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ORIGINAL RESEARCH

Genetically Supported Causality Between Immune Cells Traits and Low Back Pain: A Bi-Directional Two-Sample Mendelian Randomization Study

Jianbing Wang[1,2,*], Mengye Zhu[1,3,*], Yuhan Liu[4], Daying Zhang[1,3], Shuchun Yu[5], Jinjin Zhang[6]

[1]Department of Pain Medicine, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, People's Republic of China; [2]Department of Anesthesiology, Jiangxi Cancer Hospital, Nanchang, People's Republic of China; [3]Jiangxi Key Laboratory of Trauma, Burn and Pain Medicine, Nanchang, People's Republic of China; [4]College of Medicine, Jinggangshan University, Ji'an, People's Republic of China; [5]Department of Anesthesiology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, People's Republic of China; [6]Department of Pain Medicine, Ji'an Central People's Hospital, Ji'an, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jinjin Zhang, Department of Pain Medicine, Ji'an Central People's Hospital, No. 106, Jinggangshan Avenue, Ji'an City, Jiangxi Province, People's Republic of China, Email zhangjj12356@163.com; Shuchun Yu, Department of Anesthesiology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, No. 1, Minde Road, Nanchang, People's Republic of China, Email yscdoc@hotmail.com

Purpose: Prior studies have suggested that immune cells play a crucial role in Low Back Pain (LBP). We employed a bi-directional Mendelian randomization (MR) study to investigate the causal relationship of immune cells with the risk of LBP.

Patients and Methods: Single Nucleotide Polymorphisms (SNPs) that had a significant genetic association with immune cells were used as instrumental variables (IVs). The inverse variance weighted (IVW) method was used as the primary approach for MR analyses. To assess the robustness, sensitivity analyses were further performed using MR-Egger and MR-PRESSO.

Results: The MR analysis revealed a causal relationship between six types of immune cells and LBP ($P < 0.05$), including CD4 Treg AC (OR, 0.925; 95% CI, 0.878–0.974; $P = 0.003$), CD19 on $CD20^{-}$ $CD38^{-}$ (OR, 0.938; 95% CI, 0.898–0.979; $P = 0.003$), CD4 on HLA DR^{+} $CD4^{+}$ (OR, 0.947; 95% CI, 0.909–0.987; $P = 0.010$), CD25 on $CD39^{+}$ $CD4^{+}$ (OR, 0.954; 95% CI = 0.922–0.988; $P = 0.008$), CD14 on CD33br HLA DR^{+} $CD14^{dim}$ (OR, 0.950; 95% CI = 0.916–0.985; $P = 0.006$), and CD4RA on TD $CD4^{+}$ (OR, 1.030; 95% CI, 1.012–1.048; $P = 0.001$). Reverse MR analysis found no evidence of potential causal effects of genetically predicted LBP on the six types of immune cells.

Conclusion: This study has demonstrated a close genetic connection between immune cells and LBP, providing valuable insights for future clinical research.

Keywords: immune cells, low back pain, Mendelian randomization

Introduction

Low back pain (LBP) is a symptom characterized by discomfort and pain below the costal margin, above the buttock creases, and along the axillary midline, which may or may not be accompanied by leg pain.[1] Over the past three decades, LBP has emerged as the primary cause of disability worldwide.[2] It is a common health concern that affects a diverse population, with variations in duration, severity, pain intensity, and functional limitation.[3] According to the Global Burden of Disease Study, LBP is the most common condition with a significant societal impact.[4] The 1-year incidence of first-ever LBP ranges from 6.3% to 15.4%, while the 1-year incidence of any episode of LBP varies from 1.5% to 36%.[5] Given its high prevalence and substantial global burden, identifying potential causal risk factors for LBP is of immediate importance.

The immune system consists of a functional complex of immune cells, whose individual or collective traits may influence the development of LBP. Some observational evidence suggests that LBP could be an autoimmune disease, as it shows a higher prevalence in female patients and a significant heritability, which are common features of most

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autoimmune diseases.[6,7] Intervertebral disc degeneration is a major factor that causes persistent LBP and disability in middle-aged and elderly individuals.[8] The intervertebral disc, which lacks blood and lymph vessels and is the largest aneural structure in the body, is segregated from the immune system. However, disc herniation or degeneration facilitates the invasion of blood and lymph vessels from adjacent connective tissues, presenting disc antigens to the immune system and eliciting an adaptive immune response.[9,10] Nevertheless, the relationship between immune cells and LBP is complex and multifaceted, and the mechanisms by which altered immune cells influence the development of LBP are poorly understood.

