scientific reports

Check for updates

OPEN

Expression of miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters in peripheral blood mononuclear cells of ischemic stroke

Tingting Feng[1], Lizhen Wang[1], Ruijiao Zhou[1], Juming Yu[3], Shunxian Wang[1], Xiaodong Deng[4], Wei Zhang[4], Binru Wang[2], Fengjiao Li[1] & Ying Ma[1]✉

To estimate the correlation between miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters and ischemic stroke (IS). 83 hospitalized patients diagnosed with IS (experimental group) and 50 healthy subjects (control group) were enrolled in the Affiliated Hospital of North Sichuan Medical College from July 2020 to December 2020. The levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in peripheral blood mononuclear cells (PBMCs) were measured by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). The expressions of miRNA-1250-5p and miRNA-3065-5p were significantly higher in the experimental group compared to the control group (2.04 ± 0.22 vs. 1.54 ± 0.33, $P = 0.002$, 6.41 ± 2.17 vs. 1.42 ± 0.24, $P < 0.001$, respectively) No significant difference in miRNA-338-3p expression was observed between the experimental and control groups (1.87 ± 0.22 vs. 1.25 ± 0.11, $P = 0.309$). The expression levels of miRNA-1250-5p increased after 24 h and no more than 7 days of disease progression but decreased after 7 days compared to baseline ($P < 0.05$). The expression levels of miRNA-3065-5p and miRNA-338-3p in patients with a discharge National Institutes of Health Stroke Scale (NIHSS) score greater than 33 were higher than those in the group with a score of 3 or less ($P < 0.05$). Additionally, the expression level of miRNA-3065-5p in patients with discharged mRS scores of 3 or higher was greater than in patients with discharged mRS scores of 2 or lower ($P < 0.05$). The miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters showed a positive correlation with neutrophil percentage and a negative correlation with lymphocyte percentage ($P < 0.05$). Furthermore, miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p significantly correlated in IS ($P < 0.001$). miRNA-1250-5p and miRNA-3065-5p may be associated with IS.

Keywords Ischemic stroke, miRNA clusters, miRNA-338-3p, miRNA-1250-5p, miRNA-3065-5p

Ischemic stroke (IS) is characterized by a high incidence, elevated mortality rates, significant disability, and a high recurrence rate, which impose a substantial health and economic burden on society and families. The primary pathophysiological mechanisms involved in IS include cell death, inflammation, immune activation, and microvascular dysfunction. The prognosis of IS is closely linked to the transcriptional regulation of gene expression during ischemic events. Several studies have demonstrated that microRNAs (miRNAs) are crucial in ischemia-reperfusion processes[1–3]. Approximately 25–48% of miRNAs in the entire human genome exist as clusters, a phenomenon attributed to the principles of evolutionary biology in the coregulation of physiological processes. miRNA regulate gene expression by binding to target messenger RNA, thereby influencing protein expression and biological functions[4]. MicroRNA clusters (miRNA clusters), defined as two or more miRNA genes closely located on chromosomes, are co-expressed and functionally related, making them a focal point of research in recent years.

[1]Department of Neurology, Affiliated Hospital of North Sichuan Medical College, Sichuan, China. [2]Department of Pain, Suining Central Hospital, Sichuan, China. [3]Department of Neurology, Sichuan Lansheng Brain Hospital, Sichuan, China. [4]Department of Forensic Medicine, North Sichuan Medical College, Nanchong, Sichuan, China. ✉email: yingma1314@126.com

In the human genome, miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p belong to the same clusters and are located in the intron of the Apoptosis-Associated Tyrosine Kinase (AATK) gene on chromosome 17q25.3[4]. These microRNAs are primarily involved in the regulation of ferroptosis[5], apoptosis[6], the production of inflammatory mediators[7,8], cell proliferation[9], and neuronal differentiation[10]. Earlier studies have found that miRNA-338-3p, miRNA-1250, and miRNA-3065-5p are abundant in the human brain[10], particularly miRNA-338-3p in the white matter. Recently, miRNA-338-3p was found to be significantly downregulated in acute IS and has been shown to exert neuroprotective effects on cell autophagy via the miR-338-3p/cPKCγ axis[11]. miRNA-1250-5p may be critical for inducing apoptosis, proliferation, migration, and invasion, possibly by binding to the 3'untranslated region (3′ UTR) of Metastasis-Associated Protein 1 (MTA1) and inhibiting its expression[6]. Additionally, miRNA-3065-3p could influence the proliferation and survival of nerve cells, potentially by regulating the expression of the Cytokine Receptor-Like Factor 1 (CRLF1) gene[12]. These studies suggest that the miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p may play significant roles in IS. Faria et al. proposed that the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters may modulate the myelination program in humans and be associated with demyelinating diseases[10]. However, the expression levels of these microRNAs in patients with IS remain unknown. We hypothesized that the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters are important transcriptional regulators affecting the prognosis of IS, which could facilitate further exploration of the regulatory transcriptional mechanisms underlying ischemia-reperfusion and provide molecular evidence for new therapeutic options for IS. We tested this hypothesis by investigating the expression of the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters in IS through a case-control study derived from a Chinese population.

Materials and methods

Study population

The study population comprised 83 patients with IS (the experimental group, 52 men and 31 women, mean age: 62.6 ± 10.6 years), and 50 healthy volunteers (the control group, 34 men and 16 women, mean age: 60.0 ± 7.0 years). All patients were selected in the department of Neurology, Affiliated Hospital of North Sichuan Medical College from July 2020 to December 2020. Two neurologists diagnosed them based on the 11th Clinical Revision of International Classification of diseases (ICD-11-CM) diagnostic criteria, the 4th National Cerebrovascular Conference in 1995 and the 4th National Cerebrovascular Disease Academic Conference in 2018. Patients diagnosed with transient ischemic attacks, hemorrhagic stroke, subarachnoid hemorrhages, traumatic brain injuries, spinal cord injury, tumour, neurodegenerative disease, leukemia, autoimmune diseases, rheumatoid arthritis, asthma, demyelinating disease, severe kidney and liver function damage were excluded. The volunteers were from the physical examination center of the same hospital during the same period. Informed consents were obtained for all participants. The sex, age and race of the control group were matched as closely as possible to the patients in the experimental group. This study was approved by the Ethics Committee of the North Sichuan Medical College Ethics Committee and conformed to the guidelines of the Declaration of Helsinki. Parkinson, colorectal cancer.

