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ORIGINAL RESEARCH

Impact of Immune Cells on Stroke Limited to Specific Subtypes: Evidence from Mendelian Randomization Study

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ABSTRACT

Introduction: Stroke is one of the common diseases that pose a severe threat to human health, with immune cells playing a crucial role in its onset and recovery. However, the specific mechanisms and causal relationships of different immune cell groups in various clinical stroke subtypes are unclear. This study explored the causal relationship between immune cells and stroke and its subtypes using Mendelian randomization (MR) analysis.

Methods: Data from genome-wide association studies were analyzed using inverse-variance weighted (IVW), MR-Egger, and weighted median methods for MR analysis, along with heterogeneity tests, sensitivity analysis, and pleiotropy analysis.

Results: $CD45RA^{+}CD28^{-}CD8^{+}$ T cell %T cell (OR 1.002, 95% CI 1.001–1.003; $P_{FDR} = 0.02$), CD27 on $CD24^{+}CD27^{+}$ B cell (OR 1.127, 95% CI 1.061–1.198; $P_{FDR} = 0.04$), CD27 on $IgD^{-}CD38^{dim}$ B cell (OR 1.138, 95% CI 1.076–1.203; $P_{FDR} = 0.005$), and CD27 on switched memory B cell (OR 1.144, 95% CI 1.076–1.216; $P_{FDR} = 0.01$) were found to increase the risk of large artery stroke. Switched memory B cell %lymphocyte (OR 1.206, 95% CI 1.103–1.318; $P_{FDR} = 0.02$) increased the risk of small vessel stroke. Reverse MR analysis did not reveal any reverse causal associations. Furthermore, by substituting the outcome data, a secondary MR analysis was conducted to validate the primary findings.

Conclusion: Our study reveals several causal links between immune phenotypes and stroke and its different subtypes, highlighting the complex interactions between the immune system and stroke. These findings provide new directions for further uncovering the biological basis of stroke and assist in advancing research on early interventions and treatment strategies.

Keywords: Immune cells; Stroke; Large artery stroke; Small vessel stroke; Mendelian randomization

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Key Summary Points

A comprehensive analysis was conducted on the causal associations between 731 types of immune cells and six kinds of strokes and their subtypes.

The analysis utilized inverse-variance weighted (IVW), MR-Egger, and weighted median methods. Additionally, the MR-Egger and MR-PRESSO methods were employed to ensure the absence of horizontal pleiotropy.

After multiple testing corrections, it was found that four types of immune cells increase the risk of large artery stroke, and one type of immune cell raises the risk of small vessel stroke.

Our study data is based on European populations, which mitigates the potential impact of population stratification on the results but also limits the generalizability of our findings to other populations.

INTRODUCTION

Stroke is one of the common diseases that pose a severe threat to human health. Globally, approximately 16 million people suffer from acute stroke each year, ranking it as the second leading cause of death [1]. Stroke has a high disability and mortality rate, placing a significant burden on society and families [2]. Stroke can be categorized into ischemic and hemorrhagic types, with each subtype having distinct pathogenic mechanisms [3]. Ischemic stroke involves ischemic injury and reperfusion damage, triggering the aggregation and activation of immune cells, which promotes the release of inflammatory factors such as cytokines and chemokines, playing a role in ischemic brain tissue damage [4–6]. Monocytes can secrete pro-inflammatory cytokines and oxidative stress products, activate PTK and MAPK pathways, and damage the blood–brain barrier; macrophages can also produce NO, reactive oxygen species (ROS), and pro-inflammatory factors, regulating the inflammatory response; and adaptive immune cells such as T lymphocytes and natural killer cells are also extensively involved in ischemic brain injury [7–9].

However, the specific roles and causal relationships of different immune cell populations in various clinical stroke subtypes still need to be fully understood. No studies have systematically assessed the causal association between immune cells and different subtypes of stroke. Mendelian randomization (MR) studies, using genetic markers as instrumental variables, can effectively infer causal relationships between exposure factors and outcomes [10]. This research design overcomes the limitations of traditional observational studies that are susceptible to confounding factors and reverse causation, as genetic markers can avoid interference from environmental factors and behavioral choices. The random allocation nature of genetic markers endows the study with an internal validity similar to that of randomized controlled trials [11].