Mendelian randomization (MR) is a robust technique that uses genetic variants, mainly single nucleotide polymorphisms (SNPs), as instrumental variables (IVs) to assess the possible causal effect of an exposure on outcomes.[11] The strength of MR lies in the fact that genetic variations are randomly distributed during meiosis and remain unaltered post-fertilization, thereby enabling MR to circumvent the limitations inherent in conventional epidemiological studies, such as confounding factors, reverse causation, and selection biases. While MR provides valuable insights into genetic associations, it is important to emphasize that MR exclusively evaluates the genetic component of traits and does not account for environmental or lifestyle confounders. Despite its potential, the causal association between immune cells and low back pain has not been explored using MR. Therefore, we undertook a bi-directional two-sample MR analysis to probe this potential causal connection, with the ultimate goal of laying a theoretical groundwork for future investigations into the complex mechanisms and risk factors of LBP.

Materials and Methods

Study Design

To determine the relationship between immune cells and low back pain, we conducted a bi-directional two-sample MR study using data from publicly accessible Genome-Wide Association Studies (GWASs). The MR methodology hinges on three core assumptions: 1) The genetic variants used as IVs have a significant association with the exposure; 2) These genetic variants are not linked to any confounding factors; 3) There is no direct link between the genetic variation and the outcome, except through the exposure. The design of our study is depicted in Figure 1.

Data Sources

To conduct the MR analyses, we utilized inheritable IVs of LBP sourced from FinnGen, covering 13,178 cases and 164,682 controls. The GWAS summary statistics for each immune trait can be accessed from the GWAS Catalog

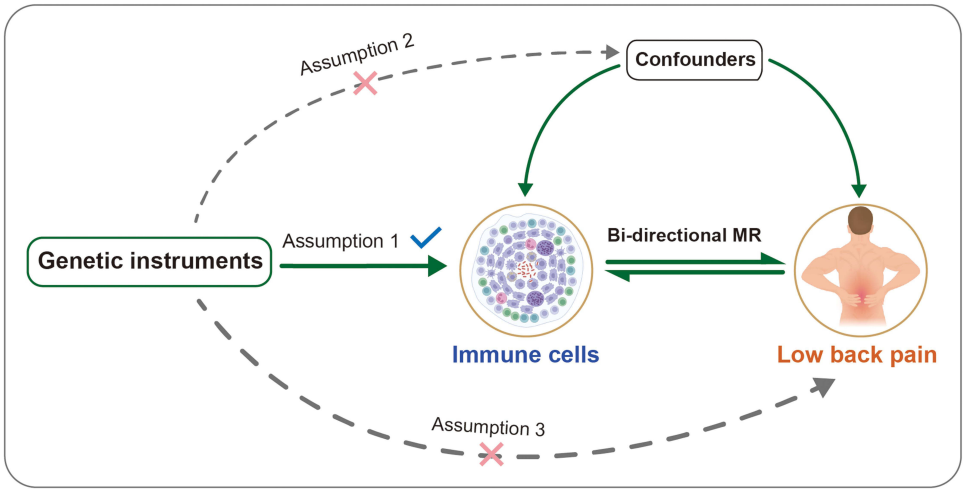

Figure 1 The overview of the study design.
Abbreviation: MR, Mendelian randomization.

(accession numbers range from GCST90001391 to GCST90002121).[12] Our research included four categories of immune signatures: Median Fluorescence Intensities (MFI), Relative Cell (RC) counts, Absolute Cell (AC) counts, and Morphological Parameters (MP). We examined a total of 731 immunophenotypes, comprising AC counts (n = 118), MFI indicating surface antigen levels (n = 389), MP (n = 32), and RC counts (n = 192). Specifically, the MFI, AC, and RC features encompass B cells, CDCs, mature stages of T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg panels. The MP feature includes CDC and TBNK panels. The original GWAS on immune traits was conducted using data from 3757 European individuals with no overlapping cohorts. Approximately 22 million SNPs genotyped with high-density arrays were imputed with a Sardinian sequence-based reference panel,[13] and associations were tested after adjusting for covariates such as sex and age. Comprehensive information on all genetic datasets used in this study can be found in Table S1.

