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ORIGINAL RESEARCH

Assessing the Causal Relationship Between Immune Cells and Temporomandibular Related Pain by Bi-Directional Mendelian Randomization Analysis

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Introduction: Even with significant progress has been made in elucidating the pathogenesis of temporomandibular disorders (TMD), the pathophysiology of temporomandibular joint (TMJ) pain is still obscure. Our study aimed to explore whether there is a causal link between immune cells and TMD-related pain.

Materials and Methods: Based on the TMD-related pain data obtained from the FinnGen Research Consortium and the 731 immune traits extracted from the GWAS Catalog and utilized a two sample Mendelian Randomization (MR) method, with immune cell as the exposure and TMD-related pain as the outcome. MR analyses were conducted employing the inverse-variance weighting method (IVW) as the primary analytical method to evaluate the causal association. Sensitivity analyses were conducted to enhance the robustness, heterogeneity and horizontal pleiotropy of the results. A reverse MR analysis was also conducted for immune cell traits identified in the initial MR analysis.

Results: After false discovery rate (FDR) correction, two immune traits were observed and found to be significantly associated with TMD-related pain: Hematopoietic Stem Cell absolute count (OR=0.954, 95% CI= 0.933~0.976), and HLA DR+ CD4+ T cell (OR=1.040, 95% CI=1.019~1.061). On the reverse MR analysis, no significantly associated results were found in causal effects of TMD-related pain on immune traits.

Conclusion: Our study showed a potential causal relationship between immune cells and TMD-related pain, eliminating reverse causality. These discoveries significantly enhance our knowledge of the interaction between immune traits and TMD-related pain, opening new possibilities for designing treatment from an immunological perspective.

Keywords: temporomandibular related pain, immune trait, immune cell, Mendelian randomization

Introduction

Temporomandibular disorders (TMD) encompass a range of medical and dental disorders that affect the function and health of the temporomandibular joints (TMJ), masticatory musculature and the accompanying structures, and are manifested by limitation of jaw movements, TMJ sounds like clicking and crepitus, as well as headaches and pain.[1] Various factors contribute to the development of TMD, including trauma to the joints and muscles, anatomical variations, psychosocial components, and the heightened sensitivity of nociceptive pathways.[2,3] Symptoms of TMD, especially pain, was considered to be a threatening stimulus in TMD patients, and generally associated with fatigue, mood and sleep disturbances, and poor quality of life.[4] Biomechanical and psychological signs and symptoms, often complicated by pain, tend to blur the fundamental cause(s) of the issue. Therefore, it is notoriously difficult to treat. Clinically, pain is often managed with symptomatic treatment, highlighting the need for more targeted treatments aimed at reducing or eliminating pain and enhancing the quality of life for these patients.[5]

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Previously, it has been thought that the production and perception of physiologic and clinical pain primarily reflects neuronal activity alone. However, it has become increasingly apparent that the majority of pain types either originate from or are shaped by the complicated interaction between the immune system and the somatosensory system.[6–8] It is considered that peripheral nociceptors can discern immune cells by recognizing the cytokines they secrete. Conversely, once activated, nociceptors release cytokines, neuropeptides, chemokines, and microRNAs from their terminals or cell bodies, thereby influencing resident immune cells such as neutrophils, macrophages, mast cells, and dendritic cells.[9,10] Extensive evidence indicates that specific pro-inflammatory cytokines, including TNF-α, IL-1, and IL-6, play a role in the development of pathological pain.[11–13] Additional studies have demonstrated that TNF-α is a key mediator of TMD-related pain, influencing both synaptic plasticity in central sensitization and the inflammatory pain response, which leads to increased nociceptive sensitization in periarticular tissues and trigeminal ganglia.[14,15] Therefore, pain needs to be recognized as a neuroimmune condition by considering neuroimmune interactions as integrative drivers and modulators, and not just a sign of inflammatory response.

As an important element of the immune system, immune cells are the initial responders of therapeutic intervention. When faced with pathogenic factors, these cells secrete cytokines or directly interact with lymphocytes to maintain the balance of tissue microenvironment and actively participate in the process of tissue injury and regeneration.[16] Despite growing interest, research exploring the relationship between immune cells and TMD-related pain remains limited, often constrained by small sample sizes and the influence of confounding variables.

Advancements in large-scale genome-wide association studies (GWAS) and Mendelian randomization (MR) techniques now allow for the investigation of causal relationships between immune traits and disease outcomes.[17] Some studies have validated the use of MR in probing causal associations in TMD, offering a method to minimize confounding factors and avoid reverse causality in causal inference.[18,19] Therefore, our study employs a two-sample MR approach, integrating GWAS data of immune traits and TMD-related pain, to explore the causal relationship between immune traits and TMD-related pain.

Materials and Methods

Study Design

In this research, a two-sample Mendelian Randomization (MR) approach was employed to investigate the causal link between 731 immune traits and TMD-related pain. Genetic variation was leveraged as instrumental variables (IVs) in the MR analysis to test a causal link between the exposure and the outcome (Figure 1). The research began by using immune traits as the exposure to assess potential causal relationships with TMD-related pain risk. In the next step, TMD-related pain was treated as the exposure to investigate possible reverse causal effects on immune traits. The selection of these IVs was guided by three primary assumptions: (1) Relevance, meaning the IVs must be linked to the exposure; (2) Independence, which required the IVs to be unrelated to confounding factors; (3) Exclusion restriction, where the IVs were expected to be independent of the outcome, conditional on the exposure. Any instrumental variables (IVs) that violate the three core assumptions will be excluded from the analysis (Figure 2). All cases included in this study have been approved by the relevant institutional review boards, and all participants have provided their informed consent.

