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ORIGINAL RESEARCH

The Causal Relationship Between Immune Cells and Neuropathic Pain: A Two-Sample Mendelian Randomization Study Based on Genome-Wide Association Analysis

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Purpose: Increasing evidence indicates that various types of immune cells are associated with different forms of neuropathic pain (NP). However, the causal relationships among these associations remain unclear. To elucidate the causal relationships between immune cells and NP, we conducted a two-sample Mendelian randomization (MR) analysis.

Patients and Methods: The exposure and outcome Genome-wide association analysis (GWAS) data used in this study were obtained from open-access databases. This study employed a two-sample MR analysis to evaluate the causal relationships between 731 immune cell traits and four types of NP, including postherpetic neuralgia (PHN), trigeminal neuralgia (TN), diabetic peripheral neuropathy (DPN), and drug-induced peripheral neuropathy (DIPN).

Results: The relative count of CD39+ CD4+ %T cells was positively associated with TN, while the mean fluorescence intensity (MFI) of CD20 on IgD+ CD38br (B cell) and forward scatter area (FSC-A) on myeloid dendritic cells (DCs) were negatively associated with TN. Additionally, the relative count of CD8br NKT %lymphocytes was positively associated with PHN, and the MFI of HLA DR on CD33br HLA DR+ CD14 (myeloid cells) was negatively associated with PHN. The MFI of CD4 on activated and secreting T regulatory (Treg) cells was positively associated with DPN. Furthermore, the relative count of B cell % CD3- lymphocytes was negatively associated with DIPN.

Conclusion: This MR study, using genetic data from individuals of European descent, provides evidence supporting the causal relationships between several specific immune cell phenotypes and various NP subtypes.

Keywords: Mendelian randomization, immune cell, neuropathic pain, causal relationship, genome-wide association analysis

Introduction

Neuropathic pain (NP) arises from direct injury or disease affecting the somatosensory nervous system. Conditions encompassed by NP include trigeminal neuralgia (TN), postherpetic neuralgia (PHN), diabetic peripheral neuropathy (DPN), and drug-induced peripheral neuropathy (DIPN).[1] NP is estimated to affect about 7% to 10% of the global population.[2] The severe and persistent nature of NP can greatly diminish patients' physical and mental health, imposing a significant economic burden.

Despite continuous research efforts, the precise mechanisms behind NP are still not fully understood. Although opioid medications are commonly prescribed, they often fail to offer adequate relief and are associated with considerable side effects.[3] Recent studies have highlighted the critical importance of the interaction between the immune and nervous systems in NP. Strong evidence indicates that neuroinflammation contributes to both peripheral and central sensitization, resulting in hyperalgesia. Almost all components and cell types within the immune system, encompassing both innate and adaptive immunity, have been linked to neuropathic pain.[4–6]

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The innate immune system comprises diverse cell types including neutrophils, macrophages, dendritic cells (DCs), mast cells, and natural killer (NK) cells. Traditionally believed that these cells recognize pathogens and danger signals via conserved molecular patterns, triggering intracellular signaling pathways that result in the release of inflammatory mediators essential for neuropathic pain development.[4] The adaptive immune system functions through antigen-specific cellular and humoral responses involving T cells and B cells. Each T and B cell clone possesses unique antigen receptors, ensuring high specificity. Traditionally, the adaptive immune system is seen as crucial in sustaining neuropathic pain.[4] Nonetheless, recent research indicates that specific subpopulations of these cells might also aid in alleviating neuropathic pain. A study exploring immune cells in the cerebrospinal fluid of patients with PHN and patients with polyneuropathy noted that the frequency of CD56+ NK cells was negatively correlated with mechanical pain sensitivity (MPS), and in patients with herpes zoster, the frequency of CD8+ cytotoxic T cells correlated with MPS, however, in polyneuropathy patients, CD8+ cytotoxic T cell frequency correlated with reduced MPS.[7] MRC1+ macrophages were found to inhibit inflammation and ameliorate mechanical pain after nerve injury in peripherally nerve-injured mice.[8] CD4+CD25+Foxp3 + regulatory T cells (Tregs), which reduce T cell proliferation and proinflammatory cytokine production, play a role in the endogenous recovery of neuropathy-induced pain.[9] However, current findings on the association between immune cells and NPs are inconsistent. It may be related to the pathophysiological mechanisms of different NPs, and therefore the relationship between different immune cell phenotypes and different pain phenotypes needs to be clarified.

