + PPH4 > 0.8) (Figure 7 and Supplementary Table S5). The high posterior probability suggests that both $CD28^-CD127^-CD25^{++}$CD8br AC of Treg and OC may have similar genetic backgrounds and are likely driven by the same genetic variant, supporting the colocalisation hypothesis.

Discussion

In this study, we conducted a two-sample MR analysis to elucidate the causal relationship between immune cell

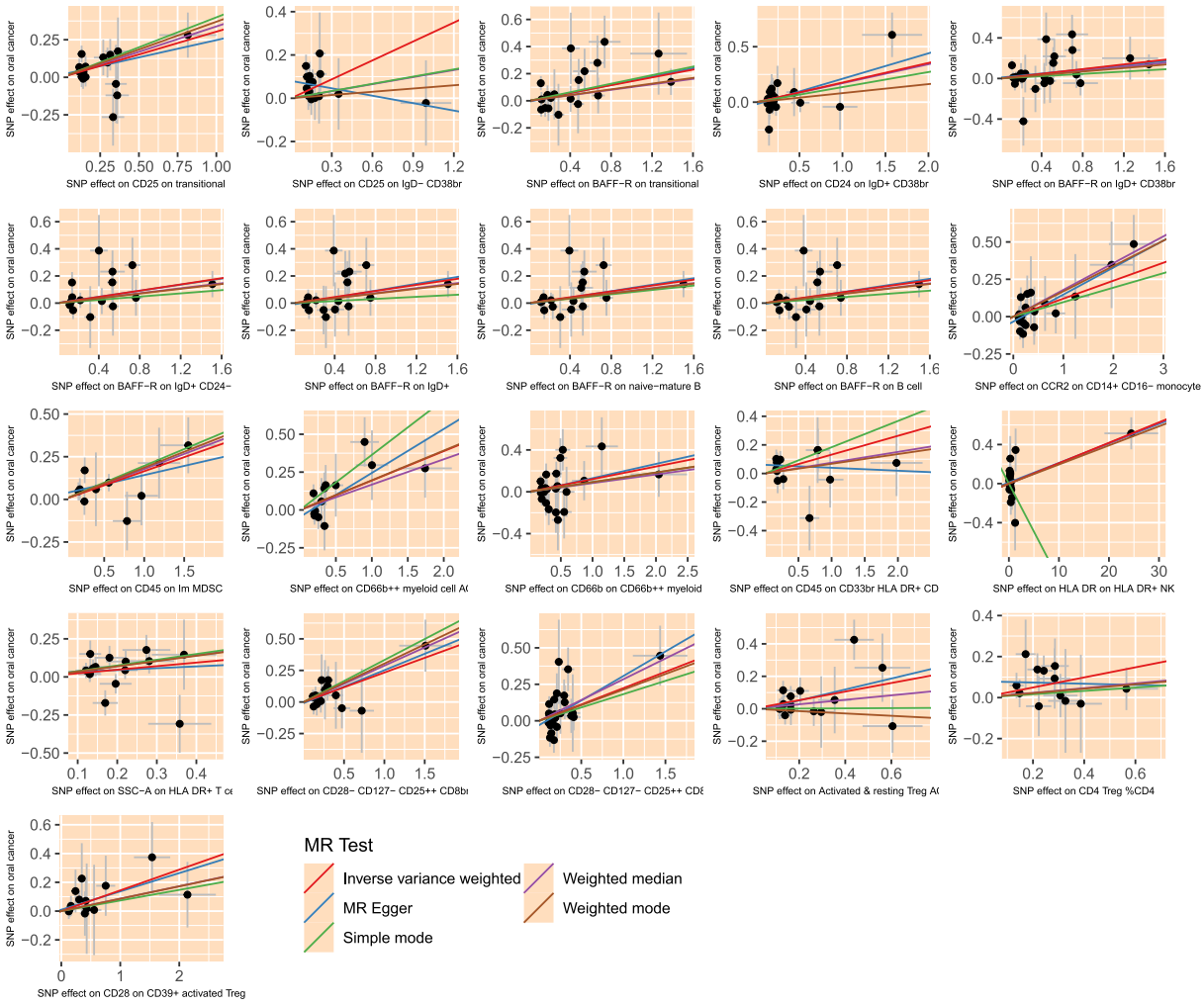


Fig. 4 – Scatter plot illustrating the relationship between 21 immune cell phenotypes and OC, which indicated that these 21 immune cell phenotypes are risk factors for OC.

