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ORIGINAL RESEARCH

Genetic Evidence for Immune Cell-Lipid Mediation Pathways Linked to Esophageal Cancer and Associated Throat/Chest Pain Phenotypes

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Purpose: Previous studies showed complex associations between immune cells and esophageal cancer (EC). EC-related pain is closely linked to immune dysregulation and abnormal lipid metabolism, but their synergistic regulatory mechanisms remain unclear. This study aimed to investigate causal relationships between immune cell traits and EC, explore plasma lipids' mediating role, supplement genetic evidence for core immune factors-lipids-pain associations, and provide targets for EC's combined " Antineoplastic-analgesic" intervention.

Methods: Genome-wide association study summary data of 731 immune cell traits, 179 lipid metabolites, 998 EC cases, and two pain-related datasets were compiled. Bidirectional MR analysis (with inverse variance weighting, MR-Egger, MR-RAPS, weighted median, weighted mode) was used to infer causal relationships, followed by sensitivity analyses for robustness and FDR correction (Benjamini-Hochberg method) to account for multiple testing. A two-step Mendelian randomization was applied to explore plasma lipids mediating role in the immune cell-EC risk link.

Results: This study identified 24 immune cell phenotypes and 14 plasma lipids suggestive causal associations with EC risk. Mediation analysis revealed that HLA DR on CD33dim HLA DR+ CD11b+ affected EC risk via Phosphatidylcholine (18:0_22:6) levels, with a suppression ratio of −82.15%, indicating an exploratory indirect effect that partially offsets the primary protection. Similarly, Phosphatidylcholine (O-18:1_20:4) exhibited a suggestive suppression effect (−10.38%) on the association between SSC-A on CD8+ T cell and EC risk. Furthermore, CD3 on HLA DR+ T cells showed a negative association with both pain-related datasets. CD33 on basophils and SSC-A on CD8+ T cells showed a positive association. Sensitivity analyses showed no significant abnormalities.

Conclusion: This study systematically identified immune cell phenotypes causally associated with EC and highlighted key lipid mediators underlying these effects. The core immune cell phenotypes and lipids are not only associated with EC risk but also show consistent genetic associations with typical EC-related pain symptoms, suggesting the existence of a co-regulatory network. These findings provide new insights into the mechanism and combined intervention of EC-related pain.

Keywords: esophageal cancer, immune cell phenotypes, Mendelian randomization, mediating factor, lipid metabolites, chest pain

Introduction

Esophageal cancer (EC) is one of the most invasive malignant tumors worldwide, ranking as the seventh leading cause of cancer-related deaths globally.[1] It accounts for 2.6% of all new cancer cases and 4.6% of cancer-related deaths, posing a severe public health challenge.[2,3] According to the latest global cancer statistics, the overall prognosis of EC remains poor, with a 5-year survival rate of approximately 20% across all stages.[4,5] EC-related pain, such as sore throat during swallowing and persistent retrosternal pain, represents a core symptom that significantly impairs patients' quality of life,

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with an incidence rate exceeding 90% in advanced stages. The severity of pain is closely associated with tumor progression and inflammatory responses.[6,7] Clinically, this pain substantially overlaps with "throat and chest pain" phenotypes (ICD-10 R07), particularly non-cardiac subtypes such as reflux-related and tumor-invasive chest pain. Given the absence of EC-specific pain GWAS data, this broader pain phenotypes serve as credible surrogates. Current neurobiological evidence supports this approach: esophageal visceral nociception—whether triggered by tumor inflammation or severe reflux, converges on shared vagal and spinal afferent pathways and involves common immune-lipid-mediated sensitization mechanisms, including algogenic mediators such as prostaglandins.[8–10] However, genetic evidence linking immune cell traits, lipids, and EC-related pain is currently lacking, limiting the development of " Antineoplastic-analgesic" targets.

In recent years, the dual role of the tumor immune microenvironment (TIME) in EC progression and pain regulation has been increasingly recognized. The TIME of EC exhibits significant heterogeneity: esophageal squamous cell carcinoma (ESCC) is dominated by exhausted T cells, regulatory T cells (Tregs), and tolerogenic dendritic cells. These immunosuppressive cells not only impair anti-tumor immunity but may also sensitize peripheral nociceptive nerves by secreting inflammatory factors such as TNF-α and IL-6, inducing cancer pain.[11,12] In contrast, the TIME of esophageal adenocarcinoma is characterized by higher levels of monocytic myeloid-derived suppressor cells (mMDSCs) and reduced infiltration of proliferative CD8+ T cells. This imbalance may simultaneously promote tumor progression and pain exacerbation by aggravating local inflammation and facilitating neural invasion.[13] Meanwhile, lipid metabolic dysregulation is a hallmark of cancer. For example, fatty acids can induce exhaustion of effector T cells or activate the immunosuppressive activity of Tregs, and lipid droplets enriched in tumor-infiltrating neutrophils can be transported to tumor cells to promote their proliferation.[14] Qin et al found that perilipin-2 and apolipoprotein E-induced lipid droplet formation promotes cytokine secretion, thereby recruiting T cells and enhancing stroma formation, which inhibits T cell activation and promotes ESCC progression.[15] Additionally, lipids play a crucial role in the regulation of pain: fatty acid metabolites, such as arachidonic acid-derived prostaglandin E2, act as potent algogenic mediators, whereas omega-3 polyunsaturated fatty acids, exert analgesic effects through anti-inflammatory actions and the inhibition of algogenic signal transduction.[16,17] These studies suggest that targeting the synergistic pathway of lipid-immune cells may provide new directions for tumor immunotherapy and cancer pain management. Although ESCC and EAC possess distinct immune landscapes, current GWAS datasets primarily evaluate EC as a combined entity. While this approach inevitably loses the resolution to identify subtype-specific immune-lipid pathways, it remains scientifically meaningful. A combined analysis maximizes statistical power and helps uncover shared pan-esophageal mechanisms—such as overarching lipid dysregulation and immune-mediated pain, providing generalized targets for broad-spectrum interventions.

