Systematic Review and Meta-Analysis

Medicine

OPEN

Association between polymorphisms in miRNAs and ischemic stroke

A meta-analysis

Yunnan Jing, MM[a,b], Siya Hu, MM[a,c], Jing Song, MM[b], Xu Dong, MD[b], Ying Zhang, MM[a,b], Xiaowei Sun, MD[a,d], Dongyan Wang, MD[b,*]

Abstract

Background: Atherosclerosis remains a predominant cause of ischemic stroke (IS). Four miRNA polymorphisms associated with arteriosclerosis mechanism were meta-analyzed to explore whether they had predictive significance for IS.

Methods: PubMed, Excerpta Medica database, Web of Science, Cochrane Library, Scopus, China National Knowledge Infrastructure, and China Wanfang Database were searched for relevant case-control studies published before September 2022. Two researchers independently reviewed the studies and extracted the data. Data synthesis was carried out on eligible studies. Meta-analysis, subgroup analysis, sensitivity analysis, and publication bias analysis were performed using Stata software 16.0.

Results: Twenty-two studies were included, comprising 8879 cases and 12,091 controls. The results indicated that there were no significant associations between miR-146a C>G (rs2910164), miR-196a2 T>C (rs11614913) and IS risk in the overall analyses, but miR-149 T>C (rs2292832) and miR-499 A>G (rs3746444) increased IS risk under the allelic model, homozygote model and recessive model. The subgroup analyses based on Trial of Org 101072 in Acute Stroke Treatment classification indicated that rs2910164 increased small artery occlusion (SAO) risk under the allelic model, heterozygote model and dominant model; rs11614913 decreased the risk of SAO under the allelic model, homozygote model, heterozygote model and dominant model.

Conclusion: This Meta-analysis showed that all 4 single nucleotide polymorphisms were associated with the risk of IS or SAO, even though the overall and subgroup analyses were not entirely consistent.

Abbreviations: 95% CI = confidence intervals, ANXA = annexin A1, AS = atherosclerosis, HWE = Hardy-Weinberg equilibrium, IS = ischemic stroke, LAA = large artery atherosclerosis, NF-κB = nuclear factor kappa-B, ORs = odds ratios, SAO = small artery occlusion, SNPs = single nucleotide polymorphisms, TOAST = trial of Org 10172 in acute stroke treatment.

Keywords: ischemic stroke, meta-analysis, miR-146a, miR-149, miR-196a2, miR-499, polymorphism

1. Introduction

Stroke is the second-leading cause of death and a major contributor to disability worldwide, which affects roughly 13.7 million people and kills around 5.5 million annually and approximately 87% of strokes are ischemic infarctions.[1–3] MicroRNAs, small molecules that controls transcription and translation,[4] is affected by single nucleotide polymorphisms (SNPs), the latter can potentially change various biological processes by affecting the maturation process, expression levels, secondary structure, or even target selection of miRNAs, leading to gene disorder or disease. Atherosclerosis (AS), one of the most important factors of ischemic stroke (IS), is a complex and long process regulated by a variety of cells and mechanisms. MiRNAs broadly regulate physiological and pathological processes in humans, including the process of AS, and may potentially affect the susceptibility to IS through SNPs. Previous evidence suggested that rs2910164, rs2292832, rs11614913, and rs3746444 affected the function of miRNAs,[5–11] which were shown to be involved in the processes of AS, such as inflammation,[12–15] endothelial injury,[16–19] lipid metabolism,[9–11] immune response,[19] and angiogenesis.[20–25] In addition, miR-146a-3p and miR-149-5p was shown to be involved in the regulation of AS plaque

This study was supported by grants from the National Natural Science Foundation of China (81473763), and was supported by grants from the Science Foundation of Heilongjiang University of Chinese Medicine (2017sit01 and 2019BS08).

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Supplemental Digital Content is available for this article.

[a] Heilongjiang University of Chinese Medicine, Harbin, China, [b] Department of Acupuncture and Moxibustion, The Second Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China, [c] Department of Obstetrics and Gynecology, The First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China, [d] Department of Acupuncture and Moxibustion, The First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China.

** Correspondence: Dongyan Wang, Department of Acupuncture and Moxibustion, The Second Affiliated Hospital of Heilongjiang University of Chinese Medicine, 411 Gogoli Dajie, Nangang District, Harbin City, Heilongjiang Province 150000, China (e-mail: doctwdy@163.com).*

How to cite this article: Jing Y, Hu S, Song J, Dong X, Zhang Y, Sun X, Wang D. Association between polymorphisms in miRNAs and ischemic stroke: A meta-analysis. Medicine 2022;101:52(e32078).

Received: 22 June 2022 / Received in final form: 3 November 2022 / Accepted: 3 November 2022

http://dx.doi.org/10.1097/MD.0000000000032078

stability.[26,27] The relationship between miRNA and AS has been verified, but whether SNPs can predict IS remains to be investigated. The results of past meta-analyses are inconsistent and inconclusive. Therefore, we collected more studies and conducted subgroup analysis according to Trial of Org 10172 in acute stroke treatment (TOAST) classification. Our research has yielded some interesting results that may provide some inspiration.

2. Methods

2.1. Study design

Our meta-analysis was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[28] This study does not require ethical approval and informed consent because it is not a clinical trial. All involved literature has passed ethical approval and informed consent.

2.2. Search strategy

We conducted a comprehensive search across 7 databases (PubMed, Excerpta Medica database, Web of Science, Cochrane Library, Scopus, CNKI, and China Wanfang Database) for studies published up to September 2022. We searched the databases using combinations of the following keywords: (ischemic stroke OR embolic stroke OR thrombotic stroke OR lacunar stroke OR cerebrovascular disease) AND (polymorphism OR SNP OR mutation OR variant) AND ((miR-146a OR miR-196a2 OR miR-149 OR miR-499) OR ("rs2910164" OR "rs2292832" OR "rs11614913" OR "rs3746444" OR "rs61270459" OR "rs57852408" OR "rs56537094" OR "rs59983323" OR "rs61492283" OR "rs386585341")). For all databases, check the options such as Map to preferred term in Emtree/Mesh, Search as broadly as possible, synonym extension, and turn off exact search to expand the search scope. Additional articles were searched by checking the references of relevant studies in the manual way.

2.3. Eligibility criteria

Eligible studies should conform with the following criteria: properly designed case-control study; the exposure was miRNAs polymorphisms; the outcome was the incident of IS; providing sufficient data on genotype frequency in the case and control groups. The main reasons for exclusion were as follows: reviews, cell or animal studies, and meta-analyses; information on genotype frequency is unclear or absent; studies contained duplicate data; repeated publications (studies recently published or with more participants were included); Chinese or English only.

2.4. Data extraction

Two researchers (Y.J. and S.H.) independently screened the literature according to the selection criteria, extracted essential data from the included studies, and then cross-checked to reach a consensus. The essential data we extracted included: the first author's name, published year, country, ethnicity, source of controls, the total number of cases and controls, the genotype frequency of case and control, and value of Hardy-Weinberg equilibrium (HWE) for controls. Discrepancy was resolved by referring to original studies in discussion with a third reviewer (J.S.).

2.5. Quality assessment

The quality of the included studies was assessed using the Newcastle-Ottawa scale,[29] which is divided into 3 dimensions, including selection, comparability and exposure and studies score below 5 will be excluded.

2.6. Statistical analysis

The extracted data were split and combined according to the 6 gene models and used to test whether polymorphisms in these miRNAs were associated with IS risk, and the strength of association was measured by odds ratios (ORs) and 95% confidence intervals (95% CIs) using the Z test. $P < .05$ were considered statistically significant. Chi-square-based Q statistic test and I^2 value were used to test heterogeneity between studies. When $P < .1$ or $I^2 > 50\%$, significant inter-study heterogeneity was indicated, and random effect model was applied for analysis; otherwise, fixed effect model was used instead. The χ^2 test was used to measure the HWE for genotypes in the controls, and P values were given in the table. When $P < .05$, it deviated from HWE; otherwise, it was consistent with HWE. Although there is no consensus about the treating of HWE-deviated studies, it may be beneficial to recommend that these studies be included in a meta-analysis.[30] We included HWE-deviated studies in the overall analysis and eliminated these studies to validate the results. Subgroup analyses based on large artery atherosclerosis (LAA) and small artery occlusion (SAO) in the TOAST classification system were performed to explore specific associations. Potential publication bias was inspected by using funnel plots and Egger's test and, if necessary, source studies of bias were excluded for meta-analysis. Sensitivity analysis was performed to verify all the results. All statistical analyses were completed by Stata software 16.0 (StataCorp, College Station, TX).

