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Causal Role of Immune Cells in Stroke: Mendelian Randomization (MR) Study

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Abstract: Objective: A two sample Mendelian randomization study was used to evaluate the causal relationship between immune cells and stroke. Methods: The data were collected from the genome-wide association study (GWAS) data set. Using the two sample Mendelian randomization method, we explored the causal relationship between 731 immune cell characteristics and stroke. There are four immune characteristics (relative cell (RC), medium fluorescence intensities (MFI), absolute cell (AC), and morphological parameters (MP)). Results: after FDR correction,13 immunophenotypes were risk factors for stroke, and 20 immunophenotypes were protective factors; 13 immunophenotypes were risk factors for ischemic stroke, and 22 immunophenotypes were protective factors; 16 kinds of immunophenotypes are risk causal relationship for cardioembolic stroke, and the other 16 kinds of immunophenotypes are protective factors; It was found that 15 immunophenotypes were risk factors for small vessel occlusion, and 29 immunophenotypes were protective factors; 21 kinds of immunophenotypes are risk factors for large artery atherosclerosis, and its protective factors are as high as 39 kinds of immunophenotypes. Conclusions: our study proved the close relationship between immune cells and stroke through Mendelian randomization study, and provided guidance for the treatment and prevention of stroke in the future.

Keywords: Stroke; Immune Cells; Causal Inference; MR Analysis; Sensitivity.

1. Introduction

Stroke is the second leading cause of death in the world. Stroke is divided into ischemic stroke and hemorrhagic stroke. Ischemic stroke refers to the acute or chronic occlusion of blood vessels supplying the brain; Hemorrhagic stroke refers to the rupture and hemorrhage of cerebral blood vessels [1].Stroke causes serious disease burden to patients. About 15% of stroke occurs in patients aged 18-50 years old. In recent years, this disease has continued to grow [2, 3]. Stroke's pathophysiology is complex, such as the traditional view that smoking, hypertension, hyperlipidemia and hyperglycemia are the recognized causes of stroke [4, 5]. However, these traditional factors can't fully explain the occurrence of stroke. Actively exploring other influencing factors of stroke is of great significance for the treatment and prevention of stroke [6]. Recently, there has been a growing interest in exploring the causal relationship between immune cells and stroke.

There are many kinds of immune cells in the brain. These immune cells and the cytokines secreted by them play an important role in the development and function of the nervous system, and many nervous system related diseases are related to the abnormal expression of immune cells, including stroke [7, 8]. In the acute and chronic stages of stroke, the interaction between the immune system and the brain has always existed. Stroke can activate the systemic immune response, and there is a complex relationship between stroke and systemic immune inflammation [9]. Immune cells and cytokines secreted by them are the medium of communication between brain and immune system. Tumor necrosis factor-α (TNF-α) after stroke, Matrix metalloproteinase 9 (MMP-9) and interleukin (IL) -1 β.In turn, these factors can lead to the aggravation of ischemic inflammation and cell death [10]. In the pathological condition after stroke, the blood-brain barrier plays a role in attracting immune cells, and a large number of white blood cells enter the brain, which further leads to the occurrence of neuroinflammation and brain edema [11, 12]. Different T cell subsets have different effects on stroke. CD4+CD25+Foxp3+T cells exist as a risk factor in the process of stroke and promote the progress of inflammation; B lymphocytes may play a protective role in early ischemic stroke [13, 14]. But so far, the research results on the causal relationship between immune cells and stroke are inconsistent. The possible reasons are the defects in the research design, the effectiveness of the sample size and the influence of other confounding factors in the research process [15, 16], Moreover, the causal relationship between 731 immune cell phenotypes and stroke has not been reported yet, so a more scientific and rigorous method is needed to explore the causal relationship between immune cells and stroke. Mendelian randomization research is a method based on Mendelian randomization distribution principle, which uses genetic variation as a tool variable to evaluate the causal relationship between exposure and outcome. This method is similar to natural randomized controlled trials, and can effectively avoid the influence of confounding factors and reverse causal relationship [17, 18]. In this study, we conducted a comprehensive two sample Mendelian randomization analysis to clarify the causal relationship between immune cell characteristics and stroke, and provide new ideas for the prevention and treatment of stroke.

2. Materials and Methods

2.1. Study Design

This study applied the genome-wide association study (GWAS) dataset to conduct a two sample Mendelian randomization study. We evaluated the causal relationship between 731 immune cell features and stroke and its clinical subtypes. Mendelian randomization studies use genetic variation as an instrumental variable, which requires meeting the three core assumptions of independence, association, and exclusivity, namely: 1. There is a strong correlation between instrumental variables and exposure factors. 2. The instrumental variable is not related to other confounding factors. 3. Instrumental variables can only affect outcome factors through exposure pathways and not through factors outside of exposure. Because all data is publicly available from the GWAS directory and has been approved by the relevant institutional review committee, no additional ethical approval is required.

2.2. Immunity-wide GWAS Data Sources (Exposure Data)

The GWAS summary data of immune cells is publicly available from the IEU GWAS database directory (https://gwas.mrcieu.ac.uk/) [19]. A total of 731 immune phenotypes (ebi-a-GCST90001391 to ebi-a-GCST90002121) are included, including absolute cell (AC) counts (n = 118), median fluorescence intensities (MFI) reflecting surface antigen levels (n = 389), morpho-logical parameters (MP) (n = 32) and relative cell (RC) counts (n = 192). AC, MFI, and RC features include CDCs, T cell maturation, B cells, bone marrow cells, monocytes, TBNK (T cells, B cells, and natural killer cells), and Treg (regulatory T cells) panels. MP features include TBNK panels and CDC (cytotoxic lymphocytes). The GWAS data of immune cells was studied using a sample of 3757 Sardinian individuals [20], Approximately 22 million SNPs affecting 731 immune cell characteristics.

2.3. Stroke GWAS Data Sources (Outcome Data)

The summary data of stroke and its clinical subtypes are publicly available from the directory of the IEU GWAS database(https://gwas.mrcieu.ac.uk/)[21], The initial dataset was sourced from the Multi Ancestor Whole Genome Stroke Association Research Alliance (METASROKE) of European ancestry participants. This data mainly includes stroke (40585 cases and 406111 controls) and Ischemic stroke (34217 cases and 406111 cases as controls). This also includes common subtypes of ischemic stroke, cardioembolic stroke (CES) (7193 cases and 204570 controls), small vessel occlusion (SVO) (5386 cases and 192662 controls), and large artery atherosclerosis (LAA) (4373 cases and 146392 controls).

2.4. Selection of Instrumental Variables (IVs)

According to recent research [22, 23], Set the significance level of each instrumental variable of immune characteristics to 1x10-5 as the screening criterion, The aggregation process in Pink software (version pink 1.90 beta) is used to trim these SNPs, Set the chain imbalance coefficient r2=0.001, set the region width to 10000, ensure that each SNP is independent of each other, and exclude the influence of pleiotropy on the results [24]. By calculating the F-statistic to remove the influence of weak instrumental variables and removing weak instrumental variables with F<10, further verifying the hypothesis of correlation. Use heterogeneity testing to eliminate significantly heterogeneous SNPs. Based on Stroke, a total of 13 to 526 independent immune phenotype IVs were identified, and these generated IVs can explain the average difference of 0.0131 in their respective immune traits. In the Ischemic stroke analysis, a total of 13 to 547 independent immune phenotype IVs were identified, and these generated IVs can explain the average difference of 0.0145 in their respective immune traits. In the CES results, a total of 4 to 529 independent immune phenotype IVs were identified, and these generated IVs can explain the average difference of 0.0233 in their respective immune traits. Based on SVO, a total of 5 to 540 independent immune phenotype IVs were identified, and these generated IVs can explain the average 0.0306 difference in their respective immune traits. Based on LAA, a total of 8 to 565 independent immune phenotype IVs were identified, and these generated IVs can explain the average 0.0325 difference in their respective immune traits.

2.5. Statistical Analysis

All analyses are in R 4.3.1 (https://www.r-project.org/) In progress.

The causal relationships between 731 immunophenotypes and stroke, Ischemic stroke, cardioembolic stroke (CES), small vessel occlusion (SVO) and large artery atherosclerosis (LAA) were evaluated by multiple MR methods with IVW as the main method. Including inverse variance weighted (IVW), MR egger, weighted median, simple mode and weighted mode. The main application of Mendelian randomization software package 0.4.3 is to conduct Mendelian randomization analysis, using OR values to evaluate the causal relationship between immune phenotype and stroke [25]. This study examines the validity and stability of IVW results through sensitivity analysis [26]. To evaluate the robustness of the results, MR Egger intercept test, Cochran's Q-statistic, and corresponding P-values were used to detect the heterogeneity of the selected IVs. In addition, the MR-PRESSO method (validity residuals and outliers) was run to exclude potential horizontal validity outliers. And finally, funnel plots and scatter plots were also used, which can demonstrate the robustness of their correlation without heterogeneity. Scatter plot display results are not affected by outlier outliers [27]. All statistical tests are conducted using a two tailed test and the estimated values are converted to OR values, with a p-value of<0.05 indicating specific statistical significance.