Genome-Wide Association Study (GWAS) Data Sources for TMD-Related Pain

The data from Genome-wide association study (GWAS) for TMD-related pain (https://r9.risteys.finngen.fi/endpoints/DENTAL_TMD) was obtained from the FinnGen Research Consortium. The diagnosis criteria followed International Classification of Diseases (ICD-10) codes M79.1, K07.60 and K07.63, which encompass various forms of TMD associated with pain, including myalgia. The dataset captured a broad range of TMD-related pain conditions without further classification into specific subtypes. This database is R9 version and conducted a genetic analysis on 377,277 Europeans, among which there were 10,303 cases (Ncase) and 366,974 controls (Ncontrol).

Genome-Wide Association Study (GWAS) Data Sources for Immune Cell Traits

The statistical summary of immune traits from GWAS was sourced from the GWAS Catalog (GWAS IDs from GCST0001391 to GCST0002121).[20] The catalog includes 731 immune traits, comprising 389 median fluorescence

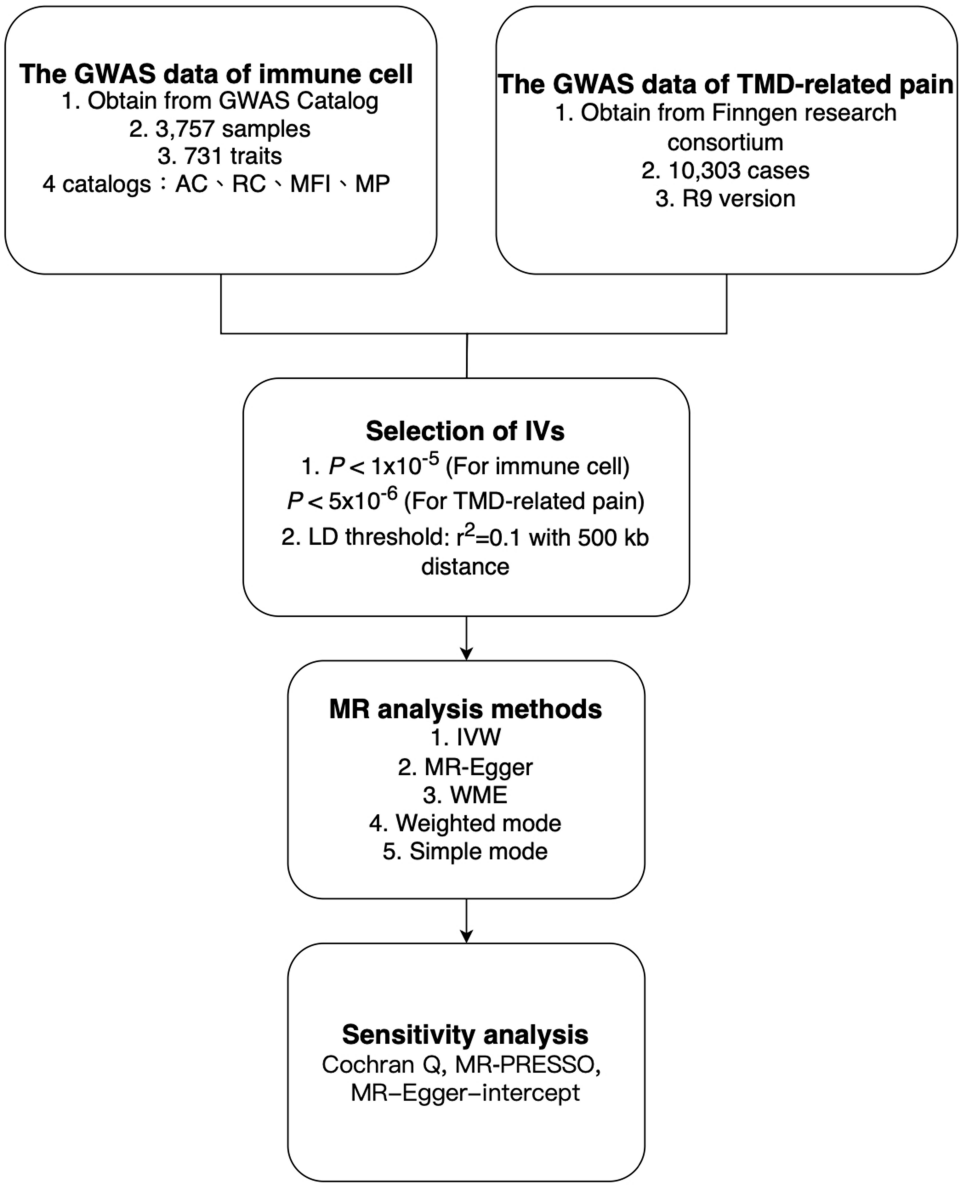

Figure 1 The flowchart of the study. GWAS, genome-wide association study.
Abbreviations: AC, absolute cell counts; RC, relative cell counts; MFI, median fluorescence intensity; MP, morphological parameters; TMD, Temporomandibular disorder; IVs, instrumental variables; LD, linkage disequilibrium; IVW, inverse-variance weighted; WME, Weighted Median; MR, Mendelian randomization; MR-PRESSO, Mendelian randomized polymorphism residual sum and outlier.