Selection and Validation of SNPs

In accordance with recent studies,[12,14] we established the significance level of IVs for each immune cell at 1×10^{-5}. We assessed the independence among the chosen SNPs based on pairwise Linkage Disequilibrium (LD). The LD across these SNPs was calculated using the 10,000 genomes LD clumping ($r^2 < 0.001$).[15] To gauge the strength of the IVs, we utilized F statistics. The R^2 and F statistic of each SNP were computed using the formulas: $R^2 = 2 \times EAF \times (1 - EAF) \times \beta^2$, and F statistic = $R^2 \times (N - 2) / (1 - R^2)$. IVs with an F-statistic less than 10 were deemed as weak instruments.[16] The IVs that were ultimately employed are presented in Table S1.

Statistical Analysis

In this research, three models were employed for MR analysis to precisely analyze causal link between immune traits and TMD-related pain, include IVW, MR-Egger and Weighted mode. The IVW method was employed as major MR analysis that leveraged the impact of Single Nucleotide. The main analysis was conducted using the IVW analysis. To detect and eliminate potential pleiotropic instruments, we utilized the Mendelian Randomization Pleiotropy Residual Sum and Outlier test (MR-PRESSO).[17] Outlier instrumental variables pinpointed by the MR-PRESSO analysis were progressively removed to reduce the influence of horizontal pleiotropy. Furthermore, we performed sensitivity analyses using the weighted median and MR-Egger methods. The weighted median method can provide valid estimates if valid IVs account for more than 50% of the information. The MR-Egger method aids in the assessment of horizontal pleiotropy of chosen IVs.[18] Cochrane's Q-value can signify heterogeneity among chosen IVs.[19] Additionally, we implemented a leave-one-out analysis to determine whether a SNP affected significant results. All statistical analyses were executed using the "TwoSampleMR" and "MRPRESSO" packages in R version 4.2.3.

Results

Genetically Predicted Immune Cells on the Risk of Low Back Pain

The analysis, post the assessment and elimination of SNPs associated with confounding, is illustrated in Figure 2. We identified protective effects of six types of immune cells on LBP: CD4 Treg AC (Treg panel), CD19 on $CD20^-$ $CD38^-$ (B cell panel), CD4 on HLA DR^+ $CD4^+$ (TBNK panel), CD25 on $CD39^+$ $CD4^+$ (Treg panel), CD14 on CD33br HLA DR^+ $CD14^{dim}$ (Myeloid cell panel), and CD4RA on TD $CD4^+$ (Maturation stages of T cell panel). Our MR analysis revealed that an increase in CD4 Treg AC was associated with a lower risk of LBP (OR, 0.925; 95% CI, 0.878–0.974; $P = 0.003$). An increase in CD19 on $CD20^-$ $CD38^-$ demonstrated a protective effect on LBP (OR, 0.938; 95% CI, 0.898–0.979; $P = 0.003$), and the presence of CD4 on HLA DR^+ CD4+ also correlated with LBP (OR, 0.947; 95% CI, 0.909–0.987; $P = 0.010$). Additionally, the OR of CD25 on $CD39^+$ $CD4^+$ on LBP risk was estimated to be 0.954 (95% CI = 0.922–0.988; $P = 0.008$) using the IVW method. Genetic components (signature) contributing to the levels of CD14 staining on CD33br HLA DR^+ $CD14^{dim}$ on risks of LBP (95% CI = 0.916–0.985; $P = 0.006$), with the weighted median also supporting this association. Interestingly, an inverse relationship was noted between CD4RA on TD $CD4^+$ and disease risk (OR, 1.030; 95% CI, 1.012–1.048; $P = 0.001$), implying a protective function of lymphocytes in LBP. Sensitivity analyses revealed no genetic polymorphism bias in any of the analyses involving genetically predicted immune cells, and