3. Results

Exploring the causal relationship between immune phenotype and stroke and its clinical subtypes

To investigate the causal effect of immune phenotype on stroke and its subtypes, we conducted a dual sample Mendelian randomization study using the IVW method as the primary method. After debugging based on FDR, many immune features were found to either promote or inhibit the progression of stroke and its subtypes. The specific results are shown below.

3.1. The Causal Relationship between Immune Phenotype and Stroke

Trails	method	nsnp	pval	OR(95%CI)	FDR
CCR2 on granulocyte	Inverse variance weighted	16	0.0063	1.05 (1.01−1.08)	0.0346
PDL−1 on CD14+ CD16− monocyte	Inverse variance weighted	14	0.0455	1.04 (1.00−1.09)	0.0487
CD4 on resting Treg	Inverse variance weighted	13	0.0284	1.04 (1.00−1.08)	0.0426
IgD− CD27− %lymphocyte	Inverse variance weighted	16	0.0440	1.04 (1.00−1.08)	0.0487
CD14 on CD14+ CD16+ monocyte	Inverse variance weighted	17	0.0255	1.04 (1.00−1.08)	0.0417
PDL−1 on CD14+ CD16+ monocyte	Inverse variance weighted	28	0.0167	1.03 (1.00−1.05)	0.0368
CD127 on CD28− CD8br	Inverse variance weighted	17	0.0481	1.03 (1.00−1.05)	0.0487
HLA DR+ CD4+ AC	Inverse variance weighted	23	0.0428	1.03 (1.00−1.05)	0.0487
CD28− CD127− CD25++ CD8br AC	Inverse variance weighted	27	0.0196	1.03 (1.00−1.05)	0.0396
CD45RA on naive CD4+	Inverse variance weighted	30	0.0122	1.02 (1.00−1.04)	0.0368
HLA DR+ CD8br %T cell	Inverse variance weighted	30	0.0487	1.02 (1.00−1.03)	0.0487
CD28 on CD39+ CD8br	Inverse variance weighted	19	0.0167	1.02 (1.00−1.03)	0.0368
HLA DR on CD14+ CD16+ monocyte	Inverse variance weighted	19	0.0472	1.01 (1.00−1.03)	0.0487
CD33 on CD33dim HLA DR+ CD11b−	Inverse variance weighted	22	0.0148	0.98 (0.97−1.00)	0.0368
CD33 on CD33dim HLA DR+ CD11b+	Inverse variance weighted	21	0.0144	0.98 (0.97−1.00)	0.0368
PB/PC %lymphocyte	Inverse variance weighted	19	0.0302	0.98 (0.96−1.00)	0.0433
CD27 on IgD+ CD24+	Inverse variance weighted	29	0.0366	0.98 (0.96−1.00)	0.0487
CD27 on IgD− CD38dim	Inverse variance weighted	28	0.0238	0.97 (0.95−1.00)	0.0417
CD25 on naive−mature B cell	Inverse variance weighted	22	0.0135	0.97 (0.95−0.99)	0.0368
CD33− HLA DR− AC	Inverse variance weighted	19	0.0265	0.97 (0.95−1.00)	0.0417
CD27 on IgD− CD38−	Inverse variance weighted	29	0.0204	0.97 (0.95−1.00)	0.0396
CD45 on CD33dim HLA DR−	Inverse variance weighted	19	0.0123	0.97 (0.95−0.99)	0.0368
CD25 on IgD+ CD38dim	Inverse variance weighted	21	<0.001	0.97 (0.95−0.99)	0.0227
CD27 on IgD+ CD38− unsw mem	Inverse variance weighted	24	0.0014	0.97 (0.95−0.99)	0.0227
Sw mem %B cell	Inverse variance weighted	13	0.0438	0.97 (0.94−1.00)	0.0487
CD27 on sw mem	Inverse variance weighted	28	0.0023	0.97 (0.95−0.99)	0.0240
SSC−A on HLA DR+ T cell	Inverse variance weighted	22	0.0378	0.97 (0.94−1.00)	0.0487
CD19 on CD24+ CD27+	Inverse variance weighted	27	0.0034	0.97 (0.94−0.99)	0.0240
CD38 on IgD+ CD24−	Inverse variance weighted	14	0.0260	0.96 (0.93−1.00)	0.0417
CD20− %B cell	Inverse variance weighted	18	0.0036	0.96 (0.93−0.99)	0.0240
Sw mem %lymphocyte	Inverse variance weighted	17	0.0122	0.95 (0.92−0.99)	0.0368
Lymphocyte AC	Inverse variance weighted	19	0.0391	0.95 (0.90−1.00)	0.0487
CD27 on IgD− CD38br	Inverse variance weighted	14	0.0156	0.93 (0.88−0.99)	0.0368

Fig 1. Forest plots showed the causal associations between immune cell traits and stroke by through the IVW method (inverse variance weighting method). CI: confidence interval

After adjusting for FDR (FDR<0.05), we detected 13 immune phenotypes as risk factors for stroke development: CCR2 on granulocyte (cDC panel), PDL-1 on CD14+CD16- monocyte (Monocyte panel), CD4 on resetting Treg (Treg panel), IgD-CD27-% lymphocyte (B cell panel), CD14 on CD14+CD16+monocyte (Monocyte panel), PDL-1 on CD14+CD16+monocyte (Monocyte panel), CD127 on CD28- CD8br (Treg panel), HLA DR+CD4+AC (TBN K panel), CD28- CD127- CD25++CD8br AC (Treg) ,CD45RA on naive CD4+(Matura stages of T cell panel), HLA DR+CD8br% T cell (TBNK panel), CD28 on CD39+CD8br (Treg panel), HLA DR on CD14+CD16+monocyte (Monocyte panel). Specifically, using the IVW method, The estimated odds ratio (OR) of PDL-1 on CD14+CD16- monocyte for stroke is 1.04 (95% CI=1.00-1.09, P=0.0455, PFDR=0.0487); The OR value of CD4 on resting Treg for stroke risk is 1.04 (95% CI=1.00-1.08, P=0.0284, PFDR=0.0426); The estimated OR for the risk ratio of IgD-CD27-% lymphocyte to stroke is 1.04 (95% CI=1.00-1.08, P=0.0440, PFDR=0.0487); The odds ratio of CD14 on CD14+CD16+monocyte to stroke was 1.04 (95% CI=1.00-1.08, P=0.0255, PFDR=0.0471); Similar results were also observed in the other four methods, with OR values in roughly the same direction (OR values>1). Detailed results can be found in Fig.1.

After adjusting for FDR (FDR<0.05), we detected 20 immune phenotypes as protective factors for stroke development: CD33 on CD33dim HLA DR+CD11b - (Myeloid cell panel), CD33 on CD33dim HLA DR+CD11b+(Myeloid cell panel), PB/PC% lymphocyte (B cell panel), CD27 on IgD+CD24+(B cell panel), CD27 on IgD - CD38dim (B cell panel), CD25 on naïve-mature B cell (B cell panel), CD33- HLA DR - AC (Myeloid cell panel), CD27 on IgD D-CD38- (B cell panel) ,CD45 on CD33dim HLA DR - (Myeloid cell panel), CD25 on IgD+CD38dim (B cell panel), CD27 on IgD+CD38- unsw mem (B cell panel), Sw mem% B cell (B cell panel), CD27 on SW mem (B cell panel), SSC-A on HLA DR+T cell (TBNK panel), CD19 on CD24+CD27+(B cell panel), CD38 on IgD+CD24- (B cell panel), CD20-% B cell (B cell panel), Sw mem% lymphocyte (B cell panel), Lymphocyte AC (TBNK panel), CD27 on IgD - CD38br (B cell panel). Specifically, use the IVW method. The odds ratio (OR) of CD33 on CD33dim HLA DR+CD11b- for stroke is 0.98 (95% CI=0.97-1.00, P=0.0148, PFDR=0.0368); The risk ratio of CD25 on naive-mature B cell for stroke is 0.97 (95% CI=0.97-1.00, P=0.0135, PFDR=0.0368); The odds ratio (OR) of CD45 on CD33dim HLA DR - for stroke is 0.97 (95% CI=0.95-0.99, P=0.0123, PFDR=0.0368); The immune phenotype results obtained from the above IVW were also observed in similar results in the other four Mendelian methods, with roughly the same direction of OR values (OR values<1). Detailed results can be found in Fig.1. In addition, the global analysis of intercepts from MRPRESSO and MR Egger further ruled out the possibility of horizontal pleiotropy in the aforementioned associations. Moreover, sensitivity analysis further confirms the observation, and scatter plots and funnel plots further demonstrate the robustness of the results.

