WHIOCE PUBLISHING PTE. LTD.
PROVIDING FIRST-CLASS SCIENTIFIC INFORMATION FOR TOP SCHOLARS

2025,Volume 10, Issue 2
Advances in Precision Medicine
Online ISSN: 2424-9106
Print ISSN: 2424-8592

Genetically Predicted Immune Cells Mediate the Association between HLA-B and Cerebral Atherosclerosis

Jiuchang Zhang[1,2], Zhongrui Yan[2]
[1]Department of Neurology, Affiliated Hospital of Qingdao University, Qingdao 266100, Shandong, China
[2]Department of Neurology, Jining No. 1 People's Hospital, Jining 270000, Shandong, China

Abstract: *Objective:* Investigating the causal relationship between HLA Class l genes and cerebral atherosclerosis (CA) and identifying the role of immune cells as a potential mediator. *Methods:* The study conducted a Mendelian randomization (MR) study using an Inverse Variance Weighted (IVW) approach to investigate the causal role of HLA Class I genes in CA and the mediating effect of immune cells on this association. *Results:* MR Analysis determined that HLA-B (IVW: odds ratio (OR) = 1.4493, 95% confidence interval (CI) = 1.1296−1.8595, $p = 0.0035$) increased the risk of CA. In addition, 39 immune cell traits were associated with CA. Notably, the proportion of genetically predicted HLA-B mediated by Unsw Mem % lymphocyte was 13.1%. *Conclusion:* This study suggests a causal relationship between HLA-B and CA, possibly mediated by immune cells.

Keywords: Immune cells; Mendelian randomization; HLA-B; Cerebral atherosclerosis

Online publication: June 28, 2025

1. Introduction

With the advent of aging, atherosclerosis poses a major threat to global health, placing a heavy burden on global healthcare systems [1]. When atherosclerotic plaque forms in the cerebrovascular system, cerebral atherosclerosis (CA) increases the risk of stroke, dementia and cognitive decline [2]. The causes of CA have been debated, such as dyslipidemia, hypertension [3], viral infection, inflammation [4] and so on. Previous studies conducted immune infiltration analysis on multiple atherosclerosis datasets and compared them from different perspectives, such as plaque location and time node. This study found that the HLA class I pathway had the highest immune score. HLA Class I genes include HLA-A, HLA-B, and HLA-C. Historically, the HLA Class I system has been associated with autoimmune diseases, infectious diseases, and diseases associated with infection [5]. Therefore, elucidating the causal relationship between HLA Class l genes and cerebral atherosclerosis can further clarify the pathogenesis of CA.

In addition, potential pathways associated with HLA Class l genes and CA have not been studied. Previous

evidence has shown the role of immune cells in CA [6,7]. However, whether HLA class l genes can affect CA by modulating immune cells remains unknown. Mendelian randomization (MR), which employs genetic variants as instrumental variables (IVs) from genome-wide association studies (GWAS), infers causal effects of exposure on outcomes [8]. The objective of this study was to ascertain whether there exists a causal relationship between HLA-Class I genes and coronary artery disease (CA), and to evaluate the extent to which immune cell-mediated HLA-class I genes contribute to CA.

2. Materials and methods

2.1. Microarray data

The datasets (transcriptome data: GSE28829, GSE43292 and GSE100927) included in this study were all obtained from GEO databases (https://www.ncbi.nlm.nih.gov/geo/).

2.2. Data processing of DEGs

The three raw datasets were pre-processed in R (version 4.1.0) using affy, including background calibration, normalization, and log2 transformation. Then, the LIMMA package was used to screen DEGs.

2.3. Immune infiltration analysis

To explore the immune association with atherosclerosis, we utilized the single-sample Gene Set Enrichment Analysis (ssGSEA) method via the Gene Set Variation Analysis (GSVA) package in R [9]. Enrichment scores for immune cells, functions, and pathways were quantified by analyzing 29 immune gene sets, which encompassed 13 immune-related activation pathways.