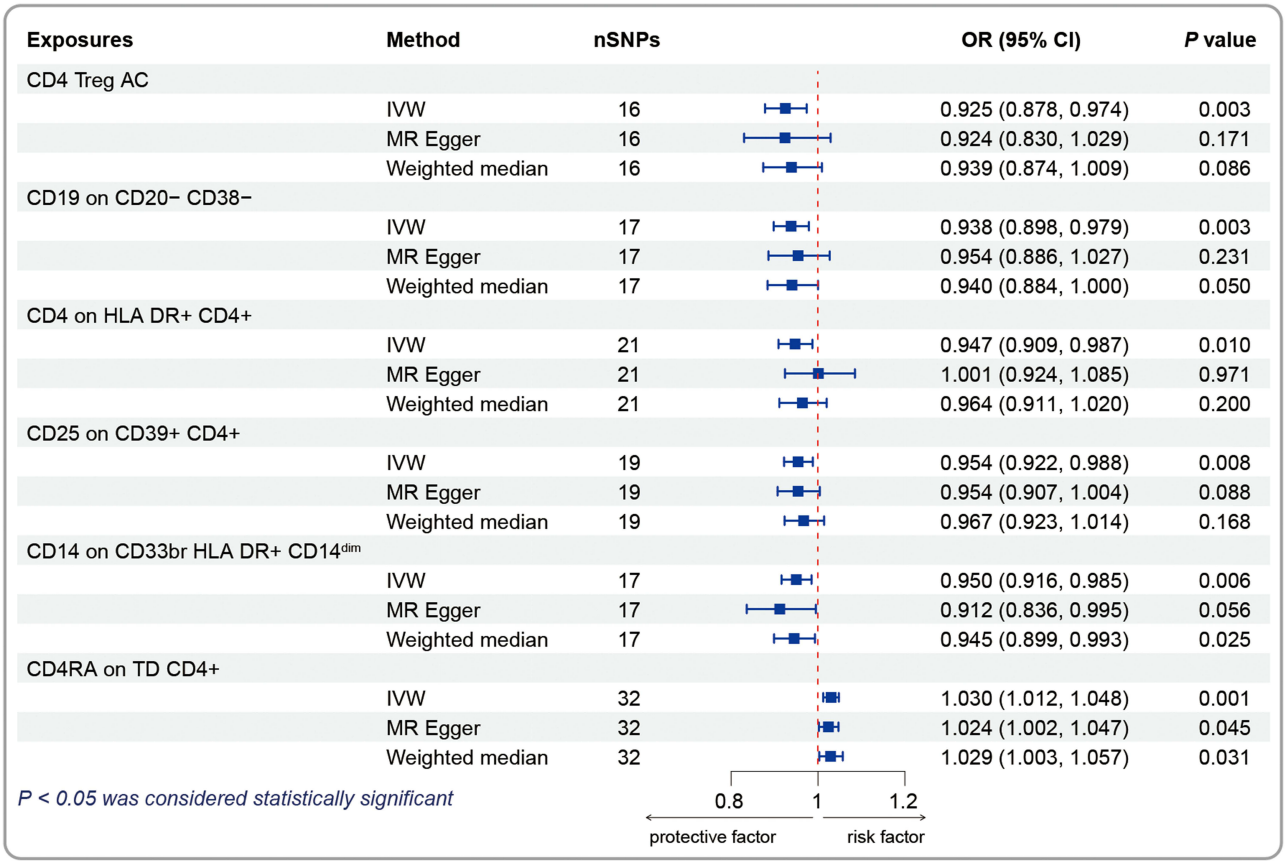

Figure 2 Forest plots summarizing the Mendelian randomization results of immune cells with a causal relationship to low back pain.
Abbreviations: nSNPs, number of single-nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization.

scatter plots affirmed the stability of the results (Figure 3A–F). To rigorously evaluate the stability of our MR estimates, we conducted a leave-one-out sensitivity analysis by iteratively excluding each individual SNP and recalculating the IVW association metrics. As demonstrated in (Figure 4A–F), this approach revealed consistently robust effect estimates across all SNP exclusion iterations, with no single variant significantly altering the pooled effect magnitude ($P > 0.05$ for all comparisons). Furthermore, no evidence of unbalanced horizontal pleiotropy was detected in any MR analysis (Table 1). These results further validate the reliability of the MR analysis against potential bias from outlier genetic variants.