Mendelian randomization (MR) is an epidemiological method that uses genetic variants as instrumental variables (IVs) to infer causal relationships between exposures and outcomes.[18,19] Compared with traditional observational studies, which are susceptible to confounding factors and reverse causation, and randomized controlled trials, which face high costs and ethical constraints, MR effectively avoids potential biases and provides reliable genetic evidence. Based on a bidirectional MR framework, this study integrated large-scale public GWAS summary data to improve research efficiency and statistical power.[20,21] Recent experimental studies have highlighted complex immune-lipid interactions in EC, these molecular findings remain highly fragmented. To systematically validate these biological hypotheses at the macroscopic genetic level, we comprehensively screened 731 immune traits and 179 lipids. This study provides genetic evidence for the "immune-lipid-EC-pain" co-regulatory mechanism, filling the gap in research on the mechanism of EC-related pain.

Material and Methods

Study Design

This study employed bidirectional Mendelian randomization in conjunction with two-step Mendelian randomization analysis to systematically investigate the genetic evidence supporting causal relationships between immune cell morphologies and endometrial cancer risk, while also assessing the potential mediation role of lipid species.[22] The study strictly adhered to three basic assumptions: (1) Single nucleotide polymorphisms (SNPs) are significantly

associated with exposure factors; (2) SNPs are independent of confounding factors; (3) SNPs affect outcomes only through exposure factors.

The main study steps are shown in Figure 1, including: (1) First, bidirectional MR was used to test the causal associations between 731 immune cell phenotypes, 179 lipid species, and EC, respectively; (2) Subsequently, immune cell phenotypes and lipid species significantly associated with EC in the above analyses were further subjected to bidirectional MR to exclude potential overlapping effects between them;[23] (3) On this basis, two-step MR analysis was used to explore the potential mediating role of lipid species in the effect of immune cells on EC risk; Bidirectional Mendelian analysis was used to explore the genetic association of core immune cell phenotypes and lipid types with typical pain. Multiple sensitivity analyses were also performed to ensure the robustness of the results.

Data Sources for Exposure, Mediation, and Outcome

Summary statistics for EC were obtained from the GWAS Catalog (ID: ebi-a-GCST90018841). This GWAS included 476,306 individuals of European ancestry, comprising 998 cases and 475,308 controls.

The pain-related phenotype ukb-a-581 was derived from the UK Biobank (UKB) (https://www.ukbiobank.ac.uk/), including 16,552 cases and 320,647 controls; the finn-b-R18_PAIN_THROAT_CHEST dataset was from the FinnGen Biobank (https://r9.risteys.finngen.fi/), including 24,609 cases and 163,123 controls.

GWAS summary data for immune cell phenotypes were obtained from the open-access database IEU Open GWAS (project IDs: ebi-a-GCST90001391 to ebi-a-GCST90002121), which originated from a cohort study of 3,757 individuals of European ancestry.[24] This study systematically analyzed the genetic basis of a wide range of immune cell characteristics, covering B cells, T cells, monocytes, natural killer cells, etc.

IVs for the lipidome were derived from a previously published genome-wide analysis of 179 lipids in human plasma (GWAS data identifiers: GCST90277238 to GCST90277416).[25] A total of 7,174 participants were included, focusing on 13 lipid classes, including four major lipid categories: triglycerides, glycerolipids, sphingolipids, and sterols.

This study was based on large GWAS and publicly available data from various groups and consortia, and informed consent was obtained from the participants for the original studies. Since this study exclusively involved the secondary