phenotypes and the risk of OC. Using GWAS data derived from an extensive European cohort, 731 immune-related traits across various immune cell types were examined. By employing SNPSs as instrumental variables, we aimed to establish causal relationships between specific immune cell traits and OC while reducing confounding influences. Our analysis identified 27 immune cell phenotypes that exhibited significant associations with the risk of OC. Among them, 21 were identified as risk factors for OC, while 6 demonstrated protective attributes. Furthermore, to substantiate the robustness of these associations, we conducted a colocalisation analysis which revealed shared genetic background (locus: rs9469077 on Chr6) between $CD28^{-}CD127^{-}CD25^{++}$ CD8Br AC of Treg and OC.

Consistent with published research concerning the role of immune cell in tumourigenesis, our findings also indicated the complex involvement of immune cell in carcinoma pathology.[25] In our study, B cells expressing CD25 and BAFF-R are reported to be associated with OC, suggesting that these cells may facilitate tumour development through mechanisms of chronic inflammation and immune tolerance. BAFF-R, a member of the tumour necrosis factor family, serves as a crucial factor in promoting B-cell survival and proliferation, potentially facilitating an inflammatory environment conducive to tumour growth.[26,27] Our observation is corroborated by investigations conducted in various haematological and solid cancers where aberrant B-cell activation induced by BAFF and BAFF-R expression has been implicated in immune evasion and disease advancement.[28] In OC, this expression of BAFF has also been identified to augment tumour cell survival and proliferation.[29] These findings indicated that targeting B-cell pathways, particularly those associated with migratory B cells, could represent a therapeutic strategy for managing OC.

MR analysis assesses whether exposure factors are associated with an outcome and determines causality. Differently, colocalisation analysis, as a method for determining whether two traits are affected by the same or different causal variants, can provide complementary insights into MR analysis for identifying potential causal associations.[30] Notably, the result of colocalisation indicated the collection between $CD28^{-}CD127^{-}CD25^{++}$ CD8Br activated Treg and OC. It is important to highlight the role of Treg, which has immunosuppressive effects and contributes to control autoimmunity and maintain immune homeostasis.[31] However, it could also