Lower Back Pain Does Not Influence Peripheral Blood Cells Characteristics

In the bi-directional analyses executed using the IVW method, we found no evidence of potential causal effects of genetically predicted LBP on immune cells, such as CD4 Treg AC (Treg panel), CD19 on $CD20^{-}$ $CD38^{-}$ (B cell panel), CD4 on HLA DR^{+} $CD4^{+}$ (TBNK panel), CD25 on $CD39^{+}$ $CD4^{+}$ (Treg panel), CD14 on CD33br HLA DR^{+} $CD14^{dim}$ (Myeloid cell panel), and CD4RA on TD $CD4^{+}$ (Maturation stages of T cell panel). The outcomes of the sensitivity analyses are encapsulated in Figure S1. Scatter plots further underscored the stability of the results (Figure S2). We detected no pleiotropy and heterogeneity in the MR-Egger intercept test and Cochran's Q test (Table S2). The leave-one-out analysis also indicated that no individual SNP had a disproportionate impact on the overall estimates (Figure S3).

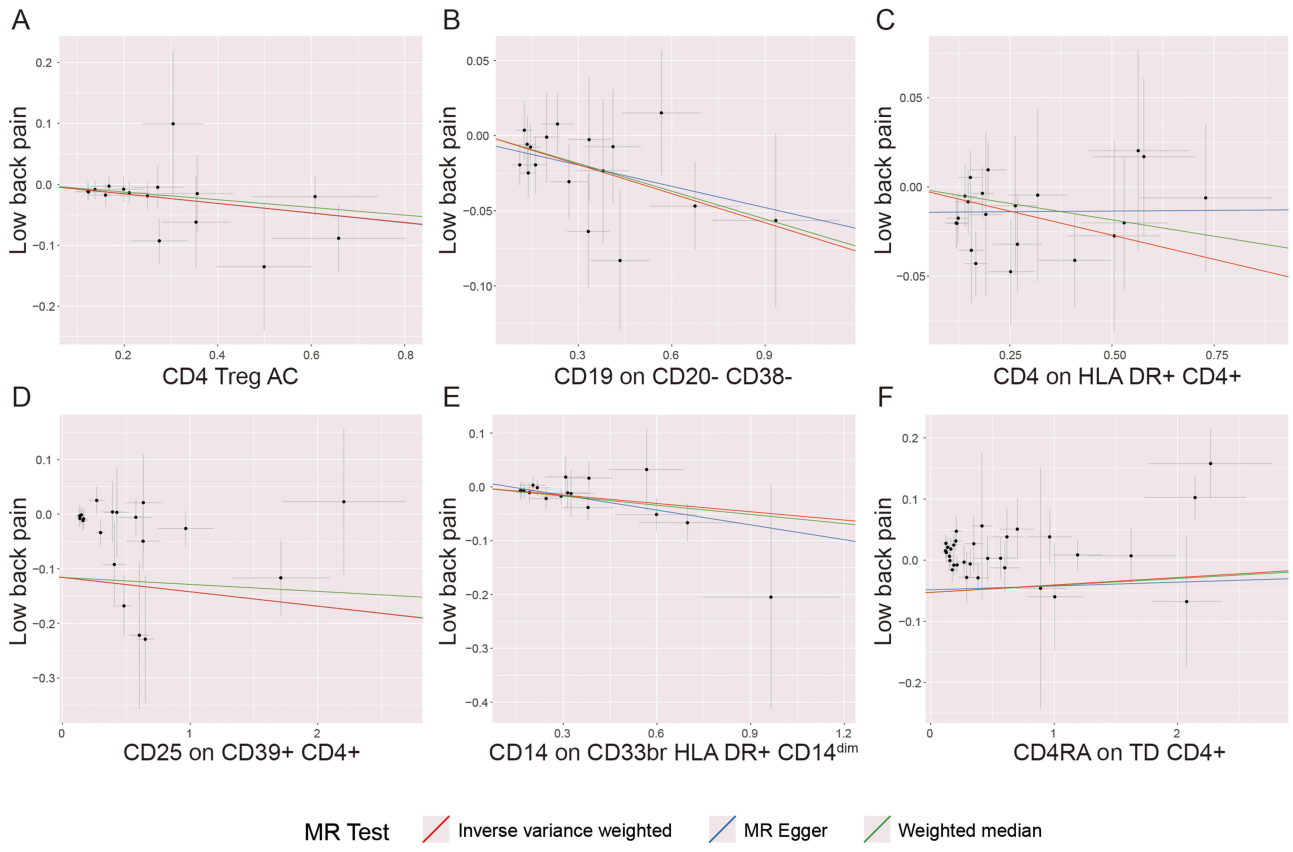

Figure 3 Scatter plots of potential effects of SNPs on immune cells and the risk of low back pain. (**A**) Scatter plot for analysis of CD4 Treg AC and low back pain; (**B**) Scatter plot for analysis of CD19 on CD20$^-$ CD38$^-$ and low back pain; (**C**) Scatter plot for analysis of CD4 on HLA DR$^+$ CD4$^+$ and low back pain; (**D**) Scatter plot for analysis of CD25 on CD39$^+$ CD4$^+$ and low back pain; (**E**) Scatter plot for analysis of CD14 on CD33br HLA DR$^+$ CD14^{dim} and low back pain; (**F**) Scatter plot for analysis of CD4RA on TD CD4$^+$ and low back pain.

Discussion