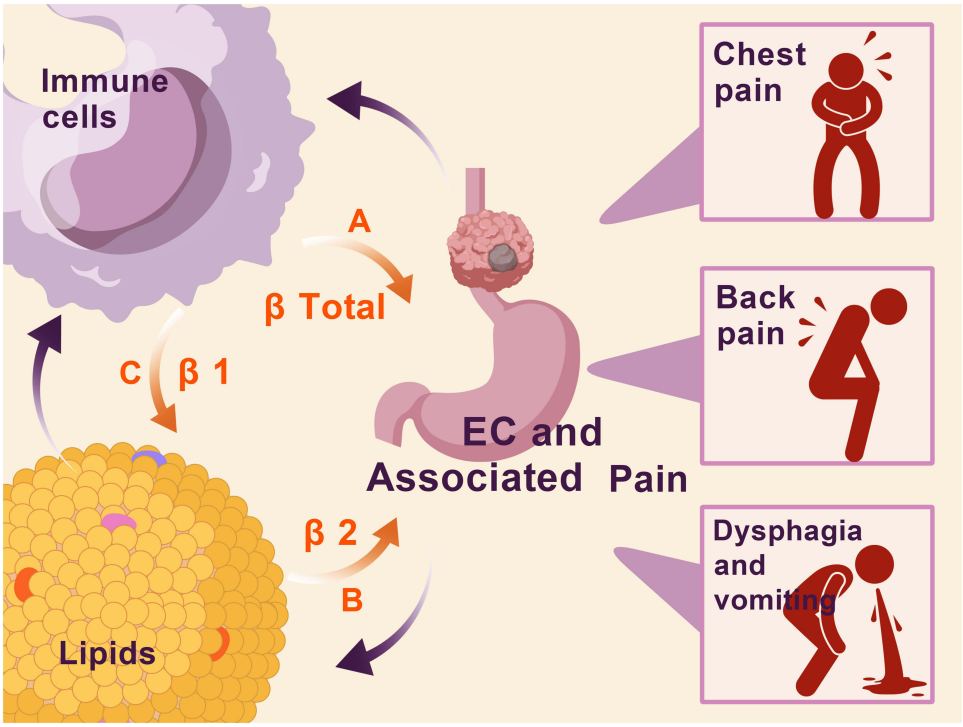

Figure 1 Design of mediation MR analysis. (**A**) The total effect (β_total) of immune cell phenotypes on EC risk. (**B**) The effect of lipid mediators on EC risk (β2). (**C**) The effect of immune cell phenotypes on lipid mediators (β1). The indirect effect (mediation or suppression) is calculated as β1×β2.

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analysis of legally obtained, publicly available, and completely anonymized data, it is exempt from ethical review by the Institutional Review Board (IRB) of Henan Provincial People's Hospital. This exemption is strictly in accordance with national legislation guidelines, specifically Items 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (promulgated on February 18, 2023, by the National Health Commission of China).

SNP Selection

To ensure an adequate number of SNPs for robust instrumental variable selection, we filtered SNPs that meet the following criteria ($p < 1e\text{-}5$ or $P < 5e\text{-}6$; linkage disequilibrium (LD): $R^2 < 0.001$, kb = 10,000).[26] Additionally, we excluded palindromic SNPs with intermediate allele frequencies. We also excluded IV with an F-statistic < 10 to minimize potential bias from weak instruments.[27]

Mendelian Randomization and Statistical Analysis

Two-sample MR analysis was performed to investigate the genetic evidence supporting causal relationships between 731 immune cell phenotypes, 179 lipids, and EC. We further explored the mediating effect of lipids, as well as the associations between core immune cell phenotypes, lipids, and EC-associated throat/chest pain. IVW regression was selected as the primary analytical method. Weighted median, weighted mode, and MR-Egger regression were also used to assess the robustness of the results. Additionally, MR-RAPS accounts for systematic and specific pleiotropy, enabling reliable inference for MR analysis with multiple weak instruments.[28] All methods were implemented in R (version 4.4.3) using the "TwoSample MR" package.[29] Relevant risk metrics were quantified as odds ratio (OR) and 95% confidence interval (CI), with $P<0.05$ considered a suggestive causal associations.

Sensitivity Analyses

A random-effects IVW method was initially used to evaluate all MR tests. Up to four MR methods were supplementary applied to generate effect estimates, each with different assumptions regarding pleiotropy. Second, Cochran's Q test was performed to assess heterogeneity; a $P<0.05$ indicated substantial heterogeneity.[30] Furthermore, MR-Egger regression intercept was used to evaluate pleiotropy; a $P>0.05$ indicated no significant pleiotropy.[31] Ultimately, a leave-one-out analysis was conducted to determine if the genetic data supporting causal effects was influenced by a singular SNP.

Mediation Analysis

We employed a two-step MR approach to investigate whether immune cell phenotypes influence the risk of EC through lipid mediation. First, we assessed the total effect (β_total) of immune cell phenotypes on EC. Subsequently, we identified lipids with a suggestive genetic association with EC and estimated their effect sizes (β2). On this basis, we further analyzed the impact of immune cell phenotypes on these lipids (β1), thereby constructing a potential mediating pathway of "immune cell—lipid—EC". The indirect effect size was calculated as $\beta1\times\beta2$. When the indirect and direct effects had opposite signs, the pathway was defined as a suppression effect, with the suppression ratio calculated as $(\beta\text{indirect}/\beta\text{direct})\times100\%$.[32] All such mediation analyses are strictly interpreted as exploratory findings to reveal suggestive genetic associations.

Results

Association Between Immune Cell Phenotypes and EC Risk

Through bidirectional MR analysis and statistical validation, this study identified 24 immune cell phenotypes significantly associated with EC risk (Figure 2), covering multiple immune cell subsets including T cells, B cells, monocytes, and dendritic cells. This indicates that multidimensional regulation of the immune system may be involved in the development and progression of EC. The IVW method was used to calculate effect sizes and quantify the strength of associations (Figure 3).