2.7. Patient and public involvement

No patients were involved in this study.

2.8. Ethics Statement

Owing to the nature of the review, ethical approval is not required.

3. Results

3.1. Literature search and study characteristics

We yielded 234 papers initially and 22 studies,[31–52] containing 8879 cases and 12,091 controls, were included in this meta-analysis finally. Figure 1 shows the progress of study selection.

Among these studies, 17 studies focused on miR-146a G>C (rs2910164),[31,32,34–41,43–47,51,52] 9 studies focused on miR-149 T>C (rs2292832),[33–35,42–44,46,49,50] 10 studies focused on miR-196a2 T>C (rs11614913),[34,36–38,42–44,46,47,50] and 11 studies focused on miR-499 A>G (rs3746444).[34,36,38,41,43,44,46–49,52] From the included studies, 17 were from China, 2 from Egypt, 2 from South Korea and 1 from Iran. Methodological quality scores for included studies are shown in Table 1. The characteristics of the included studies are presented in Table 2, classified by miRNA. HWE-deviated was found in 6 references,[31,38,40,50–52] and the others were conformed with HWE.

3.2. Publication bias and sensitivity analysis

As shown in Table 3, no significant publication bias was observed in other SNPs except miR-196a2 (rs11614913). When we excluded a study[50] focus on Caucasians (the rest studies focus on Asians), the P value was greater than .05 and the bias disappeared. All funnel plots can be found in Figure S1, Supplemental Digital Content, http://links.lww.com/MD/I18.

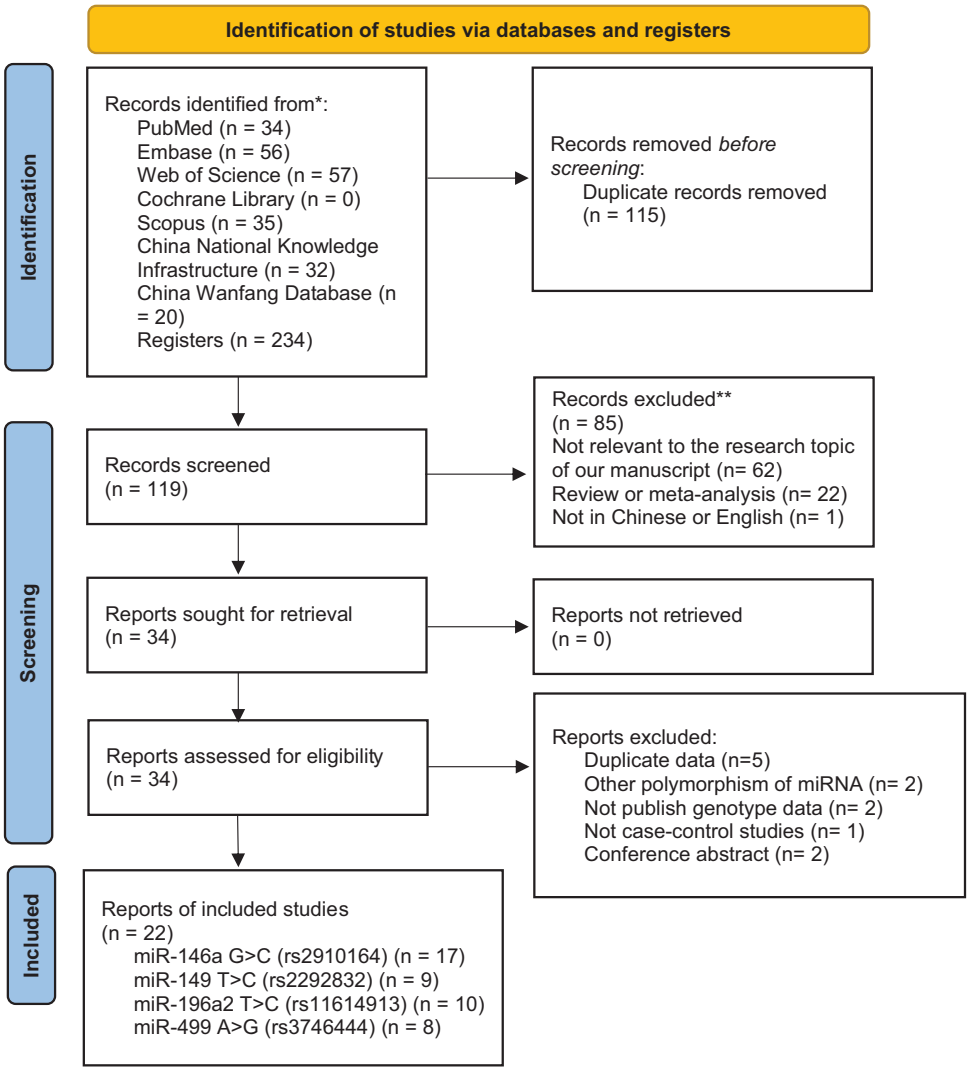

Figure 1. Flow chart of study selection process.

Sensitivity analysis results illustrated that the results of our overall analysis (including HWE-yes and re-analysis) are reliable and the results can be found in Figure S2, Supplemental Digital Content, http://links.lww.com/MD/I19.

3.3. Meta-analysis and subgroup analyses

3.3.1. MiR-146a C>G (rs2910164) and the risk of IS. No significant association was observed between rs2910164 and risk of IS under all models (allelic model: G vs C, OR = 1.06, 95% CI = 0.96–1.17; homozygote model: GG vs CC, OR = 1.19, 95% CI = 1.00–1.43; heterozygote model: CG vs CC, OR = 1.05, 95% CI = 0.96–1.14; recessive model: GG vs CG+CC, OR = 1.15, 95% CI = 1.00–1.33; dominant model: CG+GG vs CC, OR = 1.09, 95% CI = 0.98–1.21 and overdominant model: GG+CC vs CG, OR = 1.00, 95% CI = 0.94–1.07). No change was detected in the results after exclusion of the HWE-deviated study (Table 3). No significant association of rs2910164 with LAA susceptibility was observed, and rs2910164 increased SAO susceptibility under the allelic model (G vs C, OR = 1.16, 95% CI = 1.02–1.33), heterozygote model (CG vs CC, OR = 1.34, 95% CI = 1.09–1.65), dominant model (CG+GG vs CC, OR = 1.33, 95% CI = 1.09–1.62), and decreased SAO susceptibility under overdominant model (GG+CC vs CG, OR = 0.81, 95% CI = 0.67–0.97) (Table 4).

3.3.2. MiR-149 T>C (rs2292832) and the risk of IS. Rs2292832 increased the risk of IS under the allelic model (C vs T, OR = 1.16, 95% CI = 1.04–1.29), homozygote model (CC vs TT, OR = 1.40, 95% CI = 1.13–1.74), and recessive model (CC vs TC+TT, OR = 1.33, 95% CI = 1.15–1.53) but no significant association was observed for the heterozygote model (TC vs TT, OR = 1.07, 95% CI = 0.97–1.19), dominant model (TC+CC vs TT, OR = 1.14, 95% CI = 1.00–1.30), and overdominant model (CC+TT vs TC, OR = 1.00, 95% CI = 0.91–1.10). After removing the HWE-deviated literature, the result showed that the C allelic was positive in the dominant model (TC+CC vs TT, OR = 1.11, 95% CI = 1.01–1.23), and other results remained unchanged (Table 3). There was no significant association of rs2292832 with LAA susceptibility, but rs2292832 increased SAO susceptibility under the homozygote model (CC vs TT, OR = 1.65, 95% CI = 1.08–2.51) (Table 4).