3.2. The Causal Relationship between Immune Phenotype and Ischemic Stroke

Trails	method	nsnp	pval	OR(95%CI)	FDR
SSC−A on lymphocyte	Inverse variance weighted	20	0.0049	1.07 (1.02−1.12)	0.0283
CD20− CD38− %B cell	Inverse variance weighted	23	0.0085	1.05 (1.01−1.09)	0.0373
PDL−1 on CD14+ CD16− monocyte	Inverse variance weighted	14	0.0302	1.05 (1.00−1.10)	0.0449
CCR2 on granulocyte	Inverse variance weighted	16	0.0063	1.05 (1.01−1.09)	0.0313
SSC−A on T cell	Inverse variance weighted	19	0.0160	1.05 (1.01−1.09)	0.0441
CD4 on resting Treg	Inverse variance weighted	13	0.0255	1.05 (1.01−1.09)	0.0449
CD8 on TD CD8br	Inverse variance weighted	17	0.0500	1.04 (1.00−1.07)	0.0500
CD80 on CD62L+ myeloid DC	Inverse variance weighted	23	0.0207	1.03 (1.00−1.05)	0.0441
CD40 on CD14− CD16+ monocyte	Inverse variance weighted	22	0.0313	1.02 (1.00−1.04)	0.0449
HLA DR+ CD8br %T cell	Inverse variance weighted	29	0.0213	1.02 (1.00−1.04)	0.0441
CD45RA on naive CD4+	Inverse variance weighted	31	0.0321	1.02 (1.00−1.04)	0.0449
HLA DR on CD14+ CD16+ monocyte	Inverse variance weighted	19	0.0191	1.02 (1.00−1.03)	0.0441
CD28 on CD39+ CD8br	Inverse variance weighted	19	0.0227	1.02 (1.00−1.03)	0.0441
CD25 on naive−mature B cell	Inverse variance weighted	22	0.0437	0.98 (0.96−1.00)	0.0500
CD27 on IgD+ CD24+	Inverse variance weighted	29	0.0464	0.98 (0.95−1.00)	0.0500
CD28 on CD28+ CD45RA+ CD8br	Inverse variance weighted	16	0.0390	0.97 (0.95−1.00)	0.0487
CD27 on IgD− CD38dim	Inverse variance weighted	28	0.0223	0.97 (0.95−1.00)	0.0441
CD27 on IgD− CD38−	Inverse variance weighted	29	0.0476	0.97 (0.95−1.00)	0.0500
CD3 on CM CD8br	Inverse variance weighted	19	0.0492	0.97 (0.94−1.00)	0.0500
CD3 on CD28+ CD4+	Inverse variance weighted	22	0.0315	0.97 (0.95−1.00)	0.0449
CD3 on CD4+	Inverse variance weighted	27	0.0220	0.97 (0.95−1.00)	0.0441
CD16−CD56 on HLA DR+ NK	Inverse variance weighted	22	0.0295	0.97 (0.94−1.00)	0.0449
CD27 on IgD+ CD38− unsw mem	Inverse variance weighted	24	0.0020	0.97 (0.95−0.99)	0.0283
CD20 on IgD− CD38−	Inverse variance weighted	29	0.0388	0.97 (0.94−1.00)	0.0487
CD27 on sw mem	Inverse variance weighted	28	0.0038	0.97 (0.95−0.99)	0.0283
CD28 on CD39+ activated Treg	Inverse variance weighted	18	0.0338	0.97 (0.94−1.00)	0.0455
CD25 on IgD+ CD38dim	Inverse variance weighted	21	<0.001	0.97 (0.95−0.99)	0.0283
CD19 on IgD− CD27−	Inverse variance weighted	24	0.0488	0.97 (0.93−1.00)	0.0500
CD33− HLA DR− AC	Inverse variance weighted	19	0.0044	0.97 (0.94−0.99)	0.0283
CD25 on sw mem	Inverse variance weighted	20	0.0471	0.96 (0.93−1.00)	0.0500
CD19 on CD24+ CD27+	Inverse variance weighted	28	0.0025	0.96 (0.94−0.99)	0.0283
SSC−A on HLA DR+ T cell	Inverse variance weighted	22	0.0164	0.96 (0.93−0.99)	0.0441
Sw mem %lymphocyte	Inverse variance weighted	17	0.0309	0.95 (0.92−1.00)	0.0449
Lymphocyte AC	Inverse variance weighted	19	0.0174	0.93 (0.88−0.99)	0.0441
CD27 on IgD− CD38br	Inverse variance weighted	14	0.0105	0.93 (0.88−0.98)	0.0409

Fig 2. Forest plots showed the causal associations between immune cell traits and Ischemic stroke by through the IVW method (inverse variance weighting method). CI: confidence interval

After adjusting for FDR (FDR<0.05), we detected 13 immune phenotypes as risk factors for the development of ischemic stroke: SSC-A on lymphocyte (TBNK panel), CD20- CD38-% B cell (B cell panel), PDL-1 on CD14+CD16- monocyte (Monocyte panel), CCR2 on granulocyte (cDC panel), SSC-A on T cell (TBNK panel), CD4 on reseting Treg (Treg panel), CD8 on TD CD8br (Matura stages of T cell panel), CD80 on CD62L+myeloid DC (cDC panel), CD40 On CD14- CD16+monocyte (monocyte panel) ,HLA DR+CD8br% T cell (TBNK panel), CD45RA on naive CD4+(Matura stages of T cell panel), HLA DR on CD14+CD16+monocyte (monocyte panel), CD28 on CD39+CD8br (Treg panel). Specifically, applying the IVW method for Mendelian analysis is the main approach. The odds ratio (OR) of SSC-A on lymphocyte for ischemic stroke is approximately 1.07 (95% CI=1.02-1.12, P=0.0049, PFDR=0.0283); The causal ratio of PDL-1 on CD14+CD16- monocyte for ischemic stroke is approximately 1.05 (95% CI=1.00-1.10, P=0.0302, PFDR=0.0449); The OR value of CD4 on resting Treg for the risk of ischemic stroke is approximately 1.05 (95% CI=1.01-1.09, P=0.0255, PFDR=0.0449); The odds ratio (OR) of HLA DR+CD8br% T cell for ischemic stroke is approximately 1.02 (95% CI=1.00-1.04, P=0.0213, PFDR=0.0441). The immune phenotype results obtained from the above IVW were also observed in similar results in the other four methods, with roughly the same direction of OR values (OR values>1). The detailed results and can be found in Fig.2.

After adjusting for FDR (FDR<0.05), we detected 22 immune phenotypes as protective factors for the development of ischemic stroke: CD25 on naïve-matyre B cell (B cell panel), CD27 on IgD+CD24+(B cell panel), CD28 on CD28+CD45RA+CD8br (Treg panel), CD27 on IgD - CD38dim (B cell panel), CD27 on IgD - CD38- (B cell panel), CD3 on CM CD8br (Matura stages of T cell panel), CD3 on CD28+CD4+(Treg panel), CD3 on CD4+(Treg panel), CD16-CD56 on HLA DR+NK (TBNK panel), CD27 on IgD+CD38- unsw mem (B cell panel), CD20 on IgD - CD38- (B cell panel), CD27 on sw mem (B cell panel), CD28 on CD39+activated Treg (Treg panel), CD25 on IgD+CD38dim (B cell panel), CD19 on IgD - CD27- (B cell panel), CD33- HLA DR - AC (Myeloid cell panel), CD25 on sw mem (B cell panel), CD19 On CD24+CD27+(B cell panel) ,SSC-A on HLA DR+T cell (TBNK panel), Sw mem% lymphocyte (B cell panel), Lymphocyte AC (TBNK panel), CD27 on IgD - CD38br (B cell panel). Specifically, use the IVW method. The OR of CD25 on naïve-mature B cell for stroke was 0.98 (95% CI=0.96-1.00, P=0.0437, PFDR=0.0500); The OR of CD27 on IgD+CD38- unsw mem is 0.97 (95% CI=0.95-0.99, P=0.0020, PFDR=0.0283); The OR of CD20 on IgD- CD38-

is 0.97 (95% CI=0.94-1.00, P=0.0388, PFDR=0.0487); The OR of CD28 on CD39+activated Treg for stroke is 0.97 (95% CI=0.94-1.00, P=0.0338, PFDR=0.0455); The OR value of SSC-A on HLA DR+T cell is 0.96 (95% CI=0.93-0.99, P=0.0164, PFDR=0.0441); The immune phenotype results obtained from the above IVW were also observed in similar results in the other four Mendelian methods, with roughly the same direction of OR values (OR values<1). Detailed results can be found in Fig.2. In addition, the global analysis of intercepts from MRPRESSO and MR Egger further ruled out the possibility of horizontal pleiotropy in the aforementioned associations. And sensitivity analysis further confirms the observation, funnel plots and scatter plots also prove the stability of the results.