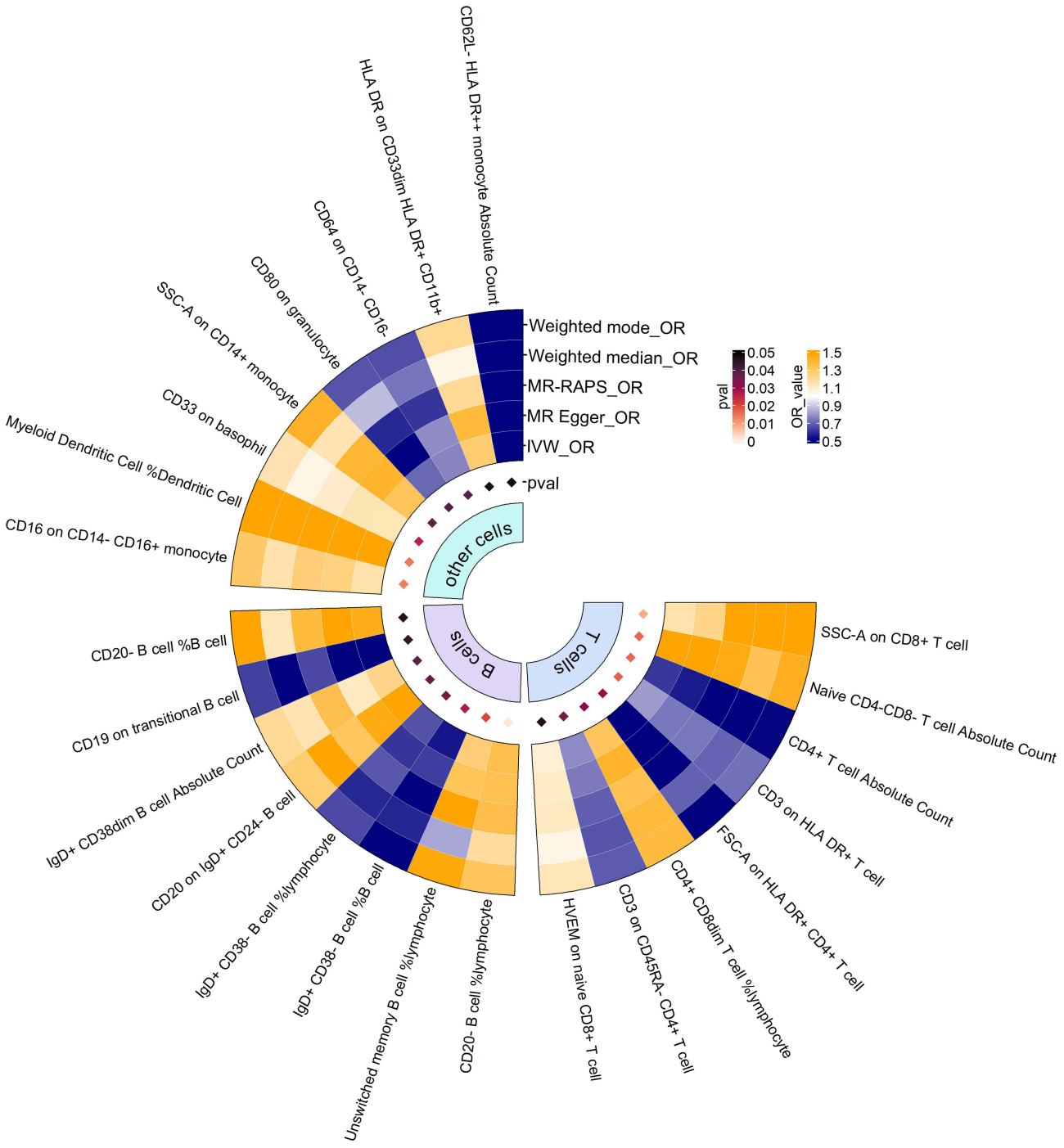

Figure 2 MR of the causal effect of immune cell phenotypes on EC. Landscape of causal associations between 731 immune cell phenotypes and EC risk. The ring heatmap illustrates the 24 immune cell phenotypes identified via the IVW method. Concentric rings (from inner to outer) represent odds ratios (OR) derived from five MR models: IVW, MR-Egger, RAPS, Weighted median, and Weighted mode. The consistent color gradients across rings indicate the robustness of the causal direction. All IVs utilized possessed F-statistics > 10, ensuring minimal weak instrument bias.

Results showed that 10 immune cell phenotypes had a protective association with EC risk. The three most protective phenotypes were CD62L- HLA DR++ monocyte Absolute Count (OR=0.879, 95% CI: 0.774~0.999, P=0.048), FSC-A on HLA DR+ CD4+ T cell (OR=0.886, 95% CI: 0.795~0.988, P=0.029), and CD4+ T cell Absolute Count (OR=0.889, 95% CI: 0.808~0.979, P=0.016). These findings suggest that genetically elevated levels of these immune cell phenotypes reduce the risk of EC. Fourteen immune cell phenotypes were positively associated with EC risk. The most prominent