3.3.3. MiR-196a2 T>C (rs11614913) and the risk of IS. No significant association was observed between rs11614913 and IS susceptibility under all genetic models (allelic model: C vs T, OR = 1.05, 95% CI = 0.99–1.12; homozygote model: CC vs TT, OR = 1.11, 95% CI = 0.97–1.26; heterozygote model: TC vs TT, OR = 1.04, 95% CI = 0.93–1.17; recessive model: CC vs TC+TT, OR = 1.07, 95% CI = 0.97–1.19; dominant model: TC+CC vs TT, OR = 1.06, 95% CI = 0.96–1.18; and overdominant model: CC+TT vs TC, OR = 1.01, 95% CI = 0.92–1.10). No

Table 1

Methodological quality of the studies included in the final analysis based on the Newcastle-Ottawa scale.

	Selection (score)				Comparability (score)	Exposure (score)			
Study	Adequate definition of patient cases	Representativeness of patient cases	Selection of controls	Definition of controls	Control for important factor or additional factor	Ascertainment of exposure (blinding)	Same method of ascertainment for participants	Non-response rate*	Total score†
Li 2010	1	1	0	1	0	0	1	1	5
Sun 2011	1	1	0	1	2	0	1	1	7
He 2013	1	1	0	1	2	0	1	1	7
Jeon 2013	1	1	0	1	2	0	1	1	7
Hu 2014	1	1	0	1	2	0	1	1	7
Liu 2014	1	1	0	1	1	0	1	1	6
Zhu 2014	1	1	0	1	2	0	1	1	7
Huang 2015	1	1	0	1	2	0	1	1	7
Zhong 2016	1	1	0	1	2	0	1	1	7
Qu 2016	1	1	0	1	0	0	1	1	5
Lyu 2016	1	1	0	1	2	0	1	1	7
Lyu 2016	1	1	0	1	2	0	1	1	7
Luo 2017	1	1	0	1	2	0	1	1	7
Zhu 2017	1	1	0	1	2	0	1	0	6
Fang 2017	1	1	0	1	1	0	1	1	6
Zhu 2018	1	1	0	1	2	0	1	1	7
Hong 2019	1	1	1	1	0	0	1	1	6
Darabi 2019	1	1	0	1	2	0	1	1	7
Zhu 2020	1	1	0	1	2	0	1	1	7
Mahmoud 2020	1	1	0	1	2	0	1	1	7
Wang 2021	1	1	1	1	2	0	0	1	7
Abdelghany 2022	1	1	0	1	2	0	1	1	7

*Non-response rate: when there was no significant difference in the response rate between both groups based on a chi-squared test ($P > .05$), one point was awarded.

†Total score: the total score was the sum of scores for each item.

HWE-deviated study here, but 1 study[50] provides significant publication bias and no change was found after eliminating this study (Table 3). There was no significant association between rs11614913 and LAA susceptibility, but rs11614913 decreased the risk of SAO under the allelic model (C vs T, OR = 0.84, 95% CI = 0.72–0.97), homozygote model (CC vs TT, OR = 0.69, 95% CI = 0.51–0.94), heterozygote model (TC vs TT, OR = 0.72, 95% CI = 0.56–0.93), and dominant model (TC+CC vs TT, OR = 0.72, 95% CI = 0.56–0.91) (Table 4).

3.3.4. miR-499 A>G (rs3746444) and the risk of IS. The overall analysis showed that rs3746444 increased the risk of IS under the allelic model (G vs A, OR = 1.18, 95% CI = 1.01–1.38), homozygote model (GG vs AA, OR = 1.43, 95% CI = 1.03–1.99) and recessive model (GG vs AG+AA, OR = 1.42, 95% CI = 1.02–1.98), but no significant association was observed in the heterozygote model (AG vs AA, OR = 1.06, 95% CI = 0.92–1.22), dominant model (AG+GG vs AA, OR = 1.13, 95% CI = 0.97–1.32), and overdominant model (AA+GG vs AG, OR = 0.98, 95% CI = 0.85–1.14). After removing the HWE-deviated studies, the allelic model lost significance, and other results were unchanged (Table 3). No significant association was observed between miR-499 A>G (rs3746444) polymorphism and risk of LAA or SAO under all genetic models (Table 4).

It is worth mentioning that except miR-499 (rs3746444), all data in the HWE-yes analysis or re-analysis and subgroup analysis were all came from Asia, which can be regarded as the study excluding ethnic differences.

All Forest plots can be found in Figure S3, Supplemental Digital Content, http://links.lww.com/MD/I20.

4. Discussion

Gene expression profiling studies have demonstrated alterations in miRNA expression in a wide range of human disease. In many cases, functional studies have linked miRNA dysregulation as a causal factor in disease progression.[53] The meta-analysis published in the past 5 years gave different results. The Du et al's[54] study in 2017 showed that rs2292832 was associated with IS risk under the allelic model, homozygote model and recessive model, while rs2910164 was not. The results of the 3 meta-analyses in 2018 were not completely consistent. Li et al's[55] study showed that rs3746444 was associated with the risk of IS under the allelic model, homozygote model and dominant model, while rs2910164, rs2292832 and rs11614913 were not. However, in the Asian population, Zou et al[56] reported that rs2910164 was associated with IS risk under recessive model, rs2292832 was associated with IS susceptibility under recessive model and homozygote model, while rs11614913 and rs3746444 were not associated with IS risk. Wang et al's[57] study supports the conclusion that rs11614913 IS not associated with IS risk in Asians. According to Bastami et al's[58] study published in 2019, rs2910164, rs11614913, and rs3746444 were not associated with the risk of IS, while rs2292832 was associated with IS risk under the allelic model, homozygote model and recessive model. Most recent meta-analysis[49] in 2020 showed that rs2292832 was significantly associated with the risk of IS in the allelic model and the recessive model, while rs3746444 was not.

The above studies only reached agreement that rs11614913 was not associated with IS susceptibility, but no consensus was reached for the other 3 SNPs. The influence of polymorphisms on miRNA function and disease susceptibility has remained elusive and controversial irrespective of intensive research,[58] meriting the need for meta-analyses.

Our overall analysis did not find a significant association between miR-146a rs2910164 and IS or LAA risk. However, the G alleles of rs2910164 increase SAO susceptibility under the allelic model (1.16-fold), heterozygote model (1.34-fold) and dominant model (1.34-fold). MiR-146a was identified as a nuclear factor kappa-B (NF-κB)-dependent inflammatory regulator, which can be up regulated in response to a variety

Table 2

Main characteristics of the studies included in the meta-analysis.