Using a large amount of publicly available genetic data, we investigated the causal associations between 731 immune cell traits and LBP. To our knowledge, this is the first two-sample MR analysis to examine the causal relationship between immune cells and LBP. Through this study, we identified causal effects of six types of immune cells on LBP. Recent studies have highlighted a strong correlation between immune cells, inflammation, and LBP, especially in the context of Lumbar Disc Herniation (LDH).[20] LDH is the most prevalent condition associated with LBP and has a significant impact on individuals' health.[21] The immune and inflammatory responses that occur in the spinal cord play a crucial role in the progression of LBP caused by LDH.[22]

Our study unveiled that an elevation in the proportion of CD4 Treg AC was linked to a reduced risk of LBP (OR, 0.925; 95% CI, 0.878–0.974; P = 0.003). CD4 Treg cells, which were initially identified by their unique cytokine production pattern, play a crucial role in maintaining peripheral tolerance.[23] These cells regulate immune responses in transplantation, allergy, and autoimmune diseases through a mechanism that is dependent on IL-10 and TGF-beta.[24] A recent study has confirmed our findings by demonstrating an association between CD4 Treg and a decreased risk of LBP.[25] CD19, a protein integral to the immune system, particularly in B cells, is often used as a marker for B cells in health and disease. This finding aligns with another study that identified a non-physiologic CD19$^+$/CD3$^+$ T cell population in a leukapheresis product undergoing chimeric antigen receptor T cell manufacturing.[26] These findings underscore the intricate relationship between immune cell populations and the risk and progression of LBP, paving the way for further research in this area.