3.3. The Causal Relationship between Immune Phenotype and Cardioembolic Stroke

Trails	method	nsnp	pval	OR(95%CI)	FDR
CD80 on myeloid DC	Inverse variance weighted	25	0.0078	1.08 (1.02−1.15)	0.0311
CD25++ CD8br AC	Inverse variance weighted	28	0.0293	1.08 (1.01−1.15)	0.0391
CD8 on naive CD8br	Inverse variance weighted	14	0.0255	1.07 (1.01−1.14)	0.0391
Activated Treg %CD4	Inverse variance weighted	18	0.0221	1.07 (1.01−1.14)	0.0391
CD80 on monocyte	Inverse variance weighted	21	0.0146	1.07 (1.01−1.13)	0.0389
CD45 on T cell	Inverse variance weighted	15	0.0308	1.06 (1.01−1.11)	0.0391
CD33br HLA DR+ CD14− %CD33br HLA DR+	Inverse variance weighted	19	0.0131	1.05 (1.01−1.10)	0.0380
Im MDSC %CD33dim HLA DR− CD66b−	Inverse variance weighted	20	0.0335	1.05 (1.00−1.10)	0.0391
CD123 on CD62L+ plasmacytoid DC	Inverse variance weighted	16	0.0069	1.05 (1.01−1.09)	0.0311
CD123 on plasmacytoid DC	Inverse variance weighted	16	0.0069	1.05 (1.01−1.09)	0.0311
CD11b on Mo MDSC	Inverse variance weighted	16	0.0165	1.05 (1.01−1.09)	0.0391
HLA DR on myeloid DC	Inverse variance weighted	16	0.0327	1.05 (1.00−1.09)	0.0391
IgD− CD38dim %B cell	Inverse variance weighted	25	0.0321	1.04 (1.00−1.09)	0.0391
CD28 on CD39+ CD8br	Inverse variance weighted	19	0.0293	1.04 (1.00−1.08)	0.0391
CD16 on CD14− CD16+ monocyte	Inverse variance weighted	19	0.0358	1.04 (1.00−1.07)	0.0395
CD28+ CD45RA− CD8br %CD8br	Inverse variance weighted	30	0.0465	1.03 (1.00−1.05)	0.0465
CD28+ CD45RA+ CD8br %T cell	Inverse variance weighted	72	0.0112	0.99 (0.98−1.00)	0.0380
CD25hi CD45RA+ CD4 not Treg %T cell	Inverse variance weighted	27	0.0343	0.97 (0.95−1.00)	0.0391
CD25hi CD45RA+ CD4 not Treg %CD4+	Inverse variance weighted	34	0.0250	0.97 (0.95−1.00)	0.0391
BAFF−R on IgD+ CD38− naive	Inverse variance weighted	27	0.0290	0.96 (0.93−1.00)	0.0391
CD45RA on resting Treg	Inverse variance weighted	24	0.0038	0.95 (0.92−0.98)	0.0307
CX3CR1 on CD14+ CD16− monocyte	Inverse variance weighted	27	0.0254	0.95 (0.91−0.99)	0.0391
CD8 on CD28+ CD45RA+ CD8br	Inverse variance weighted	22	0.0130	0.94 (0.90−0.99)	0.0380
TD CD8br %T cell	Inverse variance weighted	17	0.0437	0.94 (0.89−1.00)	0.0451
CD11c on myeloid DC	Inverse variance weighted	29	0.0214	0.94 (0.89−0.99)	0.0391
CD4 on naive CD4+	Inverse variance weighted	20	0.0297	0.93 (0.87−0.99)	0.0391
CD86+ plasmacytoid DC %DC	Inverse variance weighted	23	0.0207	0.93 (0.87−0.99)	0.0391
TCRgd %T cell	Inverse variance weighted	18	0.0056	0.92 (0.87−0.98)	0.0311
CD127 on T cell	Inverse variance weighted	18	0.0371	0.92 (0.85−0.99)	0.0396
CD33− HLA DR− AC	Inverse variance weighted	18	<0.001	0.90 (0.86−0.95)	0.0015
CD19 on IgD− CD27−	Inverse variance weighted	24	0.0029	0.89 (0.82−0.96)	0.0307
BAFF−R on IgD− CD38dim	Inverse variance weighted	4	0.0031	0.85 (0.77−0.95)	0.0307

Fig 3. Forest plots showed the causal associations between immune cell traits and cardioembolic stroke by through the IVW method (inverse variance weighting method).
CI: confidence interval

After adjusting for FDR (FDR<0.05), we identified 16 immune phenotypes as risk factors for the development of cardioembolic stroke (CES): CD80 on Myeloid DC (cDC panel), CD25++CD8br AC (Treg panel), CD8 on naive CD8br (Matura stages of T cell), Activated Treg% CD4 (Treg), CD80 on monocyte (cDC panel), CD45 on T cell (TBNK panel), CD33br HLA DR+CD14-% CD33br HLA DR+(Myeloid cell panel), Im MDSC% CD33dim HLA DR - CD66b - (Myeloid cell panel) el), CD123 on CD62L+plasmacytoid DC (cDC panel) ,CD123 on plasma point DC (cDC panel), CD11b on Mo MDSC (Myeloid cell panel), HLA DR on Myeloid DC (cDC panel), IgD - CD38dim% B cell (B cell panel), CD28 on CD39+CD8br (Treg panel), CD16 on CD14- CD16+monocyte (Monocyte panel), CD28+CD45RA - CD8br% CD8br (Treg panel). Specifically, apply the IVW method. The risk ratio of CD80 on myeloid DC to CES is approximately 1.08 (95% CI=1.02-1.15, P=0.0078, PFDR=0.03113); The risk ratio of CD80 on monocyte to CES is approximately 1.07 (95% CI=1.01-1.13, P=0.0146, PFDR=0.0389); The risk ratio of CD45 on T cell to CES is approximately 1.06 (95% CI=1.01-1.11, P=0.0308, PFDR=0.0391); The OR value of IgD- CD38dim% B cell is approximately 1.04 (95% CI=1.00-1.09, P=0.0321, PFDR=0.0391). The immunophenotypic results obtained from the above IVW were also observed in similar results in the other four methods, with OR values generally consistent (OR values>1). Detailed results can be found in Fig.3.

After adjusting for FDR (FDR<0.05), we detected 16 immune phenotypes as protective factors against cardioembolic stroke (CES): CD28+CD45RA+CD8br% T cell (Treg panel), CD25hi CD45RA+CD4 not Treg% T cell (Treg panel), CD25hi CD45RA+CD4 not Treg% CD4+(Treg panel), BAFF-R on IgD+CD38-naive (B cell panel), CD45RA on reseting Treg (Treg panel), CX3CR1 on CD14+CD16- monocyte (Monocyte panel), CD8 on CD28+CD45 RA+CD8br (Treg panel), TD CD8br% T cell (Matura stages of T cell panel), CD11c on Myeloid DC (cDC panel), CD4 on naive CD4+(Matura stages of T cell panel), CD86+plasmacytoid DC% DC (cDC panel), TCRgd% T cell (TBNK panel), CD127 on T cell (Treg panel), CD33- HLA DR AC (Myeloid cell), CD19 on Ig D - CD27- (B cell panel), BAFF-R on IgD - CD38dim (B cell panel). Specifically, use the IVW method. The OR of CD28+CD45RA+CD8br% T cell for CES is 0.99 (95% CI=0.98-1.00, P=0.0112,

PFDR=0.0380); The OR of CD45RA+CD4 not Treg% T cell for CES was 0.99 (95% CI=0.98-1.00, P=0.0112, PFDR=0.0380); The OR of CX3CR1 on CD14+CD16- monocyte for CES is 0.95 (95% CI=0.91-0.99, P=0.0254, PFDR=0.0391); The OR of TD CD8br% T cell for CES is 0.94 (95% CI=0.89-1.00, P=0.0437, PFDR=0.0451); The OR of CD19 on IgD- CD27 on CES was 0.89 (95% CI=0.82-0.96, P=0.0029, PFDR=0.0307); The immune phenotype results obtained from the above IVW were also observed in similar results in the other four Mendelian methods, with roughly the same direction of OR values (OR values<1). Detailed results can be found in Fig.3. In addition, the global analysis of intercepts from MRPRESSO and MR Egger further ruled out the possibility of horizontal pleiotropy in the aforementioned associations. And sensitivity analysis further confirms the observation, scatter plots, and funnel plots also demonstrate the robustness of the results.