This study, using the two-sample MR method, investigated the causal relationships between 731 immune cell phenotypes and six significant subtypes of stroke, including ischemic and hemorrhagic strokes. We utilized publicly available genome-wide association study data to delve into the role of different immune cells in the pathogenesis of stroke from a genetic perspective. The results of this study could provide a theoretical basis for the prevention and treatment of stroke and will expand the horizons of neuroimmunological research.

METHODS

Study Design

Our analysis based on two-sample MR evaluated the causal relationships between 731 immune cell phenotypes (across seven groups) and six subtypes of stroke. MR uses genetic variations as

Table 1 Data sources and demographic profiles

Exposures or outcome	Sample size (cases/controls)	Ancestry	Sex	Publication date (year)	Significance level	GWAS catalog ID or URL
Stroke	39,818/271,817	European	M and F	2022	5e−8	https://www.finngen.fi/fi
Intracerebral hemorrhage	3749/339,914	European	M and F	2022	5e−6	https://www.finngen.fi/fi
Ischemic stroke	34,217/406,111	European	M and F	2018	5e−8	GCST006908
Cardioembolic stroke	7193/406,111	European	M and F	2018	5e−8	GCST006910
Large artery stroke	4373/406,111	European	M and F	2018	5e−8	GCST006907
Small vessel stroke	5386/192,662	European	M and F	2018	5e−7	GCST006909
Immunophenotypes	NA	European	M and F	2020	1e−5	GCST90001391–GCST90002121

NA not acquired

proxies for risk factors. Thus, effective instrumental variables in causal inference must satisfy three key assumptions: (1) genetic variations are directly associated with the exposure; (2) genetic variations are independent of potential confounding factors between the exposure and outcome; (3) genetic variations affect the outcome exclusively through the exposure variable. All studies used in our analysis were ethically reviewed by relevant institutions, and informed consent was obtained. In conducting this study, all procedures were carried out in full accordance with the ethical standards of the Declaration of Helsinki. Our research is based on these published genome-wide association study (GWAS) summary data; hence, additional approval from an ethics review committee was not required.

GWAS Data Sources

The stroke data included stroke, intracerebral hemorrhage, ischemic stroke, and its subtypes, defined according to the Org 10172 Acute Stroke Treatment classification trial [3], including large artery stroke (LAS), small vessel stroke (SVS), and cardiogenic stroke (CES). The sources and detailed information of this data are presented in Table 1.

We downloaded the summary statistics of the 731 immune cell phenotypes from the GWAS Catalog (IDs GCST90001391 to GCST90002121) [12]. In the analysis conducted at a genome-wide significance threshold of $P < 1.28 \times 10^{-11}$, 122 independent significant association signals were identified across 70 loci, including 53 that are newly discovered. These signals elucidate the molecules and mechanisms involved in regulating 459 cellular phenotypes. Among these, 49 signals (40%) are local *cis* associations, 57 signals (47%) are distant *trans* associations, and 16 signals (13%) exhibit both *cis* and *trans* associations. These 731 immune phenotypes originated from seven major immune groups, specifically including groups such as MFI, AC, and RC containing B cells, dendritic cells, mature T cells, monocytes, granulocytes, TBNK (T cells, B cells, natural killer cells), and regulatory T (Treg) cells; the MP group contained groups such as dendritic cells and TBNK—the original GWAS of immune traits utilized data from 3757 European individuals without overlapping cohorts. Approximately 22 million single nucleotide polymorphisms (SNPs) were genotyped using high-density arrays and imputed using a reference panel based on Sardinian sequences [13], and correlations were tested after adjusting for covariates (namely sex, age, and principal components).