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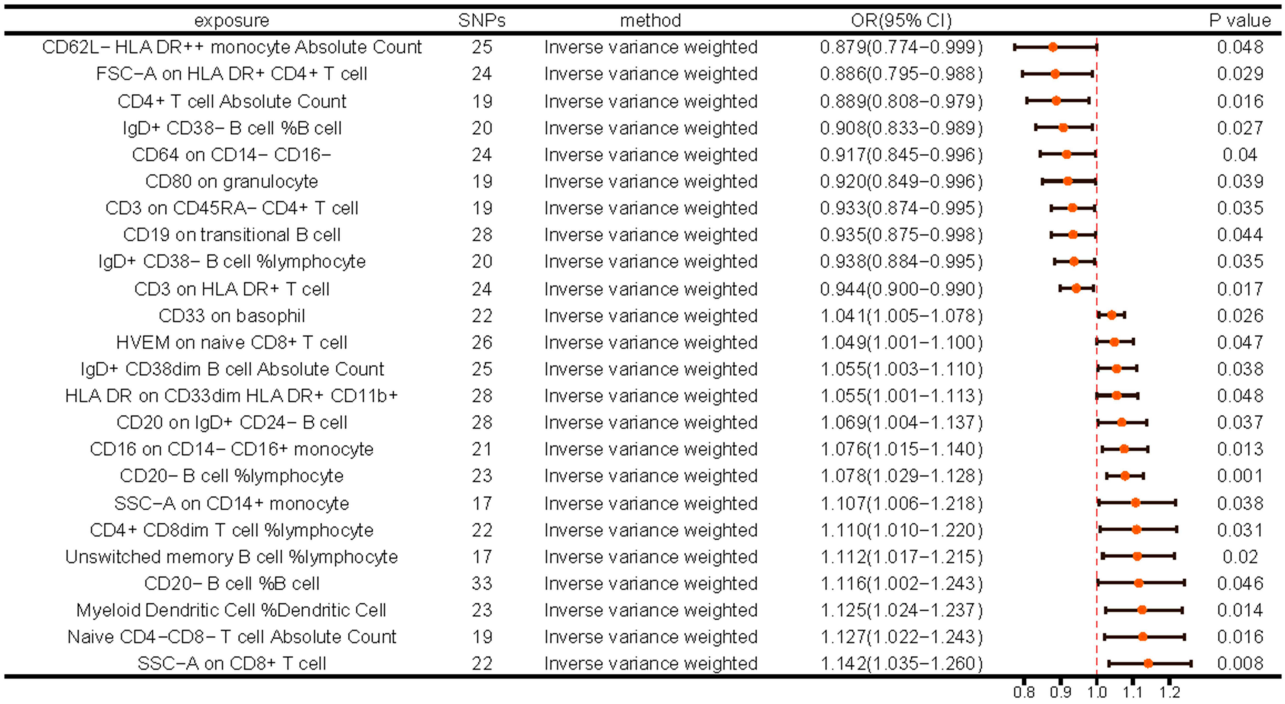

Figure 3 Forest plots of significant causal associations between immune cell phenotypes and EC risk. Effects were estimated using the inverse-variance weighted method. Data are presented as OR and 95% confidence intervals (CI). P < 0.05 was considered a suggestive causal association.

risk phenotypes were SSC-A on CD8+ T cell (OR=1.142, 95% CI: 1.035~1.260, P=0.008), Naive CD4-CD8- T cell Absolute Count (OR=1.127, 95% CI: 1.022~1.243, P=0.016), and Myeloid Dendritic Cell %Dendritic Cell (OR=1.125, 95% CI: 1.024~1.237, P=0.014). Increased levels of these immune cell phenotypes elevate the risk of EC. Sensitivity analyses, including leave-one-out and pleiotropy tests, confirmed the robustness of these findings, with all F-statistics for instrumental variables exceeding 10 (see Supplementary Figures S1, S5, and S9, and Supplementary Tables 1 and 6).

Genetic Association Analysis Between Lipid Metabolism and EC

This study screened 14 lipid molecules with suggestive causal effects on EC (Figure 4). Among them, 4 lipids had a protective association with EC risk, with phosphatidylcholine (18:2_20:3) showing the strongest protective effect (OR=0.810, 95% CI: 0.674~0.973, P=0.024). The other three protective lipids were phosphatidylcholine (O-16:0_18:2) (OR=0.816, 95% CI: 0.667~0.998, P=0.048), phosphatidylinositol (18:0_20:3) (OR=0.863, 95% CI: 0.751~0.992, P=0.039), and phosphatidylcholine (15:0_18:2) (OR=0.863, 95% CI: 0.761~0.979, P=0.022). Ten lipids were positively associated with EC risk, with triacylglycerol (56:5) (OR=1.304, 95% CI: 1.127~1.509, P<0.001) and phosphatidylcholine (O-18:1_20:4) (OR=1.311, 95% CI: 1.121~1.533, P<0.001) exhibiting the most suggestive risk effects. Elevated cholesterol levels were also associated with increased EC risk (OR=1.229, 95% CI: 1.028~1.469, P=0.023). Sensitivity analyses confirmed the robustness of these results, with all F-statistics for instrumental variables exceeding 10, and inverse MR Analyses were not significant (see Supplementary Figures S2, S6, and S10, and Supplementary Tables 2, 7, and 9).

Bidirectional Genetic Association Analysis Between Immune Cell Phenotypes and Lipids

To avoid overlapping effects on mediation analysis caused by bidirectional causal relationships between EC-related immune cell phenotypes and lipids, we examined the bidirectional associations between the two. The results showed that forward associations involved 13 immune cell phenotypes and 9 lipids (17 pairs of suggestive causal associations), while reverse associations involved 5 lipids and 4 immune cell phenotypes (7 pairs of suggestive causal associations). Notably,

exposure	SNPs	method	OR(95% CI)	P value
Phosphatidylcholine (18:2_20:3) levels	11	Inverse variance weighted	0.810(0.674−0.973)	0.024
Phosphatidylcholine (O−16:0_18:2) levels	9	Inverse variance weighted	0.816(0.667−0.998)	0.048
Phosphatidylinositol (18:0_20:3) levels	22	Inverse variance weighted	0.863(0.751−0.992)	0.039
Phosphatidylcholine (15:0_18:2) levels	25	Inverse variance weighted	0.863(0.761−0.979)	0.022
Phosphatidylcholine (18:1_20:4) levels	19	Inverse variance weighted	1.108(1.006−1.220)	0.038
Phosphatidylcholine (16:0_20:4) levels	15	Inverse variance weighted	1.114(1.020−1.217)	0.017
Phosphatidylcholine (O−16:0_20:4) levels	18	Inverse variance weighted	1.134(1.015−1.267)	0.026
Phosphatidylcholine (O−18:0_20:4) levels	12	Inverse variance weighted	1.167(1.011−1.347)	0.035
Sphingomyelin (d34:2) levels	16	Inverse variance weighted	1.201(1.014−1.423)	0.034
Cholesterol levels	20	Inverse variance weighted	1.229(1.028−1.469)	0.023
Phosphatidylinositol (18:1_20:4) levels	13	Inverse variance weighted	1.237(1.057−1.446)	0.008
Phosphatidylcholine (18:0_22:6) levels	13	Inverse variance weighted	1.279(1.035−1.580)	0.023
Triacylglycerol (56:5) levels	16	Inverse variance weighted	1.304(1.127−1.509)	...0.001
Phosphatidylcholine (O−18:1_20:4) levels	9	Inverse variance weighted	1.311(1.121−1.533)	0.001

