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Exploring causal correlations between immune cells and diabetic neuropathy: a Mendelian randomization

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Abstract

Background Circulating immune cells reportedly affect diabetic neuropathy (DN). Although associations have been previously established between numerous biomarkers and diseases, elucidating their causal relationships remains challenging. Mendelian Randomization (MR) could overcome this difficulty by applying genetic instruments to discern causal links. In this study, we conducted bidirectional two-sample MR to address this problem.

Methods We used freely available genome-wide association study summary statistics. We obtained immune cell phenotype-related summary data from a study cohort comprising 3,757 Sardinian individuals that reported data concerning 731 immune cell phenotypes. We obtained DN-related summary data from the FinnGen database and conducted sensitivity analyses. Furthermore, we assessed horizontal pleiotropy using combined MR–Egger and MR–Presso methods. We evaluated heterogeneity using Cochran's Q test and applied False Discovery Rate correction to the findings.

Results Our MR analysis significantly associated 24 immune cell phenotypes with DN. Specifically, the presence of CD45 on CD66b++myeloid cells, HLA DR on CD14+CD16- monocytes, IgD- CD24- %B cells, and CD27 on IgD- CD38br lymphocytes significantly positively correlated with the risk of DN. In contrast, the presence of CD28- DN (CD4-CD8-) %T cells, FSC-A on HLA DR+T cells, and other four T cell types negatively correlated with DN. Finally, we further confirmed the relationship between different immune cell types and DN.

Conclusions We demonstrated the immunological susceptibility of DN and clarified how immune responses influence the course of DN. These findings might help inform immunological therapy techniques as well as novel targets for DN diagnosis and treatment.

Keywords Diabetic neuropathy, Immune cells, Mendelian randomization study, Causal relationship

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Background

The most common sequelae of diabetes are a set of clinical symptoms caused by autonomic and peripheral nervous system-related damage, commonly referred to as diabetic neuropathy (DN), affecting up to half of all patients with diabetes and characterized by focal and diffuse nervous system injuries [1]. The most common form of DN is diabetic peripheral neuropathy (DPN). Other diabetic diffuse neuropathies include the constellation of autonomic neuropathies, cardiac autonomic neuropathy, and gastrointestinal dysmotility [2].

Despite the rapid elucidation of the intricate DN etiology over the past 10 years, there is no approved precise treatments remain that could target injured nerves or halting DN progression. Current evidence suggests that DN-associated pathogenesis is complex. Specifically, insulin resistance (IR), dyslipidemia, and hyperglycemia induce multiple reactions that activate the polyol, glycolysis, hexosamine, and advanced glycation end-product pathways, resulting in endoplasmic reticulum stress, mitochondrial malfunction, DNA damage, and increased inflammatory factor levels, further intensifying oxidative stress and inflammatory signals, finally resulting in DN development [3–5]. Contrary to IL-6, observed in other microvascular problems such as diabetic retinopathy (DR) and DN, the main DPN-implicated proinflammatory cytokine is tumor necrosis factor-alpha (TNF-α) [6–8]. The phagocytic role activated macrophages fulfill results in demyelination, negatively affecting signal conduction in the nerves [9, 10]. Moreover, increased TNF-α levels adversely affect oligodendrocytes, potentially causing further demyelination [11]. Furthermore, TNF-α reportedly stimulated neurite development in sensory neurons in vitro through the nuclear factor kappa-B (NF-κB) pathway. Moreover, lipopolysaccharide (LPS)-stimulated macrophages reportedly produced relevant TNF-α levels in a high-glucose environment, whereas LPS did not significantly increase TNF-α in macrophages unstimulated by glucose levels [12].

Autoimmunity has been traditionally considered the primary pathophysiology underlying type 1 diabetes. However, to date, most studies have investigated type 2 diabetes pathophysiology from a metabolic perspective. Nonetheless, an increasing body of evidence exist concerning autoimmunity involvement in type 2 diabetes, including autoantibody identification, immune cell infiltration into target organs and related organ function alteration, last but not least the response of these individuals to immunosuppressive or immunoregulatory therapeutic interventions [13].

The function of immune cells and the occurrence of diabetic neuropathy are affected by many factors such as environment, heredity and disease, Even with the use of covert grouping techniques and random allocation, completely controlling all confounding factors in practice is extremely difficult, which invariably results in bias in the results. Mendelian randomization (MR) is an important Mendelian genetic principle-based analytical method for inferring causal relationships in epidemiology. Using published summary estimates from multiple large-scale Genome-Wide Association Studies (GWASs), the two-sample MR approach provides enhanced statistical power to determine the causal relationships between exposure variables and outcomes. Mendelian randomization has been increasingly applied over recent years to predict the efficacy and safety of existing and novel drugs targeting metabolic disease, such as cardiovascular and diabetes, risk factors and to explore the repurposing potential of available drugs [14]. Accordingly, in this study, we aimed to explore the association of 731 immune cell phenotypes with DN from a genetic variation perspective. Our discoveries could potentially help further elucidate the mechanisms underlying DN.

Methods

Study design

We examined the causal relationship between 731 immune cell characteristics and DN performing a bidirectional two-sample Mendelian randomization (MR) analysis. All genetic variations used as instrumental variables (IVs) had to meet three fundamental assumptions, i.e., the (1) association hypothesis (IV closely associated with immune cell phenotypes), (2) independence hypothesis (IV independent of outcome-affecting confounding factors), and (3) exclusivity hypothesis (IV can only affect the outcome through immune cell traits) [15]. Figure 1 presents the study flowchart (Fig. 1 Overview of research design and analysis strategy). All the data we used were obtained from public databases, ruling out the requirement of an additional ethical approval.