2.4. Data source and screening IV

Publicly accessible data on genetic variants associated with HLA Class l genes were obtained from the GTEx Portal (https://gtexportal.org/home/). To minimize LD effects, the study set the significance threshold as "$P < 5\times10-8$; LD $r^2 < 0.1$" for identifying SNPs related to HLA Class l genes. CA data (R10) were sourced from the FinnGen database (https://www.finngen.fi/en/access_results). Data for 731 immune cell traits (Ebi-a-GCST0001391 to Ebi-a-GCST0002121) were retrieved from the GWAS Catalog (https://gwas.mrcieu.ac.uk/) [10]. Cochran's Q, MR-Egger, and Leave-One-Out analyses were used to assess heterogeneity, gene pleiotropy, and IV sensitivity, respectively, and mixed IVs were excluded.

2.5. Primary analysis

Using the "TwoSampleMR" package in R, we performed MR analysis to investigate the causal relationship between HLA Class I genes and CA. Methods applied included IVW, weighted median, MR-Egger, simple mode, and weighted mode [11]. The causal effect was expressed as odds ratios (OR) with 95% confidence intervals (CI), and statistical significance was defined as a P value < 0.05.

2.6. Mediation analysis

To investigate whether immune cells mediate a causal pathway from HLA-B to coronary artery (CA) outcomes, the study employed a two-step Mendelian Randomization (MR) design for mediation analysis [12]. This approach partitions the total effect of HLA-B on CA into: (1) the direct effect and (2) the indirect effect mediated by immune cells. The proportion mediated by immune cells was calculated by dividing the indirect effect by the total effect. The overall effect comprises both these components.

3. Results

3.1. Immune infiltration analysis

The study used ssGSEA to compare the enrichment scores of the activity of 13 immune-related pathways in control and atherosclerosis patients from three datasets. The study found that the activity of immune pathways was generally higher in the atherosclerosis group than in the control group. Interestingly, the HLA class I pathway was the highest rated in the three datasets (**Figure 1A**).

3.2. Expression of HLA-A, HLA-B and HLA-C

In the three datasets, HLA Class l genes were differentially expressed, and HLA-B was the most significant (**Figure 1B**).