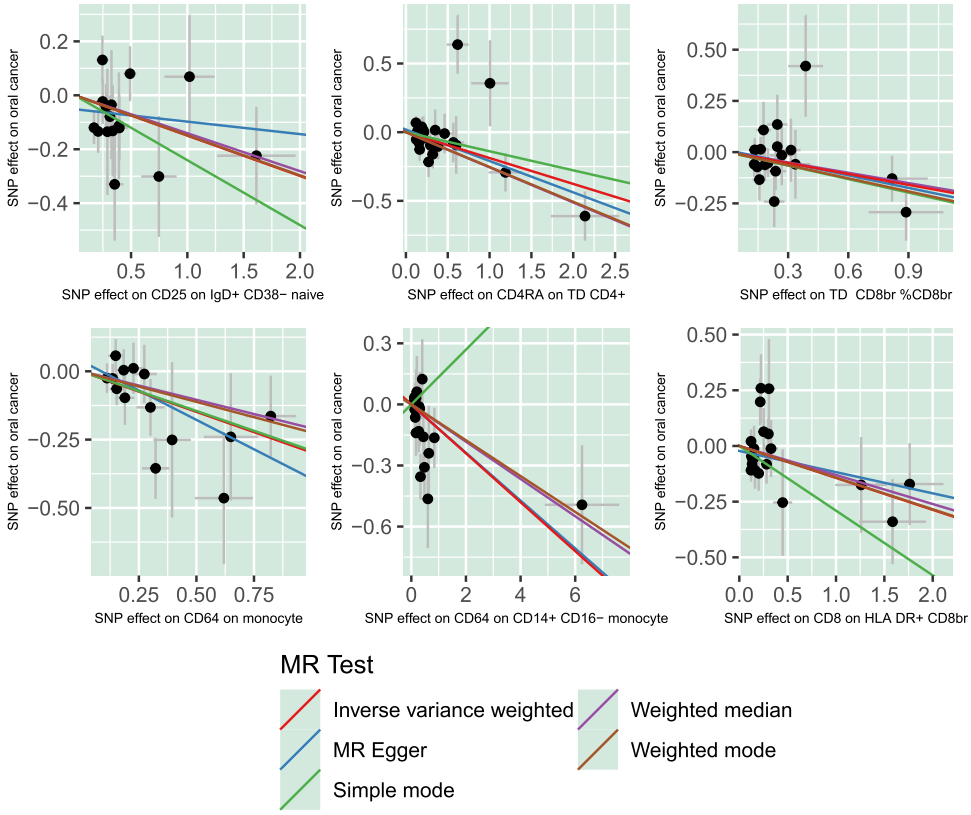


Fig. 5 – Scatter plot illustrating the relationship between six immune cell phenotypes and OC, which indicated that these six immune cell phenotypes are protective factors for OC.