Mendelian randomization (MR) is a statistical technique that leverages genetic variation as an instrumental variable to estimate causal relationships between exposure and outcome. Predominantly utilized in epidemiological causal inference, this method aids researchers in minimizing bias in experimental results, thereby enabling the derivation of causal conclusions.[10] Previous MR studies have explored the causal relationship of blood cells, gut microbiota and inflammatory factors with multiple NPs[11–13]。 In this study, we will conduct a two-sample MR analysis to detect potential causal relationships between different types of immune cells and the risk of four types of neuropathic pain (including TN, PHN, DPN, and DIPN), aiming to provide new possibilities for future therapeutic strategies.

Materials and Methods

Ethical Statement

Our study was submitted to the Ethics Committee of The First Affiliated Hospital of Fujian Medical University for review and was exempted from ethical approval as it solely involves the analysis of publicly available summary-level data from genome-wide association studies (GWAS). The GWAS summary statistics utilized in our MR study were all sourced from the Integrative Epidemiology Unit (IEU) Open GWAS Project.[14]

Study Design

This study utilizes a two-sample MR approach, leveraging single nucleotide polymorphisms (SNP) loci from genome-wide association studies (GWAS) databases as instrumental variables to examine the risk influence between immune phenotypes and NP. A total of 731 immune cell traits were identified as exposure factors associated with the occurrence of four types of NP, constituting the study outcomes. The MR analysis adheres to three fundamental principles: firstly, the exposure (immune phenotypes) must be strongly associated with the selected SNPs; secondly, these SNPs must not be influenced by external confounding factors; and thirdly, these polymorphisms should not be directly related to the outcome NP, ensuring that their causal relationship does not influence the outcome through pathways other than the immune phenotype.[15]

GWAS of Exposure Data Sources

The GWAS summary statistics for each immune trait are publicly accessible from the GWAS Catalog (accession numbers GCST0001391 to GCST0002121).[16] The data were collected from 3,757 individuals from Sardinia, encompassing 20,143,392 SNPs. In this study, 731 immune cell phenotypes were measured using flow cytometry and classified into four categories: absolute cell (AC) counts (n = 118), median fluorescence intensity (MFI) indicating surface antigen levels (n = 389), morphological parameters (MP) (n = 32), and relative cell (RC) counts (n = 192). The seven types of

immune cells analyzed in our study include T cells, B cells, DCs, monocytes, other myeloid cells, NK cells, and Treg cells. Specifically, peripheral blood samples were collected from donors via standardized venipuncture and immediately processed for antibody panel staining prior to flow cytometric analysis. Flow cytometric data acquisition was performed using two BD FACSCanto II flow cytometers with standardized configurations, followed by analysis using BD FACSDiva software (BD Biosciences). Cell populations were manually gated by trained personnel to ensure data processing consistency. All immunophenotyping procedures were completed at the designated collection center within 2 hours post-collection to minimize time-dependent analytical variability.

GWAS Data Sources for Outcome

The target NPs include TN, PHN, DPN, and DIPN. Extensive GWAS genetic data were obtained from the Finnish biobank. The TN dataset, sourced from "finn-b-G6_TRINEU", includes 800 cases and 195,047 controls, encompassing 16,380,408 SNPs. The PHN dataset, from "finn-b-G6_POSTZOST", contains 144 cases and 195,047 controls, with 16,380,406 SNPs. The DPN dataset, derived from "finn-b-DM_NEUROPATHY", comprises 1,415 cases and 162,201 controls, including 16,380,195 SNPs. The DIPN dataset, from "finn-b-G6_DRUGPOLY", includes 93 cases and 215,718 controls, encompassing 16,380,463 SNPs. All data were sourced from European populations. The FinnGen study, initiated in 2017, is a nationwide cohort aimed at collecting and evaluating genomic and health data from 500,000 participants in the Finnish biobank.[17] NPs were identified based on International Classification of Diseases (ICD) codes retrieved from the Finnish national registries.