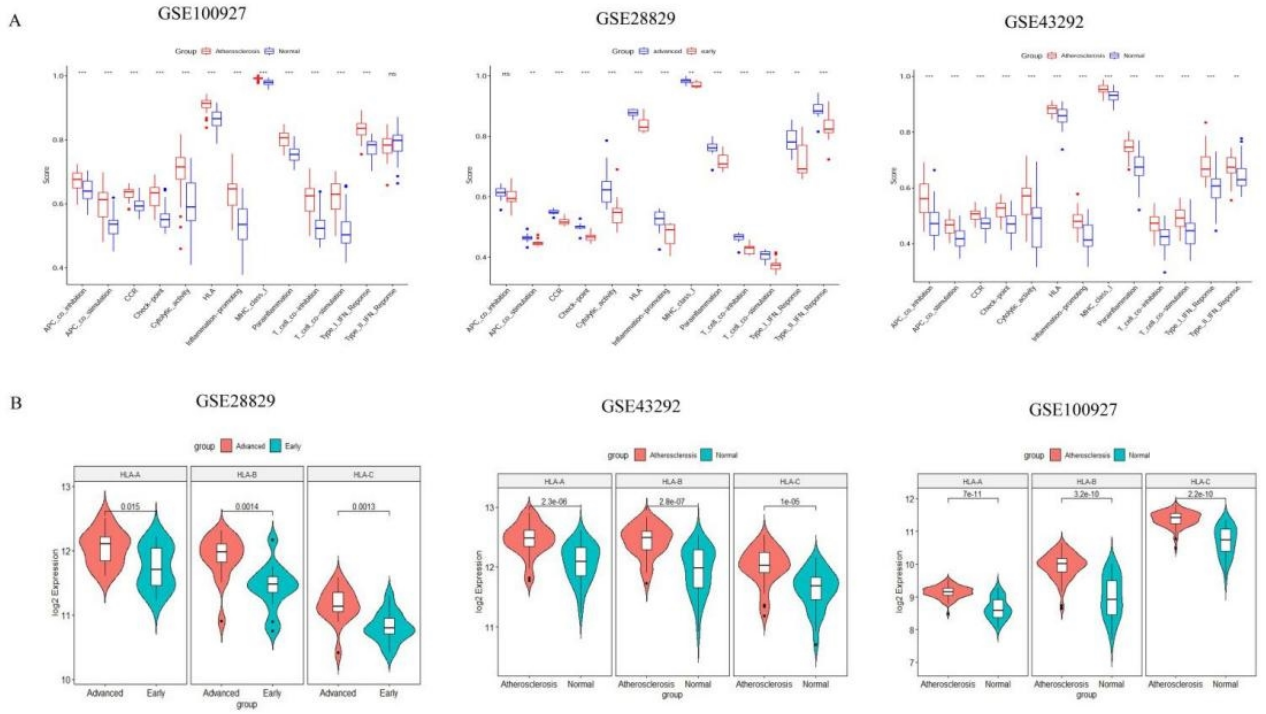

Figure 1. Comparison of the ssGSEA scores between different groups in the datasets. (A) The scores of 13 immune-related functions are displayed in box plots. (B) Expression of HLA-A, HLA-B and HLA-C in different datasets. Adjusted P values were shown as: ns, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

3.3. Association of HLA Class l genes with CA

Significant association was observed for HLA-B with CA using 17 SNPs via IVW (OR = 1.4493, 95% CI = 1.1296–1.8595, p = 0.0035; **Figure 2**), whereas no significant correlations were found for HLA-A (OR = 1.1262, 95% CI = 0.9088–1.3956, p = 0.2773) or HLA-C (OR = 1.0535, 95% CI = 0.9138–1.2145, p = 0.4728). Sensitivity analyses confirmed robustness: Cochran's Q (IVW/MR-Egger) showed no heterogeneity (**Table 1**), pleiotropy testing indicated no horizontal pleiotropy (p > 0.05; **Table 2**), and leave-one-out analysis revealed no influential SNPs (**Figure 3**).

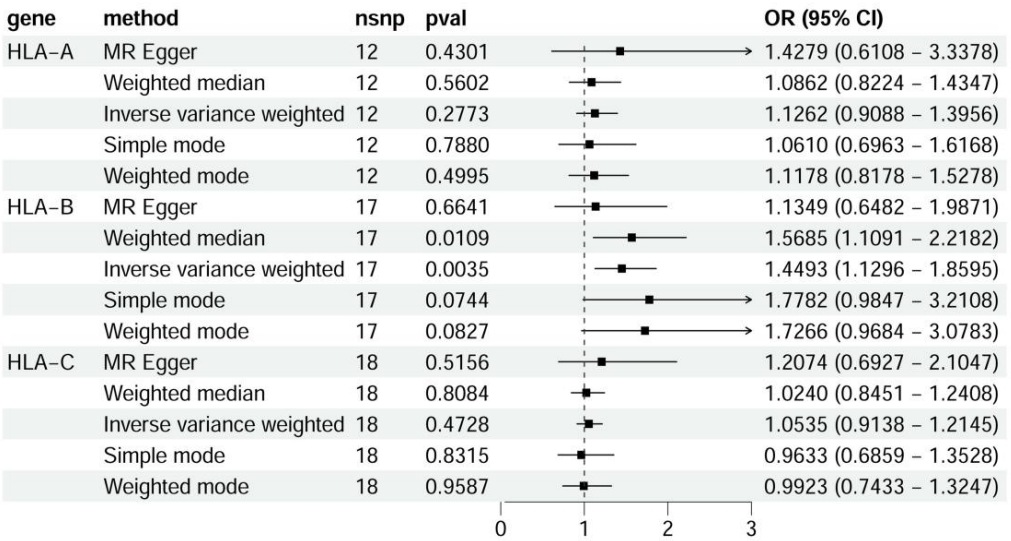

Figure 2. MR analysis showed the causal effect of HLA Class 1 genes and cerebral atherosclerosis. CI: Confidence interval; MR: Mendelian randomization; OR: odds ratio; SNP: Single nucleotide polymorphism.