Data sources

GWAS data sources for immune traits

In this study, we used a large-scale GWAS on data regarding immune cell data(https://www.ebi.ac.uk/gwas/), Which involved 731 immune cell traits in the study, ranging from GCST90001391 to GCST90002121, including 3757 non-overlapping European participants. Furthermore, it used a high-density array generated from Sardinian sequence data (57% females), comprising ≈ 22 million single-nucleotide polymorphisms (SNPs). We applied flow cytometry to obtain all the data we used in this study. The 731 immune cell phenotypes included 118 absolute cell counts (AC), 389 median fluorescence intensities (MFIs) indicative of surface antigen expression, 32 morphological attributes (MP), and 192 relative cell counts (RC) [16]. Specifically, MP characteristics comprised classic dendritic cells (cDCs) and TBNK panels,

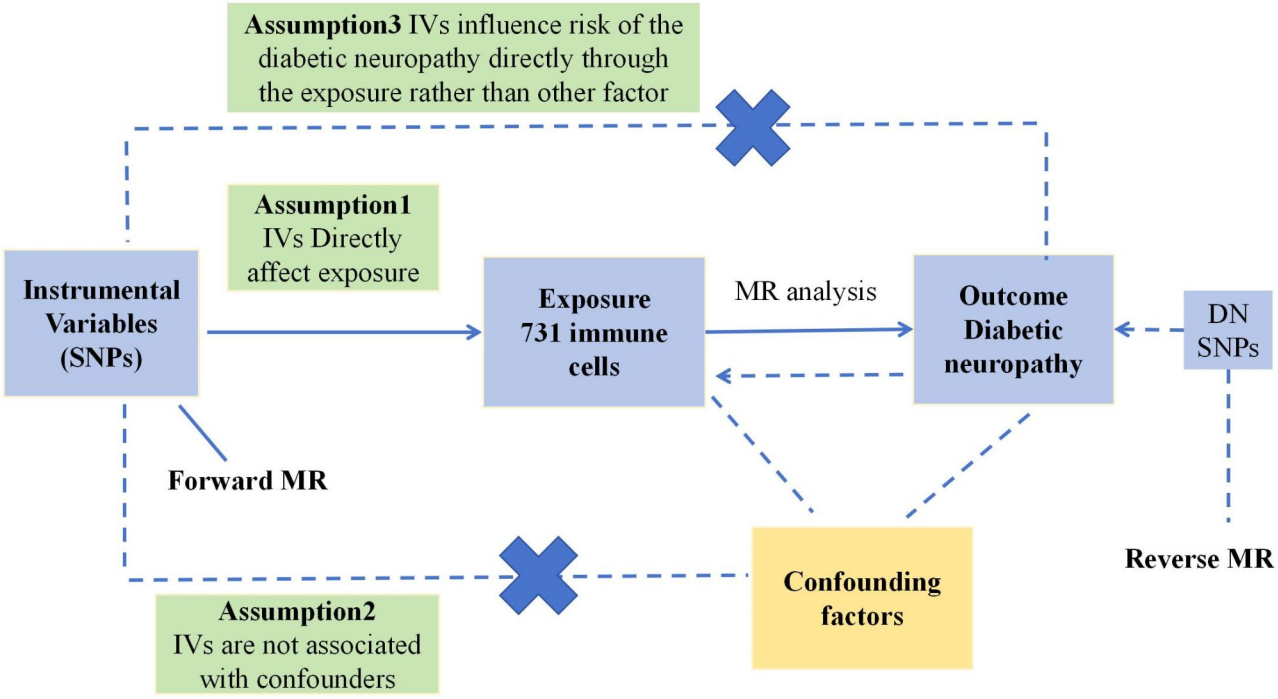

Fig. 1 Overview of research design and analysis strategy

whereas those of MFI, RC, and AC encompassed cDCs, B-cells, T-cell maturation, myeloid cells, monocytes, and TBNK (T cells, B cells, and natural killer proteins).

GWAS data sources for DN

In this study, we used a compendium of GWAS summary statistics on DN (GWAS ID: finngen_R9_DM_NEUROPATHY) extracted from the FinnGen Research Project (https://www.finngen.fi/en). This dataset comprised 274,660 samples (case number = 271817, control number = 2843) for DN, involving a total of 20,167,091 SNPs. To date, we could not obtain further stratification of gender, population age, and disease course. These data are among the few large public databases available to humanity at this stage ensures our statistical efficiency. We performed rigorous SNP quality checks to ensure data robustness and result accuracy, meeting the IV requirements.

IV selection

To ensure that the three assumptions of the two-sample MR analysis were met, we applied several quality control procedures to select the IV that fulfilled the requirements and were closely associated with immune cells. First, when the criterion for genome-wide significance was set at $P < 5e\text{-}8$, only a small number of the 731 immune cell attributes showed plausible genetic mutations. Therefore, After careful consideration, and in line with recent research trends, we adopted a more relaxed significance threshold of $P < 1 \times 10 - 5$ for our IVs selection [17]. The existing constraints of accessible genetic variations within the study domain make this criterion a practical compromise, even though it is more forgiving than traditional norms. Second, we conducted linkage disequilibrium (Used to describe a population in which alleles from two or more loci appear on one chromosome at the same time more frequently than they appear randomly, and tend to be inherited together)checks to reduce SNP connection influence (r2 = 0.001, kb = 10,000). Third, We assessed IV strengths using F-statistics. To avoid bias resulting from weak IVs, we calculated the F-statistic and retained IVs with an F-value > 10. Fourth, To meet the assumption of independence, we identified and removed potential confounding factors correlated with the exposure and outcome using PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) [18]. Hyperglycemia, hyperlipidemia, lipid metabolism disorders, vitamin deficiency, insulin resistance, and systemic autoimmune diseases might be causally related to immune cell function and DN. We carefully identified and subsequently eliminated them to effectively reduce potential confounding effects. While SNPS were not allowed to be associated with outcome events, in fact, we found no potential DN-associated confounders. Finally, to guarantee that the impact alleles for each SNP on the exposure matched their corresponding allelic effect on the outcome, we performed SNP exposure and outcome harmonization. However, we did not identify SNPs with mismatched alleles. In addition, during the harmonization phase, we identified and removed SNPs with ambiguous genotypes