Identification of SNPs

The samples from the exposure and outcome datasets did not overlap, allowing for an unbiased assessment of the causal relationship between immune phenotypes and NP. Specific screening steps were followed, utilizing the TwoSampleMR package to extract relevant SNPs.[18] The significance threshold for the instrumental variable (IV) of each immune trait was set at 1×10-5.[19] Linkage disequilibrium (LD) was checked (r^2 = 0.001, kb = 5,000), and SNPs in LD were eliminated, along with palindromic SNPs. To avoid bias from weak instruments, the F-statistic was used to estimate the IV strength for each immune phenotype, and SNPs with low F-statistics (< 10) were removed.[20]

Statistical Analysis

All analyses were conducted using R 4.3.2 (http://www.Rproject.org). To assess the causal relationship between immune cell traits and NP, we primarily used the "MendelianRandomization" package (version 0.9.0). Various methods, including Inverse Variance Weighted (IVW), MR Egger, Weighted Median (WM), Simple Mode, and Weighted Mode, were employed for robust analysis to mitigate the influence of outliers. IVW was used as the main MR method because it provides reliable causal estimates with the highest statistical power if the selected SNPs meet the instrumental variable assumptions.[21] MR Egger and Weighted Median were used to assess the robustness of the results, with the MR-Egger method offering unbiased estimates

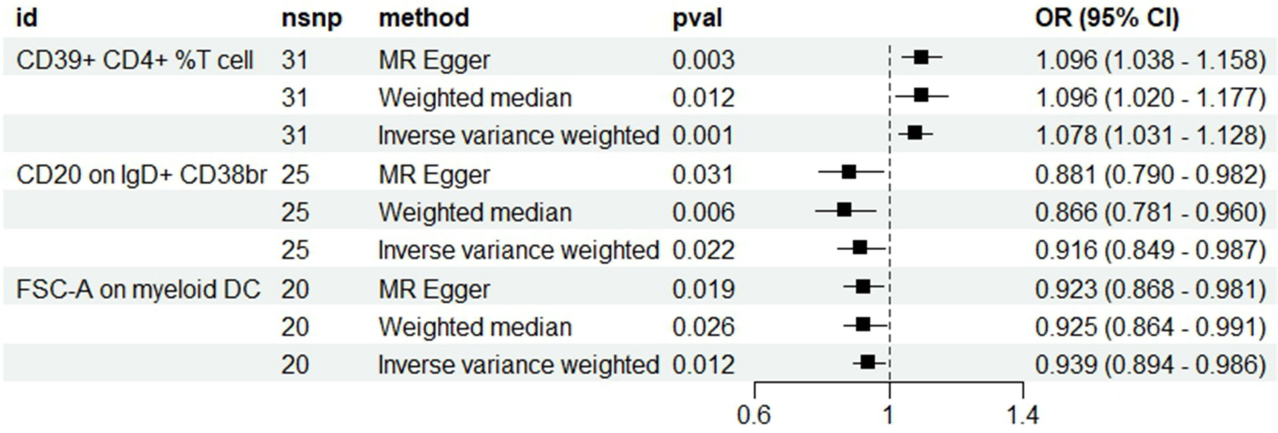

Figure 1 Forest plots showed the causal of immune cell traits on TN by using different methods.

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Table 1 Sensitivity Analysis for Immune Cell Traits on TN by MR Analysis

Exposure	Horizontal Pleiotropy		Heterogeneity	MR-PRESSO Global Test
	MR Egger-Interpreter	*p* Value	Cochran's Q *p* Value	*p* Value
CD39+ CD4+ %T cell	−0.012	0.312	0.823	0.841
CD20 on IgD+ CD38br	0.015	0.336	0.719	0.733
FSC-A on myeloid DC	0.014	0.355	0.422	0.507

Abbreviations: OR, odd radio; CI, confidence interval.

but with relatively lower statistical power.[22] The Weighted Median method requires that at least 50% of the information comes from valid instrumental variables.[23] Cochran's Q value and P value were calculated to assess heterogeneity among the estimated SNP effects. If heterogeneity was present, a random-effects model of IVW was used for causal inference.[24]

Sensitivity analyses were conducted using the MR-Egger intercept test and the MR-PRESSO method to evaluate horizontal pleiotropy.[22,25] A statistically significant intercept term in the MR-Egger intercept test indicates significant horizontal pleiotropy in the MR analysis. The MR-PRESSO method was employed to validate the IVW model results and correct for outlier influence. Finally, a leave-one-out sensitivity test was performed by systematically removing each SNP and repeating the IVW analysis to assess the consistency of the causal effect driven by individual SNPs. Funnel plots and forest plots were constructed to visualize horizontal pleiotropy in the MR analysis.