intensities (MFI) reflecting surface antigen levels, 192 relative cell counts (RC), 118 absolute cell counts (AC), and 32 morphological parameters (MP). Specific immune traits in the database were categorized into Treg panels, mature T cell panels, TBNK panels (including T cells, B cells, and NK cells), dendritic cell (DC) panels, mature B cell panels, myeloid panels and monocytes. The genomic analysis of immune traits was investigated using data from 3757 Europeans by collecting peripheral blood from blood donors, which underwent flow cytometry analysis after antibody staining, with estimates derived from the gene sequences of the Sardinian population.[21]

Selection of Instrumental Variables

For the selection of IVs for immune traits, according to recent studies,[20,22] the significance of immune traits was set to $P<1\times10^{-5}$. To ensure the independence of the SNPs, the linkage disequilibrium (LD) analysis was conducted using the

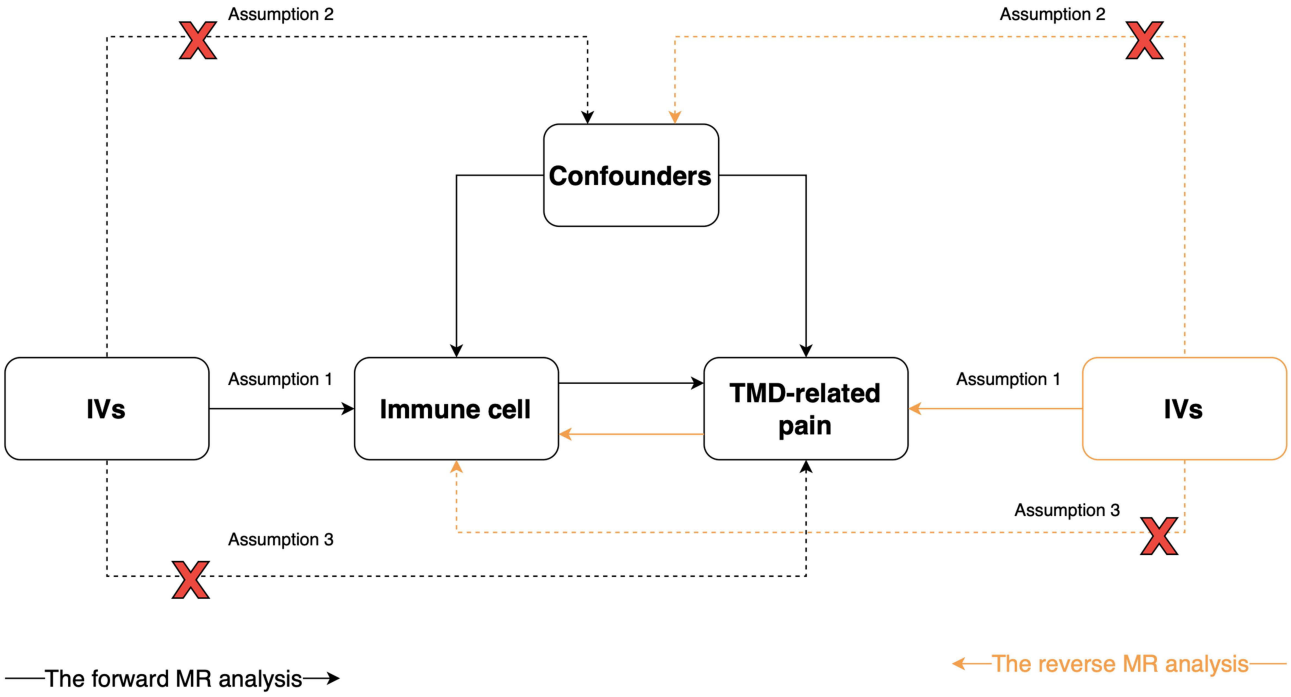

Figure 2 Schematic diagram of the assumption of two-sample Mendelian randomization.
Abbreviation: IVs, instrumental variables.

PLINK software, and the LD was defined with r^2 threshold of less than 0.1 within a 500 kb distance. The r^2 for LD was calculated using the available data from the 1000 Genomes Project.[23]

In the selection of IVs associated with TMD-related pain, the significance level was set to $P<5\times10^{-6}$, after excluding IVs with low F-statistics (F<10), and retained 18 IVs for the MR analysis. This rigorous selection process ensured that our instrumental variables are both statistically significant and robust for the subsequent MR analysis.

Statistical Analysis

In this research, five models were employed for MR analysis to precisely analyze causal link between immune traits and TMD-related pain, include Inverse Variance Weighting (IVW), MR-Egger, Weighted Median Method (WME), Weighted mode and Simple mode. The IVW method was employed as major MR analysis that leveraged the impact of Single Nucleotide.

Polymorphisms (SNPs) on the exposure for weighting, providing an estimate of the causal relationship,[24] and the False Discovery Rate (FDR) was also applied in IVW method for correction to prevent an increase in Type I errors due to multiple testing.[25] Other methods were used as complementary methods.

Moreover, several sensitivity analyses were performed for the result to analyze the horizontal pleiotropy and heterogeneity. Heterogeneity among SNPs was evaluated with the Cochran's Q test. Horizontal pleiotropy of SNPs was assessed using the MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) method[26] and MR-Egger regression (Egger-intercept) method.[27] Leave-one-out analysis was also conducted to assess the influence of each individual genetic variant.

In this study, statistical software R 4.3.2, Two Sample MR package 0.5.7, and MR-PRESSO package 1.0 were used, P-value of less that 0.05 was regarded as statistically significant.