First author	Year	Country	Ethnicity	Cases/controls	Cases			Control			HWE	Genotyping method
miR-146a (rs2910164)					**CC**	**CG**	**GG**	**CC**	**CG**	**GG**		
Li	2010	China	Asian	268/1010	79	110	79	345	455	210	0.009*	PCR-RFLP
Sun[a,b]	2011	China	Asian	358/650	136	161	61	228	304	118	0.345	PCR-RFLP
Jeon[a,b]	2013	South Korea	Asian	678/553	223	327	128	211	266	76	0.589	TaqMan
Hu	2014	China	Asian	196/205	75	87	34	97	82	26	0.193	PCR-RFLP
Liu	2014	China	Asian	296/391	85	159	52	116	198	77	0.650	PCR-RFLP
Zhu[a,b]	2014	China	Asian	368/381	145	173	50	132	185	64	0.952	PCR-RFLP
Huang	2015	China	Asian	531/531	189	261	81	219	257	55	0.106	TaqMan
Zhong	2016	China	Asian	297/300	141	128	28	113	152	35	0.133	PCR
Qu	2016	China	Asian	1139/1575	355	618	166	483	869	223	<0.001*	PCR-LDR
Lyu	2016	China	Asian	378/378	119	198	61	153	187	38	0.079	TaqMan
Luo	2017	China	Asian	298/303	129	130	39	119	139	45	0.672	PCR
Zhu	2017	China	Asian	523/510	170	267	86	204	251	55	0.085	TaqMan
Fang[a]	2017	China	Asian	314/314	112	161	41	109	150	55	0.784	SNaPshot
Zhu[a,b]	2018	China	Asian	396/378	131	194	71	154	179	45	0.521	PCR-RFLP
Hong	2019	South Korea	Asian	264/451	110	115	39	182	208	61	0.898	DNA microarray
Wang	2020	China	Asian	981/2547	269	517	195	690	1329	528	0.016*	TOFMS
Abdelghany	2022	Egypt	Caucasian	100/100	19	42	39	31	38	31	0.016*	TaqMan
miR-149 (rs2292832)					**TT**	**TC**	**CC**	**TT**	**TC**	**CC**		
He	2013	China	Asian	357/373	138	162	57	160	175	38	0.303	PCR-RFLP
Jeon[a,b]	2013	South Korea	Asian	678/553	299	303	76	262	238	53	0.921	TaqMan
Hu	2014	China	Asian	196/205	79	76	41	80	89	36	0.199	PCR-RFLP
Lyu	2016	China	Asian	378/378	142	170	66	159	178	41	0.398	TaqMan
Luo	2017	China	Asian	298/303	131	127	40	121	136	46	0.447	PCR
Zhu	2017	China	Asian	523/510	232	221	70	240	213	57	0.351	TaqMan
Zhu[a,b]	2018	China	Asian	396/378	165	179	52	190	158	30	0.720	PCR-RFLP
Zhu	2020	China	Asian	567/552	254	250	63	250	247	55	0.597	PCR-LDR
Mahmoud	2020	Egypt	Caucasian	100/100	12	31	57	29	26	45	<0.001*	TaqMan
miR-196a2 (rs11614913)					**TT**	**TC**	**CC**	**TT**	**TC**	**CC**		
Jeon[a,b]	2013	South Korea	Asian	678/553	139	352	187	105	292	156	0.126	TaqMan
Liu	2014	China	Asian	296/391	51	181	64	84	214	93	0.060	PCR-RFLP
Zhu[a,b]	2014	China	Asian	368/381	71	189	108	78	198	105	0.384	PCR-RFLP
Huang	2015	China	Asian	531/531	100	265	166	112	266	153	0.856	TaqMan
Lyu	2016	China	Asian	378/378	105	208	65	113	200	65	0.141	TaqMan
Luo	2017	China	Asian	298/303	73	138	87	75	159	69	0.385	PCR
Zhu	2017	China	Asian	523/510	150	273	100	146	260	104	0.548	TaqMan
Zhu[a,b]	2018	China	Asian	396/378	112	205	79	110	196	72	0.354	PCR-RFLP
Hong	2019	South Korea	Asian	263/455	85	108	70	139	209	107	0.101	DNA microarray
Mahmoud	2020	Egypt	Caucasian	100/100	5	41	54	21	41	38	0.120	TaqMan
miR-499 (rs3746444)					**AA**	**AG**	**GG**	**AA**	**AG**	**GG**		
Jeon[a,b]	2013	South Korea	Asian	678/553	460	195	23	365	170	18	0.740	TaqMan
Liu	2014	China	Asian	296/391	181	96	19	278	99	14	0.170	PCR-RFLP
Huang	2015	China	Asian	531/531	398	133	0	403	128	0	0.002*	TaqMan
Lyu	2016	China	Asian	378/378	257	110	11	250	113	15	0.621	TaqMan
Luo	2017	China	Asian	298/303	215	78	5	244	53	6	0.131	PCR
Zhu	2017	China	Asian	505/510	349	124	32	328	158	24	0.380	TaqMan
Zhu[a,b]	2018	China	Asian	396/378	255	123	18	249	116	13	0.910	PCR-RFLP
Hong	2019	South Korea	Asian	263/455	173	76	14	321	122	12	0.920	DNA microarray
Darabi[a,b]	2019	Iran	Caucasian	470/489	252	173	45	301	158	30	0.137	PCR-RFLP
Zhu	2020	China	Asian	567/552	414	144	9	386	153	13	0.636	PCR-LDR
Abdelghany	2022	Egypt	Caucasian	100/100	38	14	48	56	26	18	<0.001*	TaqMan

HWE = Hardy-Weinberg equilibrium.

*$P < .05$, it means that this study was deviated from Hardy-Weinberg equilibrium: the study comprises complete data of (a) large artery atherosclerosis and (b) small artery occlusion according to TOAST classification and consistent with Hardy-Weinberg equilibrium.

of immune mediators, and negatively regulates the IRAK1/TRAF6/NF-κB inflammatory cascade, thereby inhibiting the progression of AS.[12,59,60] In addition, miR-146a is involved in the regulation of multiple cell types associated with AS progression. MiR-146a is involved in the regulation of vascular smooth muscle cell proliferation, which participate in AS[21,22]; Apolipoprotein E mediated upregulation of miR146a in monocytes and macrophages could inhibit NF-κB-driven inflammation and AS[61]; MiR-146a has been showed to be overexpressed within mitochondria of aging human endothelial cells and that they could promote mitochondrial dysfunction via Bcl-2 down regulation, and affect sensitivity to apoptosis[13,16]; MiR-146a regulates transcription and translation of tumor necrosis factor-α[62] and tumor necrosis factor-α was found to be involved in AS by inducing CD47 expression and rendering vascular cells resistant to phagocytic clearance.[63] We found high heterogeneity in the conclusion that rs2910164 was not associated with IS risk and low heterogeneity in the conclusion that rs2910164 was associated with SAO risk. That led us to wonder whether unclassified disease data obscured the relationship between SAO risk and rs2910164.However, the conclusions drawn from only 4 articles may have affected our ability to draw clear inferences.

Consistent with the results of the previous meta-analysis, no association was observed between rs11614913 and the susceptibility of IS and LAA. But the C alleles may decrease

Table 3

Results of overall analyses.