Results

MR Analysis Results

We used IVW, MR Egger, and WM methods to evaluate the causal relationship between different immune phenotypes and NP. After initial screening, three immune phenotypes showed potential causal relationships with TN, two with PHN, one with DPN, and one with DIPN. All instrumental variables had F-statistics generally >10, indicating no evidence of weak instrument bias. Reverse Mendelian randomization analysis was conducted to assess the impact of TN on immune phenotypes. Despite significant differences in the results from different analytical methods, none simultaneously met the MR-Egger intercept test and MR-PRESSO method criteria, indicating the presence of horizontal pleiotropy and leading to unreliable results.

Causal Effect of Immune Cells on TN

Our findings indicate that three types of immune cells exhibit potential causal relationships with TN. Specifically, the relative count of CD39+ CD4+ %T cells was positively associated with TN. Conversely, the MFI of CD20 on IgD+ CD38br (B cells) and the MP of FSC-A on myeloid DCs were negatively associated with TN. Using the random-effects model of the IVW method, the results were as follows: CD39+ CD4+ %T cell (OR=1.078, 95% CI: 1.031–1.128,

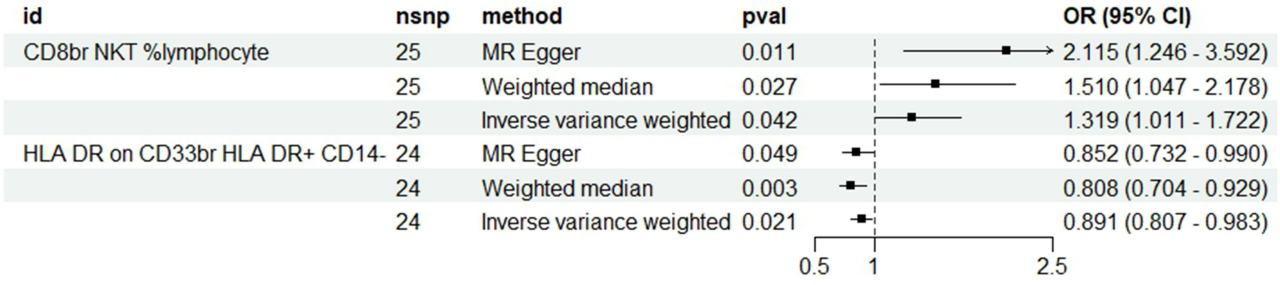

Figure 2 Forest plots showed the causal of immune cell traits on PHN by using different methods.

Table 2 Sensitivity Analysis for Immune Cell Traits on PHN by MR Analysis

Exposure	Horizontal Pleiotropy		Heterogeneity	MR-PRESSO Global Test
	MR Egger-Interpreter	*p* Value	Cochran's Q *p* Value	*p* Value
CD8br NKT %lymphocyte	−0.100	0.055	0.814	0.831
HLA DR on CD33br HLA DR+ CD14-	0.037	0.448	0.237	0.256

Abbreviations: OR, odd radio; CI, confidence interval.

p=0.001); CD20 on IgD+ CD38br (OR=0.916, 95% CI: 0.849–0.987, p=0.022); FSC-A on myeloid DC (OR=0.939, 95% CI: 0.894–0.986, p=0.012). No significant heterogeneity was observed in Cochran's Q heterogeneity test for these three causal associations (p > 0.05). The MR-Egger intercept analysis showed no significant directional pleiotropy for each immune phenotype (p > 0.05), indicating that SNPs did not significantly influence the outcome through factors other than the exposure. The MR-PRESSO analysis revealed consistent causal estimates before and after outlier correction, and the global analysis indicated no evidence of horizontal pleiotropy (p > 0.05). (Figure 1, Table 1)

Causal Effect of Immune Cells on PHN

Two immune cell traits showed significant causal associations with PHN risk. CD8br NKT %lymphocytes increased risk (IVW OR=1.319, 95% CI:1.011–1.722, p=0.042), whereas HLA DR expression on CD33br HLA DR+ CD14 myeloid cells reduced risk (OR=0.891, 95% CI:0.807–0.983, p=0.021). Sensitivity analyses confirmed robustness: no heterogeneity (Q-test p>0.05), no pleiotropy (Egger intercept p>0.05; MR-PRESSO p>0.05). (Figure 2, Table 2)