3.4. The Causal Relationship between Immune Phenotype and Small Vessel Occlusion

Trails	method	nsnp	pval	OR(95%CI)	FDR
CD25 on CD28+ CD4+	Inverse variance weighted	5	0.0300	1.19 (1.02−1.39)	0.0414
IgD on IgD+ CD38br	Inverse variance weighted	21	0.0047	1.13 (1.04−1.22)	0.0207
FSC−A on granulocyte	Inverse variance weighted	19	0.0086	1.12 (1.03−1.21)	0.0252
CD45 on HLA DR+ NK	Inverse variance weighted	13	0.0152	1.10 (1.02−1.20)	0.0318
IgD+ CD38br %lymphocyte	Inverse variance weighted	24	0.0364	1.09 (1.01−1.18)	0.0433
CD3 on EM CD8br	Inverse variance weighted	18	0.0129	1.08 (1.02−1.15)	0.0316
Granulocyte AC	Inverse variance weighted	23	0.0232	1.08 (1.01−1.15)	0.0372
CD4 on secreting Treg	Inverse variance weighted	25	0.0109	1.07 (1.02−1.13)	0.0292
HLA DR on CD14− CD16−	Inverse variance weighted	28	0.0353	1.05 (1.00−1.10)	0.0433
CD28 on CD39+ CD8br	Inverse variance weighted	18	0.0039	1.05 (1.02−1.09)	0.0207
CD123 on plasmacytoid DC	Inverse variance weighted	16	0.0492	1.05 (1.00−1.10)	0.0495
CD123 on CD62L+ plasmacytoid DC	Inverse variance weighted	16	0.0495	1.05 (1.00−1.10)	0.0495
CD11b on CD14+ monocyte	Inverse variance weighted	20	0.0362	1.04 (1.00−1.08)	0.0433
CD45 on CD8br	Inverse variance weighted	24	0.0039	1.04 (1.01−1.06)	0.0207
CD28+ CD45RA− CD8br %CD8br	Inverse variance weighted	30	0.0191	1.04 (1.01−1.07)	0.0345
CD45RA− CD28− CD8br %T cell	Inverse variance weighted	133	0.0022	1.00 (1.00−1.00)	0.0180
CD28+ CD45RA+ CD8br %T cell	Inverse variance weighted	77	0.0237	0.99 (0.98−1.00)	0.0372
CD33br HLA DR+ CD14− AC	Inverse variance weighted	25	0.0069	0.96 (0.94−0.99)	0.0234
CD28+ CD45RA+ CD8dim %CD8dim	Inverse variance weighted	25	0.0264	0.96 (0.92−0.99)	0.0388
HVEM on naive CD8br	Inverse variance weighted	19	0.0494	0.95 (0.91−1.00)	0.0495
CD20 on IgD− CD38br	Inverse variance weighted	15	0.0425	0.95 (0.91−1.00)	0.0480
CCR2 on myeloid DC	Inverse variance weighted	12	0.0480	0.95 (0.90−1.00)	0.0495
CD20 on IgD+ CD38dim	Inverse variance weighted	29	0.0340	0.95 (0.90−1.00)	0.0433
CD25 on IgD+	Inverse variance weighted	23	0.0076	0.94 (0.91−0.98)	0.0238
TD CD4+ %T cell	Inverse variance weighted	24	0.0047	0.94 (0.90−0.98)	0.0207
CD39+ CD8br AC	Inverse variance weighted	22	0.0472	0.94 (0.88−1.00)	0.0495
IgD− CD38dim %lymphocyte	Inverse variance weighted	23	0.0301	0.94 (0.88−0.99)	0.0414
CD62L on CD62L+ plasmacytoid DC	Inverse variance weighted	15	0.0393	0.93 (0.88−1.00)	0.0455
CD19 on IgD+ CD24+	Inverse variance weighted	29	0.0145	0.93 (0.87−0.99)	0.0318
CD4 Treg %T cell	Inverse variance weighted	17	0.0237	0.93 (0.87−0.99)	0.0372
EM DN (CD4−CD8−) %T cell	Inverse variance weighted	22	0.0349	0.92 (0.86−0.99)	0.0433
CD25 on IgD+ CD38dim	Inverse variance weighted	21	<0.001	0.92 (0.88−0.97)	0.0179
CD62L− monocyte AC	Inverse variance weighted	17	0.0148	0.92 (0.86−0.98)	0.0318
CD19 on IgD− CD38dim	Inverse variance weighted	25	0.0246	0.91 (0.85−0.99)	0.0373
CD11c on CD62L+ myeloid DC	Inverse variance weighted	23	0.0177	0.91 (0.85−0.98)	0.0345
DN (CD4−CD8−) NKT %lymphocyte	Inverse variance weighted	30	0.0194	0.91 (0.84−0.99)	0.0345
DN (CD4−CD8−) NKT %T cell	Inverse variance weighted	29	0.0069	0.90 (0.84−0.97)	0.0234
T cell %leukocyte	Inverse variance weighted	17	0.0025	0.90 (0.84−0.96)	0.0180
CD62L− plasmacytoid DC AC	Inverse variance weighted	21	0.0016	0.89 (0.83−0.96)	0.0179
CD19 on unsw mem	Inverse variance weighted	20	0.0196	0.88 (0.79−0.98)	0.0345
CD8br %leukocyte	Inverse variance weighted	16	0.0064	0.88 (0.80−0.96)	0.0234
CD27 on IgD− CD38br	Inverse variance weighted	14	0.0113	0.87 (0.78−0.97)	0.0292
DN (CD4−CD8−) AC	Inverse variance weighted	19	0.0012	0.86 (0.78−0.94)	0.0179
Sw mem %lymphocyte	Inverse variance weighted	17	<0.001	0.84 (0.77−0.92)	0.0029

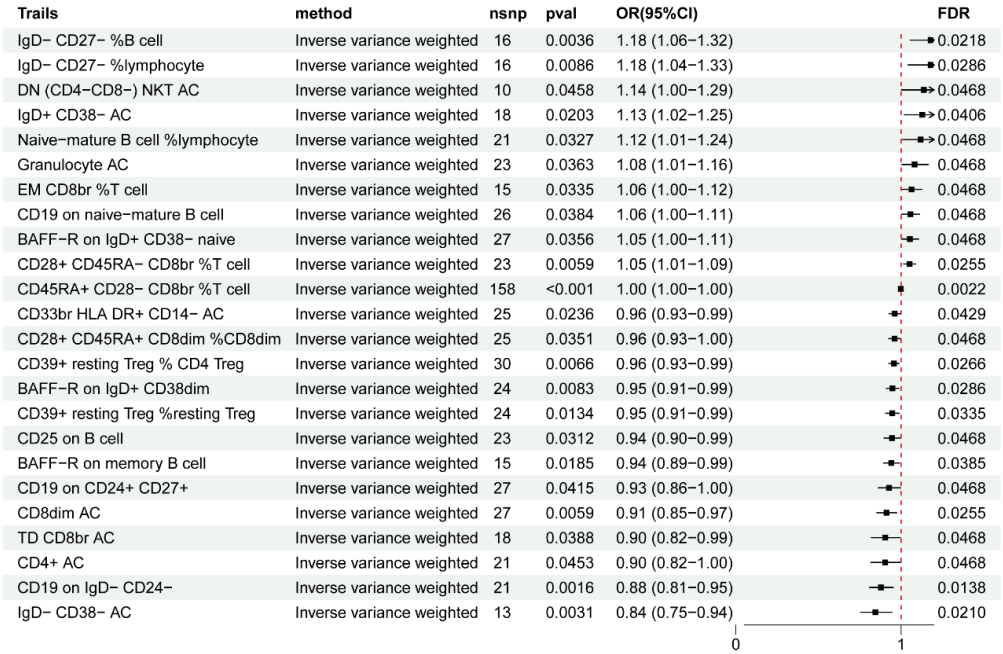
Fig 4. Forest plots showed the causal associations between immune cell traits and small-vessel occlusion by through the IVW method (inverse variance weighting method). CI: confidence interval