Selection of Instrumental Variables

When selecting instrumental variables (IVs) for immune traits, we referred to recent studies [12, 14], setting the significance level at

1×10^{-5}. The clumping procedure in PLINK software (version v1.90) was used to prune these SNPs (linkage disequilibrium [LD] r^2 threshold < 0.001 within 10,000 kb distance) [15], where LD r^2 was calculated on the basis of the 1000 Genomes Projects as a reference panel.

For selecting IVs for stroke and its subtypes, we adjusted the significance levels according to the actual conditions, with the maximum set at 5×10^{-6} and the minimum at 5×10^{-8} (see Table 1 for details). The clumping procedure was the same as that used for immune traits. Furthermore, to assess the reliability and effectiveness of each SNP as a genetic IV in MR analysis, we calculated the *F*-statistics for each SNP using the formula $F = R^2(N - 2)/(1 - R^2)$. Here, R^2 represents the proportion of variance in the exposure variable explained by the IV, and N is the sample size of the original GWAS serving as the outcome variable. Specifically, R^2 was calculated using the following formula: $R^2 = [2 \times \text{EAF}(1 - \text{EAF}) \times \beta^2]/([2 \times \text{EAF}(1 - \text{EAF}) \times \beta^2] + \{2 \times \text{EAF}(1 - \text{EAF}) \times N \times [\text{SE}(\beta)]^2\})$. If the *F*-statistic of an IV was less than 10, it was considered unreliable and excluded from subsequent MR analysis. These steps help ensure that our study uses high-quality genetic IVs for reliable analysis.

MR Analysis

This study employed the statistical analysis software R version 4.2.3, and the analyses were conducted using the Mendelian Randomization package (version 0.9.0). The primary method used was the inverse-variance weighted (IVW) method. Additional methods, such as MR-Egger and weighted median, were also applied to enhance the reliability of the results. To mitigate the impact of multiple testing, we employed the p.adjust function within the R programming environment to apply false discovery rate (FDR) correction to the *P* values generated by the IVW method. Results with corrected $P < 0.05$ were considered positive, even if the *P* values from the MR-Egger and weighted median methods were greater than 0.05, as long as the direction of effect was consistent across all three methods.

Heterogeneity, Pleiotropy, and Sensitivity Analysis

The heterogeneity test was conducted using IVW and MR-Egger methods. If the test *P* value was less than 0.05, it indicated heterogeneity among SNPs; if it was greater than 0.05, it suggested no heterogeneity. Sensitivity analysis employed the leave-one-out method to explore the influence of individual SNPs on the causal association.

To exclude the effects of horizontal pleiotropy, we searched for related SNPs on the PhenoScanner website and excluded those that might directly affect stroke risk factors such as diabetes, hypertension, and stroke. The presence of horizontal pleiotropy was assessed using the intercept from the MR-Egger method; a significant intercept implies pleiotropy. Additionally, the MR-PRESSO method was used to detect and adjust for potential outliers that might significantly influence the MR analysis results because of horizontal pleiotropy. If horizontal pleiotropy could not be eliminated through these three methods, we would abandon the MR for that particular exposure factor.

Furthermore, scatter plots and funnel plots were utilized. Scatter plots indicated that results were not influenced by outliers. Funnel plots demonstrated the robustness of the correlations and the absence of heterogeneity.

RESULTS

Exploration of Causal Effect of Immunophenotypes on Stroke and Its Subtypes

In the unadjusted IVW results, 39 immune cell types were causally associated with stroke, 19 with ICH (intracerebral hemorrhage), 44 with ischemic stroke, 55 with large artery stroke, 45 with cardioembolic stroke, and 56 with small vessel stroke.