Causal Effect of Immune Cells on DPN

Higher CD4 MFI on activated/secreting Tregs was associated with elevated DPN risk (IVW OR=1.050, 95% CI:1.008–1.093, p=0.019). The association showed homogeneity (Q-test p>0.05) and passed all pleiotropy checks (Egger intercept p>0.05; MR-PRESSO p>0.05). (Figure 3, Table 3)

Causal Effect of Immune Cells on DIPN

B cell % CD3- lymphocytes showed a protective effect against DIPN (IVW OR=0.844, 95% CI:0.730–0.975, p=0.022). Sensitivity analyses supported reliability: no heterogeneity (Q-test p>0.05), no pleiotropy (Egger intercept p>0.05; MR-PRESSO p>0.05). (Figure 4, Table 4)

Discussion

Utilizing comprehensive publicly available genetic datasets, our research investigated the causal relationships between 731 immune cell phenotypes and four types of NP. We identified potential causal associations involving: three different immune cell types and TN, two distinct immune cell types and PHN, one immune cell type and DPN, and one immune cell type and DIPN. These results were consistently validated through a range of sensitivity and quality control analyses.

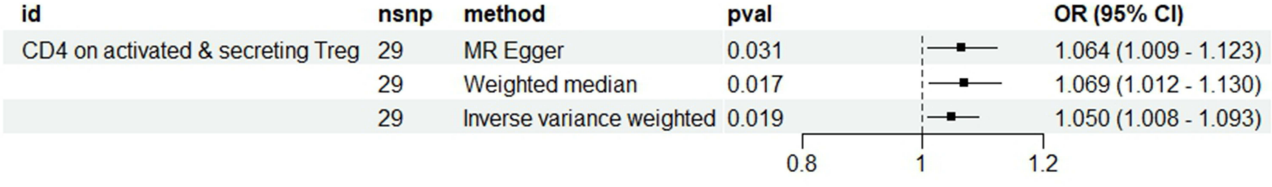

Figure 3 Forest plots showed the causal of immune cell traits on DPN by using different methods.

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Table 3 Sensitivity Analysis for Immune Cell Traits on DPN by MR Analysis

Exposure	Horizontal Pleiotropy		Heterogeneity	MR-PRESSO Global Test
	MR Egger-Interpreter	*p* Value	Cochran's Q *p* Value	*p* Value
CD4 on activated & secreting Treg	−0.010	0.448	0.350	0.396

Abbreviations: OR, odd radio; CI, confidence interval.

Among the various T cell subsets, evidence indicates that pro-inflammatory T cells play a pronociceptive role in NP, while anti-inflammatory T cells and Tregs have antinociceptive functions.[4] Our study found that the percentage of CD39+ CD4+ T cells is closely linked to TN. CD39 is generally considered a marker of Tregs but can also be expressed in conventional CD4+ T cells, often associated with Th17 effector functions.[26,27] Th17 cells primarily produce IL-17, which plays a significant pronociceptive role in mouse models of Chronic Constriction Injury (CCI) and Spared Nerve Injury (SNI).[28,29] Moreover, in a bone cancer pain (BCP) model, it was observed that during the progression of BCP, T cells infiltrate the spinal cord, causing a Th17/Treg imbalance that promotes microglial activation and increases IL-17/IL-17A levels, exacerbating BCP.[30] These findings may explain why CD39+ CD4+ T cells promote TN development. Additionally, we observed that CD4 on activated and secreting Tregs is associated with promoting DPN. This finding contradicts the traditional antinociceptive role of Tregs seen in animal models. Interestingly, studies in patients with various neuropathic pains have shown a significant increase in Tregs in their blood.[31–33] This may necessitate further investigation into the mechanism of Tregs in NP.