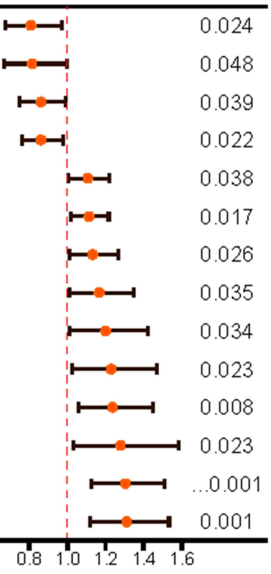

Figure 4 Forest plots of significant causal associations between lipids and EC risk. Effects were estimated using the inverse-variance weighted method. Data are presented as OR and 95% CI. P < 0.05 was considered a suggestive causal association.

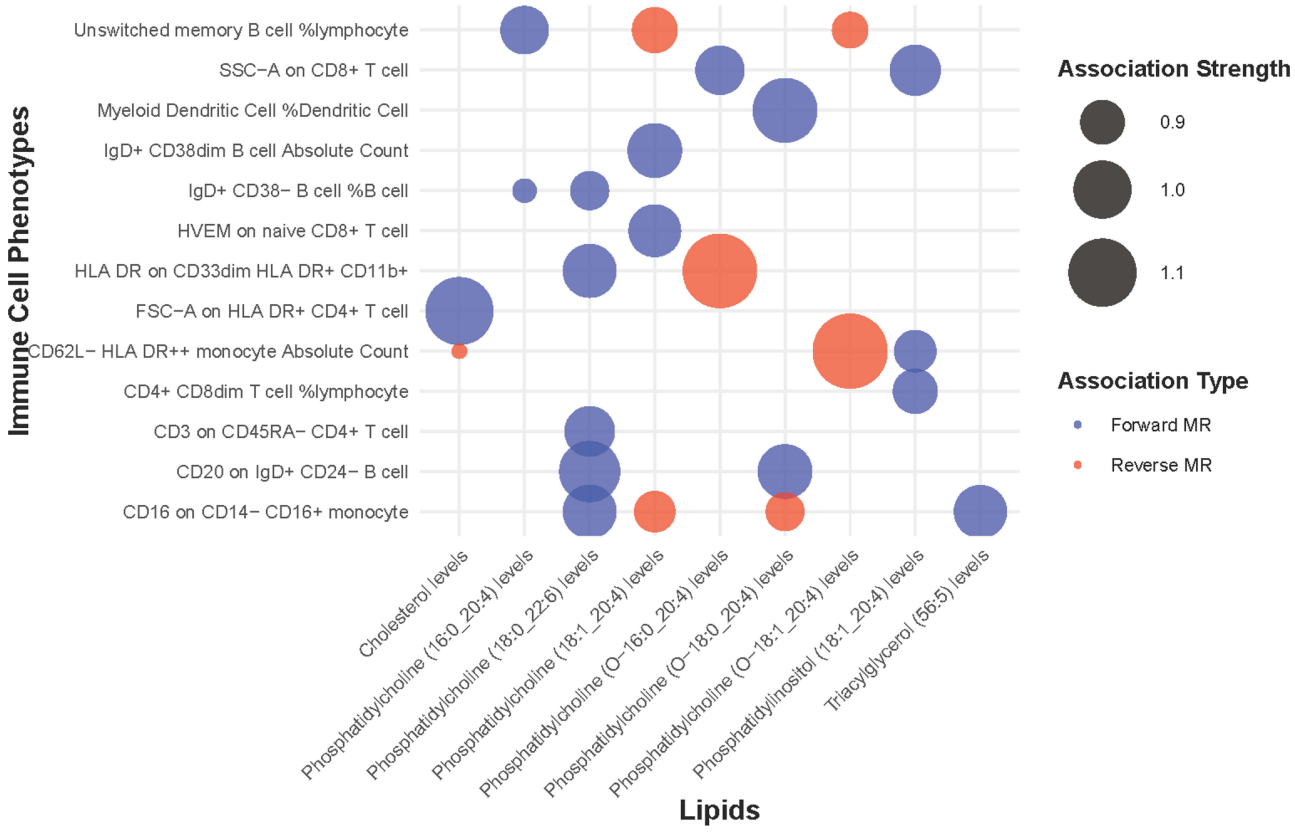

Figure 5 Matrix bubble plots showed the strength and direction of bidirectional association between EC risk related immune cell phenotypes and lipids.