Indicators and methods of detection

(1) Collection of Clinical Data: The essential characteristics of the population, medical history, IS-related scores, and laboratory indicators were collected within 24 h after admission. The parameters collected included the following: age (individuals age ≤ 45 years were classified into the young stroke group, while those age > 45 years were classified into the non-young stroke group[13]), gender, dyslipidemia (total cholesterol ≥ 5.72 mmol/L, triglyceride ≥ 1.70 mmol/L, or high-density lipoprotein cholesterol < 0.9 mmol/L), course of disease (the hyperacute phase is defined as a duration of no more than 24 h, the acute phase as lasting no more than 7 days, and the early subacute phase lasting no more than 14 days[14]), Trial of Org10172 in Acute Stroke Treatment (TOAST) classification (including five subtypes: large artery atherosclerosis, caedioembolism, small vessel occlusion, stroke of other etiology, and stroke of unknown etiology)[15], length of stay (LOS) (defined as LOS ≤ 14 days for the normal hospitalization group and LOS > 14 days for the prolonged hospitalization group)[16], Trial of Org10172 in Acute Stroke Treatment (NIHSS, an admission NIHSS score not exceeding 3 points is defined as mild stroke, while a score exceeding 3 points is classified as not mild stroke)[17], modified Rankin Scale (mRS, a discharge mRS score not exceeding 2 points is defined as the functional group, while a score exceeding 2 points is classified as the disability group[18–21]), white blood cell count, percentage of neutrophils, percentage of mononuclear cells, percentage of lymphocytes, hemoglobin, red blood cell count, and platelet count.

(2) miRNA detection: Peripheral blood mononuclear cells(PBMCs) were isolated from 3 ml anticoagulated peripheral blood samples using Ficoll-Hypaque density-gradient centrifugation. Total RNAs were extracted from the PBMCs according to the manufacturer's instructions of the miRcute miRNA Isolation Kit (TIANGEN, China). The purity and integrity of the RNA were evaluated using the NanoVue Plus spectrophotometer (BiochRom, Britain) and Agarose gel electrophoresis(BIO-RAD, America) and then stored at − 80 °C. Figure 1 showed the results of Agarose gel electrophoresis.

A total reaction volume of 20 µl, containing 8µL RNA, 10 µL 2×miRNA RT Reaction Buffer, and 2 µL miRNA RT Enzyme Mix, was carried out for cDNA synthesis of miRNA using minute Plus miRNA first-Stran cDNA synthesis kit (TIANGEN, China) at 42 °C for 60 min, followed by 95 °C for 3 min.

Real-time quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) was used for miRNA expression analysis of miRNA-338-3p, miRNA-1250-5p, miRNA-3065-5p and U6 (housekeeping miRNA). The total reaction volume was 20ul, including 0.4 µL Forward Primer (Table 1), 0.4 µL Reverse primer, 5.6 µL

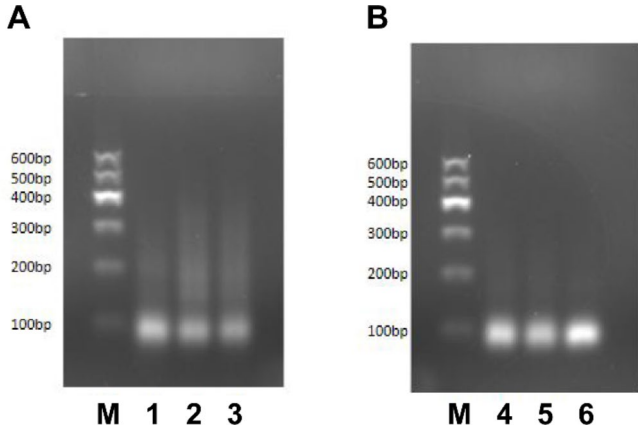

Fig. 1. Gels images of miRNA and U6.

miRNA	Primer sequences
hsa-mir-338-3p	5′-UCCAGCAUCAGUGAUUUUGUUG-3′
hsa-mir-1250-5p	5′-CCACGGTGCTGGATGTGGCCTTT-3′
hsa-mir-3605-5p	5′-GCGCGCGTCAACAAAATCACTGATGCT-3′

Table 1. miRNA primer sequences.

ddH2O, ten μL 2× miRcute Plus miRNA Premix (SYBR&ROX), 1.6 μL 50×ROX Reference Dye, two μL cDNA. The RT-qPCR conditions were 95 °C for 15 min, followed by five cycles at 94 °C for 20 s, 64 °C for 30 s, 72 °C for 34 s, and then 40 cycles at 94 °C for 20 s, 60 °C for 34 s. The CT values of target miRNA and housekeeping miRNA were recorded and calculated using the 2-ΔΔCt formula for the relative expressions. Figure 2 showed the predicted structure of miRNA-338, miRNA-1250 and miRNA-3065 from miRCarta.

PCR products were analyzed using agarose gel electrophoresis (1%) stained with Genegreen Nucleic Acid Dye. Gel images of miRNA and U6 were visualized. U6 was used as an internal reference. Lanes: M:Maker, (1)(2)(3) miRNA, (4)(5)(6)U6.

Statistical analysis

Statistical analyses were conducted using IBM SPSS 25.0, and data were plotted using Prism 7.0 (Graphpad). A double-sided significance threshold of $P < 0.05$ was applied. Normality tests were performed using the Kolmogorov-Smirnov test, histograms, P–P plots, and Q–Q plots. For continuous variables, data are presented as mean ± standard deviation ($\overline{x}$ ±s)or [median (P25, P75)]. Differences between the two groups were assessed using either a parametric test (Student's t-test) or a non-parametric test (Wilcoxon rank sum test). Differences in miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p across two or more groups were compared using the Hotelling T^2 test or Wilks Λ test. Categorical variables are presented as percentages (%), and differences were analyzed using the Chi-square test, Calibration Chip Test, or Fisher's Precision Test. Correlation analyses were performed using either Pearson or Spearman methods.

Results

Baseline characteristics of the experimental group and control group

Table 2 summarizes the clinical characteristics of the experimental and control groups. There were no significant differences in gender and age between the experimental and control groups ($P > 0.05$).

The expression of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p between the experimental and control groups

Table 3; Fig. 3 illustrate the expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in IS. There was no significant difference in the expression level of miRNA-338-3p between the experimental and control groups ($P > 0.05$). In contrast, the expression levels of miRNA-1250-5p and miRNA-3065-5p were significantly higher in the experimental group compared to the control group ($P < 0.05$). Overall, the expression levels of the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters in the experimental group were greater than those in the control group ($P < 0.05$).

Expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in the IS group

Table 4; Fig. 4 illustrate the IS population's expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p. The expression levels of miRNA-1250-5p increase during the acute phase (up to 7 days) but decrease after seven days compared to baseline ($P < 0.05$). The expression levels of miRNA-3065-5p and miRNA-338-3p

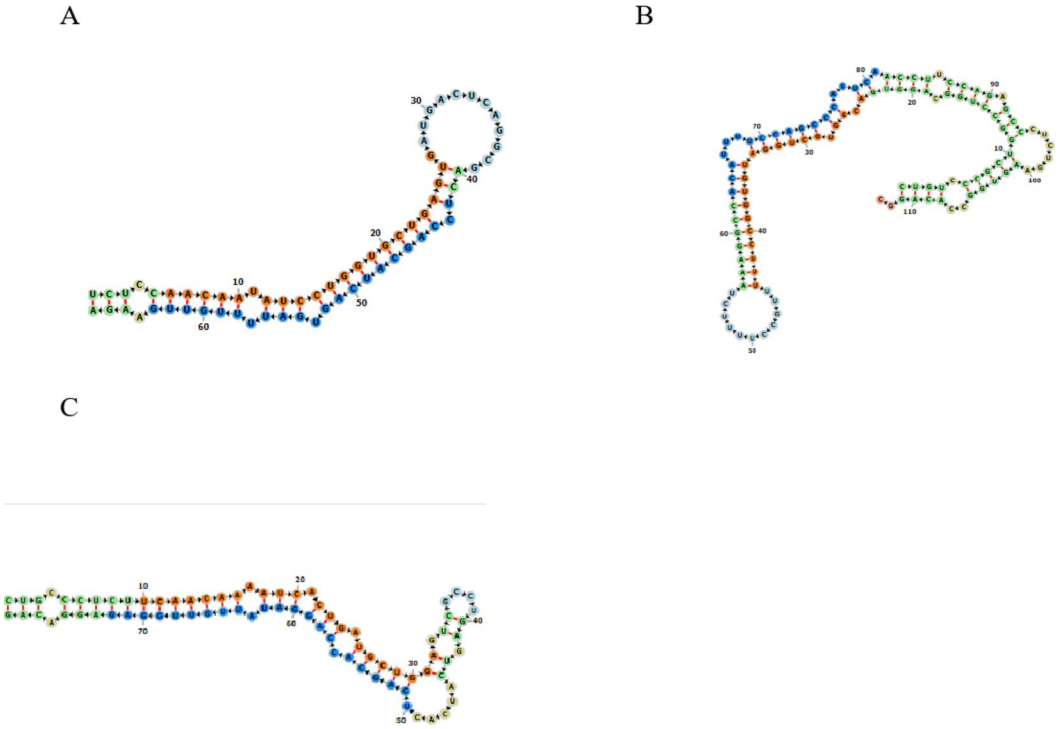

Fig. 2. The predicted structure of miRNA-338, miRNA-1250 and miRNA-3065. (**A**) miR-338(hsa-mir-338), (**B**) miR-1250(hsa-mir-1250). (**C**) miR-3065(hsa-mir-3065). miR-338, miR-1250 and miR-3065 are all locate in chr17.From miRCarta V1.1 (https://mircarta.cs.uni-saarland.de/advanced_search/).

Variables	Experimental group ($n=83$)	Control group ($n=50$)	*P*
Age (mean ± SD)	62.5 ± 10.6	60.0 ± 7.0	0.107
Sex (men: women)	52:31	34:16	0.532
Dyslipidemia, n (%)	39 (47.0%)	20 (40.0%)	0.432
Course of disease	2.6 ± 2.8	NA	NA
Admission NIHSS score	6.3 ± 7.1	NA	NA
Discharge NIHSS score	4.6 ± 6.9	NA	NA
Discharge MRS score	1.8 ± 1.7	NA	NA

Table 2. Demographic information, clinical characteristics. *n* number of subjects, *%* frequency, *NIHSS* National Institutes of Health Stroke Scale, *mRS* modified Rankin Scale.

miRNA	Experimental group ($n=83$)	Control group ($n=50$)	*P*
miRNA-338-3p	1.87 ± 0.22	1.25 ± 0.11	0.309
miRNA-1250-5p	2.04 ± 0.22	1.54 ± 0.33	**0.002**
miRNA-3065-5p	6.41 ± 2.17	1.42 ± 0.24	**< 0.001**
miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters	–	–	**0.008**

Table 3. The expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p between the experimental and control groups. Bold characters highlight all significant p-values.

in patients with a discharge NIHSS score greater than 3 were higher than those in the group with a score of 3 or less ($P<0.05$). Additionally, The expression levels of miRNA-3065-5p in the population with discharged mRS scores of 3 or higher was greater than that in the population with discharged mRS scores of 2 or lower ($P<0.05$).

The correlation between the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters and IS

The expression level of miRNA-3065-5p positively correlates with the admission NIHSS score and the length of hospital stay in patients with IS ($P<0.05$). Additionally, the expression levels of miRNA-338-3p and miRNA-

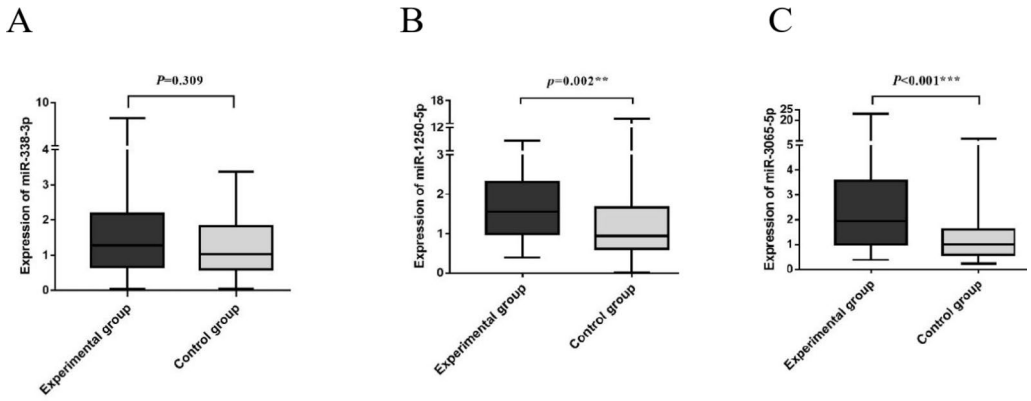

Fig. 3. The expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in PBMCs between the experimental and control groups. (**A**) The expression level of miRNA-338-3p in the experimental group was higher than in the control group. However, this difference was not statistically significant. (**B**) The expression level of miRNA-1250-5p in the experimental group was higher than that in the control group, and the difference between the two groups was statistically significant. (**C**) The expression level of miRNA-3065-5p in the experimental group was also higher than in the control group, with a statistically significant difference observed between the two groups. $^{*}P<0.05$,$^{**}P<0.01$,$^{***}P<0.001$.