Table 1. Heterogeneity results by Cochran's Q test of HLA Class l genes on cerebral atherosclerosis

Exposures	Outcomes	Cochran's Q test	Heterogeneity *p*-value
HLA-A	Cerebral atherosclerosis	IVW	9.60E-01
		MR Egger	9.48E-01
HLA-B		IVW	0.709703178
		MR Egger	0.710800693
HLA-C		IVW	8.20E-01
		MR Egger	7.83E-01

Table 2. Pleiotropy results of MR analysis of HLA Class l genes on cerebral atherosclerosis

Exposure	Outcomes	SE	Intercept	*p*-value
HLA-A		0.112250334	-0.063552389	0.583761733
HLA-B	cerebral atherosclerosis	0.071855283	0.068652298	0.3545085
HLA-C		0.085929971	-0.042767689	0.625463383

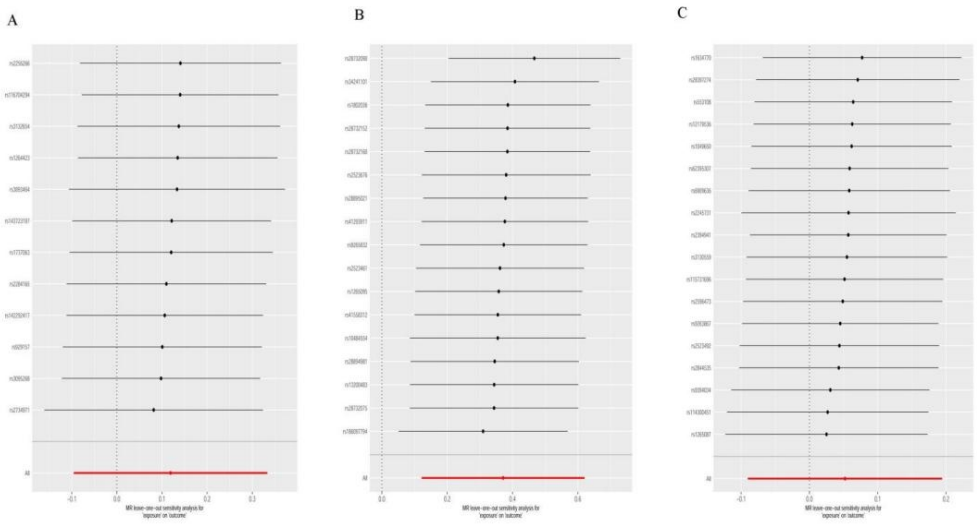

Figure 3. Leave-one-out analysis visualizing the Mendelian randomization (MR) estimates of the different exposures (A: HLA-A; B: HLA-B; C: HLA-C) with the outcome (cerebral atherosclerosis).

3.4. Association of immune cells with CA

The study finds that the inverse relationship between Unsw Mem % lymphocyte and CA is the most significant (OR = 0.6577, 95% CI = 0.5234–0.8265, $p < 0.001$) (**Figure 4**). No heterogeneity or horizontal pleiotropy was observed, and no single SNP appeared to drive the causal estimates (**Table 3**, **Table 4**, **Figure 5**).

Figure 4: MR analysis showed 16 immune cell traits had promoting effect on cerebral atherosclerosis and 23 immune cell

traits had inhibiting effect on cerebral atherosclerosis.

Table 3. Heterogeneity results by Cochran's Q test of Unsw Mem %lymphocyte on cerebral atherosclerosis

Exposures	Outcomes	Cochran's Q test	Heterogeneity *p*-value
Unsw Mem % lymphocyte	Cerebral atherosclerosis	IVW	0.527183699
		MR Egger	0.470141429

Table 4. Pleiotropy results of MR analysis of Unsw Mem % lymphocyte on cerebral atherosclerosis

Exposure	Outcomes	SE	intercept	*p*-value
Unsw Mem % lymphocyte	Cerebral atherosclerosis	0.046170232	0.014666025	0.754042196