Result

Exploration of Causal Impacts of Immune Traits on TMD-Related Pain

In this study, we conducted a two-sample MR analysis, mainly employing the IVW method. At a nominal significance level, we revealed the causal role between TMD-related pain and 75 types of immune traits (IVW $P<0.05$). Specifically,

33 types were found to decrease the risk of TMD-related pain, 41 types increased the risk, while 1 type appeared to have no effect (Figure 3). After the FDR adjusting in the IVW method, we identified a causal relationship in 2 types of immune traits: Hematopoietic Stem Cell absolute count (Myeloid panel), HLA DR+ CD4+ T cell (TBNK panel). The IVW method showed that Hematopoietic Stem Cell absolute count could decrease the level of TMD-related pain (OR=0.95, 95% CI= 0.93~0.98, P_{FDR}= 0.019, P = 4.99E-05) and HLA DR+ CD4+ T cell could increase the level of TMD-related pain (OR=1.04, 95% CI=1.02~1.06, P_{FDR} =0.024, P = 1.27E-04). We also observed similar results using four other analytical approaches. For the immune trait Hematopoietic Stem Cell, the results were as follows: the MR Egger result was OR=0.98, 95% CI= 0.95~1.01, P =0.189; the WME result was OR=0.96, 95% CI= 0.93~0.99, P=0.017; the Weighted mode result was OR=0.96, 95% CI= 0.93~1.00, P =0.04; and the Simple mode result was OR= 0.95, 95% CI= 0.90~1.00, P =0.072. For the immune trait HLA DR+ CD4+ T cell, the results were as follows: the MR Egger result was OR=1.03, 95% CI= 1.00~1.06, P =0.034; the WME result was OR=1.03, 95% CI= 1.00~1.06, P =0.059; the Weighted mode result was OR=1.03, 95% CI= 1.01~1.06, P =0.009; and the Simple mode result was OR=1.07, 95% CI= 1.02~1.14, P =0.018 (Figure 4). Scatter plots in five methods were showed in (Figure 5). Furthermore, the sensitivity analyses of the Cochran Q, MR-PRESSO, Egger-intercept, LOO analysis were showed in (Table 1 and Figure 6).

Exploration of Causal Impacts of TMD-Related Pain on Immune Traits

To investigate the reverse causality of TMD-related pain on immune traits, a two-sample MR analysis was employed, mainly using the IVW method. At a nominal significance level (IVW P<0.05), evidence of a causal relationship was identified between TMD-related pain and 24 types of immune cells. Specifically, TMD-related pain was observed to elevate the level of 10 type of immune traits, while decreasing the levels of 14 other types (Figure 7). The results showed no immune traits after FDR adjustment.

Discussion

By using publicly accessible genetic data, we conducted a systematic examination of the potential causal relationship between 731 immune cell traits and TMD-related pain, employing MR techniques. For all we know, this study represents the first application of MR to investigate a broad spectrum of immune traits in relation to TMD-related pain. We found a causal relationship in 2 types of immune traits: Hematopoietic Stem Cell absolute count and HLA DR+ CD4+ T cell. Our result offers further evidence for a causal relationship between immune traits and TMD-related pain. However, reverse MR analysis failed to identify significant causal associations.

Interestingly, our results indicate that higher absolute cell counts of HSC may be linked with reduced TMD-related pain. While there is limited-existing literature on this, the established roles of HSC such as self-renewal and the capacity to give rise to a variety of progenitor cells, including lymphoid and myeloid stem cells, as well as the ability to further differentiate into progenitor T cells, progenitor B cells and NKs, may shed light on our observations. Mast cells, also derived from HSCs, are an essential component of the innate immune system and are highly responsive to activation of the hypothalamic-pituitary-adrenal (HPA axis), the major stress response system, and their activation leads to down-stream secretion of cortisol and suppression of immune responses. These findings implied that stress and psychological factors might be crucial in the pathogenesis of TMD pain and aligned with previous research on the relationship between trait anxiety and TMD.[18]

Further, our data showed elevated expression of HLA DR+ CD4+ T cell may increase the risk of TMD pain. This may be consistent with the findings of previous research the reduced expression of HLA-DR on monocytes during chronic inflammation highlights the anti-inflammatory role of HLA-DR molecules.[28] There are few studies to explore its association with TMD pain risk and little is known about the role of HLA DR+ CD4+ T cell in its progression. When HLA-DR is low expressed, CD4 + T lymphocytes are not activated, resulting in the body can neither stimulate B cells to produce specific antibodies, nor increase the production of CD8 + T lymphocytes, and this immune pathway is impaired and can lead to immune suppression. The mechanism of immune cells related in the TMD pain may be very complex, more experimental research should be carried out to further explore the mechanism behind HLA DR+ CD4+ T cell and TMD pain risk.