as well as palindromic SNPs. In conclusion, following a thorough multistep screening procedure, we carried out MR analysis using the remaining IVs.

Statistical analysis

We performed MR analyses using the R 4.4.1 software (https://www.r-project.org/) and median-weighted analy sis using the Mendelian Randomization package (version 0.6.6). Inverse Variance Weighting (IVW), MR-Egger, Weighted Median, Weighted Mode, and MR-Presso were the primary traditional MR analytic techniques we used. In recognition of heterogeneity between studies, we prioritized positive results from the IVW approach, which enhanced heterogeneity consideration. Subsequently, we performed sensitivity tests, including heterogeneity and horizontal multiple validity to assess possible pleiotropy. We used Cochran's Q test to gauge IV heterogeneity. We used MR–Egger regression for weighted linear regression with intercepts to identify possible horizontal pleiotropy (Genetic variation directly affects multiple phenotypes through pathways unrelated to exposure, which are independent of each other. The violation of the exclusivity assumption of Mendelian randomization analysis may lead to bias in the estimation of causal effects)within the IVs. We repeated the IVW analysis after the deletion of irrelevant SNPs. If the *P*-value exceeded 0.05, we deemed heterogeneity and pleiotropy absent. To ascertain whether any one SNP significantly impacted the overall causal effect, a leave-one-out analysis was also carried out, which involved repeatedly removing each genetic variant from the analysis and recalculating the causative effect. The results are shown as 95% confidence intervals and odds ratios (OR). A *P* value of less than 0.05 was considered statistically significant. Similar techniques were applied to DN and reverse MR investigation of immune cell characteristics. Furthermore, we applied the online program Bioladder to perform False Discovery Rate (FDR) correction to address the problem with performing multiple comparisons. Based on previous studies, FDR < 0.05 was considered to indicate a significant causal association, while FDR < 0.2 was considered suggestive of a causal relationship.

Results

The causal inference of immunophenotypes on DN

After rigorous quality checks, we identified six immune cell phenotypes significantly associated with the risk of DN. Figure 2 presents the results of the analysis (Fig. 2 The forest plot illustrating the causal impact of immune cells on the risk of DN). Specifically, IgD- CD24- %B cells (odds ratio [OR] = 1.075; 95% confidence interval [CI], 1.010–1.142; *P* = 0.021; FDR = 0.048), naive CD4 + AC (OR = 1.272; 95% CI, 1.145–1.414; *P* = 7.49E-06; FDR = 0.000), CD27 on IgD- CD38br (OR = 1.178; 95% CI, 1.031–1.345; *P* = 0.016; FDR = 0.047), HLA DR on CD14 + CD16- monocytes (OR = 1.108; 95% CI, 1.023–1.194; *P* = 0.007; FDR = 0.046), and CD4 on activated and secreting Tregs (OR = 1.050; 95% CI, 1.008–1.093; *P* = 0.019, FDR = 0.046) displayed a significant positive correlation with the risk of DN. This suggests that this five particular immune cell subpopulations might have a significant impact on Increasing the risk of DN. In contrast, FSC-A on HLA DR + T cells (OR = 0.951; 95% CI, 0.916–0.988; *P* = 0.101; FDR = 0.048) showed a significant