Genetic models	Subgroup	No.	I^2 (%)	Model for analysis	P_H	OR (95% CI)	P_Z	P_E
miR-146a (rs2910164)								
G versus C	Overall	17	78.4	REM	.000	1.06 (0.96, 1.17)	.223	.582
	HWE-yes	13	80.8	REM	.000	1.04 (0.92, 1.19)	.515	.231
GG versus CC	Overall	17	70.0	REM	.000	1.19 (1.00, 1.43)	.051	.441
	HWE-yes	13	71.7	REM	.000	1.18 (0.93, 1.48)	.168	.303
CG versus CC	Overall	17	33.1	REM	.091	1.05 (0.96, 1.14)	.287	.376
	HWE-yes	13	41.5	REM	.058	1.06 (0.94, 1.18)	.345	.440
GG versus CG+CC	Overall	17	60.4	REM	.001	1.15 (1.00, 1.33)	.048	.503
	HWE-yes	13	60.7	REM	.002	1.14 (0.95, 1.37)	.148	.354
CG+GG versus CC	Overall	17	59.9	REM	.001	1.09 (0.98, 1.21)	.134	.383
	HWE-yes	13	64.1	REM	.001	1.08 (0.94, 1.24)	.272	.314
GG+CC versus CG	Overall	17	0.0	FEM	.861	1.00 (0.94, 1.07)	.902	.744
	HWE-yes	13	0.0	FEM	.779	0.99 (0.92, 1.07)	.845	.926
miR-149 (rs2292832)								
C versus T	Overall	9	51.2	REM	.037	**1.16 (1.04, 1.29)**	**.006**	.251
	HWE-yes	8	33.7	FEM	.159	**1.13 (1.05, 1.22)**	**.001**	.874
CC versus TT	Overall	9	46.3	REM	.061	**1.40 (1.13, 1.74)**	**.002**	.193
	HWE-yes	8	34.5	FEM	.153	**1.34 (1.14, 1.56)**	**.000**	.796
TC versus TT	Overall	9	17.9	FEM	.284	1.07 (0.97, 1.19)	.168	.277
	HWE-yes	8	0.0	FEM	.722	1.06 (0.96, 1.17)	.282	.260
CC versus TC+TT	Overall	9	11.9	FEM	.335	**1.33 (1.15, 1.53)**	**.000**	.409
	HWE-yes	8	18.1	FEM	.287	**1.31 (1.13, 1.52)**	**.000**	.611
TC+CC versus TT	Overall	9	42.3	REM	.085	1.14 (1.00, 1.30)	.049	.222
	HWE-yes	8	2.1	FEM	.413	**1.11 (1.01, 1.23)**	**.030**	.555
CC+TT versus TC	Overall	9	0.0	FEM	.887	1.00 (0.91, 1.10)	.949	.912
	HWE-yes	8	0.0	FEM	.885	1.01 (0.92, 1.11)	.850	.090
miR-196a2 (rs11614913)								
C versus T	Overall	10	29.5	FEM	.127	1.05 (0.99, 1.12)	.174	.001
	Re-analysis	9	0.0	FEM	.952	1.03 (0.97, 1.10)	.308	.130
CC versus TT	Overall	10	29.7	FEM	.172	1.11 (0.97, 1.26)	.124	.000
	Re-analysis	9	0.0	FEM	.944	1.07 (0.94, 1.22)	.307	.113
TC versus TT	Overall	10	23.0	FEM	.232	1.04 (0.93, 1.17)	.442	.016
	Re-analysis	9	0.0	FEM	.754	1.02 (0.92, 1.15)	.668	.597
CC versus TC+TT	Overall	10	3.0	FEM	.412	1.07 (0.97, 1.19)	.175	.077
	Re-analysis	9	0.0	FEM	.747	1.05 (0.95, 1.17)	.343	.588
TC+CC versus TT	Overall	10	29.1	FEM	.177	1.06 (0.96, 1.18)	.246	.001
	Re-analysis	9	0.0	FEM	.894	1.04 (0.94, 1.16)	.461	.168
CC+TT versus TC	Overall	10	0.0	FEM	.605	1.01 (0.92, 1.10)	.858	.842
	Re-analysis	9	0.0	FEM	.552	1.02 (0.93, 1.12)	.704	.822
miR-499 (rs3746444)								
G versus A	Overall	11	75.8	REM	.000	**1.18 (1.01, 1.38)**	**.041**	.024
	HWE-yes	9	64.7	REM	.004	1.11 (0.97, 1.28)	.131	.259
GG versus AA	Overall	11	53.5	REM	.022	**1.43 (1.03.1.99)**	**.033**	.424
	HWE-yes	9	23.8	FEM	.232	**1.31 (1.05, 1.65)**	**.018**	.277
AG versus AA	Overall	11	55.1	REM	.014	1.06 (0.92, 1.22)	.454	.490
	HWE-yes	9	63.3	REM	.005	1.07 (0.91, 1.26)	.427	.053
GG versus AG+AA	Overall	11	54.9	REM	.018	**1.42 (1.02, 1.98)**	**.036**	.340
	HWE-yes	9	6.1	FEM	.385	**1.29 (1.03, 1.62)**	**.025**	.197
AG+GG versus AA	Overall	11	65.9	REM	.001	1.13 (0.97, 1.32)	.125	.023
	HWE-yes	9	66.8	REM	.002	1.10 (0.93, 1.30)	.269	.082
AA+GG versus AG	Overall	11	58.8	REM	.007	0.98 (0.85, 1.14)	.782	.951
	HWE-yes	9	59.3	REM	.012	0.98 (0.89, 1.08)	.628	.049

Statistically significant results are bolded.

95% CI = 95% confidence interval, FEM = fixed effect model, OR = odds ratio, P_E = the P value for egger, P_H = the P value for the Q test, P_Z = the P value for the Z test, REM = random effect model.

SAO risk under the allelic model (0.84-fold), homozygote model (0.69-fold), heterozygote model (0.72-fold) and dominant model (0.72-fold). MiR-196a2 polymorphism was proved to specifically regulate ANXA1,[23,64,65] the latter can mediate endothelial cell migration and angiogenesis [Pin AL], and plays an unique role in the resolution axis as they can target both endogenous inflammatory and pro-resolving pathways.[66–68] The ANXA1-FPR2/ALX pathway has been demonstrated in several studies[69–73] to significantly mitigate and rescue adverse thromboinflammatory phenotype in cerebral microvessels, theoretically preventing the onset of IS. However, only 3 studies were included and the result needs further validation.

Our analyses showed that the G alleles of rs3746444 increase the risk of IS under the allelic model (1.18-fold), homozygote model (1.43-fold), recessive model (1.42-fold). These results were supported by HWE-yes analysis, except the allelic model lost significance. Subgroup analysis found no association between rs3746444 and risk of LAA and SAO. The rs3746444 SNP influences the expression and function of miR-499a-5p, and alters the inhibition of the target, which in turn affects lipid metabolism[9–11] and miR-499-mediated anti-apoptosis.[74] Other studies have suggested that Rs3746444 could theoretically potentially influence the structure and function of miR-499a-3p precursor[58] and mature miR-499a-3p could promote cell proliferation and migration in AS by directly targeting MEF2C.[75]

Table 4

Results of subgroup analyses.

Genetic models	No.	I^2 (%)	Model for analysis	P_H	OR (95% CI)	P_z
miR-146a C>G (rs2910164)LAA						
G versus C	5	87.5	REM	.000	0.93 (0.69, 1.24)	.618
GG versus CC		79.1	REM	.001	1.03 (0.65, 1.65)	.896
CG versus CC		64.4	REM	.024	0.91 (0.70, 1.19)	.492
GG versus CG+CC		70.4	REM	.009	1.09 (0.76, 1.56)	.650
CG+GG versus CC		76.3	REM	.002	0.94 (0.69, 1.28)	.691
GG+CC versus CG		26.4	FEM	.246	1.11 (0.96, 1.28)	.159
miR-146a C>G (rs2910164)SAO						
G versus C	4	0.0	FEM	.665	**1.16 (1.02, 1.33)**	**.025**
GG versus CC		0.0	FEM	.718	1.29 (0.97, 1.71)	.083
CG versus CC		20.8	FEM	.285	**1.34 (1.09, 1.65)**	**.006**
GG versus CG+CC		0.0	FEM	.658	1.08 (0.84, 1.39)	.563
CG+GG versus CC		3.8	FEM	.374	**1.33 (1.09, 1.62)**	**.005**
GG+CC versus CG		23.6	FEM	.270	**0.81 (0.67, 0.97)**	**.023**
miR-149 T>C (rs2292832)LAA						
C versus T	2	83.3	REM	.014	1.27 (0.85, 1.89)	.249
CC versus TT		85.0	REM	.010	1.49 (0.55, 4.05)	.435
TC versus TT		26.7	FEM	.243	1.34 (1.07, 1.68)	.012
CC versus TC+TT		81.7	REM	.019	1.28 (0.54, 3.03)	.575
TC+CC versus TT		70.2	REM	.067	1.38 (0.92, 2.06)	.117
CC+TT versus TC		0.0	FEM	.745	0.80 (0.65, 1.00)	.049
miR-149 T>C (rs2292832)SAO						
C versus T	2	23.8	FEM	.252	1.20 (0.99, 1.46)	.069
CC versus TT		0.0	FEM	.335	**1.65 (1.08, 2.51)**	**.020**
TC versus TT		0.0	FEM	.513	1.03 (0.78, 1.36)	.840
CC versus TC+TT		0.0	FEM	.445	1.62 (1.09, 2.42)	.018
TC+CC versus TT		0.0	FEM	.347	1.13 (0.87, 1.47)	.346
CC+TT versus TC		0.0	FEM	.772	1.07 (0.82, 1.39)	.617
miR-196a2 T>C (rs11614913)LAA						
C versus T	3	71.6	REM	.030	0.82 (0.64, 1.04)	.105
CC versus TT		73.3	REM	.024	0.65 (0.39, 1.10)	.107
TC versus TT		19.7	FEM	.288	0.83 (0.66, 1.03)	.087
CC versus TC+TT		60.2	REM	.081	0.75 (0.52, 1.07)	.109
TC+CC versus TT		59.5	REM	.085	0.77 (0.55, 1.06)	.112
CC+TT versus TC		0.0	FEM	.931	0.99 (0.82, 1.18)	.883
miR-196a2 T>C (rs11614913)SAO						
C versus T	3	37.6	FEM	.201	**0.84 (0.72, 0.97)**	**.021**
CC versus TT		36.7	FEM	.206	**0.69 (0.51, 0.94)**	**.016**
TC versus TT		27.8	FEM	.251	**0.72 (0.56 ,0.93)**	**.013**
CC versus TC+TT		0	FEM	.435	0.87 (0.68, 1.12)	.282
TC+CC versus TT		43.3	FEM	.171	**0.72 (0.56, 0.91)**	**.007**
CC+TT versus TC		0.0	FEM	.645	1.16 (0.94, 1.44)	.162
miR-499 A>G (rs3746444)LAA						
G versus A	3	0.0	FEM	.606	1.01 (0.84, 1.21)	.928
GG versus AA		0.0	FEM	.743	1.23 (0.75, 2.03)	.420
AG versus AA		0.0	FEM	.773	0.94 (0.76, 1.18)	.596
GG versus AG+AA		0.0	FEM	.777	1.25 (0.76, 2.06)	.373
AG+GG versus AA		0.0	FEM	.689	0.97 (0.79, 1.20)	.794
AA+GG versus AG		0.0	FEM	.810	1.08 (0.86, 1.34)	.516
miR-499 A>G (rs3746444)SAO						
G versus A	3	0.0	FEM	.409	1.10 (0.93, 1.31)	.257
GG versus AA		0.0	FEM	.784	1.42 (0.92, 2.21)	.116
AG versus AA		0.0	FEM	.557	1.03 (0.83, 1.27)	.818
GG versus AG+AA		0.0	FEM	.853	1.39 (0.90, 2.15)	.132
AG+GG versus AA		0.0	FEM	.451	1.07 (0.87, 1.32)	.505
AA+GG versus AG		0.0	FEM	.650	1.00 (0.81, 1.24)	.985