Exposure	Method	N SNPs	OR(95%CI)	Pval
Hematopoietic Stem Cell Absolute Count	IVW	27	0.95 (0.93-0.98)	4.99E-05
HLA DR+ CD4+ T cell Absolute Count	IVW	28	1.04 (1.02-1.06)	1.27E-04
CD3 on HLA DR+ CD8+ T cell	IVW	35	0.95 (0.92-0.98)	0.001
CD27 on Plasma Blast-Plasma Cell	IVW	22	1.06 (1.02-1.09)	0.001
CD8+ Natural Killer T %T cell	IVW	29	1.07 (1.03-1.12)	0.002
HLA DR+ CD4+ T cell %lymphocyte	IVW	27	1.08 (1.03-1.13)	0.002
CD39+ resting CD4 regulatory T cell %resting CD4 regulatory T cell	IVW	165	1.02 (1.01-1.03)	0.002
CD28 on activated & secreting CD4 regulatory T cell	IVW	34	0.96 (0.94-0.99)	0.002
CD3 on CD39+ activated CD4 regulatory T cell	IVW	72	0.97 (0.95-0.99)	0.002
HLA DR+ CD4+ T cell %T cell	IVW	28	1.07 (1.02-1.11)	0.003
CD39+ CD4+ T cell %CD4+ T cell	IVW	229	1.02 (1.01-1.03)	0.003
IgD- CD38dim B cell %lymphocyte	IVW	30	0.96 (0.94-0.99)	0.003
CD39 on CD39+ CD4+ T cell	IVW	170	1.02 (1.01-1.03)	0.003
Terminally Differentiated CD4-CD8- T cell %CD4-CD8- T cell	IVW	34	1.03 (1.01-1.06)	0.003
CD39 on CD39+ secreting CD4 regulatory T cell	IVW	164	1.02 (1.01-1.03)	0.004
CD45RA on CD39+ resting CD4 regulatory T cell	IVW	14	1.04 (1.01-1.07)	0.004
CD127- CD8+ T cell %T cell	IVW	26	1.06 (1.02-1.11)	0.006
CD45RA- CD4+ T cell %CD4+ T cell	IVW	37	0.96 (0.94-0.99)	0.007
CD8+ Natural Killer T %lymphocyte	IVW	31	1.06 (1.02-1.11)	0.007
CD11b on basophil	IVW	22	0.96 (0.92-0.99)	0.007
CD33dim HLA DR+ CD11b+ Absolute Count	IVW	28	0.96 (0.94-0.99)	0.007
CD127- CD8+ T cell Absolute Count	IVW	36	1.05 (1.01-1.08)	0.007
CD14 on CD33dim HLA DR+ CD11b+	IVW	18	1.04 (1.01-1.08)	0.008
CD64 on CD14- CD16-	IVW	17	1.07 (1.02-1.12)	0.008
CD28+ CD45RA+ CD8dim T cell Absolute Count	IVW	62	1.01 (1.00-1.01)	0.008
CD19 on CD20- B cell	IVW	21	0.95 (0.91-0.99)	0.008
CD39+ resting CD4 regulatory T cell Absolute Count	IVW	153	1.01 (1.00-1.03)	0.008
T cell Absolute Count	IVW	21	1.05 (1.01-1.10)	0.011
CD20 on IgD+ B cell	IVW	36	0.98 (0.96-0.99)	0.011
CD3 on naive CD8+ T cell	IVW	80	0.98 (0.96-0.99)	0.012
CD86+ plasmacytoid Dendritic Cell %Dendritic Cell	IVW	32	0.96 (0.92-0.99)	0.013
CD39+ activated CD4 regulatory T cell %CD4 regulatory T cell	IVW	156	1.02 (1.00-1.03)	0.013
CD11c+ monocyte Absolute Count	IVW	16	0.95 (0.92-0.99)	0.014
CD3 on activated & secreting CD4 regulatory T cell	IVW	77	0.98 (0.96-1.00)	0.015
CD45RA+ CD28- CD8+ T cell %CD8+ T cell	IVW	35	1.00 (1.00-1.00)	0.015
CD39+ CD4+ T cell %T cell	IVW	223	1.01 (1.00-1.02)	0.015
PDL-1 on CD14+ CD16- monocyte	IVW	18	1.07 (1.01-1.12)	0.017
CD39 on monocyte	IVW	25	0.98 (0.97-1.00)	0.017
CD28+ CD45RA+ CD8dim T cell %T cell	IVW	62	1.01 (1.00-1.02)	0.017
IgD- CD38- B cell %lymphocyte	IVW	30	0.96 (0.92-0.99)	0.017
CD127 on CD28+ CD45RA+ CD8+ T cell	IVW	31	1.05 (1.01-1.09)	0.018
CD8+ T cell %leukocyte	IVW	36	1.05 (1.01-1.09)	0.018
CD39+ resting CD4 regulatory T cell %CD4 regulatory T cell	IVW	170	1.01 (1.00-1.02)	0.019
CD39 on CD39+ activated CD4 regulatory T cell	IVW	236	1.01 (1.00-1.02)	0.020
CD3 on HLA DR+ T cell	IVW	38	0.97 (0.95-1.00)	0.020
CD39 on CD39+ CD8+ T cell	IVW	50	1.03 (1.01-1.06)	0.020
CD8 on CD39+ CD8+ T cell	IVW	60	0.97 (0.94-1.00)	0.021
CD25 on IgD+ CD24+ B cell	IVW	43	1.02 (1.00-1.03)	0.022
Transitional B cell %B cell	IVW	16	0.93 (0.88-0.99)	0.023
CD45RA+ CD8+ T cell Absolute Count	IVW	36	0.99 (0.98-1.00)	0.023
CD3 on CD4 regulatory T cell	IVW	66	0.98 (0.96-1.00)	0.023
CD3 on CD28+ CD45RA+ CD8+ T cell	IVW	78	0.98 (0.96-1.00)	0.024
CD11c+ monocyte %monocyte	IVW	24	0.96 (0.92-0.99)	0.024
CD64 on monocyte	IVW	98	0.98 (0.97-1.00)	0.025
CD25++ CD45RA+ CD4 not regulatory T cell Absolute Count	IVW	45	1.02 (1.00-1.04)	0.029
CD4+ T cell Absolute Count	IVW	24	1.03 (1.00-1.07)	0.031
CD28 on CD45RA- CD4 not regulatory T cell	IVW	29	1.04 (1.00-1.08)	0.031
CD4 on CD39+ resting CD4 regulatory T cell	IVW	14	0.96 (0.93-1.00)	0.031
CD20 on IgD+ CD24- B cell	IVW	38	0.98 (0.96-1.00)	0.032
Natural Killer %lymphocyte	IVW	34	1.03 (1.00-1.06)	0.033
Resting CD4 regulatory T cell Absolute Count	IVW	49	1.02 (1.00-1.05)	0.033
CD3 on CD39+ secreting CD4 regulatory T cell	IVW	59	0.98 (0.96-1.00)	0.033
CX3CR1 on CD14+ CD16- monocyte	IVW	55	1.03 (1.00-1.05)	0.033
TCRgd T cell Absolute Count	IVW	32	1.04 (1.00-1.07)	0.035
Terminally Differentiated CD4-CD8- T cell %T cell	IVW	35	1.03 (1.00-1.06)	0.036
CD3 on activated CD4 regulatory T cell	IVW	71	0.98 (0.96-1.00)	0.037
SSC-A on T cell	IVW	31	1.04 (1.00-1.07)	0.038
HVEM on CD4+ T cell	IVW	19	0.97 (0.95-1.00)	0.038
CD33 on CD33dim HLA DR+ CD11b+	IVW	99	1.01 (1.00-1.02)	0.042
HLA DR on CD33dim HLA DR+ CD11b+	IVW	82	0.99 (0.97-1.00)	0.042
CD25 on unswitched memory B cell	IVW	41	1.02 (1.00-1.03)	0.044
CD127 on T cell	IVW	19	1.05 (1.00-1.10)	0.044
CD20 on naive-mature B cell	IVW	48	0.98 (0.97-1.00)	0.048
CD3 on HLA DR+ CD4+ T cell	IVW	38	0.97 (0.95-1.00)	0.049
CD8 on HLA DR+ CD8+ T cell	IVW	35	0.98 (0.95-1.00)	0.049