Number	ID	Trait	method	nsnp	pval	adjustP	OR(95%CI)	heterogeneity	pleiotropy	presso
3	1413	IgD− CD24− %B cell	IVW	15	0.0213	0.04761290	1.0746(1.0108 to 1.1424)	0.847	0.908	0.908
18	1474	Plasmacytoid DC %DC	IVW	24	0.0120	0.05067406	1.0739(1.0158 to 1.1353)	0.473	0.717	0.717
23	1503	Activated & secreting Treg %CD4+	IVW	29	0.0271	0.05718886	1.0257(1.0029 to 1.0491)	0.590	0.213	0.213
33	1538	CM CD4+ %CD4+	IVW	31	0.0349	0.05527167	0.9766(0.9554 to 0.9983)	0.499	0.615	0.615
38	1540	Naive CD4+ AC	IVW	20	<0.001	0.00014231	1.2722(1.1450 to 1.4136)	0.314	0.163	0.163
48	1577	Transitional AC	IVW	31	0.0098	0.05306551	0.8911(0.8165 to 0.9726)	0.044	0.820	0.820
53	1630	CD8br NKT AC	IVW	21	0.0447	0.05478865	1.1064(1.0024 to 1.2211)	0.893	0.756	0.756
58	1673	CD28− CD127− CD25++ CD8br %T cell	IVW	17	0.0388	0.05459031	1.0711(1.0035 to 1.1433)	0.519	0.634	0.634
63	1675	CD28− CD127− CD25++ CD8br AC	IVW	26	0.0067	0.05065896	1.0658(1.0178 to 1.1160)	0.552	0.822	0.822
68	1694	CD28− DN (CD4−CD8−) %T cell	IVW	22	0.0461	0.05470891	0.9327(0.8710 to 0.9988)	0.996	0.882	0.882
88	1739	CD19 on PB/PC	IVW	22	0.0326	0.05379597	0.9026(0.8217 to 0.9915)	0.850	0.781	0.781
93	1786	CD25 on IgD− CD27−	IVW	21	0.0276	0.05529700	0.8995(0.8187 to 0.9884)	0.320	0.191	0.191
98	1803	CD27 on IgD− CD38br	IVW	15	0.0161	0.04719913	1.1776(1.0307 to 1.3454)	0.273	0.152	0.152
103	1963	FSC−A on myeloid DC	IVW	20	0.0435	0.05511479	1.0383(1.0011 to 1.0769)	0.921	0.408	0.408
108	1964	FSC−A on plasmacytoid DC	IVW	26	0.0297	0.05132348	0.9620(0.9290 to 0.9962)	0.557	0.354	0.354
113	1975	FSC−A on HLA DR+ T cell	IVW	18	0.0101	0.04815649	0.9513(0.9158 to 0.9882)	0.582	0.997	0.997
123	1988	HLA DR on CD14+ CD16− monocyte	IVW	20	0.0073	0.04645150	1.1079(1.0280 to 1.1942)	0.224	0.264	0.264
133	2041	CD45 on lymphocyte	IVW	16	0.0288	0.05214193	0.9107(0.8374 to 0.9904)	0.642	0.950	0.950
138	2048	CD45 on CD66b++ myelod cell	IVW	14	0.0158	0.05001420	1.0770(1.0140 to 1.1438)	0.898	0.873	0.873
143	2054	CD8 on CM CD8br	IVW	13	0.0368	0.05597477	1.1122(1.0065 to 1.2290)	0.917	0.903	0.903
148	2066	CD4 on activated Treg	IVW	21	0.0063	0.06005813	1.0600(1.0166 to 1.1053)	0.817	0.419	0.419
153	2070	CD4 on activated & secreting Treg	IVW	29	0.0192	0.04566767	1.0496(1.0079 to 1.0930)	0.350	0.448	0.448
158	2081	SSC−A on CD4+	IVW	24	0.0284	0.05404285	0.9065(0.8303 to 0.9897)	0.240	0.535	0.535
163	2093	CD11b on CD66b++ myeloid cell	IVW	17	0.0419	0.05489972	0.9310(0.8690 to 0.9974)	0.804	0.860	0.860

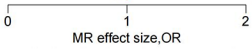

Fig. 2 The forest plot illustrating the causal impact of immune cells on the risk of DN, derived using the IVW method. OR, odds ratios; CI, confidence intervals; pval, *P*-value of the IVW method; adjustP, FDR-corrected *P*-value

negative correlation with the risk of DN. These results suggest that this immune cell type might delay DN development and play a protective role.

Because we adjusted the FDR to 0.08, we already obtained a sufficient amount of SNPS, although most studies believe that FDR = 0.2 indicates causality, so this criterion is more meaningful for our study on the basis of fully meeting the above requirements. After adjusted the FDR to 0.08, we identified associations between 18 other immune cell phenotypes and the risk of DN. Among them, plasmacytoid DC %DC (OR = 1.074; 95% CI, 1.016–1.135; $P = 0.012$; FDR = 0.050), activated and secreting Treg %CD4+ (OR = 1.026; 95% CI, 1.003–1.049; $P = 0.027$; FDR = 0.057), CD8br NKT AC (OR = 1.106; 95% CI, 1.002–1.106; $P = 0.045$; FDR = 0.055), CD28- CD127- CD25 + + CD8br %T cells (OR = 1.071; 95% CI, 1.004–1.143; $P = 0.039$, FDR = 0.054), CD28- CD127- CD25 + + CD8br AC (OR = 1.066; 95% CI, 1.018–1.116; $P = 0.007$; FDR = 0.051), FSC-A on myeloid DC (OR = 1.038; 95% CI, 1.001–1.077; $P = 0.044$; FDR = 0.055), CD45 on CD66b + + myeloid cells (OR = 1.077; 95% CI, 1.014–1.444; $P = 0.016$; FDR = 0.050), CD8 on CM CD8br (OR = 1.112; 95% CI, 1.007–1.229; $P = 0.037$; FDR = 0.056), and CD4 on activated Treg (OR = 1.060; 95% CI, 1.017–1.105; $P = 0.062$; FDR = 0.060) were positively correlated with the risk of DN. Contrastingly, CM CD4+ %CD4+ (OR = 0.977; 95% CI, 0.995–0.998; $P = 0.035$; FDR = 0.055), Transitional AC (OR = 0.891; 95% CI, 0.817–0.974; $P = 0.010$; FDR = 0.053),CD28- DN (CD4-CD8-) %T cell (OR = 0.933; 95% CI, 0.871–0.999; $P = 0.046$; FDR = 0.055),CD19 on PB/PC(OR = 0.903; 95% CI, 0.822–0.992; $P = 0.033$; FDR = 0.054),CD25 on IgD- CD27-(OR = 0.890; 95% CI, 0.819–0.988; $P = 0.028$; FDR = 0.055), FSC-A on plasmacytoid DC(OR = 0.962; 95% CI, 0.929–0.996; $P = 0.030$; FDR = 0.051), CD45 on lymphocyte (OR = 0.911; 95% CI, 0.837–0.990; $P = 0.029$; FDR = 0.052), SSC-A on CD4+(OR = 0.907; 95% CI, 0.830–0.990; $P = 0.028$; FDR = 0.054), and CD11b on CD66b + + myeloid cell (OR = 0.931; 95% CI, 0.869–0.997; $P = 0.042$; FDR = 0.055) were negatively correlated with the risk of DN. These findings highlight the importance of Treg cells in modulating immune responses and provide clues for them to explore the potential role of different T cell subpopulations in the pathogenesis of DN.