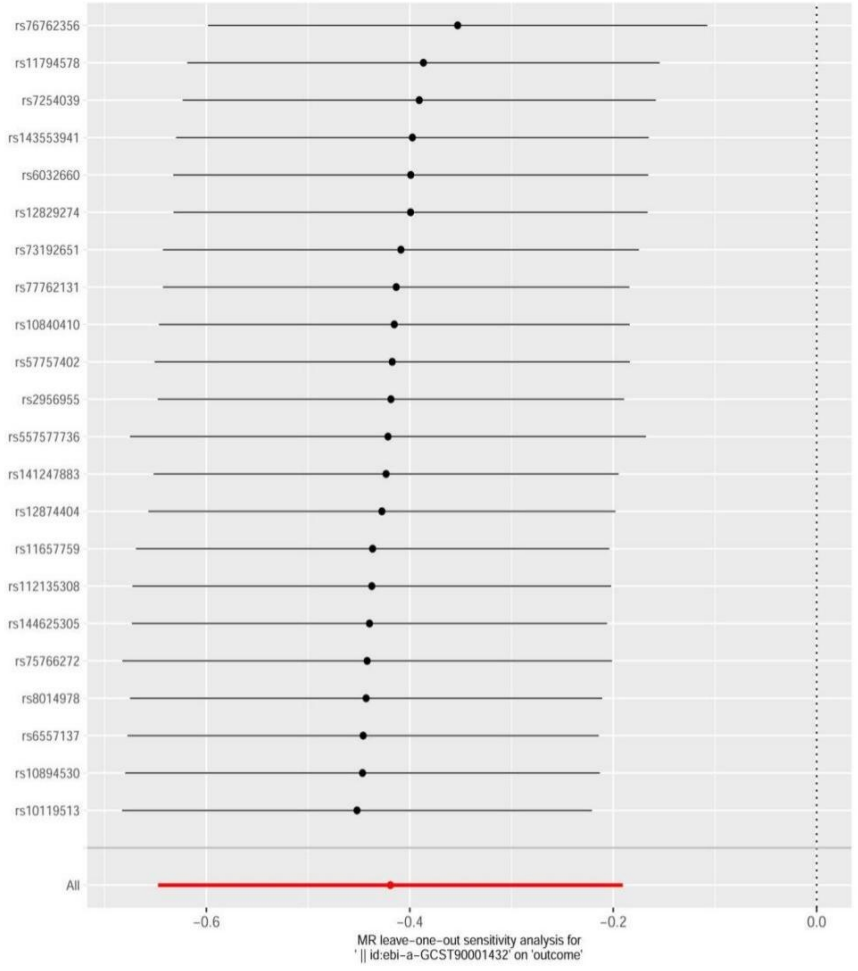

Figure 5. Leave-one-out analysis visualizing the Mendelian randomization (MR) estimates of the exposure (Unsw Mem % lymphocyte) with the outcome (cerebral atherosclerosis).

3.5. Association of HLA-B with immune cells

As shown in **Figure 6**, the MR analysis demonstrated a strong association of HLA-B with Unsw Mem % lymphocyte (OR = 0.8908, 95% CI = 0.8065–0.9840, $p = 0.0228$). Supplementary analyses confirmed no heterogeneity (**Table 5**), no horizontal pleiotropy (**Table 6**), and no influential SNPs altering the causal estimates (**Figure 7**).

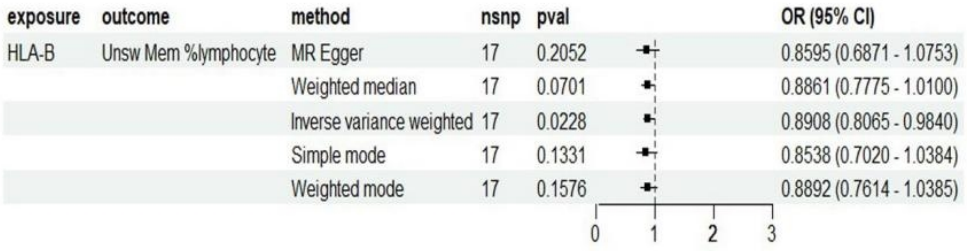

Figure 6. The MR analysis showed HLA-B was highly associated with Unsw Mem % lymphocyte.