Figure 3 Forest plots showed the causal impacts of immune traits on TMD-related pain at a nominal significance level, indicating the direction of effect (OR) and statistical significance (95% CI). OR >1 suggests increased risk, while OR <1 indicates a potential protective effect.
Abbreviations: SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; IVW, inverse-variance weighted.

Exposure	Method	N SNPs	OR(95%CI)	Pval	P_{FDR}
Hematopoietic Stem Cell Absolute Count	IVW	27	0.95 (0.93-0.98)	4.99E-05	0.036
	MR Egger		0.98 (0.95-1.01)	0.189	
	Weighted median		0.96 (0.93-0.99)	0.017	
	Weighted mode		0.96 (0.93-1.00)	0.042	
	Simple mode		0.95 (0.90-1.00)	0.072	
HLA DR+ CD4+ T cell Absolute Count	IVW	28	1.04 (1.02-1.06)	1.27E-04	0.046
	MR Egger		1.03 (1.00-1.06)	0.034	
	Weighted median		1.03 (1.00-1.06)	0.059	
	Weighted mode		1.03 (1.01-1.06)	0.009	
	Simple mode		1.07 (1.02-1.14)	0.018	

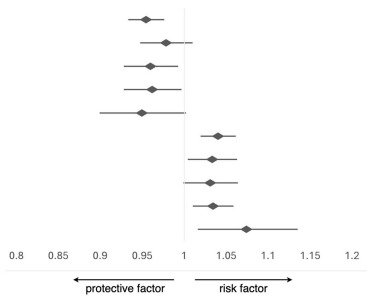

Figure 4 Forest plots showed the causal impacts of immune traits on TMD-related pain after FDR adjustment, indicating the direction of effect (OR) and statistical significance (95% CI). OR >1 suggests increased risk, while OR <1 indicates a potential protective effect.
Abbreviations: SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; FDR, false discovery rate; HLA, human leukocyte antigen; CD, cluster of differentiation; IVW, inverse-variance weighted.