Variables		Experimental group ($n=83$)					
		miRNA-338-3p		miRNA-1250-5p		miRNA-3065-5p	
		$\overline{x}\pm s$	*P*	$\overline{x}\pm s$	*P*	$\overline{x}\pm s$	*P*
Age	≤45 year (5, 6.0%)	2.0 ± 1.0	0.248	1.1 ± 0.7	0.126	2.6 ± 0.9	0.533
	>45year (78, 94.0%)	1.9 ± 2.0		2.1 ± 2.0		3.9 ± 4.9	
Course of disease	≤24 h (50, 60.2%)	1.9 ± 2.0	0.859	1.8 ± 1.7	**0.040**	3.6 ± 4.7	0.111
	7 day≥ and >1 day (27, 32.5%)	2.0 ± 2.1		2.7 ± 2.4		4.7 ± 5.3	
	14 day≥ and >7 day (6, 7.2%)	1.3 ± 0.6		1.2 ± 0.7		1.5 ± 0.6	
TOAST classification	LAA (33, 39.8%)	1.8 ± 2.0	0.505	2.0 ± 1.9	0.300	4.0 ± 4.7	0.579
	CE (37, 44.6%)	1.9 ± 1.5		2.8 ± 3.1		5.1 ± 7.6	
	SAA (11, 13.3%)	2.0 ± 2.2		2.0 ± 1.6		3.4 ± 3.9	
	SOE (2, 2.4%)	2.2 ± 0.3		0.7 ± 0.4		1.9 ± 0.3	
	SUE (0, 0.0%)	–		–		–	
Length of stay	LOS ≤ 14 day (38, 45.8%)	1.8 ± 1.9	0.909	2.0 ± 2.0	0.725	3.6 ± 5.1	0.113
	LOS > 14 day (45, 54.2%)	1.9 ± 2.1		2.1 ± 2.0		4.0 ± 4.5	
Admission NIHSS score	≤3 (43, 51.8%)	1.6 ± 2.0	0.209	2.0 ± 1.8	0.594	1.6 ± 2.0	0.209
	>3 (40, 48.2%)	2.1 ± 2.0		2.1 ± 2.1		2.1 ± 2.0	
Discharge NIHSS score	≤3 (52, 62.7%)	1.6 ± 1.9	**0.030**	2.1 ± 2.1	0.735	3.3 ± 4.4	**0.049**
	>3 (31, 37.3%)	2.4 ± 2.1		2.0 ± 1.6		4.6 ± 5.3	
Discharge mRS score	≤2 (53, 63.9%)	1.6 ± 1.8	0.070	1.8 ± 1.7	0.278	6.1 ± 23.6	**0.007**
	≥3 (30, 36.1%)	2.4 ± 2.2		2.4 ± 2.4		7.0 ± 10.3	

Table 4. The analysis of the expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in the experimental group. *TOAST* Trial of Org10172 in Acute Stroke Treatment, *LAA* large artery atherosclerosis, *CE* cardioembolism, *SAA* small vessel occlusion, *SOE* stroke of other etiology, *SUE* stroke of unknown etiology. All significant p-values are highlighted by bold characters.

3065-5p were positively correlated with the discharge mRS score ($P<0.05$). Furthermore, the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters showed a positive correlation with the percentage of neutrophils and a negative correlation with the percentage of lymphocytes ($P<0.05$) (Table 5).

The correlation of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p

Table 6; Fig. 5 illustrate the correlation among miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in IS. The expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p were significantly positively correlated in IS.

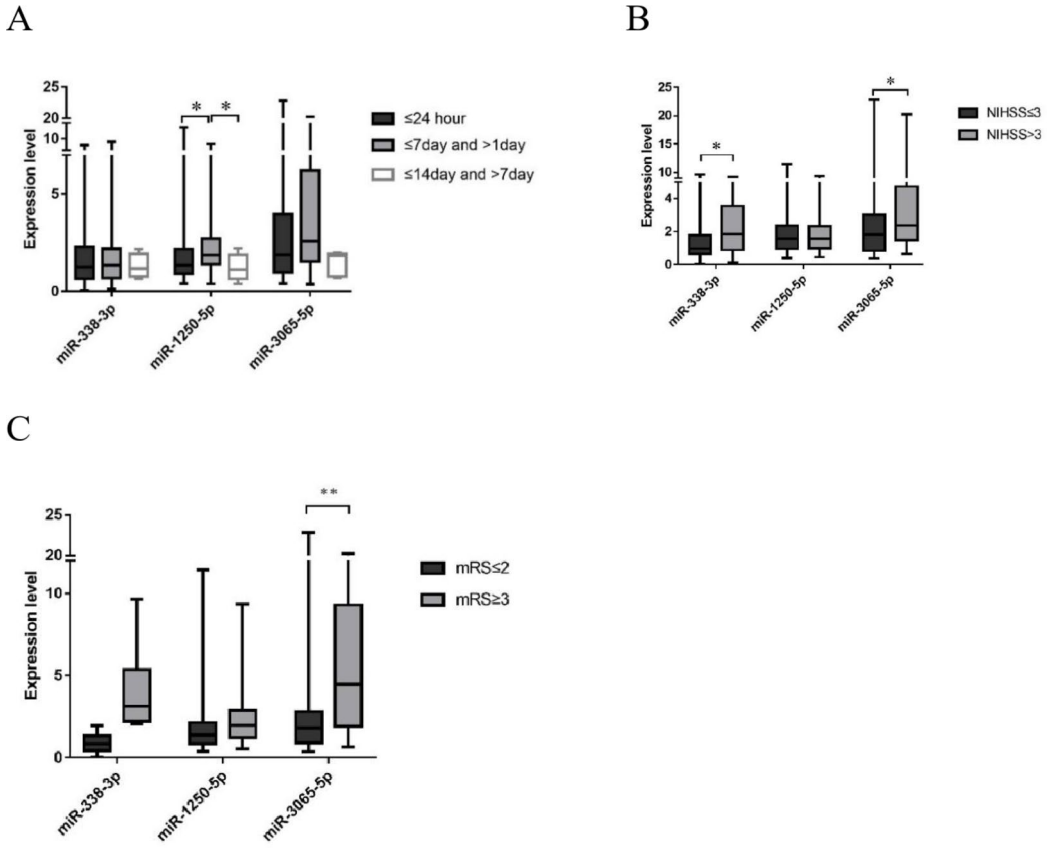

Fig. 4. The expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in different groups. (**A**) The expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p were analyzed between groups according to the stages of the disease. The difference in miRNA-1250-5p expression between groups was statistically significant. (**B**) The expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p were evaluated between groups based on the discharge NIHSS score. The differences in miRNA-338-3p and miRNA-3065-5p expression between groups were statistically significant. (**C**) The expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p were compared between groups according to discharge mRS scores. The difference in miRNA-3065-5p expression between groups was statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