Table 5. Heterogeneity results by Cochran's Q test of HLA-B on Unsw Mem % lymphocyte

Exposures	Outcomes	Cochran's Q test	Heterogeneity *p*-value
HLA-B	Unsw Mem %lymphocyte	IVW	9.92E-01
		MR Egger	9.88E-01

Table 6. Pleiotropy results of MR analysis of HLA-B on Unsw Mem % lymphocyte

Exposure	Outcomes	SE	intercept	p-Value
HLA-B	Unsw Mem % lymphocyte	0.030710641	0.010727486	0.731713187

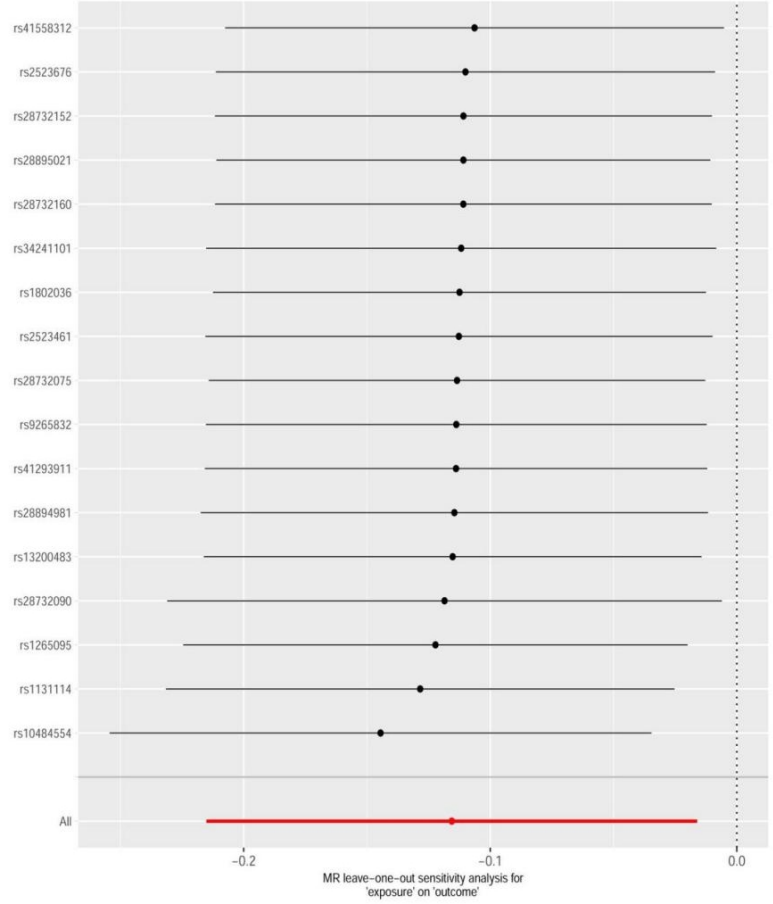

Figure 7. Leave-one-out analysis visualizing the Mendelian randomization (MR) estimates of the exposure (HLA-B) with the outcome (Unsw Mem % lymphocyte).

3.7. Proportion of HLA-B and CA association mediated by immune cells

The study conducted a mediation analysis to assess the effect of Unsw Mem % lymphocyte as a mediator between HLA-B and CA. The mediation effect of Unsw Mem % lymphocyte in the causal pathway from HLA-B to CA was 0.048, accounting for 13.1% of the total effect (**Figure 8**).

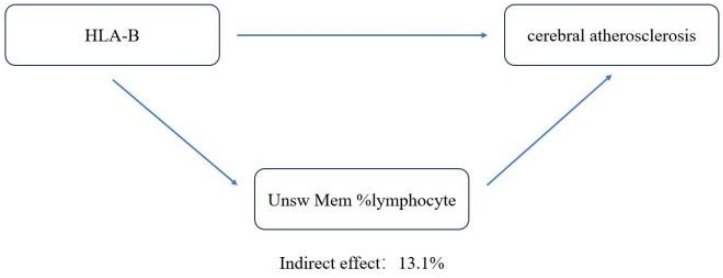

Figure 8. Schematic diagram of the mediation effect.

4. Discussion

The study conducted an MR analysis using GWAS data to investigate the associations between HLA Class I genes and CA, and to assess whether their causal relationship is mediated by immune cells. The results indicate that genetically predicted HLA-B is associated with an increased risk of CA, with 13.1% of this effect being mediated by the percentage of lymphocytes.