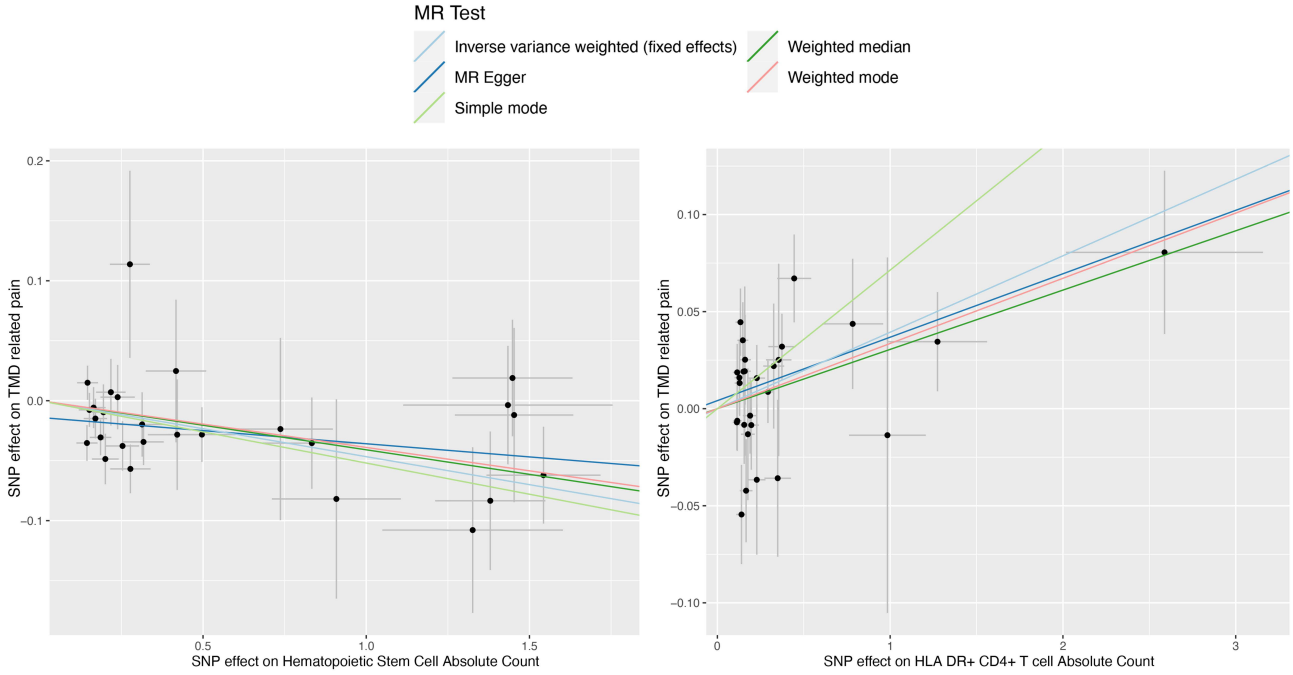

Figure 5 Scatterplot of SNP effects on the absolute counts of hematopoietic stem cells and HLADR+CD4+ T cell expression levels versus TMD-related pain. The slopes of the lines indicate the estimated causal effects, with a negative trend suggesting an inverse relationship between immune traits and TMD-related pain. The spread of the data points provides insight into the variability and consistency of these estimates.
Abbreviations: TMD, Temporomandibular disorder; MR, Mendelian randomization; SNP, single nucleotide polymorphism; HLA, human leukocyte antigen; CD, cluster of differentiation.

Despite careful selection of IVs that met various modeling assumptions and extensive sensitivity analyses to minimize the effects of possible confounders, there are limitations to our study. Firstly, this study relied on a European population database, limiting the ability to generalize the results to other ethnic groups and reducing the broader applicability of our

Table 1 Sensitivity Analyses of Causal Impacts of Immune Traits on TMD-Related Pain

Exposure	N SNPs	MR_Egger Regression		MR-PRESSO_Pval	Q_Pval
		Intercept	Intercept_Pval		
Hematopoietic Stem Cell Absolute Count	27	−0.013	0.041	7.44E-04	0.298
HLA DR+CD4+T cell Absolute Count	28	0.004	0.473	0.002	0.219

Abbreviations: SNPs, single nucleotide polymorphisms; MR-PRESSO, Mendelian randomized polymorphism residual sum and outlier; Q_Pval, Cochran Q in inverse-variance weighted method; HLA, human leukocyte antigen; CD, cluster of differentiation.

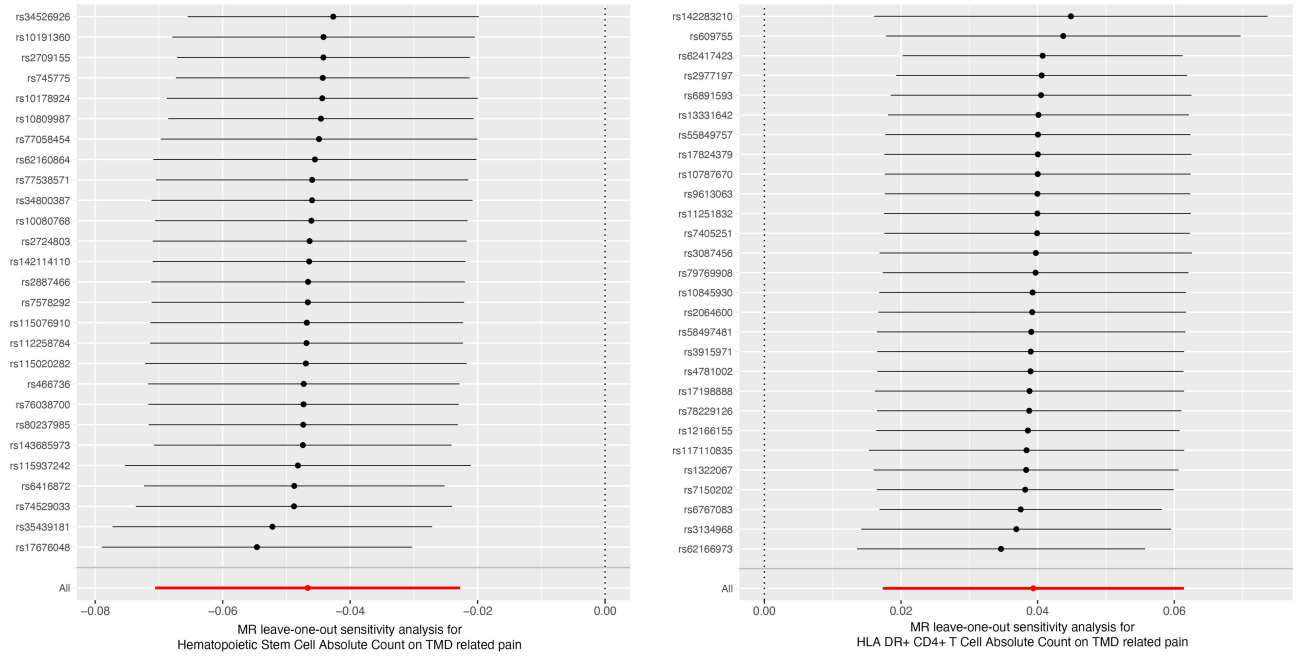

Figure 6 The leave-one-out analysis of causal impacts of immune traits on TMD-related pain. The Y-axis shows the SNP ID excluded from the analysis, and the X-axis presents the MR odds ratios. The red line reflects the MR estimate calculated with all SNPs included.
Abbreviations: TMD, Temporomandibular disorder; MR, Mendelian randomization; HLA, human leukocyte antigen; CD, cluster of differentiation.