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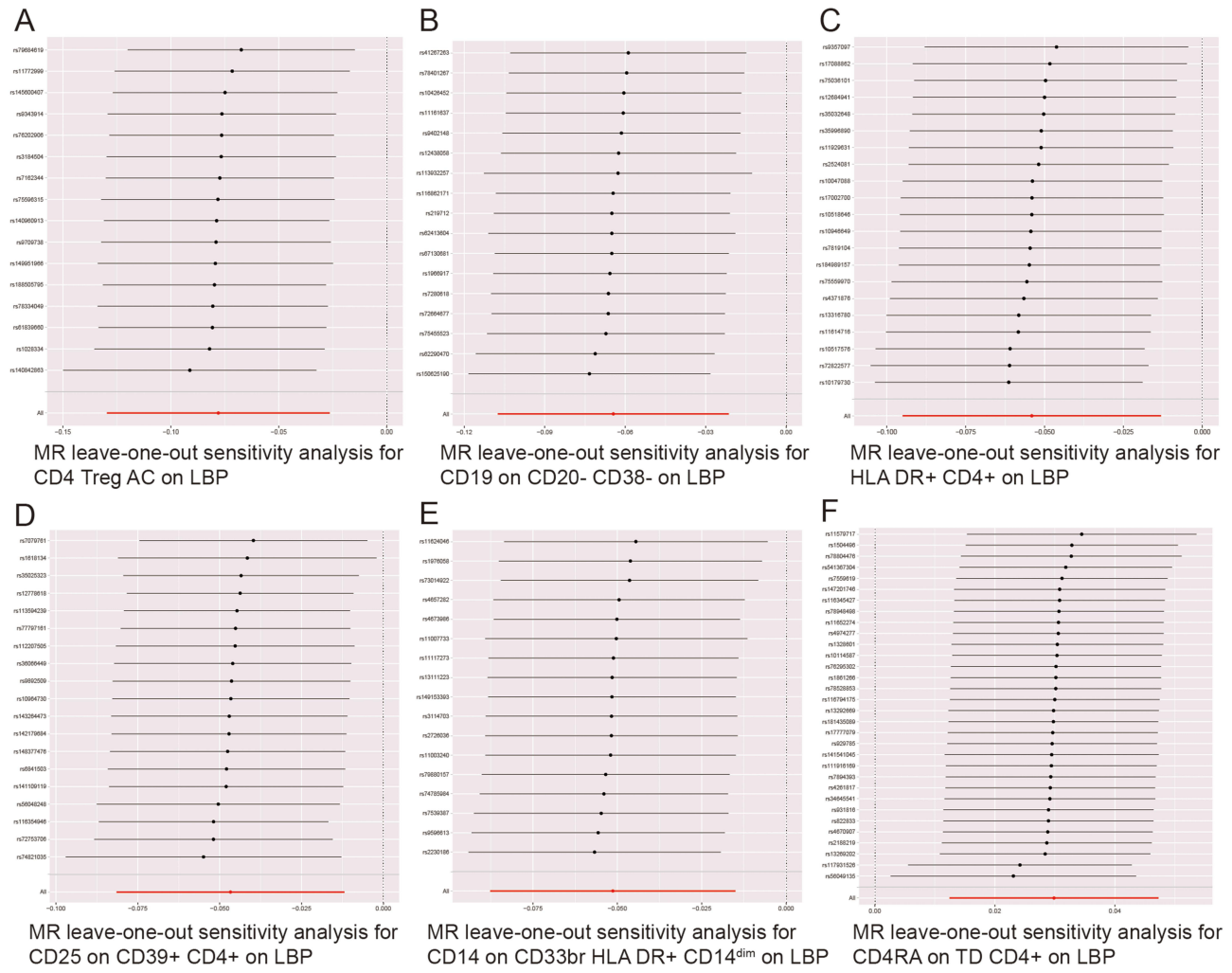

Figure 4 MR leave-one-out sensitivity analysis for immune cells on low back pain. (**A**) MR leave-one-out sensitivity analysis for CD4 Treg AC on LBP; (**B**) MR leave-one-out sensitivity analysis for CD19 on CD20$^-$ CD38$^-$ on LBP; (**C**) MR leave-one-out sensitivity analysis for HLA DR$^+$ CD4$^+$ on LBP; (**D**) MR leave-one-out sensitivity analysis for CD25 on CD39$^+$ CD4$^+$ on LBP; (**E**) MR leave-one-out sensitivity analysis for CD14 on CD33br HLA DR$^+$ CD14^{dim} on LBP; (**F**) MR leave-one-out sensitivity analysis for CD4RA on TD CD4$^+$ on LBP.

Abbreviations: MR, Mendelian randomization; LBP, low back pain.

Our study delved into the role of CD4, a protein crucial to the immune system, particularly in T cells, and HLA-DR, a component of the HLA system central to the immune system.[27,28] While these have been loosely associated with LBP in observational studies, our MR results unveiled a significant relationship between CD4 on HLA DR$^+$ CD4$^+$ and LBP

Table 1 Pleiotropy and Heterogeneity Analyses for the Association of Immune Cells With LBP

Exposures	nSNPs	Cochrane's *Q* Test		MR-Egger Pleiotropy Test			MR-PRESSO	Global *P*
		Q Value	*P* Value	Intercept	SE	*P* Value		
CD4 Treg AC	16	7.953	0.926	0.000	0.012	0.980	8.986	0.941
CD19 on CD20$^-$ CD38$^-$	17	8.736	0.924	−0.005	0.010	0.583	9.711	0.943
CD4 on HLA DR$^+$ CD4$^+$	21	14.632	0.797	−0.014	0.009	0.130	16.233	0.805
CD25 on CD39$^+$ CD4$^+$	19	18.393	0.430	0.000	0.009	0.990	19.870	0.493
CD14 on CD33br HLA DR$^+$ CD14^{dim}	17	6.769	0.977	0.012	0.012	0.331	7.748	0.977
CD4RA on TD CD4$^+$	32	30.227	0.506	0.004	0.005	0.420	33.355	0.480

Abbreviations: LBP, low back pain; nSNPs, number of single-nucleotide polymorphisms; MR, Mendelian randomization; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier test; SE, standard error.