The ROC curves of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p

Figure 6 presents the results of the ROC analysis for miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in predicting IS. The AUC values are 0.553, 0.659, and 0.737, with 95% confidence intervals of 0.455–0.650, 0.562–0.756, and 0.651–0.822, respectively.

Main association of IS with miRNA-338-3p/miRNA-1250-5p/miRNA– 3065-5p from previous analysis

The primary outcomes of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p levels were presented in Table 7 for each study.

Discussion

When ischemic damage occurs in the brain, oligodendrocytes exhibit a high sensitivity to ischemia and hypoxia. The deposition of fibrinogen, caused by the disruption-destruction of the blood-brain barrier (BBB), adversely affects oligodendrocytes' cellular state and differentiation[22]. Studies have shown that miRNA-338-3p, miRNA-1250, and miRNA-3065-5p are abundant in the human brain and associated with oligodendrocyte precursor cells' proliferation and differentiation[10]. Notably, miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p are all located within the same cluster in the intron of the AATK gene on chromosome 17q25.3. Functional analyses suggest that AATK may play a role in various pathophysiological pathways, including apoptosis, neuronal differentiation and maturation, axon formation, synaptic transmission, and glial cell proliferation (https://www.ncbi.nlm.nih.gov/gene/). However, the investigation of the miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p clusters in IS has not been thoroughly explored. In the present study, we examined the expression levels of miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in PBMCs from IS patients and healthy individuals. Our findings indicate that the expression levels of miRNA-1250-5p and miRNA-3065-5p in the PBMCs of IS patients were significantly higher than those in the control group.

Variables	Experimental group ($n=83$)					
	miRNA-338-3p		miRNA-1250-5p		miRNA-3065-5p	
	R	*P*	*R*	*P*	*R*	*P*
Age	− 0.127	0.254	0.127	0.252	0.022	0.842
Gender (female)	− 0.100	0.365	0.005	0.963	0.060	0.591
Course of disease	0.063	0.572	0.141	0.204	0.078	0.483
admission NIHSS score	0.192	0.082	0.045	0.689	0.227	**0.039**
TOAST classification (LAA)	0.129	0.244	0.051	0.644	− 0.118	0.287
Length of stay	0.071	0.525	0.140	0.207	0.243	**0.027**
Discharge NIHSS score	0.189	0.086	− 0.044	0.696	0.207	0.060
Discharge mRS score	0.261	**0.017**	0.130	0.242	0.297	**0.006**
White blood cell count ($\times 10^9$/L)	0.095	0.395	0.105	0.346	− 0.048	0.667
Percentage of neutrophils (%)	0.311	**0.004**	0.270	**0.014**	0.248	**0.024**
Percentage of mononuclear cells (%)	− 0.123	0.267	0.095	0.391	− 0.120	0.279
Percentage of lymphocytes (%)	− 0.415	**< 0.001**	− 0.314	**0.004**	− 0.219	**0.046**
Haemoglobin (g/L)	− 0.081	0.468	− 0.175	0.114	− 0.141	0.204
Red blood cell count ($\times 10^{12}$/L)	− 0.023	0.834	− 0.156	0.160	− 0.075	0.502
Platelet count ($\times 10^9$/L)	− 0.069	0.533	− 0.001	0.991	− 0.055	0.621

Table 5. The correlational analysis between miRNA-338-3p, miRNA-1250-5p, miRNA-3065-5p, and clinical parameters in patients with IS. *NIHSS* National Institutes of Health Stroke Scale, *mRS* modified rankin scale, *TOAST classification* trial of Org10172 in acute stroke treatment, *R* coefficient of correlation.

Coefficient	Experimental group ($n=83$)			Total ($n=133$)		
	miRNA-338-3p and miRNA-1250-5p	miRNA-338-3p and miRNA-3065-5p	miRNA-1250-5p and miRNA-3065-5p	miRNA-338-3p and miRNA-1250-5p	miRNA-338-3p and miRNA-3065-5p	miRNA-1250-5p and miRNA-3065-5p
Correlation coefficient	0.381	0.422	0.531	0.234	0.356	0.452
P	**< 0.001**[a]	**< 0.001**[a]	**< 0.001**[a]	**0.007**[a]	**< 0.001**[a]	**< 0.001**[a]

Table 6. The correlational analysis between miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p. [a]Based on Spearman correlation. All significant p-values are highlighted by bold characters.

In this study, we found that miRNA-1250-5p and miRNA-3065-5p were significantly different between the experimental and control groups. The results are consistent with previous findings[23], suggesting that miRNA-1250-5p and miRNA-3065-5p may be involved in the pathology of IS. In this experiment, the difference in miRNA-338-3p between the experimental and control groups was not statistically significant, which was inconsistent with some studies[11,23,24]. Whether miRNA-338-3p expression was upregulated[24] or downregulated[25,26] in ischemic injury remained controversial. A possible reason for this discrepancy was that miRNA-338 was expressed in cerebrospinal fluid (CSF) but not in serum. Additionally, miRNA-338-3p may have an independent promoter, which could account for the differences in results. Previous studies had shown that miRNA-1250-5p was critical for apoptosis induction and regulated apoptosis by upregulating MTA1 and inhibiting LncRNA ELFN1-AS1 expression by binding to the 3′ untranslated region (UTR) of MTA1[6]. Its signalling pathway may also be involved in IS by participating in endothelial angiogenesis[27]. miRNA-3065-3p was recognized as one of the critical regulators of inflammation[8]. Thus, miRNA-1250-5p and miRNA-3065-5p may play a role in the pathophysiological processes associated with IS by regulating immune cell polarization, apoptosis, inflammatory responses, or promoting angiogenesis.