Exposure	Method	N SNPs	OR(95%CI)	Pval
CD19 on switched memory B cell	IVW	17	1.21 (1.07-1.38)	0.003
CD19 on IgD- CD38- B cell	IVW	17	1.21 (1.06-1.37)	0.004
CD28 on resting CD4 regulatory T cell	IVW	9	1.29 (1.06-1.56)	0.011
FSC-A on monocyte	IVW	18	0.85 (0.75-0.96)	0.012
CD28 on CD28+ CD4-CD8- T cell	IVW	4	1.41 (1.08-1.85)	0.012
CD45 on CD33+ HLA DR+ CD14-	IVW	18	1.24 (1.05-1.48)	0.013
CD33+ HLA DR+ CD14dim Absolute Count	IVW	18	0.83 (0.71-0.97)	0.019
HLA DR on CD33+ HLA DR+ CD14dim	IVW	18	1.22 (1.03-1.45)	0.023
CD45 on CD33+ HLA DR+	IVW	18	1.22 (1.02-1.45)	0.025
CD25 on CD39+ resting CD4 regulatory T cell	IVW	18	0.88 (0.78-0.99)	0.028
CD39+ activated CD4 regulatory T cell %CD4 regulatory T cell	IVW	18	1.14 (1.01-1.27)	0.031
HLA DR++ monocyte %leukocyte	IVW	18	0.88 (0.79-0.99)	0.033
CD27 on Plasma Blast-Plasma Cell	IVW	18	0.89 (0.79-0.99)	0.036
SSC-A on HLA DR+ CD8+ T cell	IVW	18	0.88 (0.78-0.99)	0.036
CD33+ HLA DR+ Absolute Count	IVW	18	0.85 (0.73-0.99)	0.036
FSC-A on B cell	IVW	18	0.88 (0.78-0.99)	0.037
BAFF-R on CD20- B cell	IVW	17	0.89 (0.79-0.99)	0.038
HLA DR on CD33+ HLA DR+ CD14-	IVW	18	1.20 (1.01-1.42)	0.040
CD4RA on Terminally Differentiated CD4+ T cell	IVW	18	0.88 (0.77-1.00)	0.042
CD33+ HLA DR+ CD14dim %CD33+ HLA DR+	IVW	18	0.85 (0.73-1.00)	0.043
CD25 on CD39+ CD4+ T cell	IVW	18	0.88 (0.77-1.00)	0.044
HLA DR++ monocyte Absolute Count	IVW	18	0.89 (0.79-1.00)	0.044
FSC-A on HLA DR+ CD8+ T cell	IVW	18	0.89 (0.79-1.00)	0.049
CD3- lymphocyte %lymphocyte	IVW	13	1.13 (1.00-1.28)	0.049

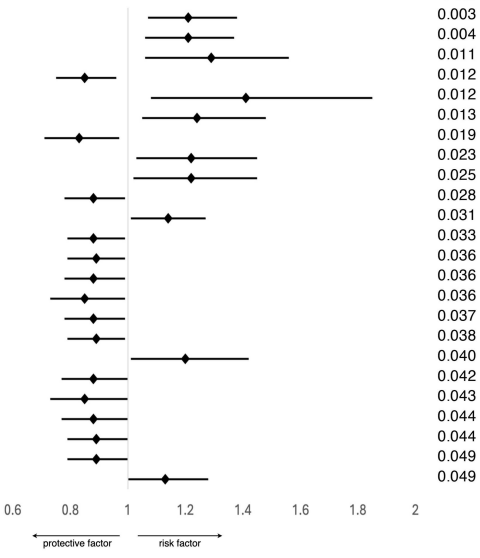

Figure 7 Forest plots showed the causal impacts of TMD-related pain on immune traits at a nominal significance level, indicating the direction of effect (OR) and statistical significance (95% CI). OR >1 suggests increased risk, while OR <1 indicates a potential protective effect.
Abbreviations: SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; IVW, inverse-variance weighted.

findings. Second, the absence of detailed individual data hindered our ability to stratify the population further. Third, due to the limited sample size, the threshold for SNP selection was loose, which may result in some degree of false positives. Therefore, we interpret the outcomes produced in this study cautiously.

Conclusion

In summary, through stringent bidirectional MR analyses, we identify a potential causal relationship between immune cells and TMD pain, eliminating reverse causality. These discoveries significantly enhance our knowledge of the interaction between immune traits and TMD-related pain, opening new possibilities for designing treatment from an immunological perspective. Further research is required to clarify the specific role of immune cells and the resulting pain in the pathogenesis of TMD.

Ethics Statement

According to local legislation (Article 32 of the document No.4 of 2023 "Notice on of the Ethical Review of Life Sciences and Medical Research Involving Humans" issued by National Health Science and Education Development) and institutional requirements, the study utilized only publicly accessible data and did not involve direct human participation, thus exempting it from the need for ethical approval or consent.

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Disclosure

The authors report no conflicts of interest in this work.

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