(OR, 0.947; 95% CI, 0.909–0.987; P = 0.010). It's important to note that our understanding of human biology is continually evolving, and future research may uncover currently unknown connections. CD25, predominantly expressed on the surface of immune-related cells including activated T cells, B cells, and NK cells, was also examined in this MR study.[29] Our research established a causal connection between CD25 on $CD39^+$ $CD4^+$ and LBP, providing genetic evidence that CD25 on $CD39^+$ $CD4^+$ serves as a protective agent against LBP. CD14, predominantly found in macrophages, is utilized as a monocyte marker. In the context of LBP, a multitude of studies have posited that macrophages are a significant source of inflammatory cytokines and pain-associated mediators.[30,31] A recent investigation demonstrated that macrophages are the main producers of inflammatory cytokines, such as TNF-α and IL-1β.[32] Miyagi M and his team discovered that $CD14^+$ cells control the expression of pain-associated molecules through inflammatory cytokines in an autocrine or paracrine manner, highlighting their importance in discogenic LBP.[33] This insinuates that $CD14^+$ cells may play a pivotal role in the pathology of chronic discogenic LBP in humans. Moreover, our MR analysis unveiled a causal connection between CD4RA on TD $CD4^+$ and the occurrence of LBP. CD4RA is a marker used to differentiate naive and memory T cells.[34] Currently, there is a scarcity of studies on the role of CD4RA in LBP. Consequently, further investigation is warranted to uncover the potential role of CD4RA in the progression of LBP.

Prior research has underscored the significance of immune cells and inflammation in the onset of LBP. However, the role of immune factors in pathogenesis and their contribution to LBP remain enigmatic, thereby hindering the assessment of pain and treatment effects. A recent investigation suggested that active immune mechanisms aid in adaptation during the acute pain phase, and a deficiency in such inflammatory responses in individuals with acute LBP heightens the likelihood of progressing to chronic pain.[35] These adaptive inflammatory responses are inherently driven by transcription, potentially impacted by both genetic and environmental elements, and can be inhibited by steroids and nonsteroidal anti-inflammatory drugs (NSAIDs). Given that LBP involves a complex interplay between the nervous and immune systems, the relationship and interaction between immune cells and LBP are expected to be further elucidated with the rapid progression of bioinformatics technology.

The strength of our research lies in its examination of the impact of immune cells on LBP through genetic analysis, leveraging data from GWAS sources. To satisfy the assumptions of MR studies, it is crucial to rigorously check for horizontal pleiotropy and heterogeneity. We employed the MR-PRESSO global test to pinpoint outliers and illustrate pleiotropy. The robustness of our significant results was further confirmed by eliminating heterogeneity using Cochrane's Q test and performing a leave-one-out sensitivity analysis. Although our study offers important insights, it has its limitations. To begin with, the GWASs we analyzed were mainly conducted on individuals of European descent, which might restrict the applicability of our results to other ethnicities, such as East Asians. Secondly, we used a more relaxed threshold to assess the results, which, while enabling a more thorough evaluation of the strong link between the immune profile and LBP, might also heighten the chance of false positives. Thirdly, a central limitation of our study lies in the MR framework itself, which isolates genetic effects but cannot disentangle these from environmental influences that may modify or confound observed associations. For instance, socioeconomic factors, lifestyle choices, or epigenetic modifications could critically shape the hematological traits under investigation. Finally, the data utilized in this study are summary statistics, implying that individual information is not accessible. This renders it unfeasible to precisely compute the sample overlap between exposure and outcome. These limitations underscore areas for further improvement in subsequent research.

Conclusion

This study represents the first MR analysis to delve into the causal relationship between immune cells and LBP. By leveraging genetic data, our MR analysis unveiled the intimate connection between immune cells and LBP. This not only enhances our understanding of the disease but also provides valuable guidance for future clinical research, potentially paving the way for novel therapeutic strategies.

Acknowledgment

We extend our gratitude to the IEU Open GWAS database for providing the necessary data for this study. We also express our appreciation to the investigators and participants of the FinnGen study for their invaluable contribution in sharing data.

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 author(s) report no conflicts of interest in this work.

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