Statistically significant results are bolded.

95% CI = 95% confidence interval, FEM = fixed effect model, OR = odds ratio, P_H = the P value for the Q test, P_z = the P value for the Z test, REM = random effect model.

Our analysis found that the C alleles of rs2292832 increase IS risk under the allelic model (1.16-fold), homozygote model (1.40-fold) and recessive model (1.33-fold). These results were supported by HWE-yes analysis while the C allele also increased the susceptibility of IS under the dominant model (1.11-fold). Subgroup analysis showed that the C alleles increase SAO susceptibility under the homozygote model (1.65-fold), even though only 2 studies were counted. Studies[8] have shown that rs2292832 affects the expression of miR-149, CC genotype have lower miR-149-5p expression level. miR-149 is widely involved in the regulation of inflammatory response in a variety of diseases[76–80] by targeting different cytokines. Existing studies have shown that miR-149 can participate in the development of advanced atherosclerotic plaques by regulating the inflammatory response of macrophages triggered by toll-like receptor[81] and by regulating the anti-phagocytic molecule CD47.[27] In addition, mir-149 is also involved in the regulation of endothelial function,[17,18] angiogenesis,[20] and the proliferation, invasion

and migration of vascular smooth muscle cells.[82,83] Therefore, rs2292832 is also considered to be potentially associated with IS risk.

In recent years, many studies established a fact that the pathological mechanisms of large vessel disease and small vessel disease are different, but both disease subtypes share the same risk factors and their processes may occur cooccurrence and could be overlapping.[84,85] Animal experiments[86,87] have also confirmed that small and large artery diseases are a continuum and their interaction is dynamic. Stiff large artery transmits the excessive flow pulsatility into the cerebral microcirculation causing diastolic hypoperfusion which both damage microvascular wall, thus leading to arteriolosclerosis and white matter damage.[88] It should be noted that the TOAST classification only classifies patients based on their first diagnosis, so the 2 diagnoses are mutually exclusive. However, in reality, the same miRNA polymorphism may affect the pathological process of LAA and SAO at the same time, as well as their susceptibility. Therefore, the results of the overall analysis may mask the relationship of miRNA to LAA/SAO susceptibility. Moreover, even if subgroup analysis did not show any correlation, it cannot be arbitrarily assumed that miRNA is not associated with LAA or SAO susceptibility, because the sample size is too small.

5. Conclusion

This study showed that all 4 SNPs were associated with the risk of IS or SAO, indicating a predictive relationship. More large-sample studies classified by disease subtypes are needed to further validate the results.

5.1. Limitations

Due to the limitations of the included literature, only a few studies have fully reported the data from SAO and LAA. The results of subgroup analysis are statistically reliable, but the sample size is insufficient, which may affect the reliability of the results. Similarly, other IS subtypes were not analyzed due to insufficient data. The included studies were case-control trials, and recall bias was inevitable. At the same time, the vast majority of studies were conducted in hospitals rather than in the community, adding to the selection bias. Confounding bias is also inevitable given the complexity of the disease. These biases are inevitable and affect the credibility of the results.

Author contributions

YJ conceived and designed the research, and wrote the first draft of the manuscript. YJ and SH have completed literature retrieval, data collection and extraction, as well as statistical analysis. JS was brought in as a third reviewer to resolve discrepancy and performed a validation of the data. XD and YZ polished the whole article and proofread it. XS and DW provided guidance and did the final proofreading of the article.

Data curation: Siya Hu.
Investigation: Yunnan Jing, Siya Hu.
Methodology: Yunnan Jing, Siya Hu.
Software: Yunnan Jing, Siya Hu.
Supervision: Xu Dong, Xiaowei Sun, Dongyan Wang.
Validation: Yunnan Jing, Jing Song.
Writing – original draft: Yunnan Jing, Siya Hu.
Writing – review & editing: Jing Song, Xu Dong, Ying Zhang, Xiaowei Sun, Dongyan Wang.

References

[1] Kuriakose D, Xiao Z. Pathophysiology and treatment of stroke: present status and future perspectives. Int J Mol Sci. 2020;21:7609.

[2] Roger VL, Go AS, Lloyd-Jones DM, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics – 2011 update: a report from the American Heart Association. Circulation. 2011;123:e18–209.

[3] GBD 2016 Stroke Collaborators. Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019;18:439–58.

[4] Wang SW, Liu Z, Shi ZS. Non-Coding RNA in acute ischemic stroke: mechanisms, biomarkers and therapeutic targets. Cell Transplant. 2018;27:1763–77.

[5] Jazdzewski K, Murray EL, Franssila K, et al. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci USA. 2008;105:7269–74.

[6] Kiselev I, Bashinskaya V, Kulakova O, et al. Variants of MicroRNA genes: gender-specific associations with multiple sclerosis risk and severity. Int J Mol Sci. 2015;16:20067–81.

[7] Ghaffarzadeh M, Ghaedi H, Alipoor B, et al. Association of MiR-149 (RS2292832) variant with the risk of coronary artery disease. J Med Biochem. 2017;36:251–8.

[8] Wei WJ, Lu ZW, Li DS, et al. Association of the miR-149 Rs2292832 polymorphism with papillary thyroid cancer risk and clinicopathologic characteristics in a Chinese population. Int J Mol Sci. 2014;15:20968–81.

[9] Chen LB, Zheng HK, Zhang L, et al. A single nucleotide polymorphism located in microRNA-499a causes loss of function resulting in increased expression of osbpl1a and reduced serum HDL level. Oncol Rep. 2017;38:3515–21.

[10] Li Q, Chen L, Chen D, et al. Influence of microRNA-related polymorphisms on clinical outcomes in coronary artery disease. Am J Transl Res. 2015;7:393–400.

[11] Giuliani A, Montesanto A, Matacchione G, et al. The association between single nucleotide polymorphisms, including miR-499a genetic variants, and dyslipidemia in subjects treated with pharmacological or phytochemical lipid-lowering agents. Int J Mol Sci. 2022;23:5617.

[12] Olivieri F, Prattichizzo F, Giuliani A, et al. miR-21 and miR-146a: the microRNAs of inflammaging and age-related diseases. Ageing Res Rev. 2021;70:101374.