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Table 1 Genetic Association Analysis Between Core Immune/Lipid Factors and Throat/Chest Pain

Expose	Outcome	P_val	OR (95% CI)
CD3 on HLA DR+ T cell	ukb-a-581	<0.001	0.997 (0.995~0.998)
CD3 on HLA DR+ T cell	finn-b-R18_PAIN_THROAT_CHEST	0.009	0.959 (0.930~0.990)
CD33 on basophil	finn-b-R18_PAIN_THROAT_CHEST	0.020	1.020 (1.003~1.038)
SSC-A on CD8+ T cell	ukb-a-581	<0.05	1.002 (1.000~1.004)
Triacylglycerol (56:5) levels	ukb-a-581	0.029	1.004 (1.000~1.008)

Notes: P ≥ 0.05 represents no significant pleiotropy.
Abbreviations: OR, odds ratios; CI, confidence interval.

none of the 7 pairs of reverse MR associations overlapped with the immune cell phenotype-lipid pairs identified in forward MR analysis (Figure 5). This indicates that the immune-lipid regulatory network has a high degree of directionality, which will not interfere with subsequent mediation studies. Details regarding the sensitivity analyses are provided in the Supplementary Material (Supplementary Figures S3, S4, S7, S8, S11 and S12, and Supplementary Tables 3, 4, and 10).

Validation of Mediation Effect of Lipid Metabolism in the Association Between Immune Cell Phenotypes and EC

Two-step MR identified two suggestive suppression (masking) pathways. Specifically, phosphatidylcholine (18:0_22:6) acted as a suppressor in the HLA DR on CD33dim HLA DR+ CD11b+ to EC link (indirect effect = −0.0238, P = 0.0462), with an exploratory suppression ratio of −82.15%. In addition, phosphatidylcholine (O-18:1_20:4) exerted a suggestive suppression effect (−10.38%) on the risk pathway triggered by SSC-A on CD8+ T cells. Detailed suppression ratios are provided in Supplementary Table 8.

Genetic Association Analysis Between Core Immune/Lipid Factors and Throat/Chest Pain

A total of 3 EC-related immune cell phenotypes and 1 immune cell-related lipid showed suggestive causal associations with pain datasets. The direction of these associations was consistent with the effect direction of each phenotype on EC, and all reverse validations were negative (Table 1 and Supplementary Table 5).

Discussion

In recent years, research has focused on the synergistic role of TIME and lipid metabolism reprogramming in the progression of EC. However, traditional observational studies are susceptible to unadjusted confounding factors and reverse causality, making it difficult to accurately establish causal associations between immune/metabolic factors and EC risk. Notably, this study used bidirectional MR combined with two-step MR to investigate the bidirectional causal effects between 731 immune cell traits and EC, as well as 179 potential lipid mediators for the first time. To ensure the identified signatures are not merely metabolic consequences of tumor progression, we conducted reverse MR analyses. The lack of significant genetic associations in the reverse direction supports the primary causal role of the "immune-lipid" axis in EC pathogenesis. Furthermore, on the basis of exploring the "immune-lipid-EC" causal network, this study validated the genetic associations between core immune cell phenotypes/lipids and pain.

Among numerous immune cell traits, this study identified multiple subsets with suggestive causal effects. These findings are highly consistent with the mechanisms of immune surveillance dysfunction and immune suppression in the pathological process of EC. Regarding risk factors, genetically elevated SSC-A values of CD8+ T cells were identified as one of the most prominent risk factors for EC (OR=1.142, P=0.008), and were closely associated with pain occurrence. Increased SSC-A values represent enhanced intracellular granularity, which in tumor immunology is specifically linked to pathological lipid droplet accumulation and lysosomal expansion within exhausted CD8+ T cells.[33,34] These

metabolically exhausted T cells, while exhibiting high internal complexity, are functionally impaired and prone to secreting pro-inflammatory cytokines such as TNF-α and IL-6, which drive both tumor progression and pain sensitization.[35] Genetic propensity to form such overactivated but functionally impaired CD8+ T cell subsets essentially weakens effective anti-tumor immune responses, thereby increasing the risk of EC at the causal level. Consistent with this risk signal, elevated proportion of myeloid dendritic cells among total dendritic cells (OR=1.125) was also positively associated with EC risk, suggesting that genetically driven myeloid cell accumulation may induce a tolerant phenotype more prone to immune escape.[36] In addition, elevated CD33 on basophils may exacerbate local inflammation and neural stimulation by releasing mediators such as histamine and IL-4, simultaneously increasing EC risk and pain susceptibility.[37]

In contrast to risk phenotypes, this study also identified key protective factors. The CD62L- HLA DR++ monocyte Absolute Count (OR=0.879) showed the strongest protective effect. The phenotypic characteristics (CD62L deficiency and high HLA-DR expression) of this cell subset indicate that they are highly activated or differentiated myeloid cells. Although they belong to the same lineage as MDSCs, high HLA-DR++ expression may represent myeloid precursor cells with strong anti-tumor activity or efficient antigen-presenting capacity.[38,39] In addition, CD3 on HLA DR+ T cell exhibited dual protective effects. This phenotype is an activated antigen-presenting T cell, and its genetically elevated levels can reduce EC risk by enhancing anti-tumor immunity, while inhibiting peripheral nociceptive neural sensitization through secreting anti-inflammatory cytokines such as IL-10.[40,41] This result was consistent across two pain-related datasets, suggesting that it may be a potential dual-target for "anti-tumor-analgesia".