After correction for FDR ($P_{\text{FDR}} < 0.05$), no causal associations were found between immune cells and stroke, ICH, ischemic stroke, or cardioembolic stroke. However, four immune cell types were causally associated with large

artery stroke and one with small vessel stroke. Specifically, the percentage of $CD45RA^{+}CD28^{-}CD8^{+}$ T cells in T cells (OR 1.002, 95% CI 1.001–1.003; $P_{FDR} = 0.02$), CD27 on $CD24^{+}CD27^{+}$ B cells (OR 1.127, 95% CI 1.061–1.198; $P_{FDR} = 0.04$), CD27 on $IgD^{-}CD38^{dim}$ B cells (OR 1.138, 95% CI 1.076–1.203; $P_{FDR} = 0.005$), and CD27 on switched memory B cells (OR 1.144, 95% CI 1.076–1.216; $P_{FDR} = 0.01$) were found to increase the risk of large artery stroke (see Fig. 1a). The percentage of switched memory B cells in lymphocytes (OR 1.206, 95% CI 1.103–1.318; $P_{FDR} = 0.02$) was found to increase the risk of small vessel stroke (see Fig. 1b). Information on SNPs involved in each immune phenotype can be found in Supplementary Tables 1–5.

Additionally, the aforementioned associations were confirmed to be free from horizontal pleiotropy, as evidenced by the MR-Egger intercept and MR-PRESSO global test (see Table 2). Leave-one-out analysis indicated that results were not influenced by any single SNP, with scatter plots and funnel plots demonstrating the stability of the results (see Supplementary Figs. 1–5).

Exploration of Causal Effect of Stroke on Immunophenotypes

In the reverse MR analysis, the unadjusted IVW results showed causal associations of large artery stroke with 15 immune cell types, ischemic stroke with 22, small vessel stroke with 18, cardioembolic stroke with 33, and intracerebral hemorrhage with 16. No causal associations were found between any stroke subtype and immune cells after FDR correction ($P_{FDR} < 0.05$).

Validation of Key Findings

Following FDR correction ($P_{FDR} < 0.05$), we identified causal associations between four types of immune cells and large artery stroke, and one type of immune cell with small vessel stroke. On the basis of peer reviewers' recommendations, we utilized the latest GWAS Catalog data for large artery stroke (GCST90104542) and small vessel stroke (GCST90104543) to validate our findings. The updated datasets, both published in 2022 and derived from European populations, included 6399 cases with 1,234,808 controls for large artery stroke,

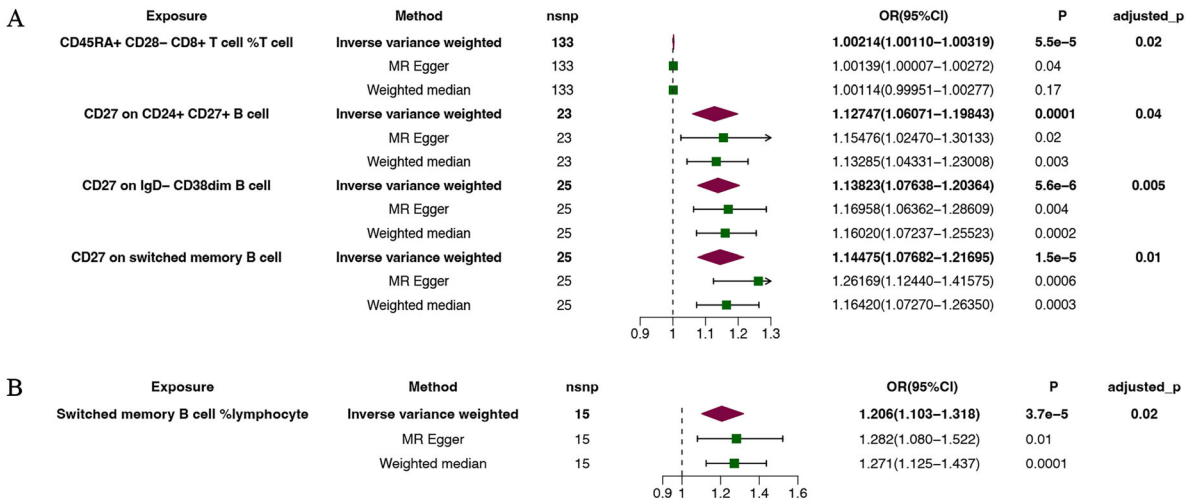

Fig. 1 Estimated causal effects of immunophenotypes on **a** large artery stroke and **b** small vessel stroke using different MR methods. *IVW* inverse variance weighted, *MR-Egger* Mendelian randomization-Egger, *nsnp* number of single nucleotide polymorphisms, *CI* confidence interval. adjusted_p, to mitigate the impact of multiple testing, false discovery rate (FDR) correction was applied to the *P* values from the IVW method