Intracranial atherosclerosis is the leading cause of stroke and vascular dementia [13,14]. Atherosclerotic plaques and arterial stenosis in intracranial arteries differ histologically from those in extracranial cerebral arteries. The onset and progression of intracranial atherosclerosis is relatively later than that of the coronary artery and the extracranial carotid artery [15]. Intracranial atherosclerosis typically forms fibrous plaques, while lipid-rich necrotic cores, common in extracranial plaques, are rarely seen or limited to the proximal intracranial segments [16]. In the extracranial blood vessels, atherosclerosis occurs and develops, and the antigens, inflammatory cells and abnormal cells produced enter the intracranial blood vessels with the blood flow. Inflammatory cells and abnormal cells express more MHC-I molecules, which are involved in the injury of vascular endothelial cells and the regulation of inflammatory response, which may cause the occurrence and development of CA. Many studies have found that EBV, SARS-CoV-2, HIV and other viruses promote the development of atherosclerosis [17–20]. Meanwhile, they have a high affinity with alleles on HLA-B, which may induce a higher risk of autoimmune reaction [21].

The study found that the risk of CA decreased as the proportion of Unsw Mem % lymphocyte increased. Unswitched memory (Unsw Mem) B cells develop in the spleen in infants and mature in the germinal centre throughout life [22]. Abnormalities in the population and function of Unsw Mem B have been reported in patients with autoimmune diseases [23]. Unsw Mem B can lead to a reduction in IgM production [24]. For example, SLE patients have low IgM levels, and a reduction in IgM levels correlates with disease duration [25]. Unsw Mem cells were protective against secondary cardiovascular events in advanced atherosclerotic disease [26]. HLA-B may be overexpressed on unswitched memory cells, activating CD8+T cells, causing quantitative and qualitative defects in Unsw Mem B that may impair the function of the protective IgM, thereby leading to the development of atherosclerosis.

Some limitations should be recognized:

(1) The generalizability of the conclusions is limited because all data were collected from the Finnish population; it is

necessary to validate the conclusions in other populations.

(2) The small sample size for CA may have introduced bias in the results.

(3) The pathway from HLA-B to CA was partly mediated by Unsw Mem % lymphocyte, with a mediation effect of 0.048 (13.1% of the total effect).

Other mediators likely exist and warrant further investigation. In the later stage, scRNA-seq can be performed on the plaques of CA patients through single-cell multi-omics verification to confirm the reduction of Unsw Mem B cells and the clonal expansion of CD8+ T cells. The HLA-B transgenic mouse + Unsw Mem B cell deletion model was constructed in the animal model to observe intracranial vascular lesions.

Disclosure statement

The author declares no conflicts of interest.

References

[1] Zhang S, Zhang Q, Lu Y, et al., 2024, Roles of Integrin in Cardiovascular Diseases: From Basic Research to Clinical Implications. Int J Mol Sci, 25(7): 4096.

[2] Chen L, Spagnolo-Allende A, Yang D, et al., 2024, Epidemiology, Pathophysiology, and Imaging of Atherosclerotic Intracranial Disease. Stroke, 55(2): 311–323.

[3] Bae H, Lee J, Park J, et al., 2007, Risk Factors of Intracranial Cerebral Atherosclerosis Among Asymptomatics. Cerebrovasc Dis, 24(4): 355–360.

[4] Napoli C, Palinski W, 2005, Neurodegenerative Diseases: Insights into Pathogenic Mechanisms from Atherosclerosis. Neurobiol Aging, 26(3): 293–302.

[5] Witt K, 2023, Role of MHC Class I Pathways in Mycobacterium tuberculosis Antigen Presentation. Front Cell Infect Microbiol, 13: 1107884.

[6] Wu X, Huang L, Huang Z, et al., 2022, The Lymphocyte-to-Monocyte Ratio Predicts Intracranial Atherosclerotic Stenosis Plaque Instability. Front Immunol, 13: 915126.

[7] Louka A, Sagris D, Ntaios G, 2022, Immunity, Vascular Aging and Stroke. Curr Med Chem, 29(34): 5510–5521.