Lipidomic disorders are a hallmark of cancer, especially metabolism-related cancers. Among the 10 risk lipids, phosphatidylcholine (O-18:1_20:4) (OR=1.311) and triacylglycerol (56:5) (OR=1.304) had the most significant pro-tumor effects. Biochemically, the sn-2 position of Phosphatidylcholine (O-18:1_20:4) typically houses an AA. In the metabolically altered tumor microenvironment, phospholipases cleave this sn-2 fatty acid chain to liberate free AA, which serves as the direct precursor for the synthesis of pro-inflammatory prostaglandins via the cyclooxygenase pathway.[42] Oncologically, they actively promote tumor growth and facilitate immune evasion by orchestrating an immunosuppressive microenvironment. Neurologically, they act as intensely potent algogenic mediators that directly sensitize peripheral nociceptors, thus exacerbating cancer-related chest pain. Therefore, genetically elevated levels of this phospholipid provide a biochemical substrate that simultaneously fuels tumor progression and pain sensitization.[43] In addition, cholesterol levels (OR=1.229) were also confirmed as a risk factor for EC. High cholesterol has been shown to induce metabolic reprogramming of immune cells, making them prone to exhibit an immunosuppressive phenotype.[44]

This study highlighted two exploratory suppression effects involving phospholipids: first, phosphatidylcholine (18:0_22:6) acted as a suppressor in the HLA DR on CD33dim HLA DR+ CD11b+ to EC link, with an exploratory suppression ratio of −82.15%. This phospholipid is rich in docosahexaenoic acid (DHA, 22:6), a recognized Omega-3 polyunsaturated fatty acid with potent anti-inflammatory and immunomodulatory functions.[45,46] This striking magnitude arises mathematically because a significant negative indirect effect nearly offsets the positive direct effect, leading to artificial inflation of the ratio when the total effect size is modest. Biologically, this identifies a potential competitive consumption mechanism where highly activated HLA-DR+ myeloid cells exhaust circulating phosphatidylcholine (18:0_22:6) to fuel anti-tumor functions, creating a metabolic feedback loop that masks the primary protection. Similarly, the −10.38% suppression effect of phosphatidylcholine (O-18:1_20:4) suggests that even within risk-promoting T-cell pathways, compensatory metabolic regulatory mechanisms may exist. Genetic propensity to form a high SSC-A activated/exhausted T cell state may result in highly active metabolism, potentially consuming certain pro-inflammatory AA-phosphatidylcholines in the circulation, leading to relatively reduced genetic levels in plasma, thereby slightly offsetting the main risk effect caused by T cell dysfunction.[47] These complex mediation effects provide potential targets and directions for future research.

This study has several limitations that must be acknowledged. First, the discovery GWAS included a relatively small number of EC cases (n = 998). While this represents a valuable dataset for EC in European populations, it is undeniably smaller than GWAS cohorts for more prevalent diseases. This sample size potentially constrains the precision of our estimates and the statistical power to detect modest causal associations, particularly in mediation analyses where indirect effects are often subtle. Consequently, some identified associations may be subject to overestimation due to limited case

numbers, while there is an increased risk of false-negative results (Type II errors) for mediators with modest impacts. Given that several identified immune traits exhibit odds ratios near 1.0, these suggestive associations remain susceptible to biases such as weak instruments and selection effects, and should be interpreted with caution as exploratory findings. Second, evaluating EC as an unstratified broad category inevitably masks subtype-specific (ESCC vs. EAC) immune-lipid pathways. Third, the broad pain proxies encompass non-cancer etiologies (eg., reflux), restricting our ability to conclusively isolate purely tumor-induced signals. Fourth, the reliance exclusively on European cohorts limits the global applicability of our findings. Given the genetic differences in lipid metabolism and HLA loci, and the higher incidence of ESCC in populations worldwide, cross-ethnic validation in large-scale Asian cohorts is urgently required.

Lastly, we anticipate that our research findings will serve as a reference that will methodically direct further mechanistic studies. Biologically, elevated SSC-A functionally reflects T cell metabolic exhaustion, characterized by severe intracellular lipid droplet accumulation. Crucially, the mediating lipid contains an AA chain, which acts as the essential precursor for synthesizing prostaglandins (eg., PGE2). Because prostaglandins actively drive tumor progression while simultaneously acting as intensely strong algogenic mediators, this specific immune-lipid pair provides a closed-loop biochemical rationale. Furthermore, given its potential exploratory value, CD3 on HLA DR+ T cells may represent a promising candidate for further investigation. This marker exhibited consistent protective genetic associations in the EC discovery cohort and was similarly observed in two independent external pain datasets (UKB and FinnGen). This cross-cohort consistency provides statistical support for its potential reproducibility.

The lipid GWAS data employed in this MR study capture circulating plasma profiles, which do not fully reflect the dynamic, localized lipid metabolism within the TIME. Accordingly, these findings should be interpreted as genetic evidence of systemic metabolic predisposition rather than confirmed local events. Mechanistic validation of the proposed mediation pathways will require future in vitro and in vivo experiments, ideally complemented by spatial metabolomics, to precisely delineate immune–lipid crosstalk within the TIME. Prioritizing these fundamental markers in subsequent functional assays will be essential to advance clinical translation.

Conclusion

In this MR Study, we found that both immune cell phenotypes and lipids have genetic evidence supporting causal relationships with EC in the European population, and some lipids play a mediating role in affecting the risk of EC through specific immune cell phenotypes. At the same time, core immune cell phenotypes and lipids also have a genetic association with EC-related throat/chest pain. These findings highlight the necessity of " Antineoplastic -analgesic" combined intervention for EC patients targeting the "immune-lipid" synergistic pathway, and lay the foundation for the clinical mechanism research and treatment practice of EC and related pain in the future.

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Disclosure

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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