In this study, miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p showed significant positive correlations with each other in IS. Notably, the changes in the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters synchronized with the percentage of inflammatory cells, exhibiting a highly significant positive correlation with the percentage of neutrophils and a highly significant negative correlation with the percentage of lymphocytes. This confirms the co-expression of miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters in IS and suggests their involvement in regulating the inflammatory response associated with IS. Faria et al. demonstrated that these miRNA clusters synergistically contribute to the proliferation and differentiation of oligodendrocyte precursor cells in demyelinating diseases[10]. Simultaneously, they synergistically downregulated the expression of Runt-related transcription factor 2(Runx2), Bone morphogenetic protein 7 (BMP7), and Wnt family member 5 A(Wnt5a) genes to promote osteoblast differentiation in osteoblast models[28]. Additionally, databases such as TargetScan and miRBase suggested that miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p share many common target genes, including Zinc Finger Proteins (ZNF), Solute Carrier (SLC), Ribosomal Protein S (RPS), and Adenosine Triphosphate (ATP), among others. Notably, ZNF and SLC genes are closely associated with

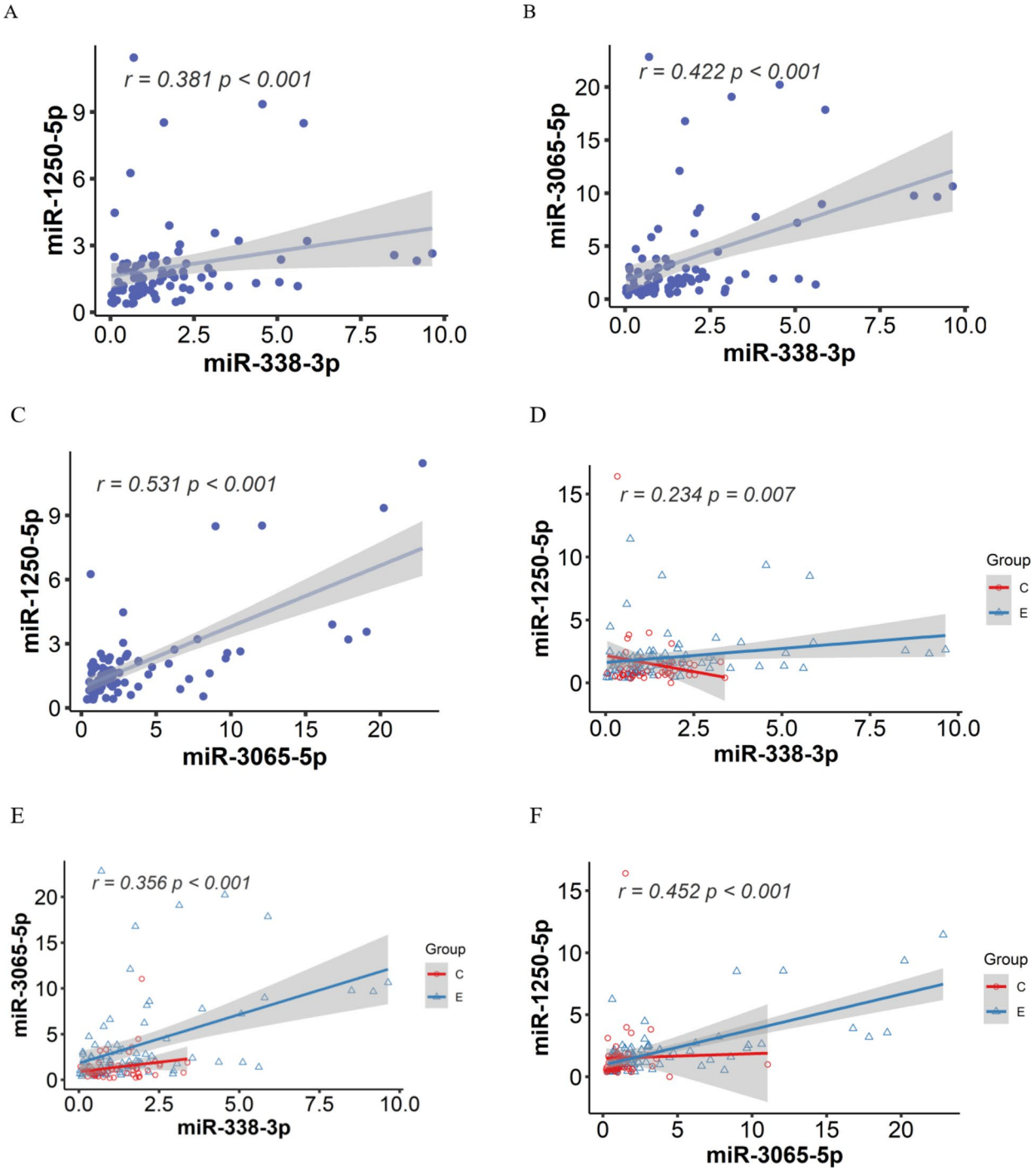

Fig. 5. The correlational analysis between miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p groups. E: Experimental group, C: Control group. (**A–C**) Illustrate the correlations between miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p in the experimental group, while (**D–F**) depict these correlations in the overall population. (**A**) In the experimental group, miRNA-338-3p was positively correlated with miRNA-1250-5p ($r = 0.381$, $p < 0.001$). (**B**) In the experimental group, miRNA-338-3p was positively correlated with miRNA-3065-5p ($r = 0.422$, $p < 0.001$). (**C**) In the experimental group, miRNA-1250-5p was positively correlated with miRNA-3065-5p ($r = 0.531$, $p < 0.001$). (**D**) In the experimental group, miRNA-338-3p was positively correlated with miRNA-1250-5p. In the control group, miRNA-338-3p was negatively correlated with miRNA-1250-5p, while a positive correlation was observed in the total population ($r = 0.234$, $p = 0.007$). (**E**) miRNA-338-3p positively correlated with miRNA-3065-5p in the experimental, control group, and total population ($r = 0.356$, $p < 0.001$). (**F**) miRNA-1250-5p was positively correlated with miRNA-3065-5p in the experimental group, control group, and total population ($r = 0.452$, $p < 0.001$).