[8] Burgess S, Davey G, Davies N, et al., 2023, Guidelines for Performing Mendelian Randomization Investigations: Update for Summer 2023. Wellcome Open Res, 4: 186.

[9] Hänzelmann S, Castelo R, Guinney J, 2013, GSVA: Gene Set Variation Analysis for Microarray and RNA-seq Data. BMC Bioinformatics, 14: 7.

[10] Orrù V, Steri M, Sidore C, et al., 2020, Complex Genetic Signatures in Immune Cells Underlie Autoimmunity and Inform Therapy. Nat Genet, 52(10): 1036–1045.

[11] Ding W, Chen L, Xia J, et al., 2024, Causal Association Between Lipid-Lowering Drugs and Cancers: A Drug Target Mendelian Randomization Study. Medicine (Baltimore), 103(18): e38010.

[12] Carter A, Sanderson E, Hammerton G, et al., 2021, Mendelian Randomisation for Mediation Analysis: Current Methods and Challenges for Implementation. Eur J Epidemiol, 36(5): 465–478.

[13] Zhang C, Shi J, 2022, 7T MRI for Intracranial Vessel Wall Lesions and Its Associated Neurological Disorders: A Systematic Review. Brain Sci, 12(5): 528.

[14] Zhang D, Jia N, Hu Z, et al., 2024, Bioinformatics Identification of Potential Biomarkers and Therapeutic Targets for

Ischemic Stroke and Vascular Dementia. Exp Gerontol, 187: 112374.

[15] Velican C, Velican D, 1982, Atherosclerotic Involvement of Human Intracranial Arteries with Special Reference to Intimal Necrosis. Atherosclerosis, 43(1): 59–69.

[16] Kang D, Kim D, Kim J, et al., 2024, Emerging Concept of Intracranial Arterial Diseases: The Role of High Resolution Vessel Wall MRI. J Stroke, 26(1): 26–40.

[17] Lu Y, Zhang X, Hu W, et al., 2021, The Identification of Candidate Biomarkers and Pathways in Atherosclerosis by Integrated Bioinformatics Analysis. Comput Math Methods Med, 2021: 6276480.

[18] Sharma H, Mossman K, Austin R, 2024, Fatal Attractions That Trigger Inflammation and Drive Atherosclerotic Disease. Eur J Clin Invest, 54(5): e14169.

[19] Fayos M, Arnaiz F, González V, et al., 2024, Progression of Subclinical Cardiovascular Disease in Patients with HIV. Rev Esp Quimioter, fayos29apr2024.

[20] Wang C, Huang C, Yeh M, et al., 2024, Hepatitis C Virus Infection Associated with Coronary and Thoracic Aortic Atherosclerosis. Am J Med Sci, S0002-9629(24)01063-2.

[21] Adiguzel Y, Mahroum N, Muller S, et al., 2023, Shared Pathogenicity Features and Sequences Between EBV, SARS-CoV-2, and HLA Class I Molecule-binding Motifs with a Potential Role in Autoimmunity. Clin Rev Allergy Immunol, 65(2): 206–230.

[22] Aranburu A, Piano E, Baban A, et al., 2017, Human B-cell Memory Is Shaped by Age- and Tissue-Specific T-Independent and GC-Dependent Events. Eur J Immunol, 47(2): 327–344.

[23] Yuuki H, Itamiya T, Nagafuchi Y, et al., 2024, B Cell Receptor Repertoire Abnormalities in Autoimmune Disease. Front Immunol, 15: 1326823.

[24] Hu F, Zhang W, Shi L, et al., 2018, Impaired CD27+IgD+ B Cells with Altered Gene Signature in Rheumatoid Arthritis. Front Immunol, 9: 626.

[25] Sivri A, Hasçelik Z, 1995, IgM Deficiency in Systemic Lupus Erythematosus Patients. Arthritis Rheum, 38(11): 1713.

[26] Meeuwsen J, Duijvenvoorde A, Gohar A, et al., 2017, High Levels of (Un)Switched Memory B Cells Are Associated With Better Outcome in Patients With Advanced Atherosclerotic Disease. J Am Heart Assoc, 6(9): e005747.