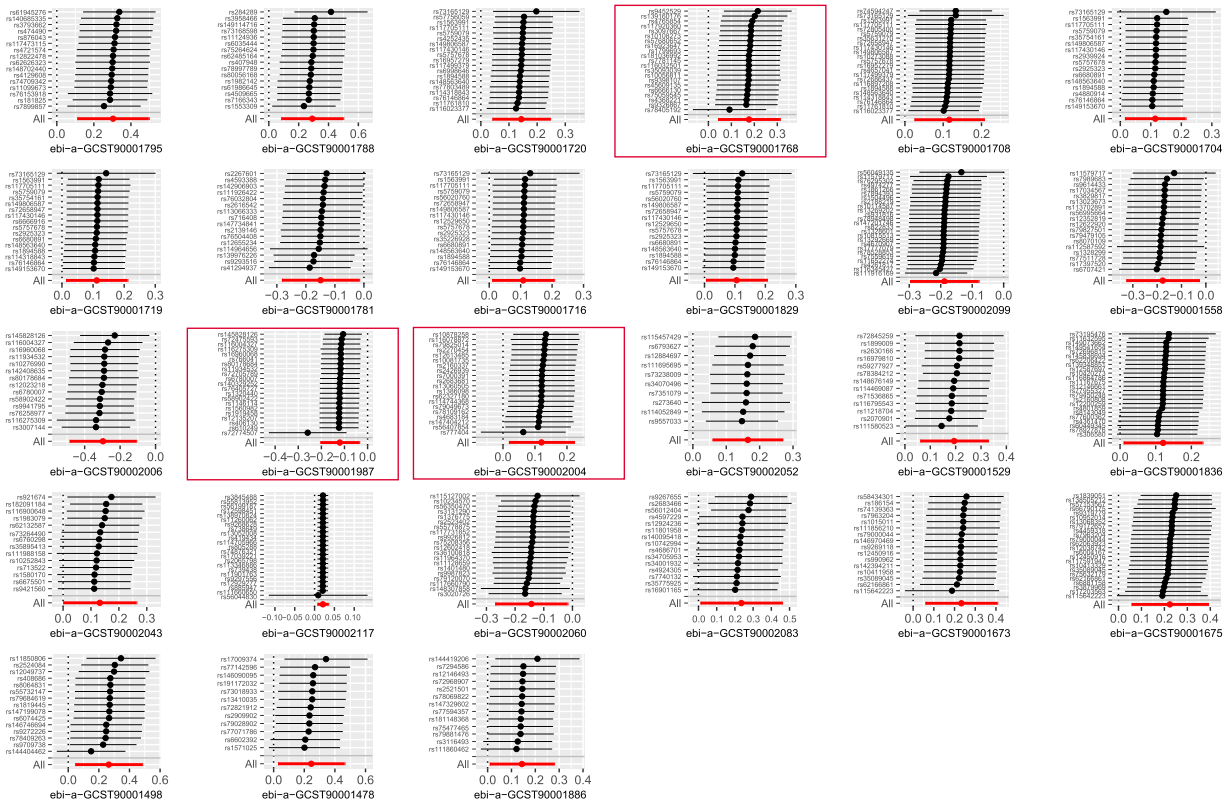


Fig. 6 – Leave-one-out analysis revealing the impact of individual SNPs on 27 causal relationships between immune cell phenotypes and OC. The red boxes indicate that these causal relationships may have poor robustness.