After adjusting for FDR (FDR<0.05), we identified 15 immune phenotypes as risk factors for the development of small vessel occlusion (SVO): CD25 on CD28+CD4+(Treg panel), IgD on IgD+CD38br (Bcell panel), FSC-A on granulocyte (cDC panel), CD45 on HLA DR+NK (TBNK panel), IgD+CD38br% lymphocyte (B cell panel), CD3 on EM CD8br (Matura stages of T cell panel), Granulocyte AC (TBNK panel), CD4 on secreting Treg (Treg panel), HLA DR on CD14- CD16- (Monocyte panel) ,CD28 on CD39+CD8br (Treg panel), CD123 on plasma point DC (cDC panel), CD123 on CD62L+plasma point DC (cDC panel), CD11b on CD14+monocyte (Myeloid cell panel), CD45 on CD8br (TBNK panel), CD28+CD45RA - CD8br% CD8br (Treg panel). Specifically, apply the IVW method. The risk ratio of CD25 on CD28+CD4+to SVO is approximately 1.19 (95% CI=1.02-1.39, P=0.0300, PFDR=0.0414); The risk ratio of FSC-A on granulocyte to SVO is approximately 1.12 (95% CI=1.03-1.21, P=0.0086, PFDR=0.0252); The risk ratio of

CD45 on HLA DR+NK to SVO is approximately 1.10 (95% CI=1.02-1.20, P=0.0152, PFDR=0.0318); The risk ratio of CD4 on secreting Treg to SVO is approximately 1.07 (95% CI=1.02-1.13, P=0.0109, PFDR=0.0292); The risk ratio of CD11b on CD14+monocyte to SVO is approximately 1.04 (95% CI=1.00-1.08, P=0.0362, PFDR=0.0433). The immunophenotypic results obtained from the above IVW were also observed in similar results in the other four methods, with OR values generally consistent (OR values>1). Detailed results can be found in Fig.4.

After adjusting for FDR (FDR<0.05), we detected 29 immune phenotypes as protective factors against small vessel occlusion (SVO): CD45RA - CD28- CD8br% T cell (Treg panel), CD28+CD45RA+CD8br% T cell (Treg panel), CD33br HLA DR+CD14- AC (Myeloid cell panel), CD28+CD45RA+CD8dim% CD8dim (Treg panel), HVEM on naive CD8br (Matura stages of T cell panel), CD20 on IgD - CD38br (B cell panel), CCR2 on Myeloid DC (CDC panel), CD20 20 on IgD+CD38dim (B cell panel), CD25 on IgD+(B cell panel) ,TD CD4+% T cell (Matura stages of T cell panel), CD39+CD8br AC (Treg panel), IgD - CD38dim% lymphocyte (B cell panel), CD62L on CD62L+plasmacytoid DC (cDC panel), CD19 on IgD+CD24+(B cell panel), CD4 Treg% T cell (Treg panel), EM DN (CD4-CD8-)% T cell (Matura stages of T cell panel), CD25 On IgD+CD38dim (B cell panel), CD62L monocyte AC (cDC panel) CD19 on IgD - CD38dim (B cell panel), CD11c on CD62L+myeloid DC (cDC panel), DN (CD4-CD8-) NKT% lymphocyte (TBNK panel), DN (CD4-CD8-) NKT% T cell (TBNK panel), T cell% leukocte (Treg panel), CD62L- plasmacytoid DC AC (cDC panel), CD19 on unsw mem (B cell panel), CD8br% Leukocyte (TBNK panel), CD27 on IgD - CD38br (B cell panel) ,DN (CD4-CD8-) AC (TBNK panel), Sw mem% lymphocyte (B cell panel). Specifically, use the IVW method. The risk ratio of CD20 on IgD- CD38br to SVO is approximately 0.95 (95% CI=0.91-1.00, P=0.0425, PFDR=0.0480); The risk ratio of CD25 on IgD+to SVO is approximately 0.94 (95% CI=0.91-0.98, P=0.0076, PFDR=0.0238); The risk ratio of TD CD4+% T cells to SVO is approximately 0.94 (95% CI=0.90-0.98, P=0.0047, PFDR=0.0207); The risk ratio of DN (CD4-CD8-) NKT% lymphocyte to SVO is approximately 0.91 (95% CI=0.84-0.99, P=0.0194, PFDR=0.0345); The immune phenotype results obtained from the above IVW were also observed in similar results in the other four methods, with roughly the same direction of OR values (OR values<1). Detailed results can be found in Fig.4. Specifically, conducting horizontal pleiotropy and sensitivity analysis demonstrated the robustness of the results, while scatter plots and funnel plots also demonstrated the stability of the observed causal associations.

3.5. The Causal Relationship between Immunophenotype and Large Artery Atherosclerosis



Trails	method	nsnp	pval	OR(95%CI)	FDR
IgD− CD27− %B cell	Inverse variance weighted	16	0.0036	1.18 (1.06−1.32)	0.0218
IgD− CD27− %lymphocyte	Inverse variance weighted	16	0.0086	1.18 (1.04−1.33)	0.0286
DN (CD4−CD8−) NKT AC	Inverse variance weighted	10	0.0458	1.14 (1.00−1.29)	0.0468
IgD+ CD38− AC	Inverse variance weighted	18	0.0203	1.13 (1.02−1.25)	0.0406
Naive−mature B cell %lymphocyte	Inverse variance weighted	21	0.0327	1.12 (1.01−1.24)	0.0468
Granulocyte AC	Inverse variance weighted	23	0.0363	1.08 (1.01−1.16)	0.0468
EM CD8br %T cell	Inverse variance weighted	15	0.0335	1.06 (1.00−1.12)	0.0468
CD19 on naive−mature B cell	Inverse variance weighted	26	0.0384	1.06 (1.00−1.11)	0.0468
BAFF−R on IgD+ CD38− naive	Inverse variance weighted	27	0.0356	1.05 (1.00−1.11)	0.0468
CD28+ CD45RA− CD8br %T cell	Inverse variance weighted	23	0.0059	1.05 (1.01−1.09)	0.0255
CD45RA+ CD28− CD8br %T cell	Inverse variance weighted	158	<0.001	1.00 (1.00−1.00)	0.0022
CD33br HLA DR+ CD14− AC	Inverse variance weighted	25	0.0236	0.96 (0.93−0.99)	0.0429
CD28+ CD45RA+ CD8dim %CD8dim	Inverse variance weighted	25	0.0351	0.96 (0.93−1.00)	0.0468
CD39+ resting Treg % CD4 Treg	Inverse variance weighted	30	0.0066	0.96 (0.93−0.99)	0.0266
BAFF−R on IgD+ CD38dim	Inverse variance weighted	24	0.0083	0.95 (0.91−0.99)	0.0286
CD39+ resting Treg %resting Treg	Inverse variance weighted	24	0.0134	0.95 (0.91−0.99)	0.0335
CD25 on B cell	Inverse variance weighted	23	0.0312	0.94 (0.90−0.99)	0.0468
BAFF−R on memory B cell	Inverse variance weighted	15	0.0185	0.94 (0.89−0.99)	0.0385
CD19 on CD24+ CD27+	Inverse variance weighted	27	0.0415	0.93 (0.86−1.00)	0.0468
CD8dim AC	Inverse variance weighted	27	0.0059	0.91 (0.85−0.97)	0.0255
TD CD8br AC	Inverse variance weighted	18	0.0388	0.90 (0.82−0.99)	0.0468
CD4+ AC	Inverse variance weighted	21	0.0453	0.90 (0.82−1.00)	0.0468
CD19 on IgD− CD24−	Inverse variance weighted	21	0.0016	0.88 (0.81−0.95)	0.0138
IgD− CD38− AC	Inverse variance weighted	13	0.0031	0.84 (0.75−0.94)	0.0210

Fig 5. Forest plots showed the causal associations between immune cell traits and large artery atherosclerosis by through the IVW method (inverse variance weighting method).
CI: confidence interval

After adjusting for FDR (FDR<0.05), we identified 21 immune phenotypes as risk factors for the development of large artery atherosclerosis (LAA): IgD - CD27-% B cell (B cell panel), IgD - CD27-% lymphocyte (B cell panel), DN (CD4-CD8-) NKT AC (TBNK panel), IgD+CD38- AC (B cell panel), CD25 on CD39+resting Treg (Treg panel), CD127 on CD4+(Treg panel), Naive -mature B cell% lymphocyte (B cell panel), PDL-1 on monocyte (Monocyte panel) ,CD8 on TD CD8br (Matura stages of T cell panel) ,CD45 on T cell (TBNK panel), Granuloceyte AC (TBNK panel), CD40 on CD14+CD16- monocyte (Monocyte panel), CD80 on CD62L+myeloid DC (cDC panel), CD8 on EM CD8br (Matura stages of T cell panel), CD40 on CD14-CD16+monocyte (Monocyte panel), EM CD8br% T cell (Matura stages of T cell panel), CD19 on naive-mature B cell (B cell panel), CD40 on CD14+CD16+monocyte (Monocyte panel), BAFF-R on IgD+CD38- naive (B cell panel), CD28+CD45RA - CD8br% T cell (Treg panel), CD40 on monocytes (Monocyte panel). Specifically, the IVW method is applied. The risk ratio of IgD- CD27% B cell to LAA is approximately 1.18 (95% CI=1.06-1.32, P=0.0036, PFDR=0.0217); The risk ratio of DN (CD4-CD8-) NKT AC

to LAA is approximately 1.14 (95% CI=1.00-1.29, P=0.0457, PFDR=0.0467); The risk ratio of PDL-1 on monocyte to LAA is approximately 1.12 (95% CI=1.00-1.24, P=0.0459, PFDR=0.0468); The risk ratio of CD28+CD45RA- CD8br% T cell to LAA is approximately 1.05 (95% CI=1.01-1.08, P=0.0059, PFDR=0.0254); The results obtained from the above IVW are similar to those of the other four methods, with OR values in a roughly consistent direction (OR value>1). Detailed results can be found in Fig.5, Fig.6.