Table 2 MR sensitivity analyses of genetically predicted immunophenotypes on large artery stroke and small vessel stroke

Outcome	Exposure	Heterogeneity tests		Directional horizontal pleiotropy test	
		Methods	Cochran's $Q(P)$	MR-Egger intercept (P)	Pleiotropy*
Large artery stroke	$CD45RA^{+}CD28^{-}CD8^{+}$ T cell %T cell	MR-Egger, IVW	140.47 (0.27), 143.85 (0.22)	0.02 (0.07)	0.24
	CD27 on $CD24^{+}CD27^{+}$ B cell	MR-Egger, IVW	25.59 (0.22), 25.85 (0.25)	− 0.009 (0.65)	0.21
	CD27 on $IgD^{-}CD38^{dim}$ B cell	MR-Egger, IVW	22.42 (0.49), 22.90 (0.52)	− 0.008 (0.49)	0.48
	CD27 on switched memory B cell	MR-Egger, IVW	23.85 (0.41), 27.65 (0.27)	− 0.03 (0.06)	0.25
Small vessel stroke	Switched memory B cell %lymphocyte	MR-Egger, IVW	6.40 (0.93), 7.07 (0.93)	− 0.01 (0.42)	0.94

*Detect by MR-PRESSO Global Test

MR-Egger Mendelian randomization-Egger, *IVW* inverse-variance weighted

and 6811 cases with 1,234,808 controls for small vessel stroke. Supplementary MR analysis corroborated our discoveries, as illustrated in Fig. 2. Additionally, pleiotropy tests were conducted to ensure the robustness of our results, detailed in Supplementary Table 6.

DISCUSSION

Main Findings

In this study, we assessed the potential causal links between 731 immune cell phenotypes and stroke and its subtypes. Following FDR correction, we found no immune cells causally associated with stroke, ICH, ischemic stroke, or cardioembolic stroke. However, we identified four immune cell types that increase the risk of large artery stroke and one that increases the risk of small vessel stroke. Through reverse MR analysis, we confirmed that there is no reverse causal relationship between stroke and its subtypes and immune cell phenotypes. Additionally, to strengthen the reliability of our primary findings, we conducted secondary MR analyses using different datasets to validate our discoveries. This approach significantly enhances the credibility of our results, an aspect often lacking in many current MR studies.

Our findings are robust, as confirmed by sensitivity analyses and pleiotropy tests. Current research suggests that although IVW is less robust, it provides more accurate estimates in the absence of horizontal pleiotropy [16]. Our MR-pleiotropy tests and MR-PRESSO Global Test results suggest no presence of horizontal pleiotropy. Therefore, we consider IVW as our primary reference method, and when IVW's P_{FDR} is < 0.05, and the other two methods concur in direction, we regard the causal association as present.

We opted for the FDR method for multiple testing correction owing to its ability to strike a good balance in controlling the FDR. This approach maintains a lower rate of false findings while preserving greater statistical power, particularly beneficial when dealing with large datasets. Its advantage lies in allowing a certain proportion of false positives, thereby reducing the likelihood of type II errors (false negatives), which is especially important in exploratory