[13] Rippo MR, Olivieri F, Monsurrò V, et al. MitomiRs in human inflamm-aging: a hypothesis involving miR-181a, miR-34a and miR-146a. Exp Gerontol. 2014;56:154–63.

[14] Drechsler M, de Jong R, Rossaint J, et al. Annexin A1 counteracts chemokine-induced arterial myeloid cell recruitment. Circ Res. 2015;116:827–35.

[15] Zhou R, Huang W, Fan X, et al. miR-499 released during myocardial infarction causes endothelial injury by targeting α7-nAchR. J Cell Mol Med. 2019;23:6085–97.

[16] Giuliani A, Cirilli I, Prattichizzo F, et al. The mitomiR/Bcl-2 axis affects mitochondrial function and autophagic vacuole formation in senescent endothelial cells. Aging (Albany NY). 2018;10:2855–73.

[17] Wang G, Li Y, Liu Z, et al. Circular RNA circ_0124644 exacerbates the ox-LDL-induced endothelial injury in human vascular endothelial cells through regulating PAPP-A by acting as a sponge of miR-149-5p. Mol Cell Biochem. 2020;471:51–61.

[18] Palmieri D, Capponi S, Geroldi A, et al. TNFα induces the expression of genes associated with endothelial dysfunction through p38MAPK-mediated down-regulation of miR-149. Biochem Biophys Res Commun. 2014;443:246–51.

[19] Tabasi F, Hasanpour V, Sarhadi S, et al. Association of miR-499 polymorphism and its regulatory networks with Hashimoto thyroiditis susceptibility: a population-based case-control study. Int J Mol Sci. 2021;22:10094.

[20] Chamorro-Jorganes A, Araldi E, Rotllan N, et al. Autoregulation of glypican-1 by intronic microRNA-149 fine tunes the angiogenic response to FGF2 in human endothelial cells. J Cell Sci. 2014;127(Pt 6):1169–78.

[21] Sun SG, Zheng B, Han M, et al. miR-146a and Krüppel-like factor 4 form a feedback loop to participate in vascular smooth muscle cell proliferation. EMBO Rep. 2011;12:56–62.

[22] Chistiakov DA, Orekhov AN, Bobryshev YV. Vascular smooth muscle cell in atherosclerosis. Acta Physiol (Oxf). 2015;214:33–50.

[23] Pin AL, Houle F, Fournier P, et al. Annexin-1-mediated endothelial cell migration and angiogenesis are regulated by vascular endothelial growth factor (VEGF)-induced inhibition of miR-196a expression. J Biol Chem. 2012;287:30541–51.

[24] Wang T, Guan R, Xia F, et al. Curcumin promotes venous thrombi resolve process in a mouse deep venous thrombosis model via regulating miR-499. Microvasc Res. 2021;136:104148.

[25] Wang X, Wu C. Tanshinone IIA improves cardiac function via regulating miR-499-5p dependent angiogenesis in myocardial ischemic mice. Microvasc Res. 2022;143:104399.
[26] Liu H, Wang H, Ma J, et al. MicroRNA-146a-3p/HDAC1/KLF5/IKBα signal axis modulates plaque formation of atherosclerosis mice. Life Sci. 2021;284:119615.
[27] Ye ZM, Yang S, Xia YP, et al. LncRNA MIAT sponges miR-149-5p to inhibit efferocytosis in advanced atherosclerosis through CD47 upregulation. Cell Death Dis. 2019;10:138.
[28] Page MJ, McKenzie JE, Bossuyt PM, et al. 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
[29] Wells GA, Shea B, O'connell D, et al. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-analyses. Ottawa, Canada: Ottawa Hospital Research Institute; 2011.
[30] Shamseer L, Moher D, Clarke M, et al.; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015;350:g7647.
[31] Li L. Association of miRNA-146a Polymorphism with Risk of Cardiovascular Disease and Ischemia Stroke and the Mechanisms [master's thesis]. Changsha, China: Central South University; 2010.
[32] Sun J. Association of miRNA-146a and EPHX2 Polymorphisms with Risk of Ischemic Stroke in Changsha Han Population and the Mechanisms [master's thesis]. Changsha, China: Central South University; 2011.
[33] He S, Han Y. Association between miR-149 polymorphism and ischemic stroke of Han population in Hanzhong of Shanxi. J Mod Lab Med. 2013;2834:3238.
[34] Jeon YJ, Kim OJ, Kim SY, et al. Association of the miR-146a, miR-149, miR-196a2, and miR-499 polymorphisms with ischemic stroke and silent brain infarction risk. Arterioscler Thromb Vasc Biol. 2013;33:420–30.
[35] Hu Y, Li S, Jiang X, et al. Study on the association of miR-146aC>G, miR-149 T>C polymorphism with susceptibility to ischemic stroke. Prog Mod Biomed. 2014;14:5648–51.
[36] Liu Y, Ma Y, Zhang B, et al. Genetic polymorphisms in pre-miRNAs and risk of ischemic stroke in a Chinese population. J Mol Neurosci. 2014;52:473–80.
[37] Zhu R, Liu X, He Z, et al. miR-146a and miR-196a2 polymorphisms in patients with ischemic stroke in the northern Chinese Han population. Neurochem Res. 2014;39:1709–16.
[38] Huang S, Zhou S, Zhang Y, et al. Association of the genetic polymorphisms in pre-miRNAs with risk of ischemic stroke in a Chinese population. PLoS One. 2015;10:e0117007.
[39] Zhong H, Cai Y, Cheng J, et al. Apolipoprotein E Epsilon 4 enhances the association between the rs2910164 polymorphism of mir-146a and risk of atherosclerotic cerebral infarction. J Atheroscler Thromb. 2016;23:819–29.
[40] Qu JY, Xi J, Zhang YH, et al. Association of the miRNA-146a SNP rs2910164 with ischemic stroke incidence and prognosis in a Chinese population. Int J Mol Sci. 2016;17:660.
[41] Lyu G, Wang S, Wang Q. Association of miR-146a rs2910164 and miR-499 rs3746444 polymorphisms with risk of ischemic stroke. New Med. 2016;47:257.
[42] Lyu G, Wang S, Wang Q. Correlation analysis of single nucleotide polymorphisms of miR-196A2 and miR-149 with ischemic stroke. Prev Med. 2016;28:933.
[43] Luo HC, Luo QS, Wang CF, et al. Association of miR-146a, miR-149, miR-196a2, miR-499 gene polymorphisms with ischemic stroke in a Chinese people. Oncotarget. 2017;8:81295.
[44] Zhu H, Zhang H, Bao L, et al. Analysis of association of genetic polymorphisms of miRNAs with ischemic stroke. Chin J Med Genet. 2017;34:261–5.
[45] Fang JB. An Association Study between Ets-1 and miR-146a Gene Polymorphism and Ischemic Stroke [Ph.D. thesis]. Zhanjiang, China: Guangdong Medical University; 2016.
[46] Zhu X, Hou R, Ma A, et al. Associations of miR-146a, miR-149, miR-196a2, and miR-499 polymorphisms with ischemic stroke in the Northern Chinese Han Population. Med Sci Monit. 2018;24:7366–74.
[47] Hong SJ, Kim SK, Yun DH, et al. Association between miRNA-4669 polymorphism and ischemic stroke in a Korean population. Dis Markers. 2019;2019:7238319.
[48] Darabi H, Salmaninejad A, Jaripour ME, et al. Association of the genetic polymorphisms in immunoinflammatory miRNAs with risk of ischemic stroke and subtypes in an Iranian population. J Cell Physiol. 2019;234:3874–86.
[49] Zhu R, Wang QW, Zhao J, et al. miR-149 and miR-499 gene polymorphism and the incident of ischemic stroke in the Asian population: from a case-control study to meta-analysis. Clin Neurol Neurosurg. 2020;193:105789.
[50] Mahmoud D, El-Sisi O, Sheta M, et al. Association of miR-196a2 and miR-149 single-nucleotide polymorphisms with atherosclerotic ischemic stroke susceptibility. Egypt J Neurol Psychiatr Neurosurg. 2020;56:87.
[51] Wang M, Wang S, Wang X, et al. Carotid intima-media thickness, genetic risk, and ischemic stroke: a family-based study in rural China. Int J Environ Res Public Health. 2020;18:119.
[52] Abdelghany WM, Zoheir N, Abd Elhamid S, et al. Pre-miRNAs single nucleotide variants (rs3746444 A > G and rs2910164 C > G) increase the risk of ischemic stroke in the Egyptian population: a case–control study. Egypt J Med Hum Genet. 2022;38:23.
[53] Hammond SM. An overview of miRNAs. Adv Drug Deliv Rev. 2015;87:3–14.
[54] Du J, Cui C, Zhang S, et al. Association of MicroRNA-146a and MicroRNA-149 polymorphisms with strokes in Asian populations: an updated meta-analysis. Angiology. 2017;68:863–70.
[55] Li CX, Weng H, Zheng J, et al. Association between MicroRNAs polymorphisms and risk of ischemic stroke: a meta-analysis in Chinese individuals. Front Aging Neurosci. 2018;10:82.
[56] Zou D, Liu C, Zhang Q, et al. Association between polymorphisms in microRNAs and ischemic stroke in an Asian population: evidence based on 6,083 cases and 7,248 controls. Clin Interv Aging. 2018;13:1709–26.
[57] Wang Y, Li Q, Mambiya M, et al. A meta-analysis of the association between Microrna-196A2 and risk of ischemic stroke and coronary artery disease in Asian population. J Stroke Cerebrovasc Dis. 2018;27:3008–19.
[58] Bastami M, Choupani J, Saadatian Z, et al. miRNA polymorphisms and risk of cardio-cerebrovascular diseases: a systematic review and meta-analysis. Int J Mol Sci. 2019;20:293.
[59] Del Monte A, Arroyo AB, Andrés-Manzano MJ, et al. miR-146a deficiency in hematopoietic cells is not involved in the development of atherosclerosis. PLoS One. 2018;13:e0198932.
[60] Park H, Huang X, Lu C, et al. MicroRNA-146a and microRNA-146b regulate human dendritic cell apoptosis and cytokine production by targeting TRAF6 and IRAK1 proteins. J Biol Chem. 2015;290:2831–41.
[61] Li K, Ching D, Luk FS, et al. Apolipoprotein E enhances microRNA-146a in monocytes and macrophages to suppress nuclear factor-κB-driven inflammation and atherosclerosis. Circ Res. 2015;117:e1–11.
[62] El Gazzar M, Church A, Liu T, et al. miRNA-146a regulates both transcription silencing and translation disruption of TNF-α during TLR4-induced gene reprogramming. J Leukoc Biol. 2011;90:509–19.
[63] Kojima Y, Volkmer JP, McKenna K, et al. CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis. Nature. 2016;536:86–90.
[64] Luthra R, Singh RR, Luthra MG, et al. miRNA-196a targets annexin A1: a miRNA-mediated mechanism of annexin A1 downregulation in cancers. Oncogene. 2008;27:6667–78.
[65] Butcher MJ, Galkina EV. wRAPping up early monocyte and neutrophil recruitment in atherogenesis via Annexin A1/FPR2 signaling. Circ Res. 2015;116:774–7.
[66] Rawish E, Nording H, Münte T, et al. Platelets as mediators of neuroinflammation and thrombosis. Front Immunol. 2020;11:548631.
[67] Lievens D, Zernecke A, Seijkens T, et al. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. Blood. 2010;116:4317–27.
[68] Stoll G, Kleinschnitz C, Nieswandt B. Combating innate inflammation: a new paradigm for acute treatment of stroke? Ann N Y Acad Sci. 2010;1207:149–54.
[69] Ansari J, Senchenkova EY, Vital SA, et al. Targeting AnxA1/Fpr2/ALX pathway regulates neutrophil function promoting thrombo-inflammation resolution in sickle cell disease. Blood. 2021;137:1538–49.
[70] Senchenkova EY, Ansari J, Becker F, et al. A novel role for the AnxA1-Fpr2/ALX signaling axis as a key regulator of platelet function to promote resolution of inflammation. Circulation. 2019;140:319–35.
[71] Vital SA, Becker F, Holloway PM, et al. Formyl-Peptide Receptor 2/3/Lipoxin A4 receptor regulates neutrophil-platelet aggregation and attenuates cerebral inflammation: impact for therapy in cardiovascular disease. Circulation. 2016;133:2169–79.
[72] Ansari J, Kaur G, Gavins FNE. Therapeutic potential of Annexin A1 in ischemia reperfusion injury. Int J Mol Sci . 2018;19:1211.
[73] Vital SA, Senchenkova EY, Ansari J, et al. Targeting AnxA1/Formyl peptide receptor 2 pathway affords protection against pathological thrombo-inflammation. Cells. 2020;9:2473.
[74] Ding W, Li M, Sun T, et al. A polymorphism rs3746444 within the pre-miR-499 alters the maturation of miR-499-5p and its antiapoptotic function. J Cell Mol Med. 2018;22:5418–28.