Subsequently, we conducted a horizontal pleiotropy test using a combination of MR–Egger and MR–Presso. The aforementioned results did not show any horizontal pleiotropy; further, Cochran's Q test revealed no heterogeneity in any outcome. Scatter plots and leave-one-method sensitivity analyses supported these findings (Supplementary Figures). We further employed a heatmap for visual analysis of the findings. Initially, we filtered out the IDs of all immune cell phenotypes with positive results based on *P*-values derived through the IVW method. The different colors in Fig. 3 represent the *P*-values of the sensitivity analysis results for each immune cell phenotype (Fig. 3 The heatmap depicting the IDs of immune cell phenotypes with positive results).

The causal inference of DN on immunophenotypes

Reverse MR Analysis showed no positive results. Although statistical analysis found that some SNPS satisfied the three core assumptions, we found no meaningful results when adjusted to FDR < 0.2. However, this does not mean that DN cannot affect the functional state of immune cells, and there may still be a lack of effective methods to discover this potential link.

Discussion

Through a two-sample bidirectional MR study, we identified a total of 24 immune cell phenotypes significantly associated with the risk of DN (FDR < 0.08). We identified no significant correlations in the reverse MR analysis. Now, we attempt to further discuss these positive results referring to the deep subpopulation classification features of immune cells by flow cytometry.

We observed that the risk of DN significantly correlated with two dendritic cell types (Plasmacytoid DC %DC and FSC-A on myeloid DC), two T cell maturation stage types (Naive CD4 + AC and CD8br NKT AC), CD45 on CD66b + + myeloid cells (neutrophile granulocyte), and HLA DR on CD14 + CD16- monocytes acting as protective factors against the risk of DN. At the same time, we also revealed a negative correlation of DN in four types of T cells (i.e., CM CD4+ %CD4+, FSC-A on HLA DR + T cells, CD45 on lymphocytes, and SSC-A on CD4+) and in FSC-A on plasmacytoid DC, CD8 on CM CD8br, and CD11b on CD66b + + myeloid cells.

Low-grade inflammation was involved in type 2 diabetes progression onset as well as in its microvascular complications, including DN [18–20]. We calculated the SII as platelet × neutrophil/lymphocyte counts [21]. Growing body of research confirms that increased SII levels are independently associated with an increased risk of DPN in Chinese patients with T2DM [22]. This discovery is consistent with our conclusion that neutrophils levels increase and that of lymphocytes decrease in patients with DN.

Dendritic cells are key cells in the immune system, responsible for capturing pathogens and transmitting their antigenic information to T cells, which initiates an adaptive immune response. In fact, dendritic cells are mainly derived from monocytes. Monocytes and macrophages are important first line defenders in immune system of body. Once inflammation occurs, monocytes and macrophages will quickly gather to lesion area, and monocytes will differentiate into macrophage to destroy pathogens or cell fragments through phagocytosis and