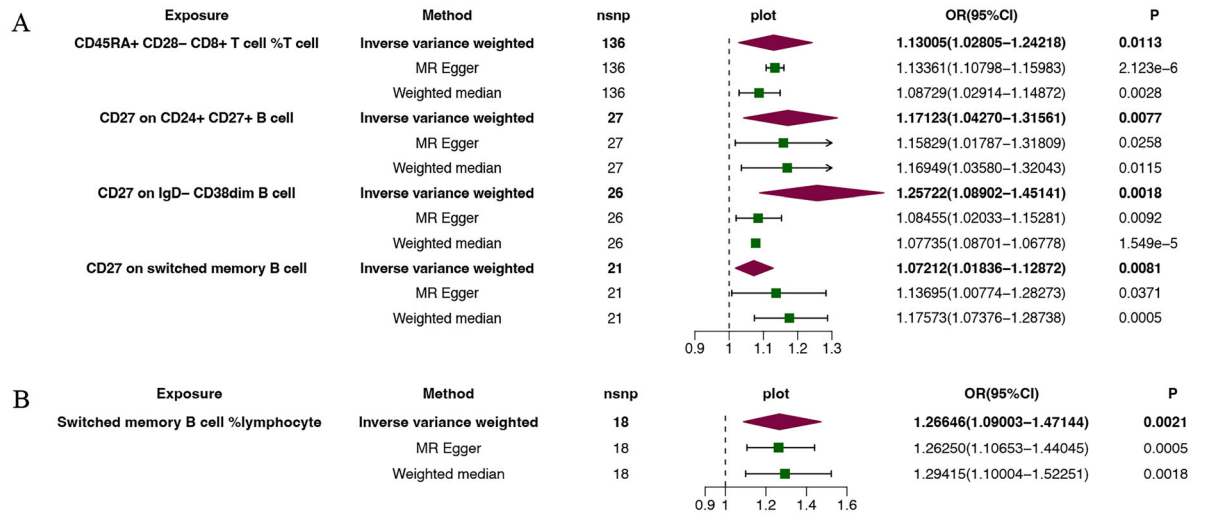

Fig. 2 Validates the primary MR analysis results based on new large artery stroke and small vessel stroke data. Estimated causal effects of immunophenotypes on **a** large artery stroke and **b** small vessel stroke using different MR methods. *IVW* inverse variance weighted, *MR-Egger* Mendelian randomization-Egger, *nsnp* number of single nucleotide polymorphisms, *CI* confidence interval

research. However, we acknowledge that no statistical method is perfect, and we cautiously consider our research conclusions to be statistically significant, albeit subject to validation by further experimental studies.

Relationship Between Immune Cells and Stroke and Its Subtypes

CD45RA and CD28 are important surface markers of T cells, with CD45RA typically associated with the differentiation of naïve and memory T cells. At the same time, CD28 is a major co-stimulatory receptor for T cell activation [17–19]. A specific T cell subset, $CD45RA^{+}CD28^{-}CD8^{+}$ T cells, may play a significant role in the immune response following stroke. T cell responses can be observed within 24 h post-ischemic stroke, and T cells remain active in the brain for at least 30 days, underscoring their importance in neuroprotection and inflammation post-stroke. The $CD45RA^{-}CD28^{-}$ phenotype may represent T cell subsets with different functions and maturation states. For instance, $CD8^{+}CD45RA^{-}$ T cells exhibit increased cytokine secretion, potentially impacting inflammation and aging processes, thus correlating with an increased risk of large artery stroke [20].

Literature informs us that cerebral small vessel disease (CSVD) is closely related to immune responses and inflammatory reactions. Inflammatory mediators and lymphocytes significantly contribute to the development of brain injury and neurological deficits post-acute ischemic stroke or cerebral hemorrhage due to large artery occlusion. It is also mentioned that immunosenescence and inflammatory responses, along with their interaction with the cerebrovascular system, may be underlying factors for CSVD [21]. This could hint at the potential role of switched memory B cells in small vessel stroke, although their specific pathophysiological roles still need further clarification.

Further exploring the association with large artery stroke, we note that CD27 is an important immunoregulatory molecule playing a crucial role in the activation and differentiation of B and T cells. From a broader perspective, the role of immune responses in CSVD, including inflammation and immune reactions associated with pathological changes in cerebral microvasculature, may provide clues for understanding the association between immune cell

phenotypes and large artery stroke [22, 23]. Additionally, CSVD is a significant cause of vascular dementia and is closely linked to cognitive decline and functional loss in the elderly [21].