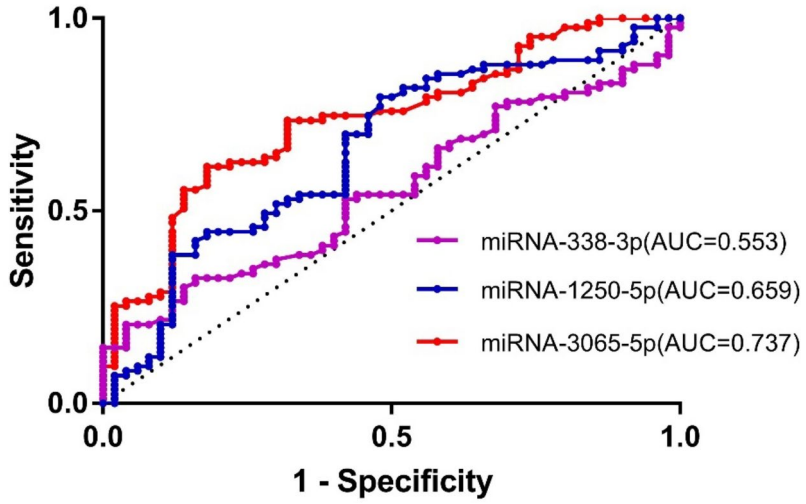

Fig. 6. The ROC of miRNA-338-3p, miRNA-1250-5p and miRNA-3065-5p.

Main outcome	Studies	Experimental/controls	*P*-values
Lower miRNA-338-3p in experimental versus controls	Wei et al.[9]	6/6	< 0.001
	Chen et al.[18]	98/85	< 0.001
	Teng et al.[19]	256/256	< 0.001
Higher miRNA-338-3p in experimental versus controls	Li et al.[17]	8/8	< 0.05
	Peng et al.[15]	72/51	0.056
miRNA-1250-5p in IS	NA		
miRNA-3065-5p in IS	NA		

Table 7. Summary of outcomes from the measurement of peripheral miR-338-3p/miRNA-1250-5p/miRNA-3065-5p in IS.

IS. Research has shown that ZNF and its associated proteins significantly enhance blood perfusion in ischemic tissue, mitigate inflammatory responses, promote angiogenesis, and address ischemic and inflammatory challenges by regulating gene transcription[29]. Zinc finger transcription factors maintain the structural integrity and functionality of the BBB by regulating the activity of closely linked proteins[30]. Moreover, they suppress endothelial angiogenesis by targeting the krüppel-associated box (KRAB) domain[31]. Thus, miRNA-338-3p, miRNA-1250-5p, and miRNA-3065-5p are hypothesized to be involved in IS by targeting the ZNF gene, which is closely associated with ischemic brain injury and repair, ultimately affecting the severity and prognosis of IS. Moreover, SLC transporters are widely distributed across the BBB, blood-cerebrospinal fluid barrier, and nerve cells[32]. They play critical roles in various biological processes, such as solute and drug transportation[33–36], phagocytic clearance[37], and the modulation of inflammation[38]. Therefore, the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters may co-transcribe and synergistically participate in the inflammatory response of IS, affecting the progression and prognosis of IS and could become one of the intervention targets for IS. However, further research is needed to understand its upstream and downstream mechanisms.

In summary, the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters may be involved in the pathophysiological processes of IS. The miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters are closely related to inflammatory markers, disease severity scores, and prognostic indicators in IS, suggesting that the miRNA-338-3p/miRNA-1250-5p/miRNA-3065-5p clusters may affect the severity and prognosis of IS by regulating inflammatory responses and other mechanisms.

Strengths and limitations of this study

There were some limitations in the study. First, the study was a single-center case-control study with a small sample and a single population structure, which did not represent the total population and may lead to statistical bias. Second, IS is a complex disease influenced by multiple factors and dynamic changes over time, and miRNAs may play different roles at different stages of the disease. However, we did not dynamically detect the miRNA clusters expression in the same individual.

Received: 16 October 2023; Accepted: 14 January 2025
Published online: 01 April 2025