Trails	method	nsnp	pval	OR(95%CI)	FDR
CD25 on CD39+ resting Treg	Inverse variance weighted	14	0.0235	1.13 (1.02−1.25)	0.0429
CD127 on CD4+	Inverse variance weighted	8	0.0452	1.12 (1.00−1.25)	0.0468
PDL−1 on monocyte	Inverse variance weighted	12	0.0460	1.12 (1.00−1.24)	0.0468
CD8 on TD CD8br	Inverse variance weighted	16	0.0422	1.11 (1.00−1.22)	0.0468
CD45 on T cell	Inverse variance weighted	15	0.0095	1.08 (1.02−1.15)	0.0300
CD40 on CD14+ CD16− monocyte	Inverse variance weighted	22	0.0109	1.08 (1.02−1.15)	0.0313
CD80 on CD62L+ myeloid DC	Inverse variance weighted	24	0.0134	1.08 (1.02−1.15)	0.0335
CD8 on EM CD8br	Inverse variance weighted	23	0.0293	1.08 (1.01−1.15)	0.0468
CD40 on CD14− CD16+ monocyte	Inverse variance weighted	23	0.0044	1.07 (1.02−1.13)	0.0237
CD40 on CD14+ CD16+ monocyte	Inverse variance weighted	18	0.0227	1.05 (1.01−1.10)	0.0429
CD40 on monocytes	Inverse variance weighted	26	0.0431	1.04 (1.00−1.07)	0.0468
CD33 on Mo MDSC	Inverse variance weighted	21	0.0460	0.96 (0.93−1.00)	0.0468
CD33 on CD33dim HLA DR+ CD11b−	Inverse variance weighted	22	0.0302	0.96 (0.92−1.00)	0.0468
CD33 on CD33br HLA DR+ CD14−	Inverse variance weighted	20	0.0308	0.96 (0.92−1.00)	0.0468
CD33 on CD33dim HLA DR−	Inverse variance weighted	19	0.0172	0.96 (0.92−0.99)	0.0381
CD33 on CD33dim HLA DR+ CD11b+	Inverse variance weighted	21	0.0255	0.95 (0.91−0.99)	0.0450
CD4 on activated & secreting Treg	Inverse variance weighted	31	0.0441	0.95 (0.90−1.00)	0.0468
CD3 on secreting Treg	Inverse variance weighted	20	0.0351	0.94 (0.89−1.00)	0.0468
HLA DR on HLA DR+ T cell	Inverse variance weighted	19	0.0459	0.94 (0.89−1.00)	0.0468
CD4 on CD39+ CD4+	Inverse variance weighted	28	0.0186	0.94 (0.89−0.99)	0.0385
CD3 on EM CD4+	Inverse variance weighted	18	0.0409	0.93 (0.88−1.00)	0.0468
CCR2 on monocyte	Inverse variance weighted	20	0.0349	0.93 (0.88−1.00)	0.0468
CD3 on HLA DR+ CD4+	Inverse variance weighted	26	0.0291	0.93 (0.88−0.99)	0.0468
CD25 on IgD+ CD38dim	Inverse variance weighted	21	0.0059	0.93 (0.89−0.98)	0.0255
CD25 on IgD+	Inverse variance weighted	24	<0.001	0.92 (0.89−0.97)	0.0059
CD27 on IgD+ CD38− unsw mem	Inverse variance weighted	24	0.0147	0.92 (0.86−0.98)	0.0340
CCR2 on CD14− CD16−	Inverse variance weighted	15	0.0491	0.91 (0.83−1.00)	0.0491
CD27 on IgD+ CD24+	Inverse variance weighted	29	0.0011	0.91 (0.86−0.96)	0.0106
CD27 on unsw mem	Inverse variance weighted	28	0.0134	0.90 (0.83−0.98)	0.0335
CD4 on CD39+ activated Treg	Inverse variance weighted	23	0.0144	0.90 (0.83−0.98)	0.0340
CD27 on memory B cell	Inverse variance weighted	23	0.0022	0.89 (0.82−0.96)	0.0163
CD27 on IgD− CD38−	Inverse variance weighted	29	<0.001	0.89 (0.83−0.95)	0.0080
CD27 on IgD− CD38dim	Inverse variance weighted	29	<0.001	0.88 (0.83−0.93)	<0.001
CD25 on IgD− CD38br	Inverse variance weighted	18	0.0110	0.88 (0.79−0.97)	0.0313
CD27 on sw mem	Inverse variance weighted	27	<0.001	0.87 (0.82−0.92)	<0.001
CD45 on CD33dim HLA DR+ CD11b−	Inverse variance weighted	9	0.0072	0.87 (0.78−0.96)	0.0271

Fig 6. Forest plots showed the causal associations between immune cell traits and large artery atherosclerosis by through the IVW method (inverse variance weighting method).
CI: confidence interval

After adjusting for FDR (FDR<0.05), we detected that 39 immune phenotypes were protective factors for the development of large artery atherosclerosis (LAA):CD45RA+CD28- CD8br% T cell (Treg panel), CD33 on Mo MDSC (Myeloid cell panel), CD33br HLA DR+CD14- AC (Myeloid cell panel), CD28+, CD45RA+CD8dim% CD8dim (Treg panel), CD33 on CD33dim HLA DR+CD11b - (Myeloid cell panel), CD39+resetting Treg% CD4 Treg (Treg panel), CD33 on CD33br HLA DR+CD14- (Myeloid cell panel), CD33 on CD33dim HLA DR - (Myeloid cell panel) ,CD33 on CD33dim HLA DR+CD11b+(Myeloid cell panel), CD4 on activated&secret Treg (Treg panel), BAFF-R on IgD+CD38dim (B cell panel), CD39+reseting Treg% reseting Treg (Treg panel), CD25 on B cell (B cell panel), CD3 on secreting Treg (Treg panel), HLA DR on HLA DR+T cell (TBNK panel), BAFF-R on memory B cell (B cell panel), CD4 on CD39+CD4+(Treg panel) ,CD3 on EM CD4+(Matura stages of T cell panel), CCR2 on monocyte (Monocyte panel), CD3 on HLA DR+CD4+(TBNK panel), CD25 on IgD+CD38dim (B cell panel), CD19 on CD24+CD27+(B cell panel),CD25 on IgD+(B cell panel), CD27 on IgD+CD38- unsw mem (B cell panel), CD8dim AC (TBNK panel), CCR2 on CD14- CD16- (Monocell panel), CD27 on IgD+CD24+(B cell panel), CD27 on unsw mem (B cell panel), TD CD8br AC (Matura stages of T cell panel), CD4 on CD39+activated Treg (Treg panel), CD4+AC (TBNK panel, CD27 on memory B cell (B cell panel) CD27 on IgD - CD38- (B cell panel), CD27 on IgD - CD38dim (B cell panel), CD19 on IgD - CD24- (B cell panel), CD25 on IgD - CD38br (B cell panel), CD27 on sw mem (B cell panel), CD45 on CD33dim HLA DR+CD11b - (Myeloid cell panel), IgD - CD38- AC (B cell panel). Mainly using the IVW method. The risk ratio of CD27 on sw mem to LAA is approximately 0.87 (95% CI=0.82-0.92, P=4.21E-06, PFDR=0.0001); The risk ratio of CD27 on memory B cell to LAA is approximately 0.86 (95% CI=0.82-0.96, P=0.0022, PFDR=0.016); The results obtained from the above IVW are similar to those obtained from the other four methods, with the OR value direction roughly consistent (OR value<1). Detailed results can be found in Fig.5, Fig.6. In addition, the global analysis of intercepts from MRPRESSO and MR Egger further ruled

out the possibility of horizontal pleiotropy in the aforementioned associations. Moreover, sensitivity analysis further confirms the observation, and scatter plots and funnel plots also demonstrate the robustness of the results.