T cell effector functions can be observed within 24 h post-ischemic stroke, with T cells remaining active in the brain for at least 30 days. Eliminating $CD4^+$ or $CD8^+$ T cell subsets within 24 h post-ischemic stroke can reduce infarct volume [24]. Human naïve and memory T cells can be distinguished on the basis of the expression of surface molecules (including CD45RA) [19]. Utilizing CD45RA and CD28 helps to examine two effector memory groups at early and late differentiation stages, which might relate to the association of $CD45RA^+CD28^-CD8^+$ T cells with stroke subtypes [25].

Stroke triggers a complex innate and adaptive immune response, including immune cell-mediated and factor-mediated vascular and tissue damage. Modulating immunity in stroke is of paramount importance [26]. There is increasing evidence of an adaptive (or maladaptive) immune response following ischemic stroke, indicating a complex role of the immune system in the pathogenesis of stroke [27]. Time is a critical determinant of whether immunity and inflammation in stroke are neuroprotective or neurotoxic, while the local inflammatory milieu significantly affects many proposed therapeutic approaches [28]. Particularly, Treg cells play a key role in immunoregulation in ischemia–reperfusion injury, a core aspect of stroke pathophysiology [29].

Strengths and Limitations

Our comprehensive analysis of the causal relationships between 731 immune cell types and six stroke subtypes used a variety of MR methods to ensure the robustness of the results, free from confounding by horizontal pleiotropy and other factors. Our study reveals partial causal relationships between certain immune cells with large artery stroke and small vessel stroke, providing a reference for subsequent prevention, treatment, and drug development related to these diseases. However, our study has limitations. Firstly, as a result of sample restrictions, our study population included only Europeans. While this avoids the potential influence of different populations on the results, it limits the generalizability of our findings to other populations. Secondly, as a result of limitations in the data, we were unable to perform further stratified analyses of the population. Furthermore, when delving into more detailed subtype analyses, such as the distinctions between ischemic and hemorrhagic types in CSVD with respect to immune cell types [30], we were unable to conduct further analysis. Finally, in some conclusions, the IVW results were positive, while the MR-Egger and weighted median methods were negative. Although we have explained this phenomenon on the basis of current theories, we cannot completely exclude the possibility of false positives in these results.

Reflections on Future Research

In this study, through the application of MR analysis, we preliminarily explored the causal associations between 731 types of immune cells and six subtypes of stroke. It is our hope that future research will build upon this foundation to delve deeper into the specific roles of different immune cells in the onset and progression of stroke, thereby contributing to a more nuanced understanding of disease mechanisms. Additionally, examining the interactions between immune cells and the recovery process in patients with stroke may provide valuable insights for enhancing rehabilitation strategies. Further understanding of how immune cells influence the long-term prognosis of stroke could significantly impact patient quality of life. On a technical level, advancements in high-throughput sequencing and single-cell technologies present us with unprecedented opportunities to study immune cells in stroke with remarkable resolution. Lastly, it is important to note the existence of certain stroke subtypes characterized by diagnostic challenges, often referred to as cryptogenic strokes or strokes of undetermined origin. These cases are not uncommon in clinical practice but pose

complexities in management and treatment due to the absence of a clear etiology. Future research could investigate the role and impact of immune cell types in these challenging stroke subtypes, potentially improving our understanding of stroke pathophysiology and paving the way for the development of new diagnostic tools and treatment strategies.

CONCLUSION

Our study, through in-depth bidirectional MR analysis, reveals several causal links between immune phenotypes and stroke and its different subtypes, highlighting the complex interactions between the immune system and stroke. These findings provide new directions for further revealing the biological basis of stroke and aid in advancing research on early intervention and treatment strategies.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval. As this research was based on previously published GWAS summary data, institutional review board approval was not needed, and prior informed consent was obtained from all participants. All procedures were carried out in full accordance with the ethical standards of the Declaration of Helsinki.

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