[75] Xu Z, Han Y, Liu J, et al. MiR-135b-5p and MiR-499a-3p promote cell proliferation and migration in atherosclerosis by directly targeting MEF2C. Sci Rep. 2015;5:12276.
[76] Chen Q, Wu S, Wu Y, et al. MiR-149 suppresses the inflammatory response of chondrocytes in osteoarthritis by down-regulating the activation of TAK1/NF-κB. Biomed Pharmacother. 2018;101:763–8.
[77] Wang W, Han X, Zhao T, et al. AGT, targeted by miR-149-5p, promotes IL-6-induced inflammatory responses of chondrocytes in osteoarthritis via activating JAK2/STAT3 pathway. Clin Exp Rheumatol. 2020;38:1088–95.
[78] Hübner K, Karwelat D, Pietsch E, et al. NF-κB-mediated inhibition of microRNA-149-5p regulates Chitinase-3-like 1 expression in human airway epithelial cells. Cell Signal. 2020;67:109498.
[79] Wang X, Xu Q, Wang S. Overexpression of miR-149-5p attenuates cerebral ischemia/reperfusion (I/R) injury by targeting Notch2. Neuromolecular Med. 2022;24:279–89.
[80] Liang WJ, Zeng XY, Jiang SL, et al. Long non-coding RNA MALAT1 sponges miR-149 to promote inflammatory responses of LPS-induced acute lung injury by targeting MyD88. Cell Biol Int. 2019;44:317–28.
[81] Xu G, Zhang Z, Xing Y, et al. MicroRNA-149 negatively regulates TLR-triggered inflammatory response in macrophages by targeting MyD88. J Cell Biochem. 2014;115:919–27.
[82] Zhang B, Dong Y, Liu M, et al. miR-149-5p inhibits vascular smooth muscle cells proliferation, invasion, and migration by targeting Histone Deacetylase 4 (HDAC4). Med Sci Monit. 2019;25:7581–90.
[83] Peng W, Li T, Pi S, et al. Suppression of circular RNA circDHCR24 alleviates aortic smooth muscle cell proliferation and migration by targeting miR-149-5p/MMP9 axis. Biochem Biophys Res Commun. 2020;529:753–9.
[84] Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. Lancet Neurol. 2013;12:483–97.
[85] Cuadrado-Godia E, Dwivedi P, Sharma S, et al. Cerebral small vessel disease: a review focusing on pathophysiology, biomarkers, and machine learning strategies. J Stroke. 2018;20:302–20.
[86] Hattori Y, Okamoto Y, Maki T, et al. Silent information regulator 2 homolog 1 counters cerebral hypoperfusion injury by deacetylating endothelial nitric oxide synthase. Stroke. 2014;45:3403–11.
[87] Kitamura A, Saito S, Maki T, et al. Gradual cerebral hypoperfusion in spontaneously hypertensive rats induces slowly evolving white matter abnormalities and impairs working memory. J Cereb Blood Flow Metab. 2016;36:1592–602.
[88] Shafi O. Switching of vascular cells towards atherogenesis, and other factors contributing to atherosclerosis: a systematic review. Thromb J. 2020;18:28.