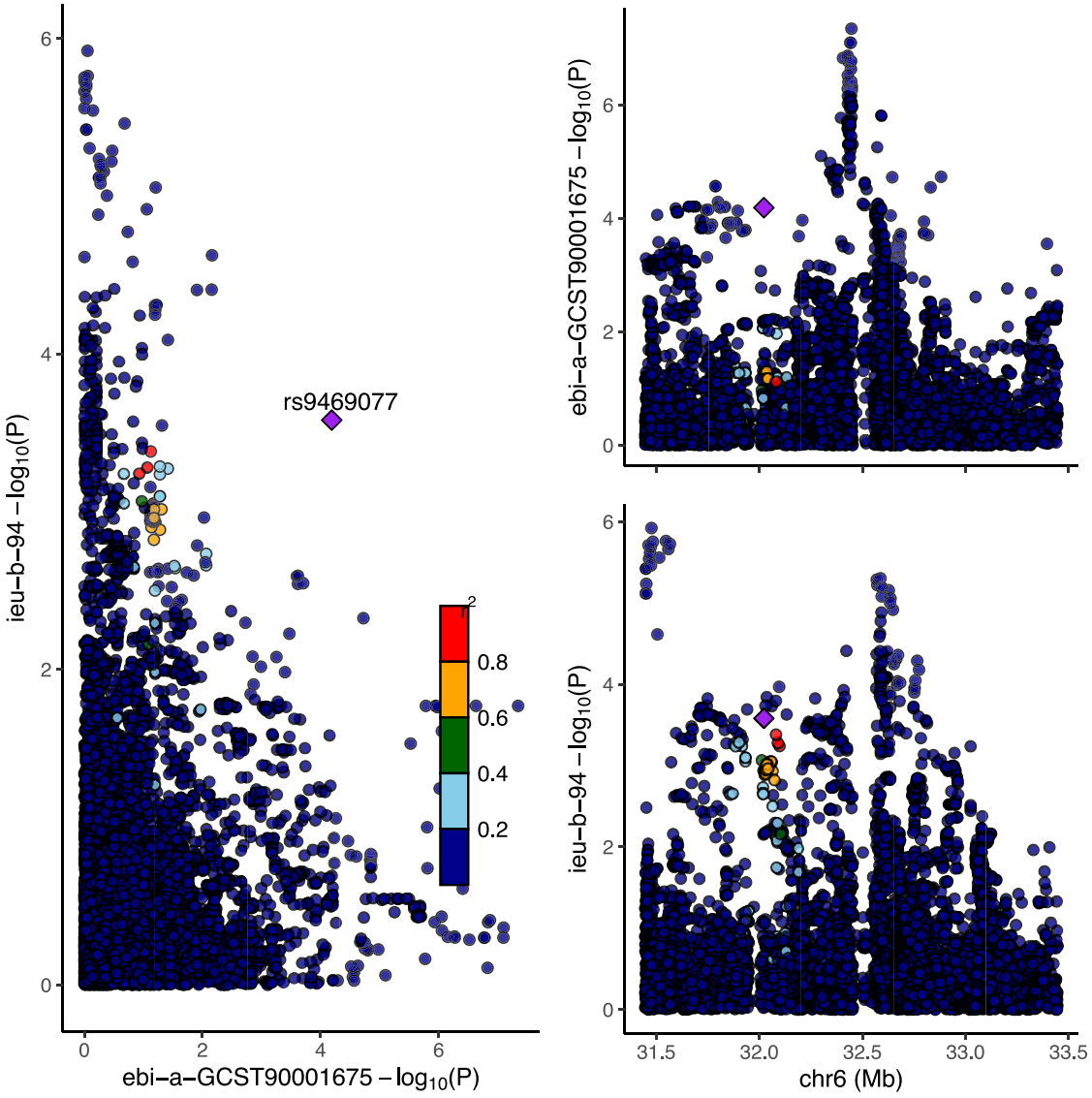


Fig. 7 – Colocalisation analysis indicating the similar genetic background between CD28^{-}CD127^{-}CD25^{++}CD8Br AC of Treg and OC.

promote the immune escape of tumours and has attracted much attention in the field of cancer research.[32] In head and neck squamous cell carcinoma including OC, elevated Treg levels correlate with tumour size and progression TME of oral malignancies, especially at the invasive margins of the tumour, indicating poor prognoses.[33,34] This correlation suggests that Treg may interrupt the body's intrinsic antitumour immune response by suppressing effector T-cell function. In addition, Tregs establish an immunosuppressive microenvironment by secreting cytokines such as IL-10 and TGF-β, contributing to the promotion of tumourigenesis.[35,36] The presence of such immunosuppressive factors in TME could also be associated with a poor prognosis in OC. From a mechanistic point of view, the presence of Treg in OC is associated with local physiological alterations. They can influence a variety of factors such as extracellular matrix composition, angiogenesis, and cell adhesion. These factors are all critical in promoting tumour invasion and metastasis. In our study, this association was highlighted by the discovery of the rs9469077 locus by colocalisation analysis, where variants in this locus may affect Treg function and may play a role in the pathogenesis of oral tumours. The link between the presence of Treg and these tumour-promoting functions suggests that targeting or modulating Treg activity may be a promising strategy to disrupt TME support for cancer proliferation.[37] In addition, the genetic correlation we found with Treg traits implies a possible genetic predisposition; particularly in OC patients, certain patients may exhibit Treg-mediated immunosuppression. This observation has implications particularly for precision medicine, because patients carrying this genetic variant may derive a therapeutic advantage from immunotherapeutic approaches that target Treg or attenuate its immunosuppressive capacity. Colocalisation analysis identified the rs9469077 locus as a shared genetic variant between CD28^{-}CD127^{-}CD25^{++} CD8Br AC of Treg and oral carcinoma. This means that rs9469077 is likely involved in both traits, suggesting a potential causal relationship. In simpler terms, this

locus acts like a genetic overlap point that connects the two traits, helping us to understand how they might influence each other through shared genetic mechanisms. This insight potentially directs future research towards the pathways influenced by this specific genetic variant. Thus, the colocalisation analysis of the rs9469077 locus suggests a genetic predisposition for oral malignancies and provides a research basis for the investigation of therapeutic strategies for oral malignancies targeting Treg. The foundation was laid.

In addition to the 21 immune cell phenotypes that may increase the likelihood of OC, our study demonstrated 6 immune cell phenotypes that produce protective effects. For example, the expression of CD64 on monocytes and CD4RA on TD $CD4^+$ of maturation stages of T cells correlates with a reduced risk of OC. CD64, one of the Fc gamma receptors expressed on immune cells, is a high-affinity receptor for immunoglobulin G, which plays an important role in phagocytosis and antigen presentation and may enhance antitumour immunity by enhancing the immune system's ability to recognise and remove malignant cell.[38,39] Similarly, the protective role of CD4RA on T lymphocytes may indicate the critical role of specific T-cell maturation stages in producing effective cytotoxic responses against tumour cell. These observations emphasise the functional heterogeneity of different immune cell populations and suggest that different immune cells may assume protective functions in the context of OC.