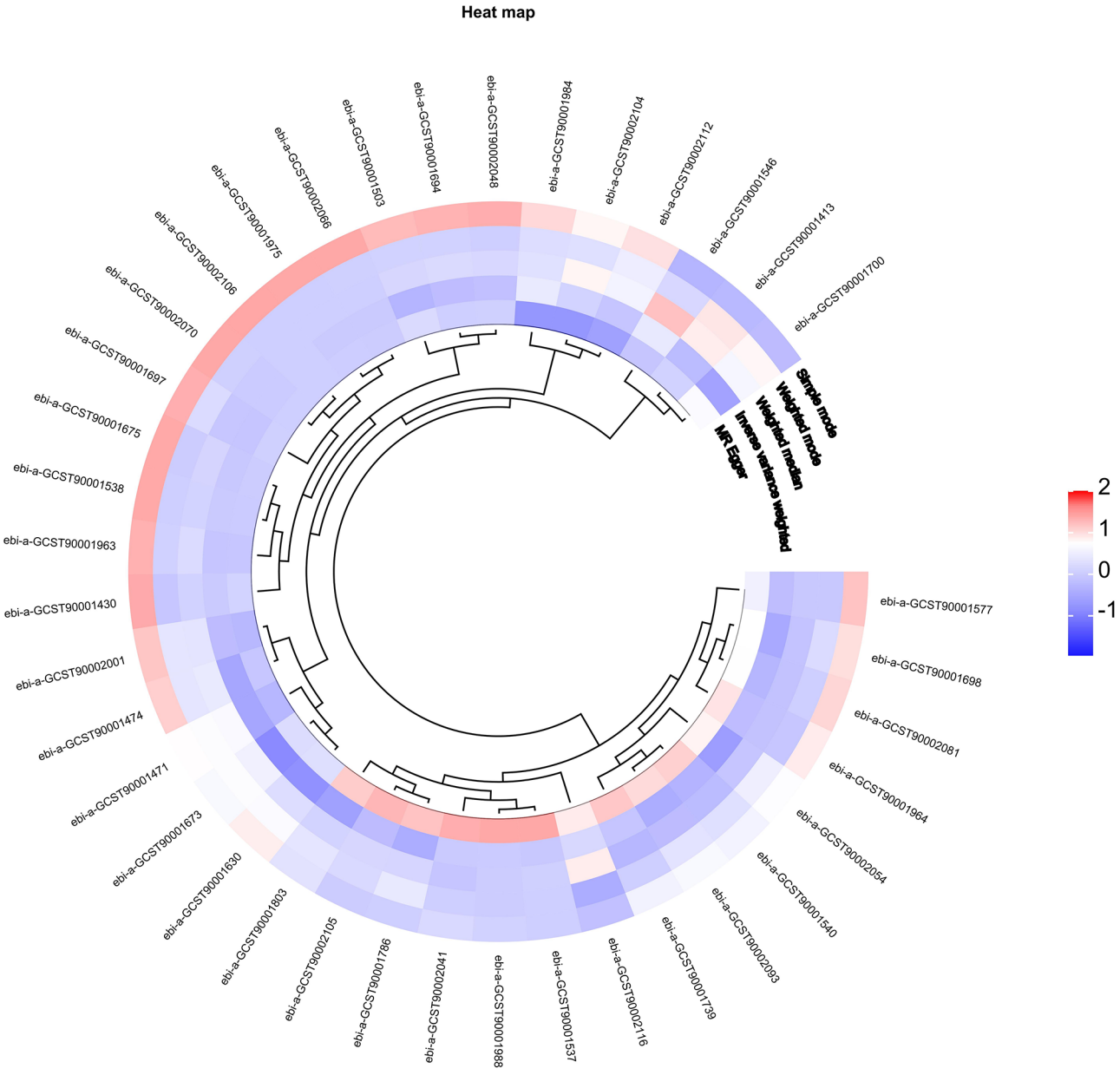

Fig. 3 The heatmap depicting the IDs of immune cell phenotypes with positive results

so on. M1 macrophages secreted a large number of proinflammatory cytokines, resulting in IR, while M2 macrophages secreted anti-inflammatory cytokines to enhance tissue repair and regeneration. Previous data showed in high hyperglycemic conditions, M1 macrophages and other immune cells are activated to express multiple inflammatory factors, potentially resulting in painful DPN and Schwann cell death. In rats with streptomycin-induced diabetes, TNF-α inhibition and MI-to-M2 macrophage phenotype conversion reportedly induces gradual axonal morphology, nerve blood flow, and nerve conduction velocity recovery [23]. Therefore, interfering with macrophages to M2 polarization has positive effect to attenuate diabetes complications. The active macrophages could be regulated by mucin domain-3 gene which can reducing the recruitment of macrophages or affecting its activation and polarization in future [24]. Our Mendelian study on immune cells is consistent with the current findings. DN is closely related to increased inflammatory factor-producing immune cell levels, including those of monocytes, macrophages, and granulocytes as well as lymphocyte redution. However, the specific pathogenesis of this disease is complex. Inflammatory mediator (e.g., TNF-α, IL-1β, IL-6, or other inflammatory factors) release would activate various inflammatory signaling pathways (e.g., NF-κB or

signal transducer, activator of transcription 3), resulting in organ damage [24]. Therefore, TNF-α downregulation likely further improves DN by affecting subsequent inflammatory pathways, thereby providing an area for more exploration. To date, TNF-α blockers have been studied using infliximab, adalimumab, and etanercept. Our findings indicated that Transitional AC (Transitional B cell type), CD19 on PB/PC (plasmacyte cell), and CD25 on IgD- CD27- (a memory B cell type) act as protective factors against the risk of DN, while another two B cell types (i.e., IgD- CD24- %B cells and CD27 on IgD-CD38br) increase the risk of DN. B lymphocyte chemotaxis in the adipose tissue recruits more immune cells to produce pro-inflammatory cytokines and autoantibodies [25], thereby creating a vicious cycle of self-specific adaptive immune responses in the adipose tissue that disrupts insulin signaling [26]. Accordingly, the B lymphocyte population is increased in order to accelerate IR development [27]. However, we also observed that three B cell types represented protective factors against DN. B lymphocytes might suggestibly play a dual role in DN, and certain regulatory B lymphocytes could exert a protective effect, which requires confirmation in future studies.

Furthermore, we discovered that up to 6 regulatory lymphocyte types, i.e., CD28- DN (CD4-CD8-) %T cells, activated and secreting Tregs %CD4+, CD28-CD127- CD25 + + CD8br %T cells, CD28- CD127-CD25 + + CD8br ACs, CD4 on activated Tregs, and CD4 on activated and secreting Tregs, exhibited a significant risk relationship with DN. Regulatory lymphocytes are reportedly implicated in type 1 diabetes pathogenesis. Recent studies described that Tregs might influence type 2 DN [28]. To preserve immunological homeostasis, Tregs (CD4 + CD25 + CD127-/lowFoxp3+), representing adaptive immune system components, might attenuate the immune response [29, 30]. Taken together, Treg modification might offer a new and promising approach for the treatment and prevention of diabetes mellitus and its sequelae [31]. Lymphocyte upregulation reportedly improves or repairs nerve damage [32]. Furthermore, doubting whether Treg plays a role by influencing inflammatory cell (e.g., monocyte, T cell, macrophage, and granulocyte) function through certain mechanisms is reasonable. Enhancing regulatory cell function could contribute to DN progression weakening. Our analysis yielded more nuanced results that paved the way for follow-up investigations. Nevertheless, whether regulatory lymphocytes undergo dynamic changes in the disease course is worth further consideration. Accordingly, further studies are warranted to validate our results and explore the potentially involved mechanisms.