Our study found that CD20 on IgD+ CD38br B cells and the percentage of CD3- lymphocytes were protective factors for TN and DIPN, respectively. The role of B cells in neuropathic pain remains unclear. Some studies suggest that B cells do not participate in the onset and progression of peripheral neuropathic pain models.[34,35] Although there is no direct evidence supporting the analgesic role of B cells, it is hypothesized that certain anti-inflammatory B cell subsets, known as regulatory B cells (Bregs), might help alleviate neuropathic pain. Bregs can produce anti-inflammatory cytokines such as IL-10, IL-35, and TGF-β, induce Treg cell differentiation, and modulate autoimmunity.[36,37]

Our study identified FSC-A on myeloid DCs as a protective factor for TN. This protective effect might be linked to the synthesis of Specialized Pro-Resolving Mediators (SPMs) by DCs. SPMs are endogenous compounds, including resolvins, protectins, maresins, and lipoxins, which play a critical role in regulating neuroinflammation and neuropathic pain hypersensitivity.[38] Numerous preclinical and clinical studies in recent years have shown that SPMs can effectively alleviate various chronic pain conditions, such as inflammatory pain, NP, chemotherapy-induced peripheral neuropathy, and bone cancer pain.[39–41] Moreover, DCs can also contribute to neuropathic pain by enhancing the kynurenine metabolic pathway in mice.[42]

Research on the role of NK cells in chronic pain outcomes indicates that these cells are involved in pain relief. In patients with postherpetic neuralgia and multiple neuropathies, the frequency of NK cells is negatively correlated with mechanical pain sensitivity.[7] Patients with fibromyalgia, a chronic pain condition of unknown etiology, have significantly lower NK cell frequencies compared to healthy controls.[43,44] Additionally, NK cells in the blood of fibromyalgia patients exhibit higher levels of the degranulation marker CD107a+ and inhibitory receptor, suggesting recent activation followed

Figure 4 Forest plots showed the causal of immune cell traits on DIPN by using different methods.

Table 4 Sensitivity Analysis for Immune Cell Traits on DIPN by MR Analysis

Exposure	Horizontal Pleiotropy		Heterogeneity	MR-PRESSO Global Test
	MR Egger-Interpreter	*p* Value	Cochran's Q *p* Value	*p* Value
B cell % CD3- lymphocyte	0.047	0.286	0.914	0.871

Abbreviations: OR, odd radio; CI, confidence interval.

by an exhausted state.[44] In mice with sciatic nerve crush injury, NK cell-mediated clearance of damaged axons leads to an initial loss of nociception due to exacerbated myelin-axon degeneration and a subsequent reduction in mechanical allodynia.[45] However, some researchers argue that excessive NK cell activation might contribute to chronic pain following nerve injury.[46] This could be linked to the increased risk of PHN associated with higher percentages of CD8bright NKT lymphocytes.

Our study indicates that elevated levels of HLA DR+ CD14- cells within the immune system are associated with a reduced risk of PHN. CD14+ cells can directly or indirectly promote the expression of inflammatory cytokines and pain-related molecules, facilitating the onset and progression of pain. Blocking CD14 or using gene knockout approaches can effectively alleviate hyperalgesia in various pain models.[47,48]

MR uses genetic data to explore the causal relationship between an exposure and an outcome. Similar to how randomized controlled trials assign participants to either the experimental or control group, MR studies "randomize" participants based on alleles influencing risk factors, determining if carriers of these genetic variations have a different disease risk compared to non-carriers. Several previous MR studies have explored risk factors for NP. Liu et al[11] found that elevated levels of IFNγ and MCP3 were causally associated with a decreased risk of PHN and elevated levels of IL-16 were causally associated with a decreased risk of TN. Pan et al[12] reported causal associations between 26 intestinal bacterial taxa and 5 NPs. As well as platelet, erythrocyte, monocyte, and neutrophil responses were significantly correlated with NP risk.[13] Our study enriches the findings of NP in MR studies.

However, MR has its limitations. One limitation of our MR study is the lack of individual-related data of NP. Another limitation is that our study only included subjects of European descent, leaving the association between NP and immune cell phenotypes in Asian populations, such as those in China, unclear. After establishing a causal relationship, the next step is to explore the mechanisms by which immune cell phenotypes influence NP. Finally, our study is only a preliminary exploration of the relationship between immune cell phenotypes and NP, but more real-world studies are still needed to elucidate the long-term effects of specific immune cell phenotypes on specific NPs across time.

Conclusion

In conclusion, this MR study, using genetic data from individuals of European descent, provides evidence supporting the causal relationships between several specific immune cell phenotypes and various NP subtypes. This study may offer researchers new avenues to explore the pathophysiological mechanisms of various NP subtypes.

Data Sharing Statement

The data of this study are available from the corresponding author upon reasonable request.

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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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