Immune cells and OC interact through multiple complex mechanisms. In general, immune cells play an anticancer role by recognising tumour-specific antigens and destroying cancer cells. However, OC cells can mutate to change their surface antigens to evade immune surveillance; tumour cells can secrete immunosuppressive factors (e.g., TGF-β, IL-10) to inhibit the activity of immune cells; tumour-associated fibroblasts and tumour-associated macrophages can secrete cytokines and growth factors (e.g., IL-6, IL-8, TNF-α) to promote tumour cell proliferation and metastasis, affecting the activity and distribution of immune cells; OC cells can upregulate the expression of immune checkpoint molecules (e.g., PD-L1, CTLA-4) and inhibit immune cell activity; Tregs and tumour-associated macrophages can be recruited to inhibit T-cell function in the tumour microenvironment, which is similar to our findings.

Our study may provide an idea for other scholars. We can study the immune microenvironment of OC in depth by combining the known or unknown immune mechanisms: for example, which site of surface antigen is altered by OC cells, which type of immunosuppressive factor is secreted, which target of tumour cells escapes from the immune system, and the distribution and activity of different immune cells in the microenvironment of OC. These types of research may reveal new therapeutic targets and discover new biomarkers for early diagnosis, prognostic assessment, or treatment response monitoring; they may also develop individualised immunotherapy protocols and exclusive treatment strategies based on the patient's immune status and tumour characteristics; and they may contribute to the study of combining immune cells with other therapeutic approaches, such as radiotherapy, chemotherapy, and targeted therapy. Based on deeper research on OC, we may be brave enough to hypothesise the production of vaccines targeting specific antigens, which may be a new way to prevent or treat OC.

Although our investigation benefits from the MR analysis's capacity to infer causal relationships, several limitations need to be discussed. The use of genetic tools can be effective in reducing heterogeneity, but it does not completely eliminate the potential for multiplicity. Results of the Steiger test and Cochran's Q test demonstrated the robustness of our findings; however, the results of Egger intercept and leave-one-out analysis indicated that four causal relationships with horizontal pleiotropy or weakness in robustness suggested the need to interpret these outcomes with caution. In addition, the GWAS dataset used in this study included individuals of European ancestry, which may limit the broader applicability of these findings to different populations. Future studies involving different cohorts will be critical to validate these associations and assess the impact of population heterogeneity on the observed relationships.

In summary, this study elucidates the multiple functions of immune cell phenotypes in OC susceptibility. The significance of these findings, especially those confirmed by colocalisation analyses, reveals specific immune pathways that may serve as prospective targets for therapeutic intervention. These loci and immune cell phenotypes require further empirical studies to confirm the underlying biological mechanisms of these associations and to explore their potential immunotherapeutic applications to inform preventive and therapeutic strategies.

Conclusion

This two-sample MR analysis provides new insights into the causal roles of specific immune cell phenotypes in OC, with certain B-cell and Treg phenotypes showing positive associations with increased carcinoma risk while others demonstrate protective effects. The colocalisation analysis identifying a shared genetic background with Treg suggests a genetic basis for immune dysregulation in OC. These findings suggest potential avenues for immunomodulatory strategies aimed at altering immune cell dynamics to mitigate OC risk. Future research should aim to validate these associations across populations and explore the underlying biological mechanisms to inform the development of targeted immunotherapies for OC prevention and treatment.

Conflict of interest

The authors declare no conflict of interests.

Author contributions

Data analysis and interpretation: Xu, Tang. *Study design*: Yang. *Writing – original draft*: Yang. *Writing – review and editing*: Lu, He.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2025.03.013.

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