4. Discussion

Based on publicly available GWAS data, we conducted a two sample Mendelian randomization study to investigate the causal relationship between 731 immune cell characteristics and different stroke. This study is the first Mendelian randomization study on the relationship between multiple immune cell characteristics and stroke. Among the four types of immune characteristics (AC, RC, MP, and MFI), 13 immune phenotypes were found to be risk factors for stroke, and 20 immune phenotypes were found to be protective factors for stroke; 13 immune phenotypes were found to have a positive correlation with ischemic stroke, while 22 immune phenotypes were found to have a negative causal relationship with ischemic stroke; 16 immune phenotypes were found to be a risk causal relationship for cardioembolic stroke, while the other 16 immune phenotypes were protective factors for cardioembolic stroke; 15 immune phenotypes were found to be risk factors for small vessel occlusion, and 29 immune phenotypes were found to be protective factors for small vessel occlusion; Finally, we found that 21 immune phenotypes were risk factors for large artery atherosclerosis, and its protective factors reached 39 immune phenotypes (FDR<0.05 for all the above analyses).

Our study found that as the proportion of CD4 on resting Treg cells increases, the risk of stroke and ischemic stroke increases, The increase in the proportion of CD4 on secretory Treg cells is also a risk factor for small vessel occlusion, while activated Treg% CD4 is a risk factor for cardioembolic stroke; On the contrary, we found that large artery atherosclerosis decreased with the increase of CD4 on CD39+activated Treg and other cells. Similarly, we found that CD45RA on resting Treg is a protective factor of cardiogenic stroke. Regulatory T cells (Treg cells) are a subset of immune regulatory cells that play a role in controlling the production of pro-inflammatory factors, influencing T cell proliferation, and maintaining immune homeostasis. The role of Treg cells in stroke may be beneficial or harmful. The function of Treg is related to the inflammatory environment in the body. Research has shown that Treg cells can act as neuroprotective modulators after stroke, and the increase of these cells can further prevent the occurrence of secondary infarction [28], The neuroprotective effect of Treg mainly exerts its immunosuppressive function through interaction with other immune cells, such as regulating the activity of neutrophils through PD-L1/PD-1 interaction, thereby exerting a protective effect on the central nervous system [29, 30]. Research has shown that IL-10 and TGF β IL-35 is an inflammatory factor secreted by activated Treg cells, which is beneficial for stroke and plays a role in neural recovery [31]. However, other research results have shown the opposite effect of Treg. In the tMACO model established by Schumann et al., it can be observed that within 24 hours after stroke, Treg cell growth is positively correlated with the size of stroke area [32]. Research has shown that the excessive activation of CD8+and CD4+effector T cells after a stroke is harmful as it can exert pro-inflammatory effects [33, 34].

Our study found that as the proportion of PDL-1 on CD14+CD16- monocyte cells increases, the risk of stroke and ischemic stroke increases. With the increase of CD40 on CD14- CD16+monocyte cells, the occurrence of ischemic stroke and large artery atherosclerosis increased. HLA DR on CD14-CD16- is a risk factor for small vessel occlusion, The expression of CD16 on CD14- CD16+monocyte is positively correlated with cardioembolic stroke, whereas we observed that CX3CR1 on CD14+CD16- monocyte is a protective factor for cardioembolic stroke. With the increased expression of CCR2 on monocyte and CCR2 on CD14-CD16-, the risk of large artery atherosclerosis is reduced. Traditional view [35], Monocytes are divided into three subgroups: classical monocytes (cd14++cd16-), intermediate monocytes (cd14++cd16+) and non classical monocytes (cd14+16cd++). Classical monocytes play a pro-inflammatory role in stroke, express high levels of CCR2 and low levels of CX3CR1, and can produce pro-inflammatory cytokines IL-6 and TNF- α, It can further affect the infarcted tissue and aggravate the degree of ischemic brain injury and stroke. Non classical monocytes exert anti-inflammatory effects in brain tissue, with high expression of CX3CR1 and no expression of CCR2. They can produce anti-inflammatory factors such as IL-10, which is beneficial for the recovery of ischemic stroke [36]. Studies have shown that in the early stage of stroke, the number of monocytes shows an increasing trend, and the phenotypic changes of monocytes are related to the different clinical processes of stroke [37, 38], CCR2 is highly expressed on the surface of classical monocytes. In the acute phase of stroke, the combination of CCL2 and CCR2 increases, which promotes the migration of classical monocytes to the brain. The severity of stroke is related to the expression of CCR2 and the number of monocyte infiltration [39]. Cx3cl1 is produced by neurons and is a member of the CX3C chemokine family. Its receptor is CX3CR1, which is mainly expressed by non classical monocytes and affects the size of the infarction area, blood-brain barrier and the recovery of neural function [40]. CD14 is a key recognition receptor of the innate immune system. It is a surface molecule involved in cell activation on monocytes and plays an important role in the acute inflammatory stage of stroke [41], CD14 deficiency can lead to low or excessive inflammation, excessive or insufficient immune cell recruitment, and further deterioration of stroke [42].

Our study found that in the B cell classification, CD27 on IgD+CD24+and CD19 on CD24+CD27+were related to the reduced risk of stroke, ischemic stroke and large artery atherosclerosis, and were protective factors. Similarly, the study found that with the increase of the proportion of CD25 on IgD+cd38dim cells, the risk of stroke, ischemic stroke, small vessel occlusion and large artery atherosclerosis decreased. CD19 on IgD - CD27- is a protective factor for cardioembolic stroke. On the contrary, some B cell subtypes are risk factors in the occurrence and development of stroke, ischemic stroke and other diseases, Examples include CD20-CD38-% B cell, IgD - CD27-% lymphocyte, and IgD - CD38dim% B cell. B cells play a protective role in the occurrence of stroke according to different cell phenotypes, and some phenotypes also play a risk factor role. Research has shown that repeated hypoxic preconditioning can significantly reduce the severity of stroke before it occurs, by inducing the immunosuppressive phenotype of B cells before stroke to exert a protective intervention effect on B cells [43]. Research has shown that B cells have a protective effect on neuronal damage after stroke, which is related to the secretion of relevant cytokines. For example, the secretion of anti-inflammatory cytokine IL-10 has the ability to limit stroke

occurrence and reduce stroke inflammation [44]. On the contrary, studies related to B cells have shown that the aggregation of B cell antibodies may be related to short-term memory decline and cognitive impairment after stroke [45]. Research reports that CD19 (+) CD86 (+) B cells are positively correlated with the risk of stroke, while CD19 (+) CD40 (+) B cells are negatively correlated with the risk of stroke [46]. CD27 is a costimulatory immune checkpoint receptor. After activation, it can promote the activity of B cell, T cell and NK cell, and promote the inflammatory immune process [47][47].

This study is the first two sample Mendelian randomization of the causal relationship between immune cells and stroke and clinical subtypes based on published GWAS cohort results. This study has the advantages that the traditional observational research does not have. The sample size is large. The use of genetic information as a tool variable can eliminate the influence of confounding factors and reverse causality. In addition, a variety of MR analysis methods and the application of level pleiotropy and heterogeneity test analysis make the results robust and comprehensive. At the same time, our research also has its limitations. First of all, the data we use are mainly European population data, which cannot represent the situation of other populations. Second, even if multiple analyses are used, level pleiotropy cannot be fully evaluated and confounding factors cannot be completely excluded. Finally, we used a more relaxed threshold to evaluate the cause and effect of immune cells and stroke, which may lead to some false positive results. Accordingly, we will more comprehensively evaluate the strong correlation between immune cells and stroke.

5. Conclusion

Our study provides genetic evidence for the causal relationship between various immune cell phenotypes and stroke and its clinical subtypes, which can highlight the complex causal relationship between the immune system and stroke. In this study, we significantly reduced the impact of confounding factors and reverse causality. This study may provide new ideas for exploring the biological mechanism of stroke, and help to explore the intervention and treatment of early stroke, which has good clinical significance.

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Authors' Contributions

QSL contributed to conceptualization and supervision—review and editing. HXZ, FH and HCZ contributed to formal analysis, statistical analysis and writing—original draft. DYL, XCP and DLQ contributed to acquisition of data. All authors contributed to the article and approved the submitted version.

Data Availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Further inquiries can be directed to the corresponding author.

Ethics Approval and Consent to Participate

All data are publicly available from the GWAS directory. All Mendelian analysis is based on the data collected by the public genome wide association study (GWAS). The data have been approved by the review committee of relevant institutions, so no additional ethical approval is required.

Competing Interests

The authors declare that they have no competing interests.

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