References

1. Bang, O. Y. et al. Circulating extracellular-vesicle-incorporated microRNAs as potential biomarkers for ischemic stroke in patients with cancer. *J. Stroke* **25** (2), 251–265 (2023).
2. Wang, Y. et al. Anti-CHAC1 exosomes for nose-to-brain delivery of mir-760-3p in cerebral ischemia/reperfusion injury mice inhibiting neuron ferroptosis. *J. Nanobiotechnol.* **21** (1), 109 (2023).
3. Deng, L. et al. Mir-671-5p upregulation attenuates blood-brain barrier disruption in the ischemia stroke model via the NF-κB/MMP-9 signaling pathway. *Mol. Neurobiol.* **60** (7), 3824–3838 (2023).
4. Boxhammer, E. et al. Deciphering the role of microRNAs: unveiling clinical biomarkers and therapeutic avenues in atrial fibrillation and associated stroke—A systematic review. *Int. J. Mol. Sci.* **25** (10), 5568 (2024).
5. Zhou, J. et al. A novel miR-338-3p/SLC1A5 axis reprograms retinal pigment epithelium to increases its resistance to high glucose-induced cell ferroptosis. *J. Mol. Histol.* **53** (3), 561–571 (2022).
6. Zhai, L-Q. et al. A long non-coding RNA, ELFN1-AS1, sponges miR-1250 to upregulate MTA1 to promote cell proliferation, migration and invasion, and induce apoptosis in colorectal cancer. *Eur. Rev. Med. Pharmacol. Sci.* **25** (14), 4655–4667 (2021).
7. Fang, X. et al. CIRC_0012535 contributes to lipopolysaccharide-induced fetal lung fibroblast apoptosis and inflammation to regulate infantile pneumonia development by modulating the mir-338-3P/IL6R signaling. *Shock* **59** (5), 820–828 (2023).
8. Martínez-Gutierrez, A. et al. A microRNA panel that regulates proinflammatory cytokines as diagnostic and prognosis biomarkers in colon cancer. *Biochem. Biophys. Rep.* **30**, 101252 (2022).
9. Woods, M. L. et al. Epigenetically silenced apoptosis-associated tyrosine kinase (AATK) facilitates a decreased expression of cyclin D1 and WEE1, phosphorylates TP53 and reduces cell proliferation in a kinase-dependent manner. *Cancer Gene Ther.* **29** (12), 1975–1987 (2022).
10. de Faria, O. et al. Regulation of miRNA 219 and miRNA clusters 338 and 17–92 in oligodendrocytes. *Front. Genet.* **3**, 46 (2012).
11. Wei, H. et al. Downregulation of mir-338-3p alleviates neuronal ischemic injury by decreasing cPKCγ-Mediated autophagy through the Akt/mTOR pathway. *Neurochem. Int.* **154**, 105279 (2022).
12. Li, Y. et al. Mir-3065-3p promotes stemness and metastasis by targeting CRLF1 in colorectal cancer. *J. Transl. Med.* **19** (1), 429 (2021).
13. Ji, R. et al. Ischemic stroke and transient ischemic attack in young adults: risk factors, diagnostic yield, neuroimaging, and thrombolysis. *JAMA Neurol.* **70** (1), 51–57 (2013).
14. Huppertz, V. et al. Impaired nutritional condition after stroke from the hyperacute to the chronic phase: a systematic review and meta-analysis. *Front. Neurol.* **12**, 780080 (2021).
15. C, J. Y. Long-term outcomes among ischemic stroke TOAST subtypes: a 12-year cohort study in China. *J. Stroke Cerebrovasc. Dis.* **33** (8), 107783 (2024).
16. Hao, R. et al. Temporal trend of comorbidity and increasing impacts on mortality, length of stay, and hospital costs of first stroke in Tianjin, North of China. *Cost Eff. Resour. Alloc.* **19** (1), 63 (2021).
17. Wang, J. et al. Safety and efficacy of tirofiban in preventing neurological deterioration in acute ischemic stroke (TREND): protocol for an investigator-initiated, multicenter, prospective, randomized, open-label, masked endpoint trial. *Brain Circ.* **10** (2), 168–173 (2024).
18. Dhillon, P. S. et al. Endovascular thrombectomy beyond 24 hours from ischemic stroke onset: a propensity score matched cohort study. *J. Neurointerv. Surg.* **15** (3), 233–237 (2023).
19. You, S. et al. Prognostic significance of international normalised ratio and prothrombin time in Chinese acute ischaemic stroke patients. *Postgrad. Med. J.* **99** (1170), 333–339 (2023).
20. Yang, W. et al. RVD2 emerges as a serological marker in relation to severity and six-month clinical outcome following acute intracerebral hemorrhage: a prospective cohort study from a single academic institution. *Clin. Chim. Acta Int. J. Clin. Chem.* **1**, 119988 (2024).
21. Zhang, Z. et al. Efficacy and safety of Ginkgolide with intravenous alteplase thrombolysis in acute ischemic stroke with large vessel occlusion: a subgroup analysis of GIANT. *Front. Pharmacol.* **15**, 1452174 (2024).
22. Hou, H. et al. Exosomal miR-128-3p reversed fibrinogen-mediated inhibition of oligodendrocyte progenitor cell differentiation and remyelination after cerebral ischemia. *CNS Neurosci. Therap.* **29**, 1405–1422 (2023).
23. Peng, G. et al. MicroRNA let-7e is a potential circulating biomarker of acute stage ischemic stroke. *Transl. Stroke Res.* **6** (6), 437–445 (2015).
24. Li, L. et al. MicroRNA-338 inhibition protects against focal cerebral ischemia and preserves mitochondrial function in vitro in astrocytes and neurons via COX4I1. *Mitochondrion* **59**, 105–112 (2021).
25. Chen, C. et al. LncRNA CASC15 promotes cerebral ischemia/reperfusion injury via miR-338-3p/ETS1 axis in acute ischemic stroke. *Int. J. Gen. Med.* **14**, 6305–6313 (2021).
26. Teng, L. & Meng, R. Long non-coding RNA MALAT1 promotes acute cerebral infarction through miRNAs-mediated hs-CRP regulation. *J. Mol. Neurosci.* **69** (3), 494–504 (2019).
27. Ishikawa, M. et al. MTA1, a metastasis–associated protein, in endothelial cells is an essential molecule for angiogenesis. *Mol. Med. Rep.* **25** (1), 11 (2022).
28. Lin, C. et al. Circulating miR-338 cluster activities on osteoblast differentiation: potential diagnostic and therapeutic targets for postmenopausal osteoporosis. *Theranostics* **9** (13), 3780–3797 (2019).
29. Wang, X. et al. Cascaded bio-responsive delivery of eNOS gene and ZNF580 gene to collaboratively treat hindlimb ischemia via pro-angiogenesis and anti-inflammation. *Biomater. Sci.* **8** (23), 6545–6560 (2020).
30. Bu, S. et al. Zinc finger proteins in neuro-related diseases progression. *Front. NeuroSci.* **15**, 760567 (2021).
31. Oo, J. A. et al. ZNF354C is a transcriptional repressor that inhibits endothelial angiogenic sprouting. *Sci. Rep.* **10** (1), 19079 (2020).
32. Mir, A. et al. SLC gene mutations and pediatric neurological disorders: diverse clinical phenotypes in a Saudi Arabian population. *Hum. Genet.* **141** (1), 81–99 (2022).
33. Latif, S. & Kang, Y-S. Blood-brain barrier solute carrier transporters and motor neuron disease. *Pharmaceutics* **14** (10), 2167 (2022).
34. Puris, E., Fricker, G. & Gynther, M. The role of solute carrier transporters in efficient anticancer drug delivery and therapy. *Pharmaceutics* **15** (2), 364 (2023).
35. Hsin, C-H. et al. In vitro validation of an in vivo phenotyping drug cocktail for major drug transporters in humans. *Eur. J. Pharm. Sci.* **186**, 106459 (2023).
36. Akashi, T. et al. L-type amino acid transporter 1 (SLC7A5)-mediated transport of pregabalin at the rat blood-spinal cord barrier and its sensitivity to plasma branched-chain amino acids. *J. Pharm. Sci.* **112** (4), 1137–1144 (2023).
37. Fazeli, G. et al. A BORC-dependent molecular pathway for vesiculation of cell corpse phagolysosomes. *Curr. Biol.* **33** (4), 607–621 (2023).
38. Sheng, L., Luo, Q. & Chen, L. Amino acid solute carrier transporters in inflammation and autoimmunity. In *Drug Metabolism and Disposition: The Biological Fate of Chemicals* (2022).

Author contributions

Conceived and designed the experiments: Y.M. Contributed experiment: TT.F, LZ.W, RJ.Z, XD.D, W.Z, BR.W, FJ. L. Analyzed the data: JM.Y., SX.W. Wrote the manuscript: TT.F . All authors reviewed the manuscript.

Funding
This Project was funded by the Special Project For Science And Technology Strategic Cooperation Between City And School, Nanchong City Research And Development Fund Project (22SXZRKX004), and the 2022 Sichuan Provincial Grassroots Health Development Research Center Funded Project of North Sichuan Medical College (SWFZ22-Z-03).

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-86841-y.

Correspondence and requests for materials should be addressed to Y.M.