Evidence suggests certain gut microbe involvement in DN occurrence and development by influencing inflammatory responses, lipid and blood sugar levels, and directly affecting the nervous system even via the gut–brain axis [33, 34]. Therefore, intestinal probiotics regulation could open an avenue of new methods and strategies for DN treatment.The GM appears to play a role at the intersection of the gut brain and the neuroimmune-endocrine axis, forming a complex network that can influence the nervous system [35]. At present, the mechanism of action between GM and DN has not been fully determined, but some previous studies have explored several possible mechanisms between GM and DN. First, it may induce inflammatory response and immune activation, and release cellular inflammatory factors and chemokines (such as toll-like receptors, NF-κB) to impair nerve function; Second, metabolites of intestinal flora, such as short-chain fatty acids (SCFAs), amino acids, trimethylamine oxide (TMAO), bile acids and indopropionic acid, are mainly involved in host metabolism and intestinal integrity, thereby regulating and affecting nerve function [36–37]. Therefore, the regulation of intestinal probiotics may be throught affect immune cells and inflammatory factors and become a new method and strategy for the treatment of DN.

Different immune cells may have opposite consequences through certain mechanisms. More research about immune cell profiling become a routine test for assessing risk in diabetic patients, or could immune-targeted therapies be explored for preventing or managing DN might be a new perspective.

In this study, we applied large sample sizes and comprehensive GWAS datasets to conduct bidirectional two-sample MR analyses, thereby significantly improving statistical efficiency. In addition, our results rely on genetic IVs and we used various reliable MR techniques to investigate causal relationships. To account for false-positive results in multi-hypothesis testing, we also applied FDR correction to adjust for multiple comparisons-related statistical bias.

However, this study has several limitations. First, despite several sensitivity studies, we could not comprehensively assess horizontal pleiotropy. Second, the lack of individual-level data impeded stratified population analysis. Therefore, we could not further investigate whether gender, age, and disease stage would differently affect the results. Third, since we based our conclusions on European databases, they cannot be directly applied to other nations without careful consideration. Finally, in this study, we applied loose criteria, which might have resulted in more false positives. However, this allowed for a more thorough assessment of the strong association between immunity and DN. Further extensive clinical studies would be required to confirm these findings and inform clinical judgments.

Conclusions

In this study, we revealed the causal relationships between immune cell phenotypes and DN. Our discoveries provide novel insights that could potentially facilitate the elucidation of the pathogenic mechanisms underlying DN as well as potential therapeutic target identification.

Abbreviations

MR	Mendelian randomization
DN	Diabetic neuropathy
DPN	Diabetic peripheral neuropathy
IR	Insulin resistance
DR	Diabetic retinopathy
DN	Diabetic nephropathy
TNF-α	Tumor necrosis factor-α
GWAS	Genome-wide association study
SNPs	Single-nucleotide polymorphism
IVs.	Instrumental variables
FDR	False discovery rate

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

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Author contributions

Lingfen Ji wrote the main manuscript text, Puyu Li, Nana Duan and Jinjin Xu Collected research data， Yijuan Song, Bohui Shu and Lijun Liang checked Figs. 1, 2 and 3. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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References

1. Callaghan BC, Price RS, Chen KS, Feldman EL. The importance of rare subtypes in diagnosis and treatment of peripheral neuropathy: A review. JAMA Neurol. 2015;72:1510–8.
2. Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, et al. Diabetic neuropathy. Nat Rev Dis Primers. 2019;5:41.
3. Cernea S, Raz I. Management of diabetic neuropathy. Metabolism. 2021;123:154867.
4. Baum P, Toyka KV, Blüher M, Kosacka J, Nowicki M. Inflammatory mechanisms in the pathophysiology of diabetic peripheral neuropathy (DN)-New aspects. Int J Mol Sci. 2021;22:10835.
5. Stino AM, Rumora AE, Kim B, Feldman EL. Evolving concepts on the role of dyslipidemia, bioenergetics, and inflammation in the pathogenesis and treatment of diabetic peripheral neuropathy. J Nerv Syst. 2020;25:76–84.
6. Ristikj-Stomnaroska D, Risteska-Nejashmikj V, Papazova M. Role of inflammation in the pathogenesis of diabetic peripheral neuropathy. Open Access Maced J Med Sci. 2019;7:2267–70.
7. Mu ZP, Wang YG, Li CQ, Lv WS, Wang B, Jing ZH, et al. Association between tumor necrosis factor-alpha and diabetic peripheral neuropathy in patients with type 2 diabetes: A meta-analysis. Mol Neurobiol. 2017;54:983–96.
8. Li X, Wu TT, Chen J, Qiu W. Elevated expression levels of serum insulin-like growth factor-1, tumor necrosis factor-alpha, and vascular endothelial growth factor 165 might exacerbate type 2 diabetic nephropathy. J Diabetes Invest. 2017;8:108–14.
9. Shi X, Chen Y, Nadeem L, Xu G. Beneficial effect of TNF-alpha Inhibition on diabetic peripheral neuropathy. J Neuroinflammation. 2013;10:69.
10. Li X, Zhu J, Liu N, Liu J, Zhang Z. TNF-alpha in peripheral neuropathy patients with impaired glucose regulation. J Diabetes Res. 2017;2017:7024024.
11. Jurewicz A, Matysiak M, Tybor K, Selmaj K. TNF-induced death of adult human oligodendrocytes is mediated by c-jun NH2-terminal kinase-3. Brain. 2003;126:1358–70.
12. Xue T, Zhang X, Xing Y, Liu S, Zhang L, Wang X, Yu M. Advances about Immunoinflammatory pathogenesis and treatment in diabetic peripheral neuropathy. Front Pharmacol. 2021;12:748193.
13. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunol Med Microbiol. 1999;26:259–65.
14. Larsson SC, Butterworth AS, Burgess S. Mendelian randomization for cardiovascular diseases: principles and applications. Eur Heart J. 2023;44:4913–24.
15. Lor GCY, Risch HA, Fung WT, Au Yeung SL, Wong IOL, Zheng W, et al. Reporting and guidelines for Mendelian randomization analysis: A systematic review of oncological studies. Cancer Epidemiol. 2019;62:101577.
16. Orrù V, Steri M, Sidore C, Marongiu M, Serra V, Olla S, et al. Complex genetic signatures in immune cells underlie autoimmunity and inform therapy. Nat Genet. 2020;52:1036–45.
17. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA. 2017;318:1925–6.
18. Verbanck M, Chen CY, Neale B, Do R. Publisher correction: detection of widespread horizontal Pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018;50:1196.
19. Yang K, Wang Y, Li YW, Chen YG, Xing N, Lin HB, et al. Progress in the treatment of diabetic peripheral neuropathy. Biomed Pharmacother. 2022;148:112717.
20. Taslamacioglu DT, Ozkul FN, Balci B. Can systemic inflammatory index predict diabetic kidney injury in type 2. Diabetes Mellitus? Diagnostics (Basel). 2023;13.
21. Yang K, Wang Y, Li Y, Chen Y, Xing N, Lin H, Zhou P, Yu X. Progress in the treatment of diabetic peripheral neuropathy. Biomed Pharmacother. 2022;148:112717.
22. Yan P, Wu Y, Dan X, Wu X, Tang Q, Chen X, Xu Y, Zhu J, Miao Y, Wan Q. Aspartate aminotransferase/alanine aminotransferase ratio was associated with type 2 diabetic peripheral neuropathy in a Chinese population: A cross-sectional study. Front Endocrinol (Lausanne). 2023;14:1064125.
23. Omi M, Hata M, Nakamura N, Miyabe M, Ozawa S, Nukada H, et al. Transplantation of dental pulp stem cells improves long-term diabetic polyneuropathy together with improvement of nerve morphometrical evaluation. Stem Cell Res Ther. 2017;8:279.
24. Zhao L, Hu H, Zhang Z, Liu L, Huang Y, Liu Q, Jin L, Zhu M, Zhang L. Inflammation in diabetes complications: molecular mechanisms and therapeutic interventions. MedComm. 2020;5:e516. 2024.
25. Wu H, Ghosh S, Perrard XD, Feng L, Garcia GE, Perrard JL, et al. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. Circulation. 2007;115:1029–38.
26. Koufakis T, Dimitriadis G, Metallidis S, Zebekakis P, Kotsa K. The role of autoimmunity in the pathophysiology of type 2 diabetes: looking at the other side of the Moon. Obes Rev. 2021;22:e13231.

27. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, et al. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med. 2009;15:921–9.
28. Xue T, Zhang X, Xing Y, Liu S, Zhang L, Wang X, et al. Advances in inflammatory pathogenesis and treatment in diabetic peripheral neuropathy. Front Pharmacol. 2021;12:748193.
29. Piconese S, Walker LSK, Dominguez-Villar M, Editorial. Control of regulatory T cell stability, plasticity, and function in health and disease. Front Immunol. 2020;11:611591.
30. Hulme MA, Wasserfall CH, Atkinson MA, Brusko TM. Central role for interleukin-2 in type 1 diabetes. Diabetes. 2012;61:14–22.
31. Qin W, Sun L, Dong M, An G, Zhang K, Zhang C, et al. Regulatory T cells and diabetes mellitus. Hum Gene Ther. 2021;32:875–81.
32. Yue W, Zhang J, Zhang W, Sun N. Alleviated diabetic osteoporosis and peripheral neuropathic pain by Rehmannia glutinosa libosch polysacchaatory T cells. Int J Biol Macromol. 2024;277:134241.
33. Naghipour A, Amini-Salehi E, Orang Gorabzarmakhi M, Shahdkar M, Fouladi B, Alipourfard I, Sanat ZM. Effects of gut microbial therapy on lipid profile in individuals with non-alcoholic fatty liver disease: an umbrella meta-analysis study. Syst Rev. 2023;12:144.
34. Vakilpour A, Amini-Salehi E, Soltani Moghadam A, Keivanlou M, Letafatkar N, Habibi A, Hashemi M, Eslami N, Zare R, Norouzi N, Delam H, Joukar F, Mansour-Ghanaei F, Hassanipour S, Samethadka Nayak S. The effects of gut Microbiome manipulation on glycemic indices in patients with non-alcoholic fatty liver disease: a comprehensive umbrella review. Nutr Diabetes. 2024;14:25.
35. Pane K, Boccella S, Guida F, Franzese M, Maione S, Salvatore M. Role of gut microbiota in neuropathy and neuropathic pain States: A systematic preclinical review. Neurobiol Dis. 2022;170:105773.
36. Kukkar A, Singh N, Jaggi AS. Attenuation of neuropathic pain by sodium butyrate in an experimental model of chronic constriction injury in rats. J Formos Med Assoc. 2014;113:921–8.
37. Bonomo RR, Cook TM, Gavini CK, White CR, Jones JR, Bovo E, Zima AV, Brown IA, Dugas LR, Zakharian E, Aubert G, Alonzo FR, Calcutt NA, Mansuy-Aubert V. Fecal transplantation and butyrate improve neuropathic pain, modify immune cell profile, and gene expression in the PNS of obese mice. Proc Natl Acad Sci U S A. 2020;117:26482–93.

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